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GATA4 represses an ileal program of gene expression in the proximal small intestine by inhibiting the acetylation of histone H3, lysine 27

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ABSTRACT

GATA4 is expressed in the proximal 85% of small intestine where it promotes a proximal intestinal ('jejunal') identity while repressing a distal intestinal ('ileal') identity, but its molecular mechanisms are unclear. Here, we tested the hypothesis that GATA4 promotes a jejunal versus ileal identity in mouse intestine by directly activating and repressing specific subsets of absorptive enterocyte genes by modulating the acetylation of histone H3, lysine 27 (H3K27), a mark of active chromatin, at sites of GATA4 occupancy. Global analysis of mouse jejunal epithelium showed a statistically significant association of GATA4 occupancy with GATA4-regulated genes. Occupancy was equally distributed between down- and up-regulated targets, and occupancy sites showed a dichotomy of unique motif over-representation at down- versus up-regulated genes. H3K27ac enrichment at GATA4-binding loci that mapped to down-regulated genes (activation targets) was elevated, changed little upon conditional *Gata4* deletion, and was similar to control ileum, whereas H3K27ac enrichment at GATA4-binding loci that mapped to up-regulated genes (repression targets) was depleted, increased upon conditional *Gata4* deletion, and approached H3K27ac enrichment in wild-type control ileum. These data support the hypothesis that GATA4 both activates and represses intestinal genes, and show that GATA4 represses an ileal program of gene expression in the proximal small intestine by inhibiting the acetylation of H3K27.

1. Introduction

Cellular identity in adult organisms is fundamentally determined by the unique panel of genes expressed within that cell. Regulation of a distinct set of genes requires precise spatial and temporal coordination of a multitude of general and specific transcription factors at *cis*-regulatory elements within DNA [1]. Recognition and binding of transcription factors to specific DNA sequences occurs within the context of chromatin, whose dynamic structural characteristics play a significant role in regulating gene expression. Cell identity is thus determined by the influences of DNA sequence, transcription factor binding, and the epigenetic chromatin state within any given cell.

The absorptive enterocyte is a highly specialized cell lineage in the small intestinal epithelium that exhibits differential identities depending on its placement along the length of small intestine. Absorptive enterocytes in jejunum express digestive enzymes, transporters, and intracellular carriers necessary for the digestion and absorption of nutrients, while absorptive enterocytes in distal ileum express a discrete set of proteins that include, among others, bile acid transporters that function in the distal re-absorption of bile acids, the first step in their necessary enterohepatic circulation. Understanding how spatial differences in absorptive enterocyte identity are determined has implications for restoring regional functions that are lost due to disease processes or intestinal resection.

GATA4, a member of an ancient family of zinc finger transcription factors that bind WGATAR motifs in DNA (W = A or T, R = A or G), is a key regulator of regional identity in absorptive enterocyte gene expression and function [2–4]. GATA4 is expressed in the proximal 85% of small intestine, but is not expressed in distal ileum, highly coincident with the demarcation in changes in intestinal gene expression and function between proximal intestine and distal ileum [2,4,5]. Conditional deletion of *Gata4* in small intestine results in the transformation

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of absorptive enterocyte gene expression and function from a proximal intestinal to a distal ileal pattern [2–4]. Specifically, the subset of genes that are expressed at high levels in proximal small intestine, but not expressed in distal ileum, are down-regulated in proximal intestine. Conversely, the subset of genes not normally expressed in proximal small intestine, but highly expressed in distal ileum, are up-regulated in proximal small intestine. For example, the lactase (*Lct)* gene that is normally expressed in jejunum and proximal ileum, but not distal ileum, is significantly down-regulated in the proximal intestine, while the solute carrier family 10, member 2 (*Slc10a2)* gene that encodes the ileal-specific, apical sodium dependent bile acid transporter is significantly up-regulated in the proximal intestine [2,4], and bile acid absorption is induced [4]. Thus, by virtue of its restricted expression to the proximal 85% of small intestine, and its functions in both activating and repressing the expression of specific intestinal genes, GATA4 promotes a jejunal identity while repressing an ileal identity in absorptive enterocyte gene expression and function. GATA4 also functions redundantly with GATA6, which is expressed throughout the length of small intestine, including distal ileum, to regulate crypt cell proliferation and secretory cell differentiation, but due to the overlapping functions with GATA6, these processes are not altered in the proximal intestine of single *Gata4* knockout mice [6]. How GATA4 confers a 'jejunal' identity while repressing an 'ileal' identity on absorptive enterocytes of the proximal small intestine is the topic of this investigation.

Chromatin structure is determined by histone proteins, which undergo a multitude of covalent modifications that influence chromatin architecture and gene expression. One such modification is acetylation of histone H3, lysine 27 (H3K27ac), a histone modification mark that is highly correlated with open chromatin and gene transcription [7–10]. In cardiac and hematopoietic systems, GATA factors have been shown to interact with CBP/p300 [11–14], a transcriptional coactivator which has intrinsic histone acetyl-transferase activity and H3K27 as its substrate [8,9]. Chromatin occupancy of GATA1 in hematopoietic cells is highly correlated with H3K27ac enrichment [15–17] but little is known about the relationship between GATA4 and H3K27ac. In the present study, we tested the hypothesis that conditional deletion of *Gata4* results in the 'ilealization' of the H3K27ac chromatin mark. To test this hypothesis, we determined the global occupancy of GATA4 in mouse jejunal epithelium using an efficient in vivo biotinylation approach, mapped this occupancy to genes down- and up-regulated by conditional *Gata4* deletion, and compared H3K27ac enrichment at these loci in wild-type control jejunum to conditional *Gata4* knockout jejunum, and to wild-type control ileum. Our data implicate GATA4 as both an activator and repressor of specific subsets of target genes within the small intestine, and show that GATA4 activates a subset of genes by a process that is independent of H3K27ac modification, but represses a different subset of genes by inhibiting the acetylation of H3K27. These data implicate novel mechanisms of gene regulation by GATA factors, and contribute to our understanding of transcriptional regulatory mechanisms in the intestinal epithelium.

