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Gastric Parietal Cell Physiology and *Helicobacter pylori*–Induced Disease

Xuebiao Yao[1,2], **Adam J. Smolka**[3]

[1]MOE Key Laboratory of Cellular Dynamics, CAS Center for Excellence in Molecular Cell Science, University of Science and Technology of China, Hefei, China;

[2]Keck Center for Cellular Dynamics and Organoids Plasticity, Morehouse School of Medicine, Atlanta, Georgia;

[3]Gastroenterology and Hepatology Division, Department of Medicine, Medical University of South Carolina, Charleston, South Carolina

Abstract

Acidification of the gastric lumen poses a barrier to transit of potentially pathogenic bacteria and enables activation of pepsin to complement nutrient proteolysis initiated by salivary proteases. Histamine-induced activation of the PKA signaling pathway in gastric corpus parietal cells causes insertion of proton pumps into their apical plasma membranes. Parietal cell secretion and homeostasis are regulated by signaling pathways that control cytoskeletal changes required for apical membrane remodeling and organelle and proton pump activities. *Helicobacter pylori* colonization of human gastric mucosa affects gastric epithelial cell plasticity and homeostasis, promoting epithelial progression to neoplasia. By intervening in proton pump expression, *H pylori* regulates the abundance and diversity of microbiota that populate the intestinal lumen. We review stimulation–secretion coupling and renewal mechanisms in parietal cells and the mechanisms by which *H pylori* toxins and effectors alter cell secretory pathways (constitutive and regulated) and organelles to establish and maintain their inter- and intracellular niches. Studies of bacterial toxins and their effector proteins have provided insights into parietal cell physiology and the mechanisms by which pathogens gain control of cell activities, increasing our understanding of gastrointestinal physiology, microbial infectious disease, and immunology.

Keywords

Gastric Organoids; Acid Secretion; Bacteria; Syntelin; *H pylori*

Infection with *Helicobacter pylori* is the most clearly identified risk factor for gastric cancer—the third leading cause of cancer mortality worldwide in men, and the fifth in women.[1] In 2017, there were an estimated 950,000 cases worldwide, and 723,000 deaths.[2] The risk of gastric cancer involves interactions among *H pylori* strain–specific virulence factors, patient

Address requests for reprints to: Xuebiao Yao, PhD, MOE Key Laboratory for Cellular Dynamics, CAS Center for Excellence in Molecular Cell Science, University of Science & Technology of China, Hefei, China. yaoxb@ustc.edu.cn.

Conflicts of interest

The authors disclose no conflicts.

genotype, and environmental factors. Perturbation of gastric acid secretion is an acute and chronic outcome of *H pylori* infection that promotes gastric carcinogenesis.[3–5] The acute inhibitory effects of *H pylori* on acid secretion are transitory and normal acid secretion can be restored after *H pylori* is eradicated.[6] In contrast to acute *H pylori* infection, which induces hypochlorhydria, chronic infection can induce an antrum-predominant phenotype associated with gastrin-mediated acid hypersecretion or a corpus-predominant phenotype associated with acid hyposecretion—this results from *H pylori*–induced proton pump suppression and activities of cytokines produced by infiltrating immune cells.[7] *H pylori*'s ability to reduce proton pump expression allows it to regulate gastric luminal acidity and alter the persistence or transit of other gastrointestinal microbes.[7]

Acid secretion is regulated by differentiated parietal cells located in secretory glands formed by invagination of the epithelial monolayer that lines the fundus and body of the mammalian stomach. The secretory glands also contain other highly differentiated cell types that protect the stomach and support its function. These include mucus neck cells, which secrete mucus; chief cells, which secrete pepsinogen; and oxyntic enterochromaffin-like (ECL) cells, which regulate acid secretion by secreting histamine in response to paracrine gastrin stimulation originating in antral G cells. Acid secretion by parietal cells is mediated by the membrane-bound enzyme H^+,K^+-ATPase, found in high density on intracellular tubulovesicular and secretory canalicular membranes.[8,9] The enzyme is a heterotetramer of 2 ATP4A subunits, which have catalytic ATPase and cation transport activity, and 2 ATP4B subunits, which are smaller and glycosylated.

In parietal cells, ATP4A and ATP4B are translated on endoplasmic reticulum–bound ribosomes into integral membrane polypeptides that localize to cytoplasmic tubulovesicles with limited K^+ permeability.[10,11] ATP4B subunits facilitate assembly of functional proton pumps and migration of the nascent enzyme complex to tubulovesicular and canalicular membranes and protect the complex from degradation.[12–14] The relative K^+ impermeability of tubulovesicular membranes ensures that ATP4A activity, responsible for the H^+-K^+ counter-transport functions of the enzyme, remains latent.[10] A distinct set of intracytoplasmic RAB11-positive vesicles express the K^+ voltage–gated channels KCNQ1 and KCNE2.[15] Nutrient-induced neural, hormonal, and paracrine stimulation of parietal cells, in the form of acetylcholine activation of calcium-dependent signaling pathways, gastrin activation of histamine release from ECL cells, and histamine-mediated increases in intracellular adenosine 3′,5′-cyclic monophosphate (cAMP), activates protein kinases. These kinases cause fusion of H,K-ATPase–containing tubulovesicles and K^+ channel–containing vesicles with collapsed invaginations of the parietal cell apical membrane. The resulting expanded membrane-delimited pan-cellular microvillous secretory canalicular compartment is characteristic of actively secreting parietal cells. Delivery of K^+ channels (KCNQ1, KCNE2, KCNJ10, and KCNJ15)[16–19] to canalicular membranes provides access of cytoplasmic K^+ to luminally oriented K^+-binding sites on ATP4A, initiating ATP binding and hydrolysis on its cytoplasmic side, which promotes electroneutral exchange of luminal K^+ for cytoplasmic protons with unitary H^+:K^+:ATP stoichiometry.[20] Under maximal secretory conditions, H,K-ATPase activity establishes a million-fold proton gradient across the canalicular membrane.[21–23] Recent high-resolution (2.8 Å) crystal structures of H,K-ATPase complexed with the K^+-competitive acid secretory inhibitors vonoprazan or

SCH28080 indicate that the conformational change of the cation-binding site, induced by ATP hydrolysis, lowers the pK_a value of Glu820 in ATP4A, enabling release of protons into the pH 1.0 environment of the stomach.[24]

Regulation of Gastric Acid Secretion

Stimulants of parietal cell acid secretion include gastrin, secreted by antral G cells (hormonal pathway)[25,26]; histamine, secreted from oxyntic ECL cells (paracrine pathway)[27]; and acetylcholine, secreted from oxyntic (gastric body and fundus) and antral intramural post-ganglionic neurons (neural pathway).[28] Parietal cells express specific receptors for each of these secretagogues (gastrin or CCK_2, H_2, and M_3, respectively). Gastrin is the major hormonal stimulant secreted during meal ingestion, inducing release of histamine from ECL cells. Gastrin also stimulates the parietal cell directly, acting via CCK_2 receptors coupled to an increase in $[Ca^{2+}]_i$. Parietal cell histamine H_2 receptors are coupled to adenylate cyclase, which catalyzes formation of cAMP. In rat hepatoma-derived cells transfected with canine histamine H_2 receptor, histamine elicited transient increases in intracellular calcium ($[Ca^{2+}]_i$) with generation of inositol trisphosphate.[29] Acetylcholine stimulates parietal cells directly through M_3 muscarinic receptors coupled to an increase in $[Ca^{2+}]_i$, and indirectly by inhibiting M_4 muscarinic receptor-mediated somatostatin secretion from oxyntic D cells.[30] Somatostatin, secreted from oxyntic and antral D cells, is the primary physiological inhibitor of acid secretion. Although there are few D cells in oxyntic mucosa, compared to parietal cells, D cells are functionally coupled to parietal and ECL cells via cytoplasmic processes or via local circulation.[31,32] Under resting conditions, somatostatin places tonic restraint on parietal cell acid secretion, ECL cell histamine secretion, and G cell gastrin secretion. Removal of this restraint (disinhibition) by activation of cholinergic neurons stimulates acid secretion. Direct evidence for Ca^{2+} signaling in histamine-dependent acid secretion in mice was provided by organelle-targeted Ca^{2+} imaging and direct patch-clamping of apical vacuolar membranes of gastric parietal cells.[33] This study revealed that a lysosome calcium channel, mucolipin 1 (MCOLN1 or TRPML1), co-localized with tubulovesicles that express ATP4A, was required for histamine-induced PKA-dependent release of Ca^{2+} from tubulovesicular stores and for acid secretion.

