Role of the Homeodomain Transcription Factor Bapx1 in Mouse Distal Stomach Development

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Background & Aims: Expansion and patterning of the endoderm generate a highly ordered, multiorgan digestive system in vertebrate animals. Among distal foregut derivatives, the gastric corpus, antrum, pylorus, and duodenum are distinct structures with sharp boundaries. Some homeodomain transcription factors expressed in gut mesenchyme convey positional information required for anterior-posterior patterning of the digestive tract. Barx1, in particular, controls stomach differentiation and morphogenesis. The Nirenberg and Kim homeobox gene Bapx1 (Nkx3-2) has an established role in skeletal development, but its function in the mammalian gut is less clear.

Methods: We generated a Bapx1 Cre knock-in allele to fate map Bapx1-expressing cells and evaluate its function in gastrointestinal development. Results: Bapx1-expressing cells populate the gut mesenchyme with a rostral boundary in the hindstomach near the junction of the gastric corpus and antrum. Smooth muscle differentiation and distribution of early regional markers are tentially normal in Bapx1 Cre/Cre gut, but there are distinctive morphologic abnormalities near this rostral Bapx1 domain: the antral segment of the stomach is markedly shortened, and the pyloric constriction is lost. Comparison of expression domains and examination of stomach phenotypes in single and compound Barx1 and Bapx1 mutant mice suggests a hierarchy between these 2 factors; Bapx1 expression is lost in the absence of Barx1.

Conclusions: This study reveals the nonredundant requirement for Bapx1 in distal stomach development, places it within a Barx1-dependent pathway, and illustrates the pervasive influence of gut mesenchyme homeobox genes on endoderm differentiation and digestive organogenesis.

Mechanisms responsible for organizing the mammalian stomach into fundus, corpus, and antral-pyloric segments are poorly understood. Corpus epithelium typically carries numerous oxyntic and zymogenic cells that produce acid and digestive enzymes, respectively. The distal stomach, which encompasses the antrum and pylorus, lacks these cell lineages but is marked in mouse and man by presence of endocrine cells that secrete gastrin and mucous cells that produce mucin 6. Muscle cells in the outer pylorus create a sphincter that controls passage of food into the duodenum.

The digestive tract differentiates in response to signals from adjacent mesenchyme. Expression of homeobox genes is often segmental along the anterior-posterior axis of the developing gut and may be especially important in relaying rostro-caudal position. Clustered Hox genes, for example, are expressed in the gut in overlapping domains, reminiscent of patterns observed along the skeletal axis; they are implicated in regional identity and in formation of intestinal sphincters and the cecum. Homeodomain proteins participate in mesoderm-endoderm signaling.

The homeobox gene Barx1 is confined to embryonic stomach mesenchyme and is required for proper stomach development. In its absence, the stomach is markedly small, abnormally shaped, lacks a pyloric constriction, shows mixing of cells from different segments, and carries intestinal villi distally. Some homeobox genes regulate fibroblast growth factor expression in the hindgut, and overexpression of NKX2.5 in chick embryos inhibits Wnt5a and Bmp4 expression during formation of the hindstomach (gizzard) and pylorus; Barx1 acts in part by limiting the duration of Wnt signaling in early stomach development. Many other factors that regulate genetic and tissue interactions in stomach development remain unknown.

The homeodomain of mammalian Nkx3-2 (Bapx1) shares ~87% identity with Drosophila BAGPIPE, a NK2 subfamily member that specifies gut smooth muscle in flies. Viral misexpression studies in the chicken suggest that Bapx1 functions in development of the gizzard, a muscular, keratinized structure in the posterior stomach. In mouse embryos, Bapx1 messenger RNA (mRNA) appears first in lateral plate mesoderm, adjacent to gut endoderm, around embryonic day (E) 8.5.

