

Transcription Factor Foxq1 Controls Mucin Gene Expression and Granule Content in Mouse Stomach Surface Mucous Cells

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Background & Aims: The gastric mucosa provides a stringent epithelial barrier and produces acid and enzymes that initiate digestion. In this regenerating tissue, progenitors differentiate continually into 4 principal specialized cell types, yet underlying mechanisms of differentiation are poorly understood. We identified stomach-restricted expression of the forkhead transcription factor FOXQ1. **Methods:** We used a combination of genetic, histochemical, ultrastructural, and molecular analysis to study gastric cell lineages with respect to FOXQ1. **Results:** Within the developing and adult gastrointestinal tract, *Foxq1* messenger RNA (mRNA) is restricted to the stomach and expressed predominantly in foveolar (pit) cells, the abundant mucin-producing cells that line the mucosal surface. Mice carrying *Foxq1* coding mutations show virtual absence of mRNA and protein for the backbone of the major stomach mucin MUC5AC. These observations correspond to a paucity of foveolar cell secretory vesicles and notable loss of stomach but not intestinal mucus. Transcriptional profiling identified a surprisingly restricted set of genes with altered expression in *Foxq1* mutant stomachs. MUC5AC is a highly tissue-restricted product that similarly depends on FOXQ1 in its other major site of expression, conjunctival goblet cells. **Conclusions:** Taken together, these observations imply that promotion of gastric MUC5AC synthesis is a primary, cell-autonomous function of FOXQ1. This study is the first to implicate a transcription factor in terminal differentiation of foveolar cells and begins to define the requirements to assemble highly specialized organelles and cells in the gastric mucosa.

The gastric mucosa contains 4 highly specialized cell types that differentiate from a common progenitor and help execute the stomach's digestive functions. Although much is known about the morphology and physiology of these cell types, few transcriptional regulators that govern their differentiation have been characterized.¹⁻³ Surface mucous cells, also known as foveolar or pit cells, line the lumen of the glandular stomach in the corpus and antrum, including mucosal pits that vary in depth in different areas.⁴ Acid-secreting parietal cells

dominate the corpus mucosa, which also houses zymogenic chief cells. These 2 cell lineages are excluded from the gastric antrum, where the proportion of foveolar cells is accordingly increased. Foveolar cells constitutively secrete a viscous mucus that protects the gastric epithelium from damage.

Secretory epithelial cells in different organs utilize distinct mucin polypeptides as the backbone for extensive glycosylation and mucus synthesis. Although more than one mucin may be present in a cell lineage, single mucin types tend to predominate and represent the major product of specialized secretory cells. Intestinal goblet cells produce only MUC2; in the stomach, mucous neck cells and basal cells in the antrum produce MUC6, whereas foveolar cells produce MUC5AC.⁵ Whereas production of particular mucin peptides is a hallmark of gastric mucous cells, other markers distinguish these cell lineages further: pit cells express gastrophilin-1 and Trefoil factor (Tff) 1, whereas mucous neck cells produce Tff2.^{6,7} MUC5AC is also produced by goblet cells in the conjunctiva, the stratified columnar epithelial lining of the eye.⁸ Regulation of individual mucin genes can thus provide important clues about lineage-specific cell differentiation in surface epithelia.

In a screen for transcription factor (TF) genes that are differentially expressed in developing mouse stomach and intestine,⁹ we observed that *Foxq1* messenger RNA (mRNA) is excluded from the intestine and expressed selectively in the stomach. Previous studies have noted *Foxq1* expression in the stomach of various species, but its exact function in this organ is unknown.¹⁰⁻¹³ To understand these functions, we studied *Satin* mice, a radiation-induced mutant strain that is homozygous for a null *Foxq1* allele.¹⁴ We observed a specific and significant defect in gastric mucin production and secretory granule biogenesis in gastric foveolar cells and traced these defects to virtually complete absence of MUC5AC and its glycosylated end products. We confirmed the findings in independent strains of *Foxq1* mutant mice

Abbreviations used in this paper: ENU, ethylnitrosourea; PAS, periodic acid-Schiff; TF, transcription factor.

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and also found absence of MUC5AC in conjunctival goblet cells. A combination of studies in vitro and in animals indicated that the forkhead protein FOXQ1 has a limited but essential function in *Muc5ac* gene regulation. FOXQ1 is the first TF to be implicated in terminal differentiation of stomach foveolar cells.

Materials and Methods

Mice

Mice were housed under pathogen-free conditions and handled according to protocols approved by an institutional Animal Care and Use Committee. *Satin* (SB/LeJ), *Beige*, 129/Sv, and C57/BL6 mice were obtained from The Jackson Laboratories (Bar Harbor, ME) and CD1 mice from Charles River Laboratories (Wilmington, MA), and mixed genetic background mice were generated by interbreeding. *Foxq1*^{ENU/+} mice were resurrected from cryopreserved sperm at the Mutant Mouse Regional Resource Center at the University of California, Davis. Genotyping for *Foxq1* alleles was done by polymerase chain reaction (PCR) as described previously.¹⁴

Expression Analyses, Histology, Immunohistochemistry, Electron Microscopy, and Transcriptional Reporter Assays

Details on expression analyses, histology, immunohistochemistry, electron microscopy, and transcriptional reporter assays are all included in the Supplementary Materials (see Supplementary Materials online at www.gastrojournal.org).

Microarray Expression Analysis

Stomachs from age-matched C57BL/6, *Beige*, and SB/LeJ mice were harvested immediately after mice were killed, then washed, and the antrum isolated. RNA was extracted with Trizol reagent, labeled, and hybridized to 430A2.0 Mouse Expression Arrays (Affymetrix, Santa Clara, CA). Data were analyzed using dChip software¹⁵ and deposited in the GEO public database, with accession number GSE8943.