Proton pump inhibitors, such as omeprazole, lansoprazole, rabeprazole, and esomeprazole, have revolutionized the treatment of gastroesophageal reflux disease, peptic ulcer disease, and Zollinger-Ellison syndrome (gastrinoma). Proton pump inhibitors are substituted benzimidazoles that inhibit gastric acid secretion by binding covalently to luminally exposed cysteine residues on ATP4A, altering its conformation. Vonoprazan is a lipophilic, weak base pyrrole derivative that blocks acid secretion by accumulating in acidic parietal cell canaliculi and prevents proton pump activation by competing with K^+ on the luminal (intracanalicular) surface of ATP4A.[34] Advantages of using vonoprazan vs conventional proton pump inhibitors include rapid onset of action, longer duration of acid suppression, and less variation among individuals, at the possible cost of increased hypergastrinemia.[35]

Parietal Cell Activation

Parietal cells have characteristic morphology closely related to their function.[36] The cells are generally pyramidal in shape and are packed with mitochondria and other membrane-bound structures. The apex of the pyramid, which faces the glandular lumen, is surrounded by tight junctions to adjacent cells. Three-dimensional nano-tomography analysis of mouse parietal cells revealed intricate networks of mitochondria, which are evenly distributed throughout the cytoplasm, and abundant roughly spherical lysosomes, which are mostly adjacent to mitochondria.[37] Tubular and vesicular membrane organelles (tubulovesicles) that express ATP4A and ATP4B in high density are distributed throughout the parietal cell cytoplasm. Resting or non-secreting cells have a limited area of apical membrane in direct apposition to the gland lumen, but more extensive luminal exposure is provided by a distinctive invagination of the apical membrane deep into the cytoplasm, forming a residual collapsed secretory canaliculus. Further studies are needed to determine whether parietal cell organelle interactions involved in histamine-induced acid secretion are affected by *H pylori* infection.

Changes in parietal cell morphology that accompany stimulation of acid secretion result from fusion of intracellular tubulovesicles with the residual secretory canalicular membranes, leading to elongation of intra-canalicular microvilli and the concomitant disappearance of cytoplasmic tubulovesicles.[11] These changes in vesicle trafficking, membrane interactions, and actin cytoskeleton arrangement are mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), which are found in different membranes and intracellular locations. Initial searches for parietal cell SNARE proteins identified 6 SNAREs: VAMP; syntaxins 1, 2, 3, and 4; and SNAP25.[38,39] Live-cell imaging with fluorescently labeled VAMP2 demonstrated the translocation of VAMP2 from tubulovesicular membranes to the apical canalicular membrane of parietal cells upon stimulation of acid secretion.[40] The functional importance of VAMP2 in stimulating acid secretion was demonstrated by concomitant inhibition of acid secretion by parietal cells exposed to tetanus toxin, a Zn-dependent proteinase that specifically cleaves VAMP2.[40,41]

Although identification of VAMP2 as a v-SNARE in parietal cells was anticipated, the identification of syntaxin 3 on tubulovesicles was unexpected. This prototypical t-SNARE localizes to vesicular membranes of parietal cells and may mediate homotypic fusion of tubulovesicles, accounting for the rapid apical morphologic changes associated with active acid secretion. Parietal cell stimulation was accompanied by translocation of co-localized syntaxin 3 and ATP4A from tubulovesicles to the apical membrane.[42] The importance of syntaxin 3 in acid secretion was demonstrated in studies with streptolysin O–permeabilized gastric glands. In these studies, recombinant syntaxin 3 competed for endogenous protein.[43] Ezrin, a membrane-cytoskeletal linker with sequence homology to talin and erythrocyte band 4.1, has been associated with the remodeling of parietal cell apical membrane that occurs with cAMP-dependent protein kinase stimulation. Atomic force microscopy studies revealed that ezrin phosphorylation and conformational change allowed binding of syntaxin 3 to the N-terminus of ezrin.[44] SNARE proteins therefore mediate recognition and docking events, but additional mechanisms, such as partition of a hydrophobic domain of a membrane protein into an adjacent closely apposed membrane, could promote thermodynamic fusion of membranes.[11,45]

Other molecular effectors of parietal cell morphologic transformation are Rab GTPases, which are members of the Ras GTPase superfamily that regulate many steps of membrane trafficking. Rabs are often tethered to membranes through 2 C-terminal prenyl groups,[46] and switch between GDP-bound and GTP-bound forms depending on activation, dissociation, displacement, and exchange factors.[47] RAB11 is involved in regulating recycling endosomes in transferrin recycling models and is also required for trafficking from trans-Golgi network to the plasma membrane.[48] Initial screening of parietal cells found a high level of *RAB11* mRNA[49] and RAB11 protein localized to tubulovesicles that contain ATP4A.[50] Expression of a dominant-negative form of RAB11 ($RAB11^{N124I}$) in parietal cells inhibited acid secretion.[51] Inhibition correlated with impaired membrane translocation from tubulovesicles to the apical plasma membrane. Interestingly, RAB11 interacts with another small GTPase, ARF6.[52] Like ATP4A, native ARF6 redistributes from predominantly cytoplasmic membranes to apical canalicular membranes when cells are stimulated.[53] In parietal cells, ARF6 is activated by an Arf-GAP containing a coiled-coil ACAP4.[54] ACAP4 interacts with ezrin, which is regulated by protein phosphorylation.[55] Histamine stimulation of parietal cell acid secretion activates PKA signaling via STK26 (or MST4),[56] leading to phosphorylation of ACAP4 at Thr^{545}. This phosphorylation enables ACAP4 to interact with ezrin.[57] Given the location of Thr^{545} between the GTPase-activating protein domain and the first ankyrin repeat of ACAP4, it is likely that MST4 phosphorylation induces a conformational change that allows ACAP4 to interact with ezrin (Figure 1*A* and *B*).

Although somatostatin, EGF, and TGFα signals inhibit acid secretion, withdrawal of the secretory stimulus alone appears to be sufficient to turn off acid secretion.[36,58] Initial studies of the mechanisms of ATP4A recovery from secretory membrane and its recycling led to discovery of a tyrosine-based internalization motif at the cytoplasmic, N-terminus of ATP4B.[59] In studies of transgenic mice, substitution of this tyrosine with an alanine resulted in continued localization of ATP4B to the parietal cell surface and sustained secretion of acid. These mice develop gastric ulcers and hypertrophic gastropathy. Post-secretory endocytosis of the proton pump involves the tetraspanin CD63, a membrane protein whose expression and co-localization with ATP4B in apical membrane induces redistribution of ATP4B to intracellular compartments.[60] CD63 interacts, through a tyrosine-based motif, with adaptor protein complexes 2 and 3, linking its interaction partners to the endocytic machinery of the cell.[60] Dynamin and clathrin also associate with the apical canalicular membrane in resting and stimulated cells.[42,61]

The complex apical membrane-cytoskeletal structure of parietal cells gives rise to innumerable intracellular canalicular membrane microvilli. Electron microscopy has shown that, in the resting state, abundant short (approximately 0.5 μm) parietal cell microvilli on the apical canalicular membrane have distinct F-actin bundles radially organized along the length of the microvilli, in addition to filamentous extensions that project from the base of each microvillus 1–2 μm into the cytosol.[36,62] Upon parietal cell stimulation, apical membrane surface area is increased significantly due to mass tubulovesicle fusion, which leads to expansion of the canalicular membrane surface into the cytoplasm, displacing and crowding together the numerous mitochondria that energize acid secretion. The radial F-actin bundles, now in significantly elongated microvilli, have the same appearance as F-actin bundles in resting cells, providing structural support for the increased microvillous surface

extensions. After withdrawal of the stimulus, the canalicular spaces remodel over the next 15–30 minutes as HCl secretion ceases. Highly ordered longitudinal F-actin filaments within the collapsed microvilli are disassembled into short, disordered bundles that extend into the cytoplasm.[63]

