HELICOBACTER PYLORI-MEDIATED DYSREGULATION OF P120^{CTN} AND MATRIX METALLOPROTEINASE-7

By

Seth Rayborn Ogden

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements for

the degree of

DOCTOR OF PHILOSOPHY

in

Cancer Biology

May, 2009

Nashville, Tennessee

Approved:

Professor Richard M. Peek, Jr.

Professor Timothy L. Cover

Professor Barbara Fingleton

Professor Albert B. Reynolds

To Kristen, my best friend and partner, for the best 5 years of my life

and

To my family, without you, none of this would have been possible

ACKNOWLEDGEMENTS

I am very grateful to my mentor, Dr. Richard M. Peek, Jr. (Rick) for providing me the opportunity to be a part of his laboratory. Rick has provided generous financial, educational, social, and emotional support during the 5 years I have known him. He has been consistently supportive no matter in what direction I have chosen to take my research. His role as a mentor has been invaluable in training me to be an independent and logical thinker, giving me the confidence to pursue my personal scientific interests and teaching me to confidently present my findings, whether through lectures or manuscripts. His unique ability to judge the character and ability of others has fostered an environment of fun, friendliness, and hard work within the laboratory. I would like to thank all of the members of the Peek lab, past and present, for their help and support, as well. Uma Krishna, Judy Romero-Gallo, and Dawn Israel have not only been a wealth of *H. pylori* knowledge, they have been extremely patient and understanding throughout my training. Aime Franco, Toni Nagy, Lydia Wroblewski, and Shannon Allen have been a joy to work with and always willing to help out in a pinch. Finally, Daniel O'Brien has made one contribution after another to my life with regard to science, relationships, and our future careers as intellectual property lawyers.

I am also very grateful to all of my collaborators both here in Nashville and around the world. Thanks to Dr. Stuart Hobbes and Dr. Jinxiong Wei for letting me in on their projects. Thanks to Dr. Kaye Washington and Dr. Elizabeth Harris for all your help scoring inflammation. Thanks to Dr. Meredith Vaughn and Nicole Lobdell for help with $p120^{ctn}$ and its many nuances. Thanks to Dr. Mark Freye for helping me separate soluble

compartments from insoluble ones. Thanks to Dr. Barbara Fingleton for about 8 rounds of staining on one set of slides. Thanks to Dylan Patel for quantitating proliferation for about 200 fields of view. Thanks to Dr. Howard Crawford (SUNY-Stoneybrook) for constructs and MMP-7 advice. And, thanks to Dr. Silja Wessler and Dr. Christiane Weydig (Magdeburg, Germany) for helping out with the Kaiso luciferase assays.

I would also like to thank all of the other members of the Vanderbilt community that have made this journey from grad student to PhD possible. Thank you to my committee members for good advice, a little bit of pressure, and continued support. Thank you to all of the administrative staff in GI (Ruby, Sue, Myra, Dorothy, and Philly) and Cancer Biology (Debbie, Tracy, and Vanessa) for dealing with all of my questions, paperwork, and reimbursement requests. Thanks to all of the people who make the Integrated Graduate Program in Biological Sciences possible, especially Dr. Jim Patton and Dr. Robert Chalkley. And, thanks to the multiple core facilities, including immunohistochemistry, tissue acquisition, and microscopy for the training and services provided.

Finally, I would like to thank the people who give me a reason to get up and do science every day—my friends and family. There are too many to name, but I hope you all know you are, whether you hail from Paducah, went to school at the University of Virginia or James Cook University, or you know me from Music City, USA. Thanks to my family, Mom and Dad, Granny and Granddaddy, and everyone else who has been with me every step of the way, you have given me the knowledge, values, and strength to become the man I have today. Last, but certainly not least, Kristen, I love you so much. I am so grateful that I came to Vanderbilt for my studies. Otherwise, I would have never

had the opportunity to meet you. Thanks for going out on a limb and giving your phone number to this weird scientist guy at the Stage wearing a wide-brimmed hat, boots, and a buckle.