Results

Stomach-Restricted Foxq1 Expression

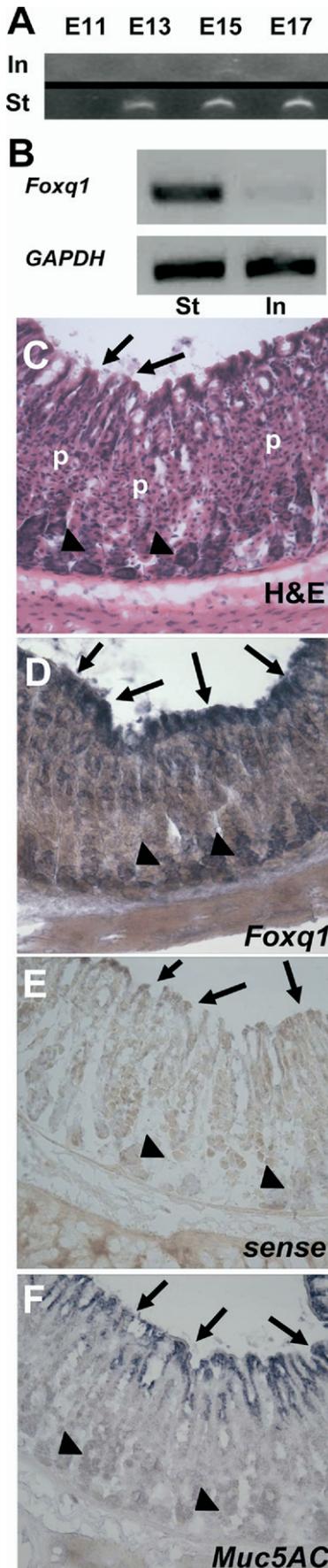
Transcriptional regulation of cell-specific gene expression in the gastrointestinal (GI) tract is not well understood. To take steps toward identifying relevant pathways, we recently surveyed the temporal and spatial expression of all known and predicted TF genes in developing mouse gut.⁹ Among mRNA that are restricted to the stomach, we identified the forkhead TF *Foxq1*. *Foxq1* transcripts first appear on embryonic day 13, and stomach-restricted expression is maintained throughout development (Figure 1A). Similarly, *Foxq1* transcripts are restricted to the adult stomach and absent from adult intestine (Figure 1B). Further inspection of *Foxq1* expres-

sion in the stomach corpus (where foveolar, parietal, and chief cells are evident by histochemical staining; Figure 1C) by in situ hybridization indicated that *Foxq1* transcripts in the adult stomach are restricted largely to surface mucous (pit or foveolar) cells (Figure 1D), which are characterized by robust expression of the lineage marker *Muc5AC* (Figure 1F). *Foxq1* transcripts were also detected in pepsinogenic chief cells at the base of gastric gland units (Figure 1D), although signals were considerably weaker than in surface mucous cells and may represent nonspecific background staining. Control (sense) probes typically gave no staining (Figure 1E). Previous studies using laser capture microdissection found foveolar cells enriched and chief cells lacking in *Foxq1* transcripts, consistent with our results.^{3,16}

Delineation of Foxq1^{sa/sa} Stomach Abnormalities

To study FOXQ1 functions, we took advantage of an existing recessive mutant mouse strain, *Satin* (*Sa*), which was generated by radiation mutagenesis¹⁷ and recognized originally by a shiny pelage resulting from disorganized hair-shaft medullae.^{14,18} The genetic defect maps to a *Foxq1* nonsense mutation that eliminates the C-terminal 112 amino acids of a 400-residue protein.¹⁴ Serial backcrosses isolated the mutation on a homogeneous genetic background with tight linkage to an additional mutation, *beige*; the resulting strain, SB/LeJ, is thus homozygous for both *Foxq1*^{sa} and *Lyst*^{bs} alleles.¹⁷ *Beige*, a mutant allele of the *Lyst* lysosomal transport gene,^{19–23} increases susceptibility to infection owing to immune dysfunction but has no known role in stomach mucosa. The hair follicle defect in the *Satin* strain is well characterized,^{14,24} but the animals seem otherwise normal and stomach defects have not been investigated.

SB/LeJ mice (which we designate *Foxq1*^{sb/sb}) show normal activity, feeding, growth, fertility, and life span; and gross stomach morphology is intact, without mucosal ulceration or tumors. Histologic examination of *Foxq1*^{sb/sb} stomachs revealed a normal mucosa (Figure 2A), with differentiated cell types present in normal numbers and distribution, judging by the following immunohistochemical markers: H/K-ATPase for parietal cells, gastrin for antral G-cells, and pepsinogen and intrinsic factor for chief cells (Figure 2I, and data not shown). Alcian blue staining for acidic mucins also showed the typical weak signal in basal mucous cells in the antrum (data not shown). By contrast, periodic acid-Schiff (PAS) staining, which identifies the neutral mucins secreted by foveolar cells, revealed a dramatic and completely penetrant defect (Figure 2B and C vs Figure 2F and G; n = 10): some glands retained a faint rim of extracellular signal, but none displayed the prominent intracellular apical staining characteristic of control animals. The full scope of the defect can be appreciated in low-magnification photomicrographs (see Supplementary Figure 1A–D online



at www.gastrojournal.org) and quantitation of stained gland units (see Supplementary Figure 2A online at www.gastrojournal.org). Defective mucin expression was confined to the stomach surface; PAS staining of intestinal goblet cells was unaffected (Figure 2D and H).

High-resolution light microscopy revealed absence of the apical zone of PAS staining in *Foxq1^{sb/sb}* pit cells (Figure 3A and B), and we used transmission electron microscopy to characterize the defect further. We observed normal size, shape, and polarity of *Foxq1^{sb/sb}* foveolar cells (Figure 3E), but apical mucous granule numbers were markedly reduced compared with controls (Figure 3C and D). In mutant animals, a few of these granules showed the typical morphology and electron density, but most were reduced in both size and density. The prominent reduction in PAS staining can thus be attributed to a significant defect in the organelles that store and release neutral gastric mucin.