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out mice were therefore predicted to have gut musculature defects, but the intestine in 3 separate mutant lines is largely intact, and investigation has centered on Bapx1’s role in spleen and skeletal development. One group commented on abnormal gastro-duodenal morphology without investigating molecular details. Although the nature and possible reasons for the defect are unknown, Bapx1 is cited as being required to generate pyloric sphincter muscle. We created a targeted mouse line that marks Bapx1-expressing cells and eliminates gene activity. Here, we report that Bapx1 is necessary for proper antral-pyloric morphogenesis and development of antral-type epithelium. We also show that Bapx1 expression in the distal stomach requires Barx1. These studies reveal a focal requirement for Bapx1 in hindstomach organogenesis and outline a transcriptional hierarchy in mammalian stomach development.

Materials and Methods

**Mouse Gene Targeting**

A λ-phage clone from a 129/Sv mouse genomic library was provided by Drs. K.-I. Yoshiura and J. Murray, University of Iowa (Iowa City, IA). A 3.6-kilobase (kb) BgII-SacII fragment containing 5’ flanking sequences and the first 46 codons of Bapx1 exon 1 served as the 5’-homology arm; a 1.6-kb SmaI fragment containing codon 112 through the end of exon 2 served as the 3’-homology arm. A PGK-Neo cassette and Cre recombinase complementary DNA (cDNA) were inserted in frame with Bapx1 coding sequence at the SacII restriction site (Figure 1A). The construct was electroporated into AB2.2 embryonic stem cells. Two targeted cell lines were used to produce chimeras and Bapx1+/Cre mice. For Southern genotyping, the probe was a [α-32P] dCTP-labeled BgII-SacI fragment from the 3’ segment of the gene, which identifies 5-kb and 7.5-kb bands for wild-type and Cre knock-in alleles, respectively (Figure 1B). To demonstrate correct targeting at the 5’ end, we used primers complementary to the Cre insert (CRE3: GCCCGATAACCTGAAACAGCAT-TGC) and to genomic DNA ~4.5-kb 5’ to the Bapx1 gene (GTTATGATGACGCCTGGAGC) to amplify a 5.7-kb DNA fragment in the targeted allele (Supplementary Figure 1). Identity of this fragment was confirmed by BgII/ClaI digestion and sequence analysis using internal primers GGTGTTCAAAATGAGGCTC and CATGTATGAATGTGGAAACCTGG. For subsequent genotyping, we used CRE3 along with CTCGG- TCTCTGTGCTCAGGCTAG and CCAAGCGATCTC- CAACAGAAGAGG in a coupled polymerase chain reaction (PCR) reaction with a 56°C annealing step (Figure 1C). Bapx1 targeted mice were maintained on the C57BL/6 background.

![Figure 1](image-url).

**Figure 1.** Generation of Bapx1Cre targeted mice and their application to examine Bapx1 gene expression. (A) Molecular strategy to generate ES cells with Cre recombinase targeted to the first Bapx1 exon between the SacII and SmaI sites. (B, C) Mice were genotyped by Southern hybridization using probes corresponding to the 3’ half of exon 2 and part of the 3’ untranslated region. (D) Identity of this fragment was confirmed by BgII/ClaI digestion and sequence analysis using internal primers GGTGTTCAAAATGAGGCTC and CATGTATGAATGTGGAAACCTGG. Identity of this fragment was confirmed by BgII/ClaI digestion and sequence analysis using internal primers GGTGTTCAAAATGAGGCTC and CATGTATGAATGTGGAAACCTGG. For subsequent genotyping, we used CRE3 along with CTCGG-TCTCTGTGCTCAGGCTAG and CCAAGCGATCTCAACAGAAGAGG in a coupled polymerase chain reaction (PCR) reaction with a 56°C annealing step (Figure 1C). Bapx1 targeted mice were maintained on the C57BL/6 background.
Expression Analyses

β-galactosidase activity was determined on whole-mount preparations using published methods. For histology, embryos were embedded in paraffin, sectioned at 5 μm, and stained with H&E. Bapx1Cre/+ and Barx1+/− mice were intercrossed to obtain compound homozygotes. Organs from crosses with Nkx2.5-GFP transgenic mice, described previously, were visualized under a Leica MZ FLIII fluorescent dissecting microscope.