The mechanisms underlying actin filament plasticity during parietal cell activation and membrane recycling have been studied using small molecule inhibitors, such as latrunculin B (Lat B), which binds to actin monomers. These analyses identified a pool of actin that binds Lat B and mediates motility of cultured cells, and a Lat B-resistant pool of actin that may constitute microvillous filaments essential for secretion. Furthermore, cell stimulation appears to increase the effects of Lat B—most likely through Lat B binding to monomeric actin generated by turnover of the actin filament pool.[64] Dynamic pools of actomyosin and F-actin are required to maintain apical membrane structures in resting cells and for trafficking of tubulovesicles to the apical plasma membranes during histamine stimulation.[65]

Ezrin also has important functions in parietal cell apical membrane remodeling. In mice with conditional knockdown of ezrin, there is no fusion of parietal cell tubulovesicles with the apical canalicular membrane, preventing acid secretion.[66] In addition, proteolysis of parietal cell ezrin by activation of calpain I prevented histamine-stimulated acid secretion, measured by decreased uptake of [^{14}C]-amino-pyrine, a membrane-permeable weak base (pK_a = 5.0) that diffuses into and is protonated in intracellular acid compartments.[67] Importantly, persistent expression of the Thr567 phospho-mimicking ezrin mutant redirected ATP4A to basolateral membrane,[68] inhibiting acid secretion and inducing an unexpected repolarization of the cells. Ezrin, F-actin, and ATP4A all accumulated at the basolateral membrane in the form of long and extensive microvillous or filopodial extensions.[68,69] Several groups, using various cell systems, found that ezrin Thr567Asp mutagenesis increased surface projections, [70,71] but specific membrane localization was not described in detail. A membrane fixation effect of ezrin Thr567Asp was demonstrated by fluorescence recovery after photo-bleaching, indicating that mutagenized ezrin, in a permanently unfolded conformation, binds tightly to the basolateral membrane, resulting in repolarization of epithelial cells.[72] These data led to a model in which turnover of ezrin Thr567 phosphorylation is required for optimal plasticity in membrane remodeling.[45,73,74] Studies are needed to delineate how ezrin links parietal cell stimulation[29] to apical membrane remodeling.

Effects of *H pylori* on Gastric Acid Secretion

Colonization of gastric mucosa by *H pylori* perturbs the primary function of gastric parietal cells, secretion of hydrochloric acid at concentrations sufficient to sterilize nutrient intake. The discovery of *H pylori* as an etiologic agent of gastritis and peptic ulceration was accompanied by studies associating acute *H pylori* infection with transient hypochlorhydria, including reports of iatrogenic *H pylori* inoculation through contaminated gastric endoscopes and nasogastric tubes. Gastric pH, 1–4 weeks after initial infection, was reported to range from 6.4 to 7.6,[75–78] with acid secretion returning to baseline levels within a few weeks or months.[75–82] Infection of dogs, ferrets, and Mongolian gerbils with Helicobacter species inhibited acid secretion.[83–85] Co-incubation of various *H pylori* species or *H pylori* sonicates with rabbit and ferret gastric epithelial cells, gastric glands, or isolated guinea pig

or human parietal cell populations inhibited acid secretion.[86–92] In most studies, decreased cellular uptake of [^{14}C]-aminopyrine was a marker of acid secretory inhibition.

These findings associated *H pylori* infection with gastric hypochlorhydria, consistent with acid inhibition being mediated by *H pylori*–induced interruptions of interactions between gastrin-secreting G cells, histamine-secreting ECL cells, somatostatin-secreting D cells, and parietal cells. However, there could also be a direct interaction of *H pylori* with parietal cells. *H pylori* have been observed, by transmission electron microscopy of human gastric biopsies, to penetrate the cytoplasm of epithelial cells, the intraepithelial intercellular spaces, and the underlying lamina propria.[93,94] More specifically, *H pylori* penetration into gastric glands and even the cytoplasm of parietal cells was observed in histologic, immunohistochemical, and ultra-structural analyses of *H pylori*–infected cats and humans. *H pylori* were found clustered within gastric glands on the apical surfaces of parietal cells and even sequestered within parietal cell secretory canaliculi.[95–98] Contrary to common belief, [97–99] *H pylori* survive at pH <3.0, which is characteristic of the gastric environment. *H pylori*'s adaptation to this acidic microenvironment has been extensively documented,[100–103] and involves a proton-gated inner membrane channel that allows diffusion of urea from the gastric lumen into the bacterium, where it is hydrolyzed by cytoplasmic urease into ammonia and CO_2. Subsequent diffusion of NH_3 into the periplasm creates a protective higher pH microenvironment essential for survival of the bacteria. This mechanism, one of several strategies deployed by bacteria to facilitate survival and colonization of acidic environments,[104] is particularly relevant in the context of potential direct *H pylori* inhibition of parietal cell acid secretion, previously considered unlikely, given the bactericidal properties of gastric acid.

It is important to understand the mechanisms of acute *H pylori*–induced hypochlorhydria because they recapitulate the effects of anti-secretory drugs. *H pylori*–induced hypochlorhydria does not involve loss of parietal cells or atrophy because normal numbers of parietal cells were found in the stomachs of acutely infected Mongolian gerbils and in gastric biopsies from patients.[75,80,85] An alternative hypothesis is that *H pylori* interferes with transcription of *ATP4A* in parietal cells. Gastric adenocarcinoma (AGS) cells were transfected with a plasmid carrying the 5′ promoter sequence of human or rat *ATP4A* fused to a luciferase reporter gene.[105] Addition of histamine to these cells produced a dose-dependent increase in cAMP, $[Ca^{2+}]_I$, and activity of the *ATP4A* promoter. Addition of *H pylori* to the transfected cells reduced basal and histamine-stimulated activity of the *ATP4A* promoter, in a dose-dependent manner. *H pylori* also inhibited *ATP4A* promoter activity induced by phorbol myristate acetate or the diacylglycerol analog 1-oleoyl-2-acetyl-*sn*-glycerol, both of which were sensitive to staurosporine and calphostin C. The data indicated that *H pylori* inhibits expression of *ATP4A* via PKA and PKC signaling pathways.[105] Interestingly, human gastric epithelial cells transfected with a human *ATP4A–Luc* reporter construct were more sensitive to *H pylori*–induced repression than cells transfected with a rat *ATP4A–Luc* reporter construct. These findings are consistent with the observation that human and non-human primate stomachs are the principal foci of *H pylori* infection.[106]

H pylori–induced repression of *ATP4A* gene expression was later observed in human gastric biopsies. Co-culture of biopsies with *H pylori* for 15 or 24 hours reduced levels of *ATP4A*

mRNA, caused virtual disappearance of ATP4A protein, and prevented acid secretion.[107] The findings could not be attributed to cell death because control (mock-infected) gastric biopsies had normal levels of ATP4A protein and acid secretory capacity. These findings are consistent with those from a microarray analysis of parietal cell mRNAs from germ-free and *H pylori*–infected mice, which found a 5.3-fold down-regulation of *ATP4A* gene expression in mice infected with *H pylori*.[108] However, the comprehensive acute *H pylori*–induced elimination of ATP4A expression and acid secretory capacity observed in human biopsies[107] indicates that, in addition to regulating transcription of *ATP4A*, *H pylori* also affects post-transcriptional phases of ATP4A synthesis and activation. These findings support the direct intervention of *H pylori* virulence factors with parietal cell mechanisms of acid secretion.