Selective Loss of MUC5AC in the Absence of Foxq1 Function

To distinguish whether paucity of apical granules in *Foxq1^{sb/sb}* pit cells reflects absence of MUC5AC synthesis or a failure to glycosylate and store the protein, we used a specific antibody. MUC5AC protein was completely absent from *Foxq1^{sa/sa}* stomach samples (Figure 4A, n = 5), indicating that FOXQ1 is required to produce the polypeptide backbone for neutral stomach mucin. Judging by other stains, including Alcian blue, synthesis of other mucins, Muc2 and Muc6, is intact in the absence of FOXQ1 function (data not shown). Gastrokine-1, another secreted and granule-bound pit cell product of unknown function,⁷ is also expressed normally in *Foxq1^{sb/sb}* surface mucous cells (Figure 4A). Finally, quantitative reverse transcription (RT)-PCR analysis of *Foxq1^{sb/sb}* stomach revealed normal *gastrokine-1*, *Mucin 1*, and stomach trefoil-family factor *TFF1* mRNA levels, whereas *Muc5ac* transcripts were reduced to <3% of levels observed in control samples (Figure 4B).

These results reveal virtual absence of *Muc5ac* mRNA and hint at selective loss of MUC5AC among apical granule contents. To determine the potentially broader

Figure 1. Restricted *Foxq1* expression in fetal and adult stomach. (A) RT-PCR results from the GIFT database.⁹ RNA from wild-type murine stomach (St) and intestine (In) at 4 different fetal stages (E11, E13, E15, E17) probed using *Foxq1*-specific primers. *Foxq1* is expressed in the stomach with increasing abundance as the embryo approaches birth but not detected in intestine. (B) RT-PCR in adult mouse stomach shows robust *Foxq1* mRNA levels relative to the intestine; *GAPDH* was used as a loading control. (D–F) In situ hybridization on adult wild-type murine stomach using *Foxq1* antisense probe (D), sense control (E), or *Muc5AC* antisense probe (F). The *Foxq1* probe gave strong signal in surface epithelium (arrows) throughout the glandular stomach (images shown here are taken from the corpus, with an H&E-stained section as a reference [C]) and considerably weaker signal in chief cells (arrowheads); *Muc5ac* is specific to surface mucous cells (arrows in F). p, Parietal cells.

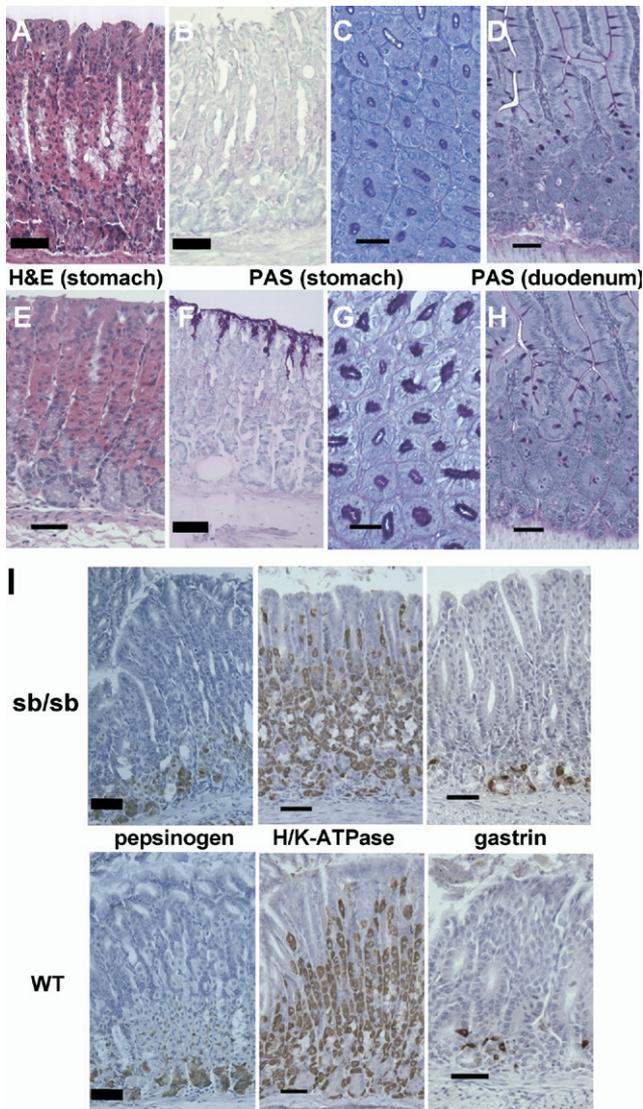


Figure 2. *Foxq1^{sb/sb}* stomachs are deficient in surface mucin. (A–H) Adult mouse stomach or duodenal tissue (*Foxq1^{sb/sb}* in top 4 panels and controls in lower 4 panels) stained with H&E or periodic acid-Schiff (PAS), as indicated. H&E staining reveals normal character of gastric glandular mucosa; images are taken from the gastric corpus. PAS stain reveals marked attenuation of surface epithelial mucous staining in *Foxq1^{sb/sb}* stomach (B and C) but normal duodenal goblet-cell staining (D). (I) Immunohistochemistry of adult murine gastric mucosa (*Foxq1^{sb/sb}* in top 3 panels and wild type in lower 3 panels), showing normal distribution of markers for chief (pepsinogen) and parietal (H/K-ATPase) cells in the corpus and enteroendocrine G (gastrin) cells in the antrum. Scale bars, 60 μ m for PAS-stained cross sections (C and G), 120 μ m for all others. Low-power photomicrographs are shown in Supplementary Figure 1A–D (see Supplementary Figure 1A–D online at www.gastrojournal.org).