RNA was reverse transcribed using SuperScript (Invitrogen, Carlsbad, CA). cDNA was detected by PCR using Bapx1 primers aga tgtcagccagcgtttc and gcagag- cgcggagcgctgcg. Fetal stomach lysates were resolved by SDS-PAGE. Binding of Bapx1 mouse antiserum (H00000579-A01; 1:500; Abnova, Taipei, Taiwan) was detected with horseradish peroxidase-conjugated goat anti-mouse antibody (Ab).

Embryos were fixed overnight in 4% paraformaldehyde at 4°C. Eight-micrometer-thick paraffin sections were dried, deparaffinized in xylene, and rehydrated. For antigen retrieval, slides were immersed in 10 mmol/L sodium citrate, pH 6.0, and treated in a pressure cooker for 2 minutes at 15 psi. Endogenous peroxidase activity was blocked with 3% H2O2 in methanol for 15 minutes and nonspecific Ab binding with 5% fetal bovine serum for 1 hour at 25°C. Primary Ab: SMαA (Sigma A2547 [1A4], 1:1000; Sigma-Aldrich, St. Louis, MO), PGP9.5 (1:2500; Chemicon AB1761), H/K-ATPase (D032-3; 1:1000; MBL International Woburn, MA), intrinsic factor (1:24,000; gift from Dr. D. Alpers; Washington University, St. Louis, MO), Pdx1 (1:6000, gift of Dr. C. Wright, Vanderbilt University, Nashville, TN), BMP4 (1:300, Chemicon MAB1049), and Nkx 2.5 (1:200, SC14033; Santa Cruz Biotechnology, Santa Cruz, CA) were applied for 3 hours at 25°C or overnight at 4°C. After treating with anti-β-gal antibody, sections were incubated in BCIP tablets; Roche) for 2–4 hours.

For expression arrays, RNA was extracted from the distal stomach of E18.5 Bapx1Cre/Cre embryos and wild-type littermates using the RNeasy kit (Qiagen, Valencia, CA). After confirmation of RNA quality, samples were processed and hybridized to CodeLink mouse bioarrays (Amersham Biosciences, Piscataway, NJ). Raw data were normalized on a log scale and filtered to reduce noise. Differential gene expression and functional gene groupings were analyzed using MatchMiner (http://discover.nci.nih.gov/matchminer/), GoMiner (http://discover.nci.nih.gov/gominer/), and GeneSpring (Agilent Technologies, Santa Clara, CA) software and are deposited in the GEO database (GSE 13935).

Results

Tracing Bapx1 Expression

We used homologous recombination to replace Bapx1 at codon 46 (exon 1) with in-frame Cre cDNA (Bapx1Cre, Figure 1A). Two independent mutant lines showed evidence for correct gene targeting (Figure 1B and 1C) loss of Bapx1 mRNA (Figure 1D) and no material effect on expression of the 2 flanking genes (Supplementary Figure 1C). We crossed these mice with ROSA26 reporter mice, in which a floxed translation-stop sequence restricts LacZ gene expression to Cre-expressing cells and their progeny. Bapx1Cre activity first appeared in Bapx1Cre;ROSA26R embryos at E9.5 in gut mesoderm and weakly in somites (Figure 2A and 2B; data not shown). Between E10.5 and E12.5, β-gal activity was prominent in the splanchnic mesoderm, somites, calvarium, Meckel's cartilage, and spleen anlage (Figures 1E, 2C and 1D, and Supplementary Figure 2A–2G). By E13.5, Bapx1 expression was evident in the cardiac outflow tract (Figure 1F) and condensing cartilage of the ribs, skull (Supplementary Figure 2I–2N), and long bones. Staining in the digestive tract was confined to mesodermal derivatives and excluded from endoderm at all stages (Figures 1G and 2E and 2F). These findings agree with previous reports of Bapx1’s role in developing skeleton and spleen17–20 and establish the fidelity of Bapx1Cre mice to mark Bapx1-expressing cells and elucidate Bapx1 function in other organs.