2. Materials and methods

2.1. Mice

Wild-type mice and previously established and confirmed *Gata4*^{flapflap} [18] transgenic *VillinCre*ER^{t2} [19], *Gata4*$^{flbio/flbio}$ [20] *and Rosa26*$^{BirA/BirA}$ [21] mice were used to establish the following groups of mice:

WT Ctl	*Gata4*$^{wt/wt}$
G4ΔIE	*Gata4*$^{flap/flap}$, Villin*Cre*ER^{T2}-positive
BirA Ctl	*Rosa26*$^{BirA/BirA}$
G4flbio	*Gata4*$^{flbio/flbio}$, *Rosa26*$^{BirA/BirA}$

Wild-type control (*WT Ctl*) mice express wild-type GATA4 under the control of the endogenous *Gata4* gene; *Gata4ΔIE* mice, after treatment with tamoxifen to excise floxed (fl) *Gata4* and induce expression of alkaline phosphatase (ap) from the *Gata4*^{flap} allele [20], do not express *Gata4* in the intestinal epithelium (IE); BirA control (*BirA Ctl*) mice express the bacterial biotinylation ligase enzyme BirA under the control of the *Rosa26* gene; and *Gata4flbio* mice express a modified GATA4 containing a FLAG (fl) and biotin ligase tag (bio) on its COOH-terminus that is efficiently biotinylated by BirA in vivo [22]. DNA was extracted from tail biopsies, and genotypes were determined by semiquantitative polymerase chain reaction (PCR) using previously validated primers [19–21]. Adult (6–8 weeks of age) *WT Ctl* and *Gata4ΔIE* mice were injected intraperitoneally with 1 mg tamoxifen (Sigma Chemical Co. Inc., St. Louis, MO) in sunflower seed oil (Sigma) once per day for 5 days to induce recombination of conditional alleles. At the time of tissue collection, mice were anesthetized and tissue was dissected as previously described [23]. Approval was obtained from the Institutional Animal Care and Use Committee.

2.2. RNA isolation and qRT-PCR

Mouse intestinal epithelial cells were isolated for RNA extraction by incubating freshly dissected segments (cut into 1 cm pieces) of small intestine in 15 ml conical tubes containing 30 mM ethylenediaminetetraacidic acid (EDTA) in 1X Weiser solution A [24], and intestinal epithelial cells were released from the lamina propria by vigorously shaking three times for 30 s and collected by centrifugation at 4 °C [25]. RNA was isolated from the small intestinal epithelial cells using the RNeasy kit (Qiagen Sciences, Germantown, MD) according to the manufacturer's instructions. To quantify mRNA abundances, quantitative reverse transcriptase (qRT)-PCR was conducted as described previously [23]. GAPDH mRNA abundance was measured for each sample and used to normalize the data. All data were expressed relative to the averages of the wild-type samples.

2.3. Nuclear extracts, biotin–streptavidin pull-down assays and Western blot

Intestinal epithelial cells were isolated from the middle 8 cm of the mouse small intestine (jejunum) as described above, and nuclear extracts were prepared as reported previously [26]. For biotin–streptavidin pull-down of GATA4, nuclear extracts were pre-cleared using protein A/G magnetic beads (Life Technologies), and incubated with streptavidin magnetic beads (M280, Life Technologies) overnight at 4 °C. Beads were washed 3 x 5 min in 1 ml buffer B (0.1% NP-40, 5 mM Tris–HCl, 100 mM KCl and 10% glycerol) at 4 °C and boiled in 30 μl 2x sample buffer for 10 min. Western blotting was performed as previously described [5] by transferring nuclear extracts to nitrocellulose membranes (Invitrogen) that were previously blocked with 5% nonfat dried milk in PBS. Membranes were then incubated with anti-mouse GATA4 (1:5000; SC-25310; Santa Cruz Biotechnology, Santa Cruz, CA) or anti-mouse β-actin (1:5000; A5441; Sigma) overnight at 4 °C, washed, incubated with horseradish peroxidase secondary antibodies and developed with SuperSignal West Femto ECL chemiluminescence solution (Thermo Fisher Scientific, Inc., Waltham, MA).

2.4. Biotin–streptavidin chromatin pull-down assays chromatin immunoprecipitation assays, and deep sequencing analysis

GATA4 chromatin occupancy was defined using biotin–streptavidin chromatin pull-down (BioChIP) assays [27], and H3K27ac enrichment was determined using chromatin immunoprecipitation (ChIP) assays [25]. In both assays, epithelial cells were isolated from the middle 8 cm (jejunum) and/or the distal 6 cm just proximal to the ileocecal junction. Epithelial fractions were cross-linked using 1% formaldehyde in phosphate-buffered saline (PBS) for 15 min at 4 °C, then for 28 min at room temperature, followed by sonication in lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl pH 8.1) using a Branson Sonifier (Branson Ultrasonic Corporation, Danbury, CT) fitted with a microprobe

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