scope of *Foxq1* function in foveolar cells, we used oligonucleotide microarrays to profile mRNA expression in the gastric antrum. The epithelium of the antrum, the most distal portion of the stomach, carries no zymogenic or parietal cells and a correspondingly high fraction of pit cells⁴; accordingly, differences in gene expression re-

lated to absence of *Foxq1* function should be most evident in this region. Because *Foxq1^{sb/sb}* mice carry tightly linked mutations in the *Foxq1* and *Lyst* genes, we also used expression profiling to compare *Foxq1^{sb/sb}* stomach with that from the *Beige* strain, which carries a mutation only in the *Lyst* gene and is congenic with the C57BL/6 line.²⁵ Analysis of antral RNA from *Foxq1^{sb/sb}*, *Beige*, and C57BL/6 control mice disclosed fewer than 20 genes with >4-fold reduction in *Foxq1^{sb/sb}* samples, and *Muc5ac* was 1 of only 4 transcripts reduced >10-fold in *Foxq1^{sb/sb}* antrum (Figure 4C). RT-PCR on independent stomach samples validated the change in *Fabp1* mRNA recorded in expression profiling (see Supplementary Figure 2B online at www.gastrojournal.org). These results together impli-

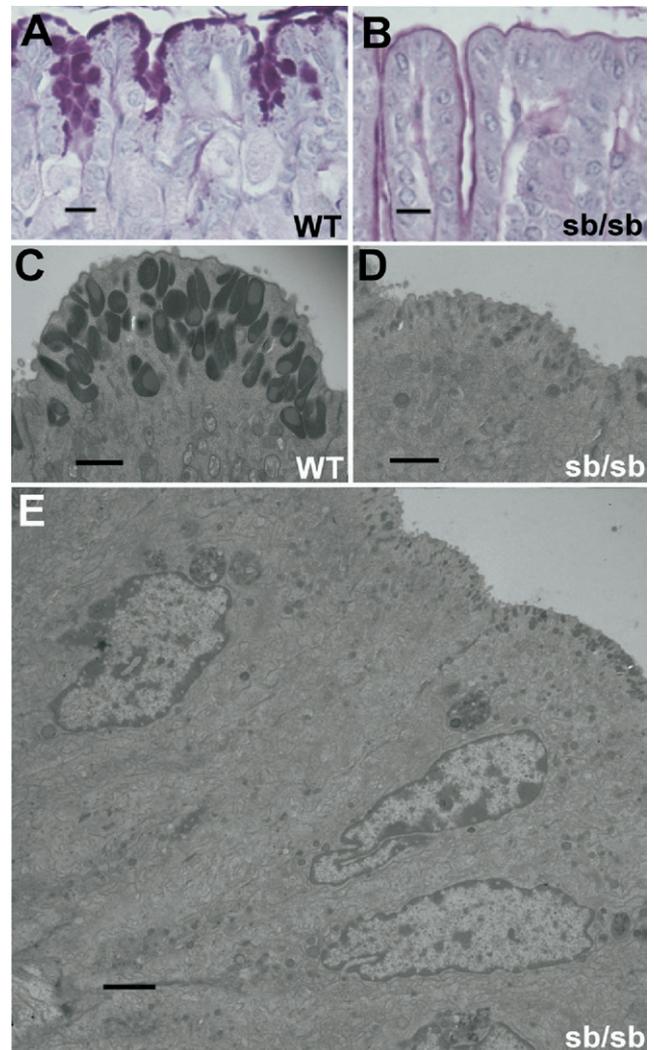


Figure 3. Foveolar-cell apical granule deficiency in *Foxq1^{sb/sb}* stomach. (A and B) High-magnification view of PAS-stained gastric foveolar cells from adult wild-type (A) and *Foxq1^{sb/sb}* (B) mice. (C and D) Transmission electron microscopy of adult gastric foveolar cells, emphasizing the apical granule zone in wild-type (C) and *Foxq1^{sb/sb}* (D) cells. (E) Full ultrastructural profile of *Foxq1^{sa/sa}* gastric foveolar cells, indicating intact architecture except for apical granule deficiency. Scale bars, A and B, 60 μ m; C and D, 1 μ m; E, 2 μ m.

cate stomach *Foxq1* function in a restricted range of activities; *Foxq1^{sb/sb}* surface mucous cells seem intact in most respects but deficient in apical granules and their principal protein product, MUC5AC, likely reflecting substantially reduced *Muc5ac* gene transcription.

Validation of the Role of *Foxq1* in Foveolar Cell Function

Because the parental strain on which the *Foxq1^{sb/sb}* mutations appeared is no longer available, in the forego-

ing histochemical analyses we used a panel of laboratory mouse strains (CD1, C57BL/6, and 129/Sv-C57BL/6 hybrids) as controls. In contrast to *Foxq1^{sb/sb}* stomach, we observed abundant apical mucus staining in every control (Figure 5A, and data not shown), suggesting that strain background is unlikely to account for the *Foxq1^{sb/sb}* phenotype. The only other mutation in this strain maps to the closely linked *Lyst* gene,^{19-21,25} which regulates biogenesis and transport of membranous organelles and enables lysosome-mediated plasma membrane repair.^{26,27} In hierarchical analysis of mRNA expression profiles, *Beige* and C57BL/6 antra clustered together, whereas *Satin* samples clustered separately (Figure 4C; the cluster dendrogram reflects the complete array data set). To exclude further the possibility that pit cell defects in *Foxq1^{sb/sb}* mice might reflect *Lyst* gene inactivity, we examined *bg/bg* mice more closely. PAS staining and MUC5AC immunohistochemistry of adult *bg/bg* stomach were similar to congenic C57BL/6 controls, with abundant signal in surface mucous cells (Figure 5A and see Supplementary Figure 3 online at www.gastrojournal.org). Thus, *LYST* deficiency alone cannot account for the foveolar cell defect, which likely results from the *Foxq1^{sa}* mutation rather than the *Lyst^{bg}* allele.