Definition of gene expression along the long axis of the embryonic stomach is confounded by rotation of the organ from an initial lie parallel to the body’s anterior-posterior axis to a final position that is nearly perpendicular. We examined serial embryo sections with the attention required to distinguish the stomach’s antero-posterior and radial axes. LacZ expression in Bapx1Cre/+ embryonic gut initiated in the distal stomach. Staining at E10.5 was intense in the caudal foregut and stomach-intestine junction but absent were equilibrated in 100 mmol/L NaCl; 100 mmol/L Tris, pH 9.5; and 50 mmol/L MgCl2, and stained (NBT/BCIP tablets; Roche) for 2–4 hours.

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from the rostral foregut and stomach (Figure 2E, sections from the same embryo in a rostral to caudal series). At E11.5, expression remained evident in the hindstomach but faint or absent in forestomach (Figure 2F), extended into the full-length of intestine, and included the spleen anlage (Figure 2F). LacZ staining involved all cells in the full thickness of the mesenchyme (Supplementary Figure 3). In older embryos, β-gal activity was present in much of the stomach, with a persistent caudal-to-rostral gradient (data not shown).

Early chick embryos express Bapx1 in the prospective gizzard (posterior stomach) but not the proventriculus (anterior stomach).15 Our Cre-based lineage analysis in mice confirmed Bapx1 expression in tissues with known functions and disclosed an anterior boundary previously unappreciated in mammalian stomach. The rostral limit of earliest Bapx1 expression corresponds roughly to the junction between corpus and antrum.

Abnormal Stomach Development in Bapx1Cre Homozygotes

Crosses between Bapx1+/Cre mice yielded null mutants in Mendelian proportions until E18.5 (25.6% Bapx1+/+, 48% Bapx1Cre+/+, and 26.4% Bapx1Cre/Cre). Mutant homozygotes typically died 1 to 3 days after birth, and ~90% of weanlings were wild-type or heterozygote. Perinatal lethality, similar to that reported with other Bapx1-null alleles,17–20 likely reflects skeletal malformation (Supplementary Figure 2O and 2P). The spleen was absent or markedly hypoplastic in Bapx1Cre/Cre mice, as judged grossly (Figure 3B) and by expression of a Nkx2.5-
GFP transgene (Supplementary Figure 4), a marker of the developing spleen.12,25 Bapx1Cre/Cre stomachs were modestly reduced in size. Nearly all of this reduction occurred in the distal segment, which was also dilated and lacked constriction at the gastro-duodenal junction, the site of the pyloric constriction and ostensibly intact enteric nerve and smooth muscle. Specialized, Alcian blue-avid mucous cells found at the base of antral gland units are normally absent from the corpus; conversely, the antrum lacks chief and oxyntic cells, the dominant lineages in the body.1 Normal hindstomach hence corresponds to the Alcian blue-staining region between the zone of chief and parietal cells in the corpus and the villous duodenal epithelium (Supplementary Figure 5A). Bapx1Cre/Cre stomachs carried few, and in many cases, no glandular units with basal Alcian blue avidity (Figure 4A and 4E vs 4B and 4F), whereas intestinal goblet cells stained readily with Alcian blue (Figure 4F). Additionally, the distance between H/K-ATPase- (Figure 4C and 4G vs 4D and 4H) or gastric intrinsic factor (Supplementary Figure 5B–E) expressing cells in the stomach body and the villous intestinal epithelium was markedly reduced. These corpus lineage markers frequently abutted the intestine (Figure 4H, Supplementary Figure 5E), indicating diminution or loss of mature antral character. Scrutiny of Alcian blue, H/K-ATPase, and gastric intrinsic factor stains revealed normal cell composition in distal corpus glands and absence of mixed corpus-antral units (Supplementary Figure 5F).

Thus, absence of Bapx1 leads to significant hindstomach truncation and loss of the pyloric constriction. The normal appearance of gastric smooth muscle (Figure 3H) and ostensibly normal intestine and gastric corpus point to a localized defect in antral-pyloric development. Although histologic examination sometimes gave the impression that antral hypoplasia was more severe along the lesser than the greater curvature of the stomach (e.g., Figure 4C and 4D), most samples lacked such disparity (e.g., Figure 3C and 3D, Supplementary Figure 6B), which we attribute to subtle variation in tissue orientation. Indeed, objective quantitation of multiple samples confirmed that both aspects of the antrum were affected (Supplementary Figure 6A).