Some of the mechanisms underlying *H pylori*–induced perturbation of *ATP4A* expression and translation have been determined.[109,110] *H pylori* strains expressing a type IV secretory system (T4SS) can transfer virulence factors through the T4SS-associated pilus into gastric epithelial cells. Structural T4SS proteins are encoded by a cytotoxin-associated gene (*cag*) pathogenicity island (PAI). The architecture of the T4SS has been elucidated by electron cryotomography[111] (Figure 2). The *H pylori* T4SS protein, CagL, interacts with $\alpha_5\beta_1$ integrins[112] to facilitate injection of the oncogenic bacterial protein CagA into cells. This activates multiple signaling pathways and up-regulates expression of the pro-inflammatory transcription factor NF-κB.[113] The $\alpha_5\beta_1$ integrins are expressed on basolateral membranes of epithelial cells, where they mediate morphologic and signaling interactions with cells in the lamina propria. *H pylori* disrupts the tight junction barriers that separate the luminal and serosal sides of the gastric epithelium[114–117] and protect the submucosa against gastric acid. This disruption allows *H pylori* to interact with parietal cell basolateral membrane receptors, activating intracellular signaling pathways, which are inaccessible from the epithelial apical surface. *H pylori* infection of cultured gastric epithelial cells inhibits expression of *ATP4A* by CagA oncoprotein- and peptidoglycan (soluble lytic transglycosylase)-dependent, ERK1- and ERK2-mediated binding of the NF-κB p50 homodimer to the *ATP4*A promoter.[118] Acute *H pylori* infection causes CagL to dissociate ADAM17 from $\alpha_5\beta_1$ integrins. This activates ADAM17-dependent, NF-κB–mediated repression of *ATP4A*.[119] The requirement for T4SS structural integrity for repression of *ATP4A* was confirmed in studies of *H pylori cag*PAI isogenic mutants, which are deficient in *cagE*, *cagM*, or *cagL*. Levels of *ATP4A* mRNA in human gastric biopsies were unaffected by co-culture with these mutants.[107] However, co-culture of biopsies with wild-type *H pylori* for 15 hours inhibited basal and histamine-stimulated acid secretion, measured by microphysiometry, confirming dependency on a functional bacterial T4SS, and consistent with direct *H pylori*–mediated activation of NF-κB in parietal cells. However, the observation does not exclude paracrine influences from other cells in the biopsies.[107]

H pylori also perturbs post-transcriptional regulation of *ATP4A* mRNA. In silico analysis of the *ATP4A* mRNA 3′ untranslated region identified microRNA 1289 as a highly conserved potential regulator of ATP4A mRNA translation.[120] *H pylori* infection of AGS cells transfected with *ATP4A* 3′ untranslated region-*Luc* reporter significantly reduced luciferase activity, whereas infection with Δ*cagA* or Δ*slt H pylori* had no effect on luciferase activity. Gastric biopsies from patients infected with *cagA*-positive *H pylori* had a significant increase in microRNA 1289 compared with biopsies from uninfected patients or those

infected with *cagA*-negative *H pylori*.[120] Only parietal cells express *ATP4A* mRNA, so the finding that *H pylori* up-regulates microRNA 1289 is consistent with direct interaction of specific *H pylori* virulence factors with parietal cells.

Virulence factors other than those that mediate CagA translocation or interleukin (IL)-8 secretion can repress expression of ATP4A by activating NF-κB.[121] AGS cells transfected with *ATP4A* promoter–*Luc* reporter constructs containing an intact or mutant NF-κB binding site were infected with wild-type *H pylori* strain 7.13, isogenic mutants that lack the *cag* PAI genes responsible for CagA translocation and/or *IL8* induction (*cagA*, *cagζ*, *cagε*, *cagZ*, and *cagβ*), or strains of *H pylori* that lack genes encoding 2 peptidoglycan hydrolases (*slt* and *cagγ*). Measurement of *H pylori*–induced activity of the *ATP4A* promoter in AGS cells, translocation of CagA, IL-8 secretion, and acid secretion in human oxyntic biopsies showed that *ATP4A* repression was independent of IL-8 expression. Furthermore, the data showed that CagA translocation and *H pylori* transglycosylases, encoded by *slt* and *cagγ*, participate in NF-κB–dependent repression of *ATP4A* and acid inhibition.[121]

Another virulence factor secreted by *H pylori*, vacuolating toxin (VacA), contributes to *H pylori*–induced hypochlorhydria.[122] Incubation of epithelial cells with VacA causes formation of anion-selective pores in the plasma membrane and large intracellular vacuoles,[123–125] apoptosis,[126] epithelial monolayer permeabilization,[122] and stimulates the autophagy pathway.[127] Exposure of isolated rabbit gastric glands to VacA induced an influx of extracellular calcium, which activated calpain, leading to proteolysis of ezrin. The loss of ezrin from the parietal cell apical membrane disrupted recruitment of tubulovesicles that contain ATP4A and thereby inhibited acid secretion (Figure 3).[128] In addition to direct suppression of acid secretion by transfer of virulence factors into parietal cells, *H pylori* also mobilizes cell proteins that inhibit acid secretion. Gastric *H pylori* infection increases T helper type 1 cell secretion of IL-1β, a pleiotropic pro-inflammatory cytokine that is a potent inhibitor of acid secretion.[129] Also, studies of rat oxyntic mucosa mounted in Ussing chambers showed that *H pylori* perfusion activated intramural calcitonin gene– related peptide sensory neurons in oxyntic mucosa that are coupled to somatostatin secretion; this subsequently suppresses acid secretion by inhibiting ECL cell histamine secretion and G cell gastrin secretion.[130]

H pylori's activation of neural pathways may explain how initially patchy colonization of superficial gastric mucosa acutely and profoundly inhibits acid secretion. In any event, direct virulence factor–dependent or indirect neural-dependent acid secretory inhibition probably occurs when parietal cells are in a resting state, when bacterial penetration into oxyntic glands and adherence to parietal cells is not impeded by the intra-glandular high-pressure counter-flow that is generated during active secretion.[131]

Progression to Neoplasia

Once regarded as a sterile environment, the gastric mucosa is now understood to host many bacterial genera collectively known as the gastric microbiota.[132] Molecular genotyping identifies the most abundant bacterial phyla in the healthy human gastric microbiota as Proteobacteria, Firmicutes, Bacteroidetes, Fusobacteria, and Actinobacteria. The most

prominent genera are *Provotella*, *Streptococcus, Rothia*, *Pasteurellaceae*, *Fusobacterium*, *Actinomyces*, *Neisseria*, *Hemophilus*, and *Porphyromonas*.[132–134] In individuals infected with *H pylori*, this organism becomes the single most abundant gastric phylotype,[135] and the relative abundance of non–*H pylori* Proteobacteria, Spirochaetes, and Acidobacteria increases, whereas that of Actinobacteria, Bacteroidetes, and Firmicutes decreases.[136] Depending on an individual's environmental, genetic, and bacterial risk factors, *H pylori* infection may initiate a sequence of pathologies, starting with chronic inflammation, that leads to atrophic gastritis (including autoimmune gastritis with pernicious anemia), intestinal metaplasia, dysplasia, and eventually gastric adenocarcinoma.[137]

Chronic infection with *H pylori* also reduces acid secretion, which may alter the abundance, diversity, and relative prevalence of bacterial species in the gastric microbiota. The role of acid in shaping the composition of the gastric microbiota is evident from studies of patients who use proton pump inhibitors or H_2-antagonists. These patients develop gastric bacterial overgrowth and have increases in non–*H pylori* bacteria.[138,139] *H pylori*–positive patients with and without gastric cancer and *H pylori*–negative patients all have significant differences in their gastric microflora.[140–142] These changes can alter the plasticity of the gastric mucosa and contribute to malignancy. Specific communities of the gastric microbiota other than *H pylori* might therefore contribute to *H pylori*–induced pathogenesis, in addition to *H pylori* virulence factors, host genetic polymorphisms, and diet.[140,143] Next-generation gene profile analyses of gastric microbial compositions revealed that patients with gastric carcinoma have a microbial dysbiosis, characterized by lower diversity, decreased abundance of *H pylori*, and higher genotoxic potential, compared to the gastric microbiota of patients with chronic gastritis.[144] Eradication of *H pylori* increased bacterial diversity and restored the relative abundance of other bacteria to levels similar to *H pylori*–negative individuals.[145]