These results do not, however, exclude the formal possibility that combined mutation of the *Foxq1* and *Lyst* genes is required to produce the stomach pit cell phenotype. Because tight linkage of the *satin* and *beige* loci prohibits their separation, we asked whether another *Foxq1* mutant strain, generated independently by ethylnitrosourea (ENU) mutagenesis,¹⁴ carries the same foveolar cell defect. In a previous study, *Foxq1^{enu}* mice phenocopied the hair defect when crossed to *Satin* mice, but *Foxq1^{enu/sb}* stomachs were not examined.¹² Because *Foxq1^{enu/enu}* mice were not viable at weaning in our crosses

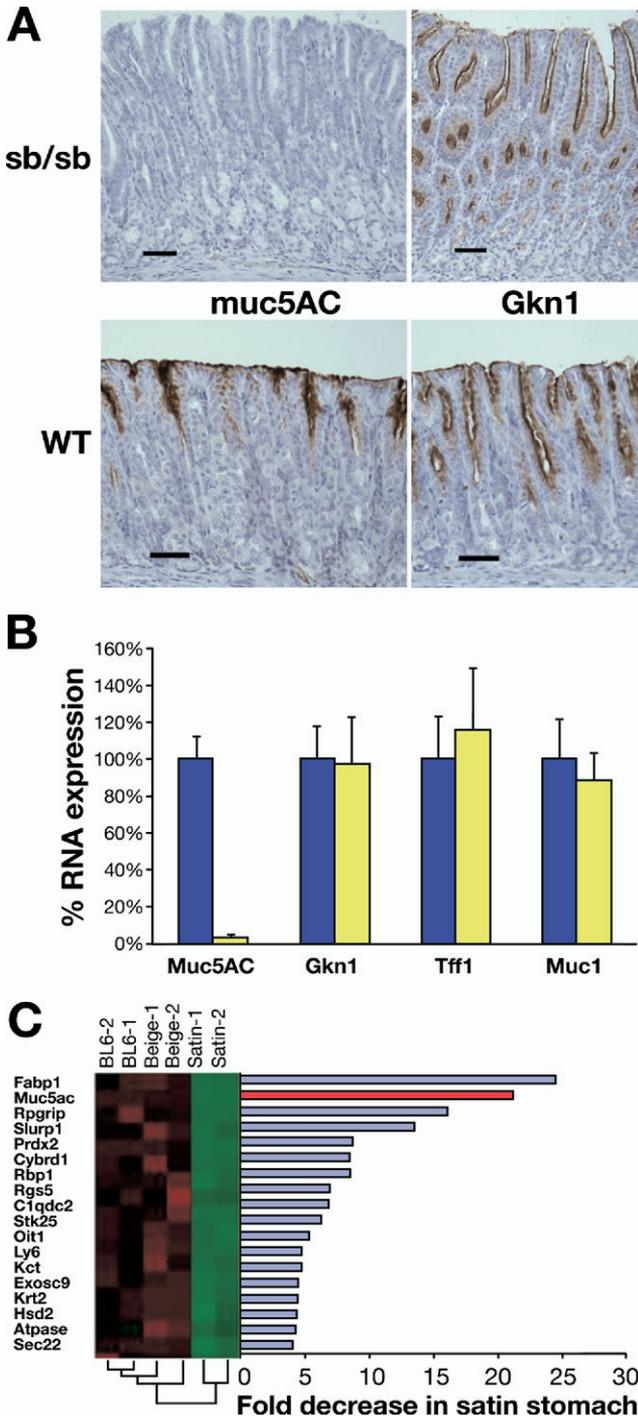


Figure 4. *Foxq1^{sb/sb}* fail to produce MUC5AC protein or mRNA. (A) Immunohistochemistry of adult *Foxq1^{sa/sa}* (top) and wild-type (bottom) mouse gastric mucosa for MUC5AC (left) and gastrokine-1 (Gkn1, right), indicating absence of MUC5AC but preserved Gkn1 expression in *Foxq1^{sb/sb}* stomach; scale bars, 120 μm. (B) qRT-PCR analysis of selected mucins and secreted foveolar cell products: *Muc5AC*, *Gkn1*, *trefoil factor 1* (*Tff1*), and *Muc1*. mRNA expression was first normalized against *Gapdh*, and the levels in *Foxq1^{sb/sb}* stomach (yellow bars) are expressed as a percentage of wild-type levels (blue bars, 100%). The results reveal marked attenuation of *Muc5ac* transcripts, with intact expression of all other tested markers. (C) Graphic depiction of relative mRNA levels revealed in expression microarray analysis of stomach antra from age-matched wild-type C57BL/6 (BL6), *Beige*, and *Foxq1^{sb/sb}* (*Satin*) adult mice. Most transcripts showed nearly identical expression in the 3 strains; data for all mRNA with >4-fold decrease are shown in expression-heat maps (red, high expression; green, low expression) and as a bar graph. *Muc5ac* was the second most differentially expressed transcript (red bar). The dendrogram below the heat map depicts results of hierarchical clustering analysis of the samples for all probes and indicates greatest similarity first between duplicate samples and secondly between BL6 and *Beige*; *Foxq1^{sb/sb}* (*Satin*) samples cluster separately.