**Molecular Correlates of Bapx1 in Hindstomach Development**

Indian Hedgehog (Ihh) mRNA is enriched in fetal mouse corpus and antrum, whereas Sonic hedgehog (Shh) is enriched in the forestomach.28 In a sign that early patterning is preserved in Bapx1Cre/Cre stomach, the boundaries of Ihh (Figure 5A and 5B) and Shh (data not shown) expression were intact at E11.5. Expression of the homeobox gene Pdx1 is normally limited to the antral-pyloric segment, providing a reliable marker of this stomach region.29 Pdx1 expression also was similar in Bapx1Cre/Cre and wild-type stomach early in development (E11.5 and E14.5 shown in 5C–5F). Consistent with the observation of antral hypoplasia, the Pdx1 expression domain was substantially smaller at E18.5 (data not shown). However,
the typical transition in staining pattern between antrum and corpus, and the symmetry across greater and lesser curvatures, were preserved. Antral hypoplasia in the absence of Bapx1 hence occurs on the background of correct anterior-posterior stomach patterning.

In chick embryos, Nkx2.5 and Bapx1 are expressed in the distal stomach (gizzard), whereas Bmp4 and Wnt5a appear in the proximal proventriculus and are excluded from the gizzard.\textsuperscript{15,30} Nkx2.5 may regulate pyloric sphincter development, and forced Bapx1 expression in the proventriculus inhibits endogenous Bmp4 expression.\textsuperscript{15} In mouse embryos, by contrast, we observed Bmp4 expression throughout stomach and intestinal mesenchyme (data not shown); Nkx2.5 mRNA and protein were also expressed widely in mesoderm at the gastroduodenal junction but clearly enriched in pyloric sphincter muscle, as predicted (Figure 5G and Supplementary Figure 2). Levels and distribution of both Nkx2.5 (Figure 5H, Supplementary Figure 4) and Bmp4 (data not shown) were unaltered in Bapx1\textsuperscript{Cre/Cre} stomach, indicating that Bapx1 loss does not interfere with their expression.

Next, we surveyed changes in Bapx1\textsuperscript{Cre/Cre} antral gene expression, using microarray analysis followed by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) confirmation of representative results (data not shown). Because early stomach pattern seems intact, we reasoned that antra from older embryos would better reveal aberrant gene expression. The distal stomach in

Figure 4. Regional markers verify hindstomach epithelial defects in Bapx1\textsuperscript{Cre/Cre} mice. Critical examination of antral Alcian blue staining and of the gastric corpus marker H/K-ATPase. (A–D) Low-power microscopic views of the gastro-duodenal junction in wild-type (A and C) and Bapx1\textsuperscript{Cre/Cre} (B and D) neonates. Brackets mark the region expressing antral-pyloric markers along the greater curvature (An, antrum-pylorus), separating the stomach (Stom) corpus from intestine (Int). Boxes outline regions of the gastro-duodenal junction shown at higher magnification in panels E–H. Alcian blue staining in mutant antral epithelium, which marks characteristic mucous cells at the gland base (arrows) in wild-type mice (A and E), is significantly reduced along the greater curvature and missing from the lesser curvature. Intestinal goblet cells (arrowheads), which also stain with Alcian blue, are unaffected. Conversely, H/K-ATPase immunostaining marks parietal cells in wild-type gastric corpus and is absent from normal antral-pyloric mucosa (C and G). In Bapx1\textsuperscript{Cre/Cre} mice, H/K-ATPase-expressing cells reside immediately adjacent to the intestine (D and H), again disclosing loss of antral mucosa. Similarly, expression of intrinsic factor, a marker of corpus-resident zymogenic cells, abuts the intestine along the lesser curvature and comes close to the intestine along the greater curvature in Bapx1\textsuperscript{Cre/Cre} stomach (Supplementary Figure 3). Scale bars, A–D, 300 μm; E–H, 60 μm.
organism-specific H/K-ATPase and Gif transcripts and a corresponding decrease in antrum-specific Muc6 mRNA (Supplementary Table 1A). These changes are consistent with the loss of antral, and distal extension of corpus, character. Considering functional gene classes (Gene Ontology), we noted increased expression of transcripts in groups related to epithelial-mesenchymal transition and regulation of endocytosis, whereas groups associated with Smad proteins, nuclear protein import, and vesicle membranes were expressed at lower levels (Supplementary Table 1B). These molecular changes in distal Bapx1Cre/Cre stomach represent an unknown combination of additional regional markers and possible underpinnings of antral hypoplasia.