During homeostasis, turnover and regeneration of gastric epithelial cells are mediated by differentiation of stem cells located in the isthmus region.[146,147] Upon infection with *H pylori*, mature parietal and chief cells are replaced by proliferating cells that secrete mucins and wound repair proteins in a lesion called spasmolytic polypeptide-expressing metaplasia (SPEM).[148,149] In studies of mice, SPEM was found to arise from a subpopulation of chief cells that express the G protein-coupled receptor Lgr5, a stem cell marker regulated by Wnt signaling.[150] In gastric pyloric epithelium, $Lgr5^+$ cells are recruited back into the cell division cycle to serve as stem cells that repair gland damage in the short term, and in the longer term can also promote SPEM that persists as a precancerous lesion.[151] Chief cells that have not attained full functional maturation are impaired in the ability to trans-differentiate into SPEM, and lose that ability as they age.[152] However, the mechanisms by which differentiated cells gain re-entry into the cell division cycle are poorly understood. It is important to learn more about the mechanisms that regulate *H pylori*–perturbed parietal cell homeostasis and progression into neoplasia. Several lines of evidence indicate that *H pylori* and the secreted oncoprotein CagA have the potential to reprogram epithelial cells and induce stem cell–like properties in gastric epithelial cells, exemplifying the complex interactions between *H pylori* and progenitor cells in the gastric mucosa.[153–155] Interestingly, CagA-induced gastric carcinogenesis occurs via iterative mechanisms, whereas the oncogenic activities of CagA are successively replaced by a series of genetic and/or

epigenetic alterations in pre-cancer cells during long-term infection with CagA-positive *H pylori*.[155]

Given the disparity of gastric cancer incidence between Asian/Pacific Islanders and African Americans,[156] studies of cell phenotypes in gastric biopsy samples from these populations might provide insights into the mechanisms that mediate the interactions among gastric microbiota, *H pylori*, and parietal cells (Figure 4).[157] These studies would require precise analyses of cell proliferation, chromosome stability during cell division, and acid secretory activity in parietal cells. An example of this approach is the comprehensive evaluation of 295 primary gastric adenocarcinomas included in the Cancer Genome Atlas Research Network that classified gastric cancer into 4 subtypes, based on 6 types of molecular features, including DNA methylation profile, Epstein-Barr virus status, whole-exome sequence, and changes in copy number of somatic genes.[158] Samples were also examined for microsatellite instability, a common feature of gastric tumors, which can be used to determine mechanisms of pathogenesis and the contributions of the gastric microbiome and genetic factors.

New Systems and Technologies to Study Parietal Cells

The gastric mucosal epithelium constantly renews itself and the stem cells that mediate this process reside in the gastric glands.[146,147] As observed during renewal of mouse enterocytes,[159] symmetric divisions of stem cells and neutral drift lead to monoclonal antral gastric units.[151] It is unclear whether symmetric divisions of stem cells are responsible for epithelial renewal in other gastric regions, such as the corpus. Identification of markers of gastric stem cells in mice, including LGR5 and Troy (a member of the tumor necrosis factor receptor superfamily), has facilitated understanding of the gastric epithelial cell lineage.[150,160] Lineage tracing revealed $Lgr5^+$ cells to be self-renewing, multipotent stem cells responsible for the long-term renewal of the gastric epithelium. Using an in vitro culture system, long-lived organoids resembling mature pyloric epithelium have been generated in culture from single $Lgr5^+$ cells.[147] In addition, temporal manipulation of the FGF, WNT, BMP, retinoic acid, and EGF signaling pathways allowed for de novo generation of 3-dimensional human gastric organoids from human pluripotent stem cells.[161]

To date, studies of host–pathogen or host–commensal interactions have relied on experimental models, such as primary or transformed cell lines and infected animals.[162] These models have elucidated unprecedented details of *H pylori* pathophysiology, but the organism's classification as an A1 carcinogen has necessarily limited experimental infection of humans. The mechanisms of *H pylori*'s persistence and ability to colonize the stomachs of about half of the world's population[163] are poorly understood. Although chronic *H pylori* infection can have serious pathological sequelae, including gastric ulceration and gastric adenocarcinoma, the temporal profile of *H pylori*–mediated pathogenesis has not been established. The development of human gastric organoids therefore provides a powerful tool to study human gastric epithelial development, gastric stem cell plasticity, and the response of gastric epithelial cells to *H pylori* infection and other ecological perturbations (Figure 4).

Light sheet fluorescence microscopy provides an advanced approach for rapid acquisition of 3-dimensional images over large fields of view and over long durations.[164] In essence, 3-dimensional images are compiled from successive light sheet illuminations of thin, 2-dimensional optical sections. The high spatial and temporal resolution of the imaging enables tracking of every cell in relatively thick samples, such as embryos over long periods of time.[164] A light sheet microscopy experiment provided high spatial resolution of mitotic stem cells in murine gastric organoids labeled with α-tubulin antibody and the nuclear dye 4′,6-diamidino-2-phenylindole (Figure 4*A*–*C*). This study provided a proof of principle for applying light sheet microscopy in real-time imaging of mitotic cellular dynamics in organoids combined with gene editing. Future studies using patient-derived gastric organoids will afford insights into the molecular mechanisms underlying *H pylori*–elicited pathogenesis, and provide valuable personalized drug discovery opportunities. In this case, the strategy of synthetic lethality can be used to screen for better regimens of precision medication.

Live fluorescence imaging of mouse gastric organoids exposed to the lipophilic weak base acridine orange, which accumulates in acid spaces in parietal cells (eg, Figure 5*A*; *b*', *arrows*), has been used to study acid secretion. Using light sheet fluorescence microscopy of gastric organoids, researchers can visualize acid secretion by individual parietal cells as a shift in acridine orange fluorescence from green to red in response to the secretagogues. When organoids preincubated with acridine orange were exposed to 100 μM histamine, red fluorescence intensity in parietal cells increased within 5 minutes, indicating the onset of histamine-induced parietal cell acid secretion (Figure 5*A*; *b*). Researchers assessed acid secretion in response to *H pylori* infection by injecting *H pylori* into the lumen of gastric organoids (Figure 5*B*; *b*). Two hours after the injection of *H pylori* into the lumen, gastric organoids were loaded with acridine orange for live fluorescence imaging, followed by histamine stimulation. Acute *H pylori* infection of gastric organoids suppressed acid secretion from parietal cells, visualized as little or no change in residual green fluorescence after addition of histamine (Figure 5*B*). These observations indicate that human gastric organoids in culture might be used to study *H pylori*–induced pertubations of parietal cell biology leading to disease. Such organoids might also provide opportunities for parietal cell and other gastric cell phenotype–based screening of small molecule libraries for potential therapeutic applications.

Future Directions

A recently developed lattice light-sheet microscopy with adaptive optics has enabled non-invasive aberration-free imaging of subcellular processes, including endocytosis, organelle contacts, and remodeling during mitosis in live human intestinal organoids and even live zebrafish.[165] This technology reveals the phenotypic diversity within cells from different organisms and developmental stages and might be used to determine how cells adapt to different physiologic settings.[166] Combinations of advanced optical imaging protocols, such as lattice light-sheet microscopy with adaptive optics and photoactivatable complementary fluorescence,[167] spectral imaging analyses,[165] and correlative light and cryo-electron microscopic tomography,[168] could increase our understanding of parietal cell organelle interactions and the cellular adaptations to *H pylori* infection. In this context, inclusion of

immune cell lineages in gastric epithelial organoids would allow study of host–pathogen interactions with great precision in vitro. The expanding collection of gene-edited mice, (epi)genetically defined tissues from patients,[169] and gastric organoids derived from gene-edited mice, will allow modeling of cellular and molecular events underlying pathogenesis of gastric cancer, and development of optimal therapeutic interventions.