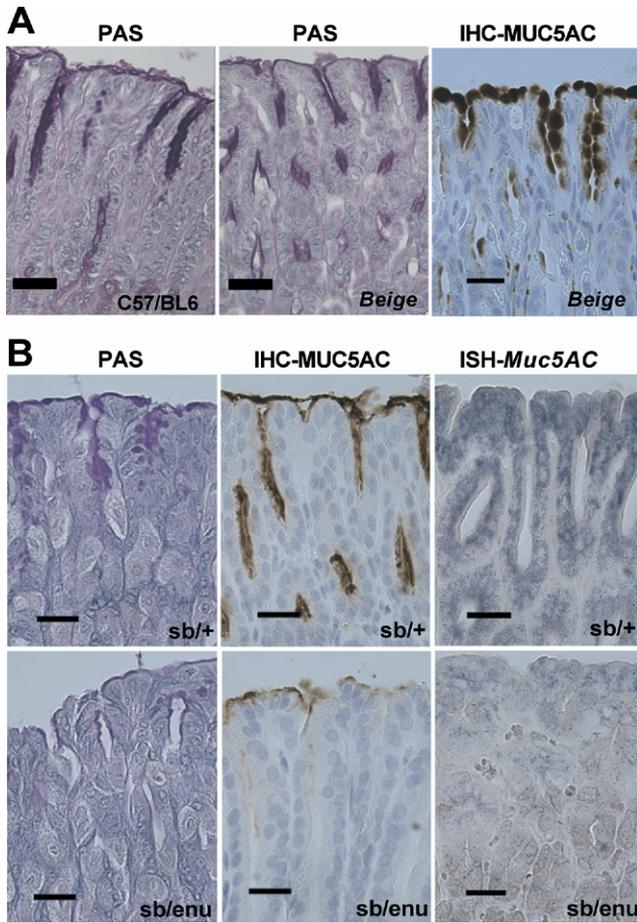


Figure 5. *Foxq1*^{sb/sb} stomach defects specifically reflect *Foxq1* gene mutations. (A) Staining of gastric mucosa from C57BL/6 (left) and *Beige* (right 2 panels) mice for PAS (left 2 panels) and MUC5AC immunohistochemistry (IHC, far right panel), showing normal expression of neutral stomach mucins and MUC5AC. Images are taken from the gastric corpus, and additional data are shown in Supplementary Figure 3 (see Supplementary Figure 3 online at www.gastrojournal.org). (B) Complementation analysis of the *Foxq1* gene: examination of *Foxq1*^{sb/+} (top row, control) and *Foxq1*^{sb/enu} (bottom row) stomach corpus for PAS stain (left), stomach antrum for MUC5AC immunostain (middle), and *Muc5ac* in situ hybridization (right). The *Foxq1*^{sb/enu} strain effectively phenocopies *Satin* (*Foxq1*^{sb/sb}) mice, and the results hint that the *Foxq1*^{enu} allele may be hypomorphic. Additional data are shown in Supplementary Figure 4 (see Supplementary Figure 4 online at www.gastrojournal.org). Scale bars, 60 μ m.

($P = .008$), we generated *Foxq1*^{enu/sb} mice. Unlike *Foxq1*^{enu/+} or *Foxq1*^{sb/+} littermates, compound heterozygous *Foxq1*^{enu/sb} mice showed loss of PAS staining (Figure 5B and see Supplementary Figure 4 online at www.gastrojournal.org, $n = 3$), similar to SB/LeJ, and hence provide genetic proof that *Foxq1* is responsible for the phenotype. Very weak MUC5AC expression in *Foxq1*^{enu/sb} stomachs, which we detected by in situ hybridization and immunohistochemistry (Figure 5B and see Supplementary Figure 4 online at www.gastrojournal.org), implies that the *Foxq1*^{enu} allele is hypomorphic for stomach func-

tion. Such a hypomorphic phenotype may result from the relatively subtle missense mutation in the *Foxq1*^{enu} allele (Ile128Ser in the winged helix domain) compared with the nonsense mutation in the SB/LeJ strain.¹⁴ These results collectively indicate that Muc5AC production in gastric pit cells depends on *Foxq1*.

The Role of FOXQ1 in *Muc5ac* Gene Regulation

Because FOXQ1 is a forkhead protein with a likely role in transcriptional regulation and *Muc5ac* mRNA levels are low in its absence, we asked whether FOXQ1 may regulate the *Muc5ac* gene directly. Previous study of the mouse *Muc5ac* promoter found that it could be activated by transforming growth factor- β signaling, along with Smad- and Sp1-family TFs.²⁸ Whereas FOXQ1 is reported to bind an AT-rich sequence in the *telokin* promoter, its consensus DNA recognition sequence is unknown.¹³ However, most forkhead TFs recognize the sequence RYMAAYA,²⁹ and the binding preference for FOXF2, which is closely related to FOXQ1,³⁰ has been determined empirically.³¹ We identified an evolutionarily conserved forkhead consensus binding sequence in the mouse *Muc5ac* promoter, 100 base pairs upstream of the transcriptional start site (Figure 6A). To assess the function of this site, we cloned the *Muc5ac* promoter sequence from -199 to $+3$ upstream of the firefly luciferase gene and tested its ability to activate reporter gene expression. Compared with a promoterless reporter construct, this *Muc5ac* promoter fragment induced robust expression of the reporter gene in CMT-93 colonic epithelial cells, a cell line chosen on the basis of demonstrated *Muc5ac* promoter activity,²⁷ as well as in the human gastric cancer cell line Kato-III (Figure 6B). However, deletion of 2 core nucleotides in the putative forkhead element (TGTTTAC \rightarrow TG-TAC) had little effect on promoter activity (Figure 6B), and cotransfection of a *Foxq1* expression plasmid did not increase it (data not shown).

To identify potential *Muc5ac* enhancers that may fall under FOXQ1 control, we searched for conserved intergenic sequences. Only 2 conserved regions (75% and 72% homology between mouse and human) contained forkhead consensus sequences (Figure 6C); no other regions are conserved in either direction until the next structural genes. We cloned these regions upstream of the *Muc5ac* promoter-reporter and tested FOXQ1-dependent transcriptional activation but observed no enhancement over promoter activity alone (data not shown). Thus, despite identification of putative FOXQ1 *cis*-elements, we gathered no conclusive evidence for direct *Muc5ac* gene regulation by FOXQ1. It is, however, important to note that Fox proteins may remodel chromatin, a function not accurately reflected in plasmid-based reporter assays.^{32,33}