During my training, I was supported by the Multidisciplinary Basic Research Training in Cancer T32 CA09592 and NIH RO1 Grants DK050587, CA077955, and DK073902.

ORIGINAL PUBLICATIONS

Wroblewski LE, Shen L, **Ogden SR**, Romero-Gallo J, Lapierre LA, Israel DA, Turner JR, Peek RM Jr. Helicobacter pylori Dysregulation of Gastric Epithelial Tight Junctions by Urease-Mediated Myosin II Activation. 2009. Gastroenterology **136**:236-246.

Ogden SR, Wroblewski LE, Weydig C, Romero-Gallo J, O'Brien DP, Israel DA, Krishna US, Fingleton B, Reynolds AB, Wessler S, Peek RM Jr. p120 and Kaiso regulate Helicobacter pylori-induced expression of matrix metalloproteinase-7. 2008. Mol Biol Cell. **19**(10):4110-21.

O'Brien DP, Romero-Gallo J, Schneider BG, Chaturvedi R, Delgado A, Harris EJ, Krishna U, **Ogden SR**, Israel DA, Wilson KT, Peek RM Jr. Regulation of the Helicobacter pylori cellular receptor decay-accelerating factor. 2008. J Biol Chem. **283**(35):23922-30.

OBrien DP, Israel DA, Krishna U, Romero-Gallo J, Nedrud J, Medof ME, Lin F, Redline R, Lublin DM, Nowicki BJ, Franco AT, **Ogden SR**, Williams AD, Polk DB, Peek RM Jr. The role of decay-accelerating factor as a receptor for Helicobacter pylori and a mediator of gastric inflammation. 2006. J Biol Chem. **281**(19):13317-23.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Figures Page