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Abbreviations used in this paper:

cAMP	adenosine 3′,5′-cyclic mono-phosphate
ECL	enterochromaffin-like
IL	interleukin
Lat B	latrunculin B
SNARE	soluble N-ethylmaleimide-sensitive factor attachment protein receptor
SPEM	spasmolytic polypeptide-expressing metaplasia
T4SS	type IV secretory system

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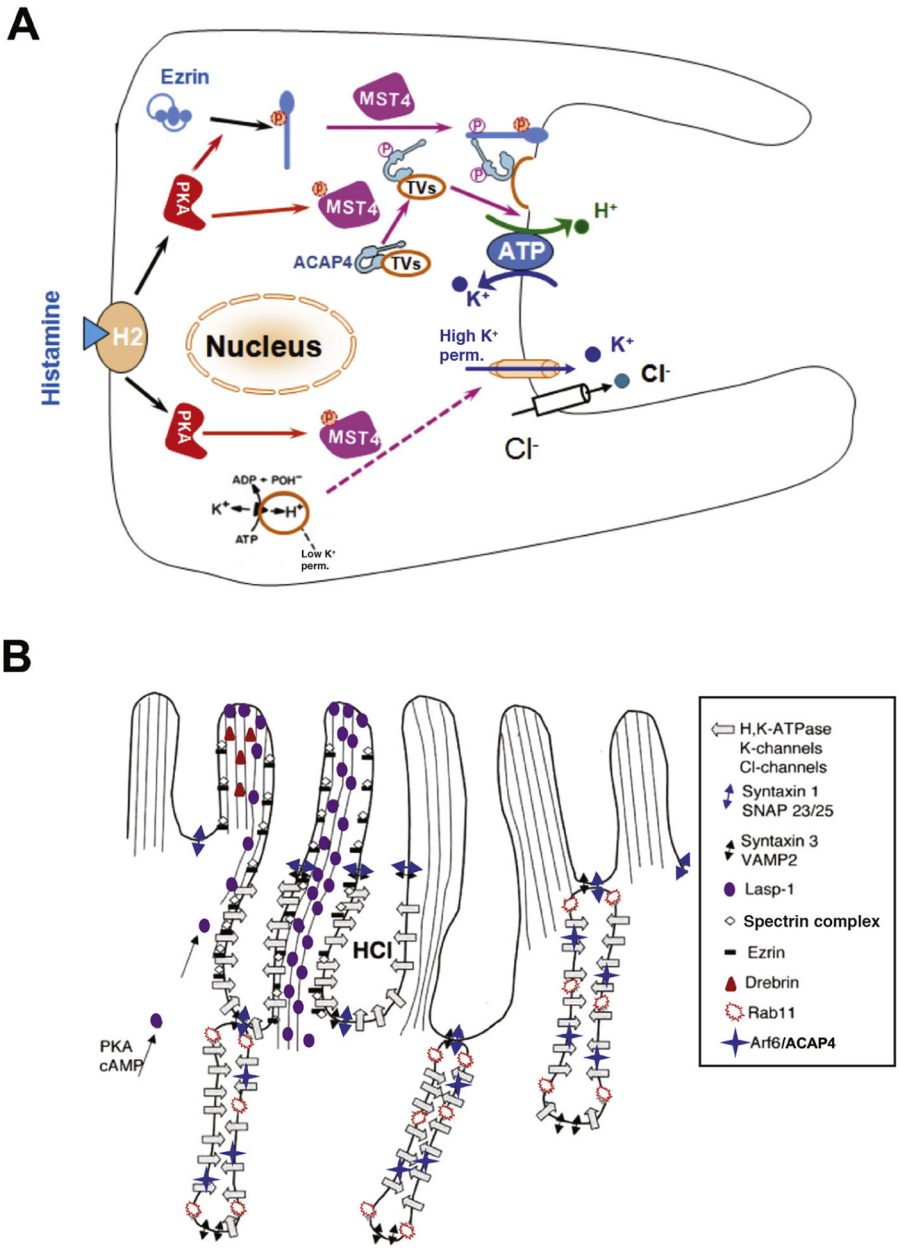

Figure 1.

Mechanisms of parietal cell acid secretion. (*A*) In the resting state, H,K-ATPase and ACAP4 are sequestered in tubulovesicles with low K^+ permeability. Histamine stimulation activates PKA signaling via MST4, resulting in conformational changes in ezrin and ACAP4 that allow them to interact and induce tubulovesicle translocation. MST4 then phosphorylates ACAP4 at Thr545, promoting its association with ezrin. Phosphorylation of ezrin by PKA at Ser66 causes a conformational change, which promotes docking and anchoring of H,K-ATPase- and ACAP4-containing tubulovesicles at the apical plasma membrane, where K^+ permeability is high. MST4 might mediate K^+ channel activation, but the mechanisms of this process have not been determined. (*B*) Model of apical membrane cytoskeletal remodeling. Histamine stimulation activates PKA, resulting in phosphorylation and activation of MST4

kinase. Activated MST4 mediates formation of the apical signaling machinery, comprising ARF6, ACAP4, and ezrin, which docks tubulovesicles to the apical membrane by interactions with Lasp-1, drebrin, spectrin, and other proteins, such as syntaxin 1 and SNAP23/25. This leads to reorganization of apical microvillous structures with extended apical surface and onset of proton pumping. The exact mechanisms underlying tubulovesicle fusion with the apical membrane and insertion of proton pump into the apical membrane remain less illustrated.