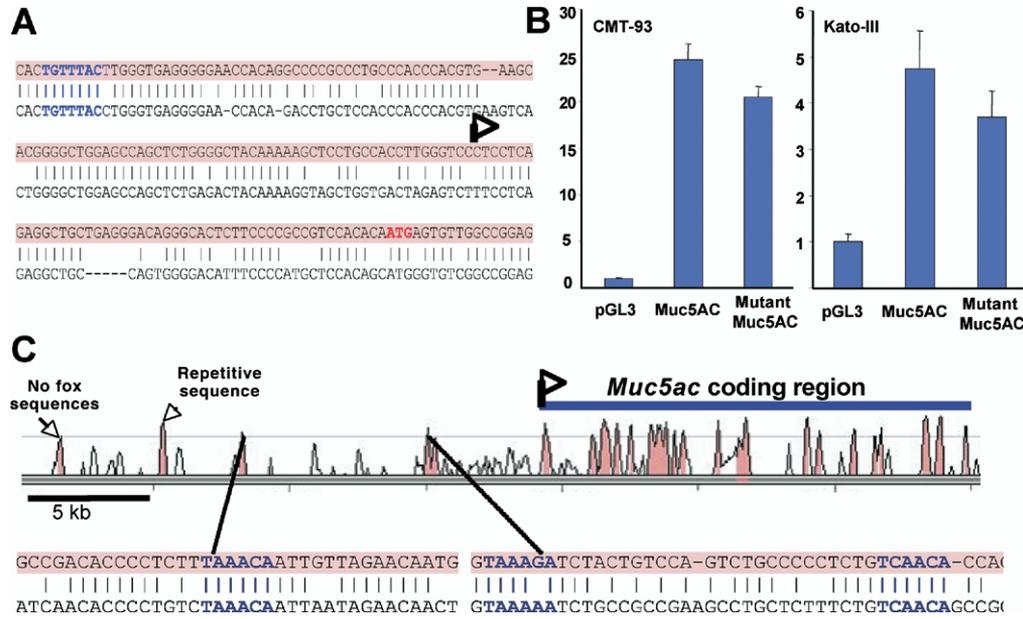


Figure 6. Conserved regions in the mouse *Foxq1* locus contain consensus forkhead-binding sites, but these may not activate *Muc5ac* gene transcription directly. (A) Comparison of *Muc5ac* promoter sequences from mouse (top, shaded) and human (bottom) reveals a conserved Foxf2 consensus element (blue type) within a region previously shown to be essential for *Muc5ac* promoter activity.²⁸ (B) A firefly luciferase reporter under control of the native or FOX site-mutant murine *Muc5ac* promoter was transfected into CMT-93 or Kato-III cells, and luciferase activity was compared with pGL3 promoterless control. Both constructs activated the reporter gene equally, indicating lack of a requirement for the putative FOX-binding activity in reporter assays on this promoter. (C) Sequence conservation profile of the murine *Foxq1* locus, adapted from Vista Browser (<http://pipeline.lbl.gov/cgi-bin/gateway2>). Conservation was highest in the coding region, but narrow peaks (>70% identity, pink shading) were also identified in the promoter and 4 distant regions, 1 that contains repetitive elements and 2 that carry consensus forkhead-binding elements, T(A/c)AA(c/t)A. Sequence from these 2 regions is shown below, with alignment of mouse (top, shaded) and human (bottom) sequences and bold highlighting of putative forkhead-binding elements.

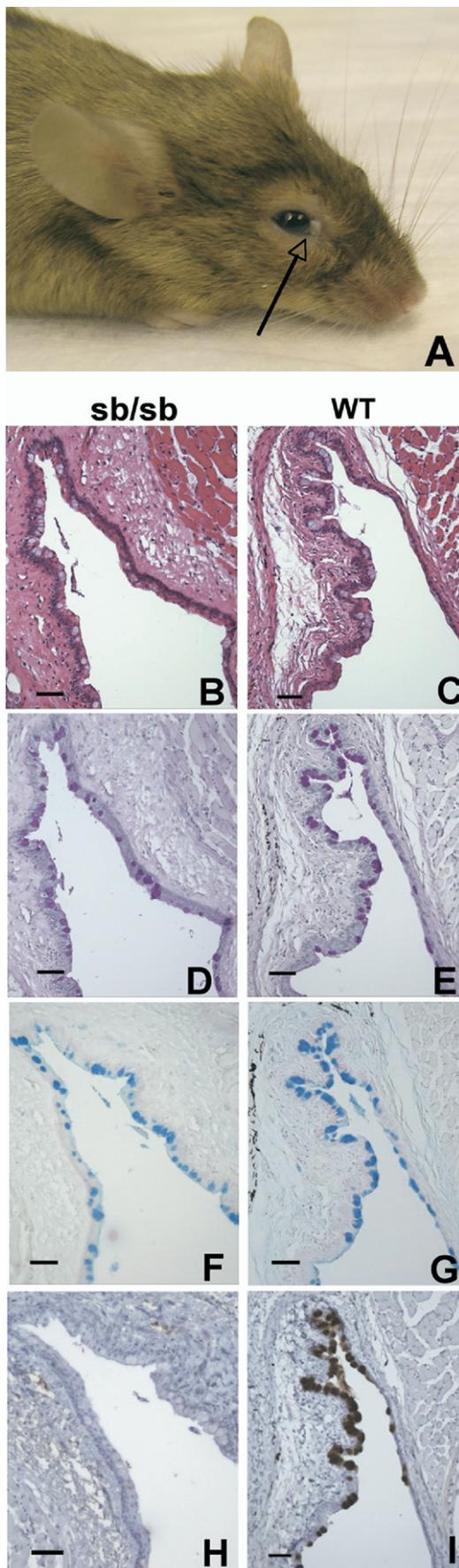
Muc5ac Expression Defects in Satin Mice Are Not Restricted to the Stomach

More than half of *Foxq1*^{sb/sb} mice over 9 months old developed ocular surface abnormalities and accumulated surface debris, which impaired opening of one or both eyes (Figure 7A). Other animals maintained in the same colony never showed the same pathology, and we noted that *Muc5ac* is also present in conjunctival goblet cells.⁸ Most conjunctival epithelial cells produce the membrane-spanning mucins Muc1 and Muc4.⁸ Goblet cells occupy the conjunctival fornix; resemble intestinal goblet cells in morphology; and, like mucus in the GI tract, secrete products that function to lubricate the surface, clear debris, and provide antimicrobial defense.³⁴ Initial inspection revealed the typical frequency and appearance of goblet cells in *Foxq1*^{sb/sb} conjunctivae, and mutant goblet cells stained with both PAS and Alcian blue, similar to controls (Figure 7B–G); these findings differ from the dramatic loss of PAS signal in *Foxq1*^{sb/sb} stomach (Figure 2A). However, conjunctival goblet cells differ from gastric pit cells, which express only MUC5AC, in that they express multiple mucin glycoproteins.³⁴ Indeed, immunostaining revealed striking absence of MUC5AC in *Foxq1*^{sb/sb} conjunctival goblet cells (Figure 7H). *Muc5ac* gene expression thus appears to depend on *Foxq1* function in more than one tissue, and its absence

from the conjunctiva is sufficient to produce an overt ocular phenotype.