LIST OF ABBREVIATIONS

ADAM ..... A Disintegrin and Metalloproteinase

AP-1 ..... Activator Protein-1

APC ..... Adenomatosis Polyposis Coli

BMDC ..... Bone Marrow-derived Cell

BSA ..... Bovine Serum Albumin

BTB/POZ ..... Broad Complex, Tramtrak, Bric a Brac/Pox Virus and Zinc Finger

Cag ..... Cytoxin Associated Gene

CARD4 ..... Caspase Recruitment Domain 4

CFU ..... Colony Forming Units

ChIP ..... Chromatin Immunoprecipitation

CRE ..... cAMP Response Element

Csk ..... Carboxy-terminal Src Kinase

Ctn ..... Catenin

DMEM ..... Dulbecco's Modified Eagle's Medium

DPBS ..... Dulbecco's Phosphate Buffered Saline

DTT ..... Dithithreitol

ECM ..... Extracellular Matrix

EGFR ..... Epidermal Growth Factor Receptor

ERK1/2 ..... Extracellular Regulated Kinase 1/2

ES ..... Embryonic Stem

FasL ..... Fas Ligand

FBS ..... Fetal Bovine Serum

GEF ..... Guanine Nucleotide Exchange Factor

GAPDH ..... Glyceraldehyde 3-phosphate Dehydrogenase

GSK-3β .....Glycogen Synthase Kinase-3β

HEK293..... Human Endothelial Kidney 293

HB-EGF ..... Heparin-binding Epidermal Growth Factor

HBSS.....Hank's Balanced Salt Solution

IGF .....Insulin-like Growth Factor

IGFBP .....IGF Binding Protein

IL..... Interleukin

INS-GAS .....Insulin-gastrin

LEF-1 .....Lymphoid Enhancer Factor-1

MALT ..... Mucosa Associated Lymphoid Tissue

MAPK ..... Mitogen-activated Protein Kinase

Min..... Multiple Intestinal Neoplasia

MIP .....Macrophage Inflammatory Protein

MMP ..... Matrix Metalloproteinase

MMTV .....Mouse Mammary Tumor Virus

MNNG .....*N*-methyl-*N*-nitro-*N*'-nitrosoguanidine

MOI.....Multiplicity of Infection

MPO.....Myeloperoxidase

NF-κB ..... Nuclear Factor-κB

NLS..... Nuclear Localization Sequence

Nod1 ..... Nucleotide-binding Oligomerization Domain Containing 1

PAS ..... Protein A Sepharose

PBS ..... Phosphate Buffered Saline

PEA3 ..... Polyomavirus Enhancer Activator 3

PG ..... Peptidoglycan

PKC ..... Protein Kinase C

PPAR ..... Peroxisome Proliferator-activated Receptor

PTP ..... Protein Tyrosine Phosphatase

PVDF ..... Polyvinylidene Difluoride

RhoA ..... Ras Homolog Gene Family, Member A

RICK ..... RIP-like Interacting CLARP Kinase

RIPA ..... Radio Immunoprecipitation Assay

RPMI ..... Royal Park Memorial Institute

RT-PCR ..... Reverse Transcription Polymerase Chain Reaction

SDS ..... Sodium Dodecyl Sulfate

SEM ..... Standard Error of the Mean

SH2 ..... Src Homology 2

SHP-1 ..... Src Homology Phosphatase-1

Slt ..... Soluble Lytic Transglycosylase

SRE ..... Serum Response Element

SRF ..... Serum Response Factor

TCF ..... T Cell Factor

Th1 ..... T Helper 1

TIMP ..... Tissue Inhibitor of Metalloproteinase

TLR ..... Toll-like Receptor

TNF-α ..... Tumor Necrosis Factor-α

VacA ..... Vacuolating Cytotoxin A

CHAPTER I

INTRODUCTION

Helicobacter pylori

Helicobacter pylori is a Gram-negative, urease-, catalase-, and oxidase-positive curved bacillus that possesses 4-5 sheathed polar flagella required for motility (**Figure 1**). It is one of the most genetically diverse bacteria known, as evidenced by the fact that virtually every isolate is unique regardless of whether cultured from different individuals or simply different gastric biopsies from a single individual (136). *H. pylori* is specifically adapted for survival within its gastric niche, and the majority of strains express factors that have evolved to affect host cell signaling pathways, resulting in enhanced virulence. Approximately 50% of the world's population is colonized with *H. pylori*, but only a subset of infected persons develops disease (242). Of these infected individuals, approximately 10% develop peptic ulcer disease, 1% develop gastric adenocarcinoma, and less than 0.1% develop mucosa associated lymphoid tissue (MALT) lymphoma. *H. pylori* infection is most commonly acquired at a young age and is thought to be passed from parent to child (84), and, although infection can be found in all regions of the world, the prevalence of *H. pylori* colonization is higher in developing regions than in developed countries (83, 84).