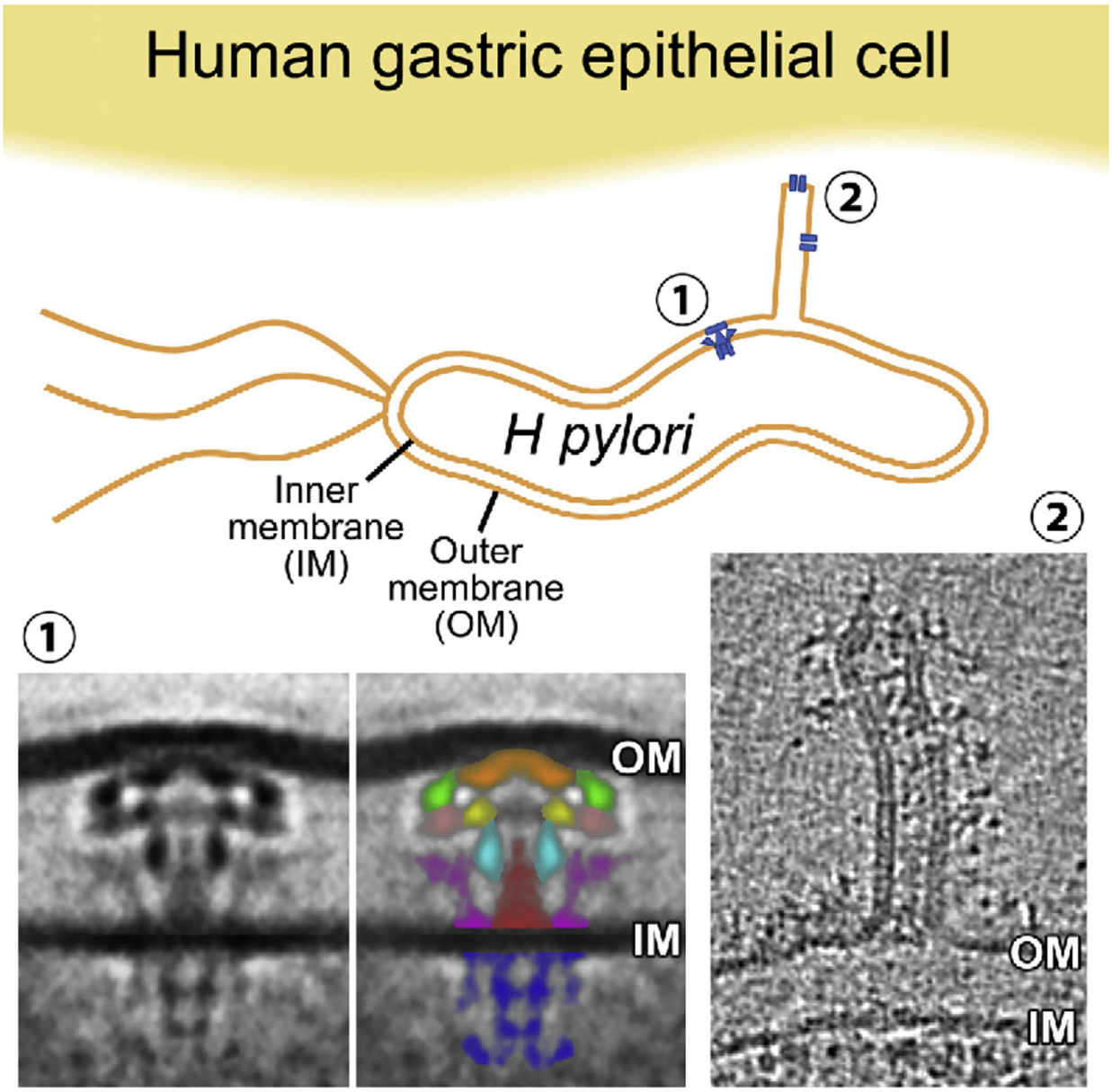

Figure 2.

Electron micrograph of the *Helicobacter pylori* Cag T4SS.[111] Using electron cryotomography, Chang et al[111] determined the structure of the *H pylori* Cag T4SS, a transmembrane multimeric translocation apparatus assembled when *H pylori* encounters gastric epithelial cells. The *cag* T4SS is a 5-barrel structure (1) in close proximity to a tube-like structure that projects from the bacterium (2) and may serve to translocate virulence factors into parietal cells. OM, *H pylori* outer membrane; IM, *H pylori* inner membrane; *green*, α-subunit; *light brown*, β-subunit; *cyan*, γ-subunit; *purple*, wing subunit; *brown*, stalk subunit; and *blue*, cytoplasmic Cag5/VirD4 coupling protein. Perturbation of the structural integrity of T4SS complexes might obviate pathogenesis. Integration of cryo-electron tomography and correlative fluorescence microscopy[163] can be used to visualize the 3-

dimensional ultrastructural features during *H pylori* infection of gastric parietal cells in organoids. Comparisons of ultrastructural changes in gastric organoids after exposure to different strains of *H pylori* could be used to study mechanisms of *H pylori* infection and develop therapeutic interventions.

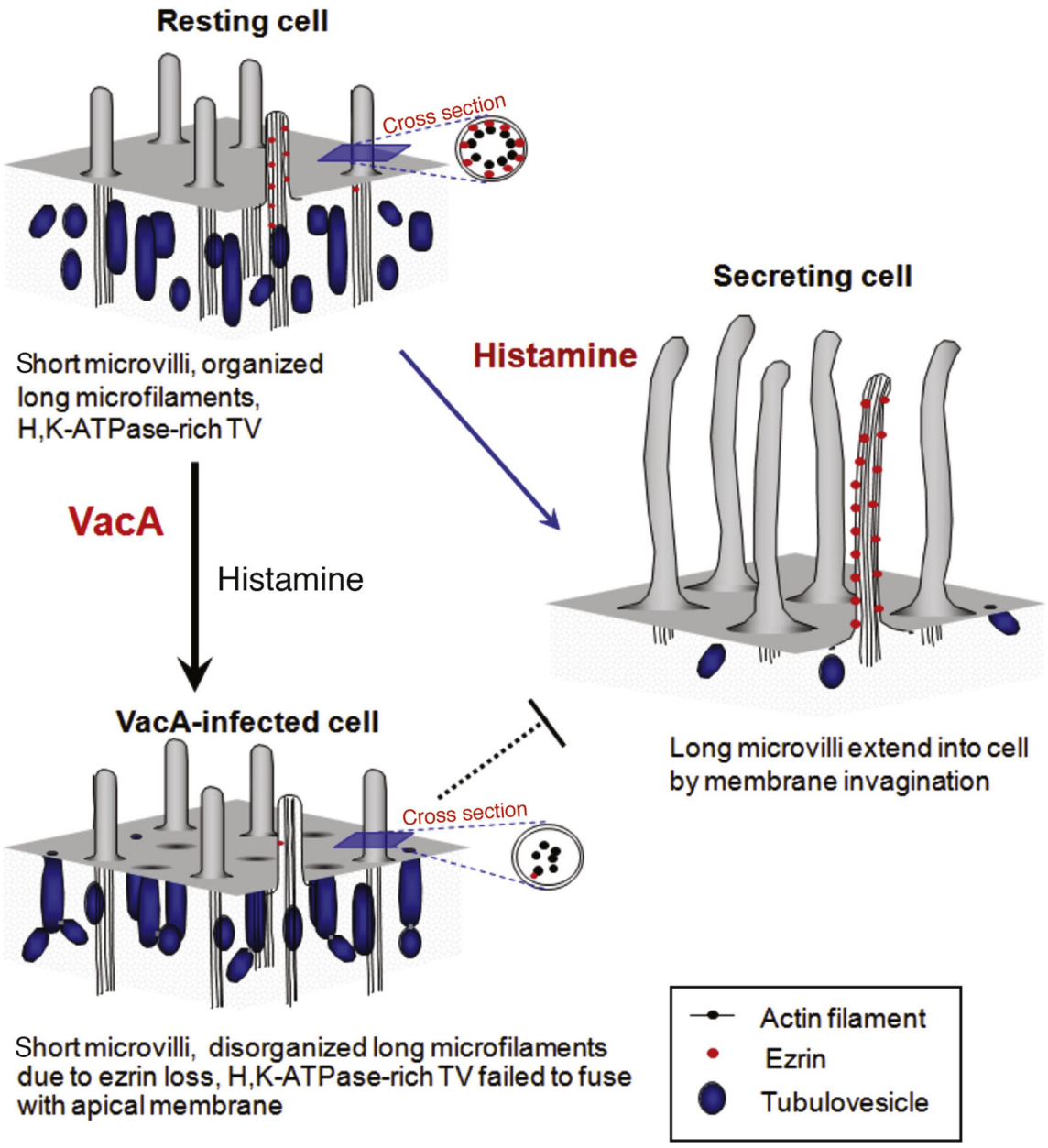

Figure 3.

Modeling changes in the cytoskeleton at the apical membranes of gastric parietal cells. Model of VacA-induced hypochlorhydria. In resting cells, apical microvilli are supported by microfilaments that extend deep into the cytoplasm. Ezrin (*red dots*) links actin filaments with the apical plasma membrane. Histamine stimulation leads to docking and fusion of ATP4A-rich tubulovesicles to the apical plasma membrane, expanding the apical surface. Interactions between the apical membrane and microfilaments, via ezrin, reorganize the expanded surface into long microvilli. VacA induces proteolysis of ezrin, disrupting the interactions between the apical membrane and microfilaments, and preventing recruitment of ATP4A to the apical membrane. The ensuing parietal cell inability to secrete acid results in hypochlorhydria.