Discussion

Surface GI epithelia engage in continuous self-renewal and differentiation of highly specialized cells. Few genetic studies have identified TFs that are responsible for particular cell features, especially in the stomach.^{1–3} Here, we report the characterization of a TF required for normal gastric foveolar cell differentiation. We show that *Foxq1* mutations in mice severely limit foveolar cells’ ability to synthesize MUC5AC and to fill the secretory granules that characterize this unique cell lineage. Besides MUC5AC, other foveolar cell products such as TFF1 and Gkn1, which are also stored in mucous granules,^{7,35–37} are unaffected by loss of *Foxq1* function, and expression profiling revealed a narrow spectrum of dys-regulated genes. In this light, we expected to find normal numbers and appearance of pit cell mucous granules, but *Foxq1*^{sb/sb} stomach ultrastructure disclosed fewer and smaller granules. This suggests that absence of MUC5AC, the backbone for the major content of these granules (neutral stomach-specific mucin), may preclude normal mucous granule formation or stability, similar to the effect of MUC2 loss on intestinal goblet cells.³⁸ On the other hand, mutant pit cells are not devoid of granules,



and gastrokine-1 immunostaining localizes correctly to the cell apex. The sum of these findings is explained most conservatively by failure of granule filling when cellular MUC5AC levels are limiting, which suggests that *Foxq1* may have evolved to fulfill a limited but essential function in pit cells. RNA microarray analysis revealed a handful of additional genes that are dysregulated in *Foxq1* mutant stomach, including *Rpgrip*, *Sec22*, and *Stk25*, which have known roles in assembly and function of membranous organelles.³⁹⁻⁴¹ These data raise the particular possibility that FOXQ1 makes additional contributions toward assembly, stability, or the structure of pit cell apical granules.

Although our data establish that *Foxq1* is required for *Muc5ac* expression in diverse tissues, the underlying mechanisms remain unresolved. Our analysis of a limited promoter region and putative enhancers do not support the elementary possibility that FOXQ1 directly activates *Muc5ac* gene transcription. On the one hand, cell transfection assays may limit the ability to determine FOXQ1 functions if, for example, an essential cofactor is missing or the epigenetic state is nonpermissive. On the other hand, FOXQ1 may regulate *Muc5ac* transcription through a distant enhancer that eluded our detection or via intermediary effectors; FOXQ1 may also be just one of several TFs that regulates *Muc5ac*. These possibilities will require specific antibodies and other tools to resolve definitively. One group previously suggested that FOXQ1 may repress transcription,¹³ in which case, its effects on *Muc5ac* gene expression could be complex.

The gastric phenotype of *Foxq1*^{sb/sb} and *Foxq1*^{sb/enu} mice notably resembles that reported in mice with *Slp2a* gene mutations,⁴² specifically in the reduced number of pit cell mucous granules. *Slp2a* is a synaptotagmin-like protein that interacts selectively with Rab27, a small GTPase known to regulate intracellular vesicle transport in diverse cells,⁴³ and *Slp2a*-Rab interaction seems to be essential for mucous granule formation and exocytosis.⁴² Quantitative RT-PCR analysis of *Foxq1*^{sb/sb} stomach did not uncover altered RNA levels of *Slp*- or *Rab*-family genes (data not shown). Stable synthesis of foveolar cell mucous granules thus requires at least 2 independent processes: intact *Slp2a*-Rab function and *Foxq1*-dependent synthesis of MUC5AC and a small number of other gene products.

Elaboration of mucus is an essential function of certain epithelia, and properties of the mucus secreted in

Figure 7. Absence of MUC5AC expression in *Foxq1*^{sb/sb} conjunctiva. (A) Ocular phenotype of >50% of aged (>6 months) *Foxq1*^{sb/sb} mice: accumulation of debris (arrow) around the eye. (B-I) Histochemical analysis of serial tissue sections of adult murine conjunctiva from *Foxq1*^{sb/sb} (B, D, F, H) and age-matched wild-type (C, E, G, I) mice. B and C: H&E stained; D and E: PAS stained; F and G: Alcian blue stained; H and I: MUC5AC immunostain. Scale bars, 60 μm.

different tissues are dictated in part by the mucin polypeptide. MUC5AC is by far the predominant mucin produced in gastric pit cells; secretion of the mature, glycosylated product protects the epithelium from acid, enzymatic, or physical damage. *Foxq1* is selectively expressed in surface mucous cells, and our data argue for its requirement in *Muc5ac* gene regulation. Nearly complete absence of MUC5AC does not produce overt disease in the stomach, including ulcers, inflammation, or tumors, which may reflect the artificial environment of animals maintained in the laboratory; by contrast, aging *Foxq1^{sb/sb}* mice develop ocular symptoms that can be attributed in principle to chronic MUC5AC deficiency. In some human dry-eye (keratitis sicca) conditions such as Sjögren's syndrome, reduced *Muc5ac* mRNA and protein levels correlate with disease severity.⁴⁴ Future studies might thus apply *Foxq1* mutant mice to investigate pathophysiology of gastric and ocular mucus deficiency.

Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: [10.1053/j.gastro.2008.014.019](https://doi.org/10.1053/j.gastro.2008.014.019).

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