**Bapx1 May Function Downstream of Barx1 to Mediate Antral-Pyloric Development**

Bapx1 is coexpressed in embryonic hindstomach mesenchyme with Barx1, although the domain of Barx1 expression encompasses nearly the whole stomach (Figure 6A). Both genes influence differentiation of overlying stomach endoderm and formation of the pyloric sphincter; the antral segment is abbreviated in Bapx1Cre/Cre mice (Figures 3 and 4) and likely lost in Barx1−/− mice.12 We crossed mice to produce compound homozygote mutants, which we studied immediately after birth because Barx1−/− mice die of respiratory failure in the perinatal period.12 Stomach anomalies in Barx1−/−;Bapx1Cre/Cre and Barx1−/−;Bapx1l/− mice were identical (Figure 6B and 6C); there was no worsening of the isolated Barx1 mutant phenotype, which is more severe than the Bapx1Cre/Cre antral defect.

To evaluate further the relationship between these coexpressed factors, we investigated gene expression in each individual knockout strain. Levels and distribution of Barx1 mRNA were not reduced or altered in fetal Bapx1Cre/Cre stomachs and may even increase slightly (Figure 6D and 6E, Supplementary Table 1A). Thus, Barx1 does not require Bapx1 for its expression and acts either upstream or independent of Bapx1. Conversely, we detected Bapx1 transcripts in wild-type hindstomach and spleen (Figure 6F, arrow and arrowhead) but not in the caudal Barx1−/− stomach (Figure 6G, arrow), indicating that hindstomach Bapx1 expression requires Barx1 function. Bapx1 expression was equally robust in wild-type and Barx1−/− somites (Figure 6G, inset), ruling out trivial reasons for lack of a stomach signal. qRT-PCR and immunoblot analyses confirmed that Bapx1 mRNA levels were markedly reduced or absent in Barx1−/− stomachs (Figure 6H and 6I). These observations collectively suggest that Bapx1 expression depends on Barx1 and that antral dysmorphogenesis in Barx1−/− stomachs might potentially reflect the attendant Bapx1 deficiency.

**Discussion**

Organogenesis requires positional cues to specify cell and tissue types correctly. Homeobox genes play a vital role in regulating developmental processes and imparting positional identity.31,32 We used homologous recombination to drive Cre expression from the mouse Bapx1 locus, thus creating a new null allele to define expression and study gene function in the developing gut. Bapx1+/Cre;ROSa26R mice confirmed Bapx1 expression domains reported previously in cartilage and spleen and revealed that, early in digestive tract development, Bapx1-expressing cells and their progeny are confined to
the intestine and prospective hindstomach. In line with this observation, Bapx1Cre/Cre mice show significant shortening of the antral segment and virtual apposition of the gastric body to the duodenum. Pdx1 and Ihh, 2 posterior markers, show correct regional expression, implying that certain elements of early stomach patterning are preserved. Thus, Bapx1Cre/Cre hindstomach defects seem to reflect a failure of proper expansion and morphogenesis.
of the antral-pyloric segment. Because the affected region corresponds to that where Bapx1 expression initiates in the digestive tract, we infer that Bapx1 activity is uniquely responsible for these aspects, even if the precise molecular mechanism is presently unknown.