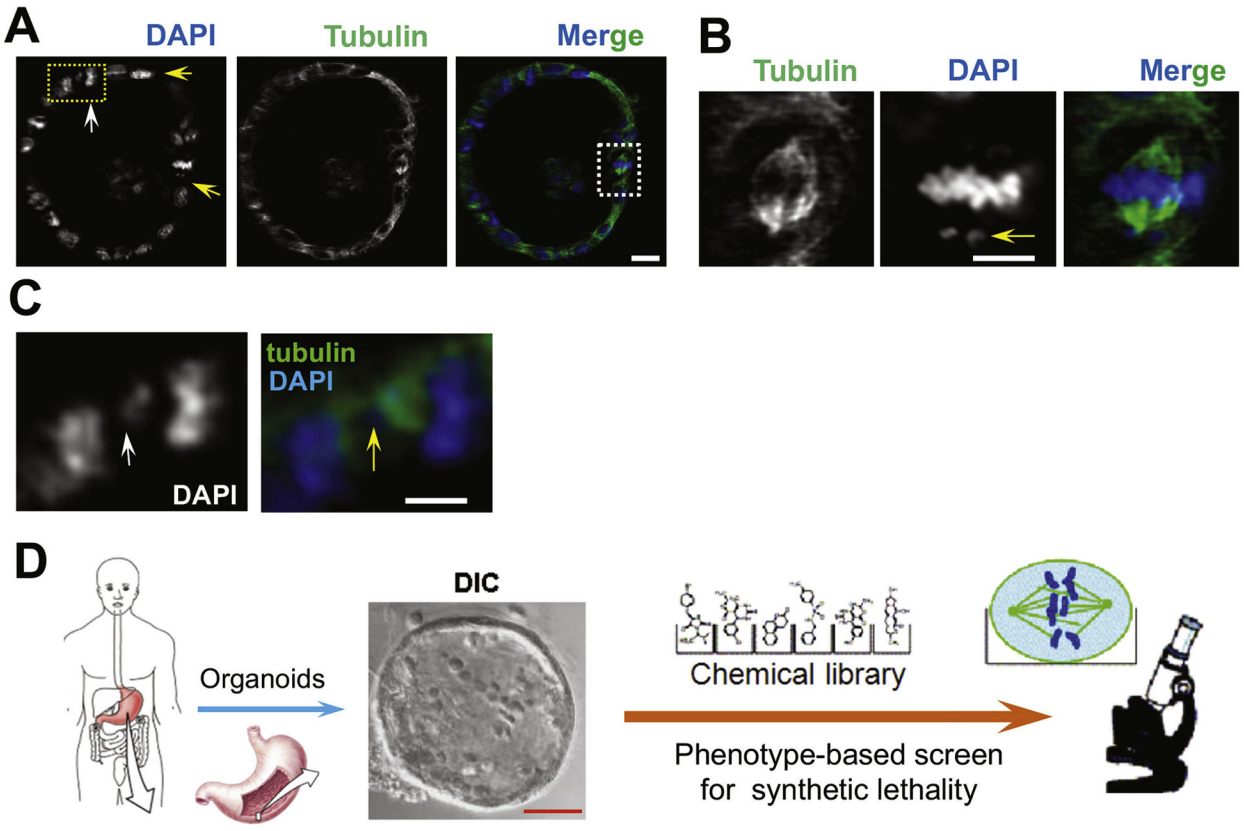

Figure 4.

Gastric stem cell division and plasticity and parietal cell functions in gastric organoids. (*A*) Non-secreting mouse gastric organoids were maintained in cimetidine and fixed for immunocytochemical staining of α-tubulin (*green*), and 4′,6-diamidino-2-phenylindole (DAPI) (*blue*). The light-sheet micrograph shows 4 mitotic cells with apparent different spindle orientations. Two metaphase-like cells are indicated by 2 yellow rows. Insets show 1 prometaphase (*yellow arrow*) and 1 aberrant anaphase-like cell with apparent perturbations of chromosome segregation (indicated by *yellow arrow*). Visualization of chromosome segregation in gastric organoids can be used to study chromosome stability and morphology of cancer cells. Light-sheet micrography of gastric epithelial and stem cells can be used to study their responses to different extracellular cues or therapeutic agents. For example, organoids derived from gastric cancer cells of patients might be used to identify the most effective treatments. Real-time spectral imaging could be used to study the effects of different combinations of agents. Scale bar = 20 μm. (*B*) Magnified image from (*A*). This mitotic cell has a lagging chromosome and spindle orientation error (*arrow*). Scale bar = 10 μm. (*C*) Magnified image from (*A*). This anaphase cell has a chromosome bridge readily apparent in DAPI staining (*white arrow*) and merged image with tubulin. Scale bar = 10 μm. (*D*) Effects of different combinations of therapeutic agents on gastric cancer cells. The schematic illustration shows how patient-derived samples can be used to screen for compounds and/or regimens that specifically tailored to specific cancers with different genetics and epigenetics.

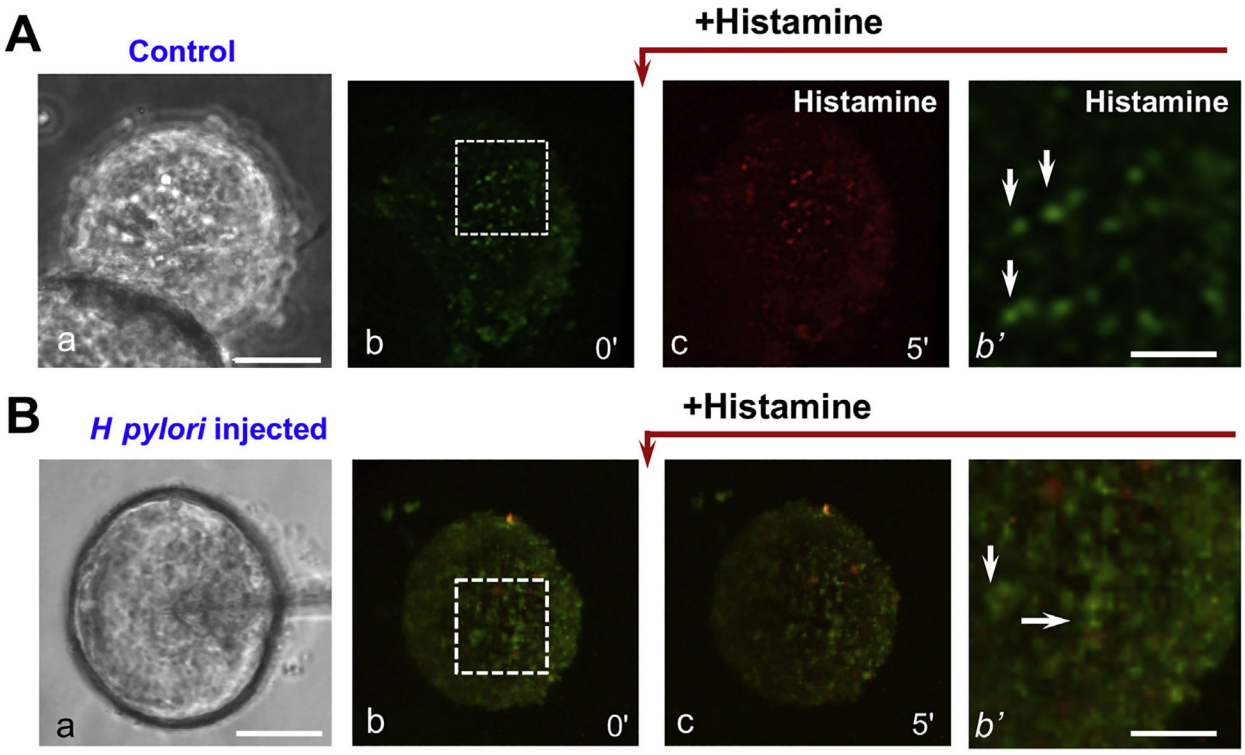

Figure 5.

Effects of acute *Helicobacter pylori* infection on acid secretion in gastric organoids. Control mouse gastric organoids or organoids exposed to microinjected *H pylori* for 4 hours were loaded with acridine orange (AO) and exposed to histamine. (*A*) Differential interference contrast (DIC) light micrograph of control organoid (*a*), and fluorescence micrographs of organoids before and after histamine stimulation (*b*) and (*c*) were collected and presented. Parietal cells are readily identified in the organoids by the AO labeling in magnified area (*b*', *arrows*). (*B*) DIC micrograph of a gastric organoid undergoing microinjection with *H pylori*, and fluorescence micrographs of injected organoids before and after histamine stimulation (*b*) and (*c*). Parietal cells are identified by AO labeling (*b', arrows*). *H pylori* infection suppressed the parietal cell response to histamine, as indicated by minimized AO fluorescence shift from green to red. Light micrograph scale bars = 100 μm. Fluorescence micrograph scale bars = 25 μm.