Despite ostensibly normal smooth muscle differentiation and preserved expression of Nkx2.5, a gene implicated in chick gizzard development,30 Bapx1Cre/Cre mice also lack normal pylorus morphology. Mice deleted for nearly the full Hoxd gene cluster lose multiple gastrointestinal valves, including the pyloric sphincter, with associated changes in regional smooth muscle and mucosa; the pyloric constriction is also missing in Barx1−/- mice.12 These findings may be relevant to hypertrophic pyloric stenosis, a common congenital disorder.33 Future efforts should aim to understand how these homeobox genes interact to generate the pyloric sphincter.

The stomach corpus and intestine developed normally, indicating that the antrum-pylorus is the only gut segment that requires Bapx1 for proper development. Alternatively, Bapx1 may function redundantly with other homeobox genes elsewhere. Less likely, abnormal hindstomach development could reflect dysmorphogenesis of the spleen and pancreas. Around E8.5 in mouse development, Bapx1 mediates lateral growth of the splanchic mesodermal plate and coupled leftward growth of the dorsal pancreas, associated with control of Fgf10 expression.34 However, anomalies akin to those we identify in Bapx1Cre/Cre stomach are not seen with a wide range of defects in spleen and pancreas development.12,25 In chondrocytes, Bapx1 serves both proliferative and antiapoptotic roles,19,35 and one reason the antrum and pylorus may develop aberrantly in its absence is if hindstomach progenitors are disadvantaged relative to anterior cells programmed for corpus differentiation. Immunostaining for cleaved caspase 3 did not reveal excess apoptosis in E11.5 hindstomachs (data not shown).

Forced Bapx1 expression in the chick proventriculus (forestomach) suppresses Bmp4 and Wnt5a expression and region-specific differentiation; conversely, forced expression of Bapx1-VP16, which artificially converts a presumed repressor into a transcriptional activator, promotes occasional expression of BMP4 and Wnt5a in the gizzard.15,36 By contrast, Bmp4 expression does not appear to be compartmentalized in the mammalian stomach nor did we detect aberrant expression of Bmp4 or Nkx2.1 in the mutant organ. Thus, despite similarities in Bapx1 expression and function in developing posterior stomach, phenotypes and affected pathways in chick and mouse seem different. These could reflect different mechanisms to create the keratinized avian gizzard vs the glandular mammalian antrum.

Although Barx1 and Bapx1 appear in different compartments in the developing spleen and their mutant phenotypes in that organ are distinct,12,18,19,21,37 their expression in distal stomach mesenchyme is overlapping. Absence of Barx1 markedly disrupts stomach development, producing aberrant morphogenesis, intestinal homeosis, and pyloric sphincter agenesis. Additional loss of Bapx1 does not worsen this phenotype, and the greater severity of antral-pyloric defects in the Barx1 mutant hints at actions upstream of Bapx1. Indeed, Bapx1 expression is virtually lost in Barx1-null stomach, and its absence could potentially account for some part of the Barx1−/- phenotype in the distal organ. Mice with tissue-specific loss of a third stomach transcription factor, the nuclear hormone receptor COUP-TFI1, also show a mild patterning defect.38 Besides expansion and disorganization of circular smooth muscle and enteric neurons, the margin between forestomach and corpus is shifted anteriorly, and the glandular stomach accordingly occupies a larger relative space. Although expansion of the corpus is a common feature of the 2 phenotypes, they occur at opposite ends: anteriorly in the case of COUP-TFI1 deficiency and posteriorly in Bapx1Cre/Cre animals.

Barx1 is expressed throughout stomach mesenchyme, whereas Bapx1 is initially confined to the caudal region. Thus, although Barx1 seems to be required for stomach Bapx1 expression, it cannot be sufficient to restrict expression to the hindstomach; other factors may promote Bapx1 expression caudally or repress it rostrally. We are presently investigating Barx1’s role in COUP-TFI1 expression. Our results meanwhile implicate Barx1 and Bapx1 within an essential pathway for mammalian hindstomach development.

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.2009.01.009.

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Conflicts of interest
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