H. pylori identification and association with gastric disease

Although the presence of spirochaetes in human gastric mucosa had been noted

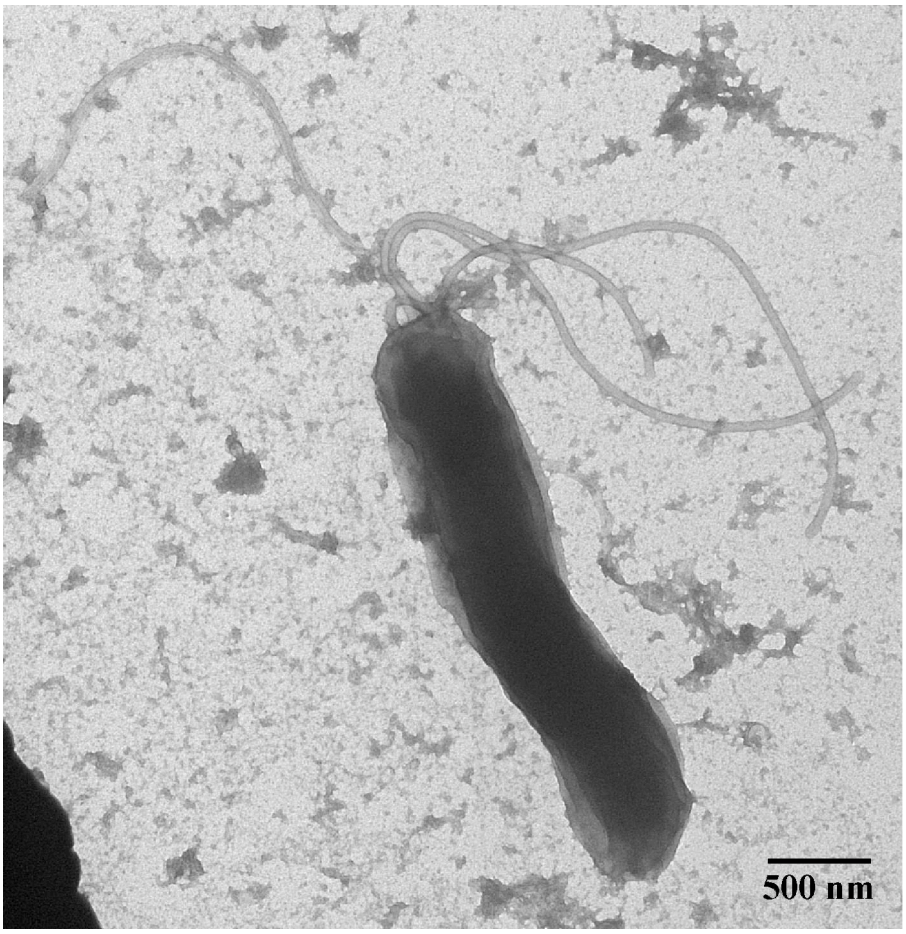

Figure 1. *H. pylori* is a gram-negative, curved bacillus that possesses 4 to 5 polar flagella required for motility. Electron micrograph provided by and reprinted with the permission of Aime T. Franco.

previously (98, 236), Marshall and Warren first conclusively demonstrated the presence ofwhat is now known as *H. pylori* in 1983 by staining gastric biopsy sections using the Warthin-Starry silver method (189). They described the bacterium as a curved bacillus possessing polarized sheathed flagella found in close association with the surface epithelium (189). It was noted that *H. pylori* was more prevalent in patients suffering from chronic gastritis, although its presence was reported in about half of all routine gastric biopsy specimens (189). Further study revealed that *H. pylori* was present in 100 out of 100 patients suffering from a duodenal ulcer (188). Although spiral gastric bacteria had been described as commensal organisms in other mammals (78, 174, 320), the "highly suspicious" association of this bacterium with polymorphonuclear infiltration in the human antrum and a direct correlation to duodenal ulceration led these investigators to pursue a hypothesis that this organism is pathogenic, playing a direct role in the development of gastritis and duodenal ulceration (188, 189).

The pair of investigators successfully isolated and cultured *H. pylori* from a gastric biopsy in 1984, initially naming it *Campylobacter pyloridis* (187). Despite significant skepticism about his hypothesis by leaders in the gastroenterology field, Marshall pressed on in his pursuit to prove that *H. pylori* is the causative agent of gastritis and duodenal ulceration. Realizing the need for indisputable evidence, he went so far as to test *H. pylori*'s capacity to fulfill Koch's postulates by drinking a liquid broth culture of the bacterium, documenting the resulting pathology by serial endoscopies, and eradicating the infection via antibiotic treatment (185). In 1988, Marshall and Warren published their most convincing data yet, a prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pyloridis*, in which it was demonstrated

that treatment with colloidal bismuth subcitrate in combination with the antibiotic tinidazole could eradicate *C. pyloridis* in up to 70% of patients, with clearance resulting in healing of 92% of ulcers and a mere 21% rate of relapse during a 12-month follow-up period (186). Rauws and Tytgat gave credence to this work in a follow-up study in 1990 that concluded, "*H. pylori* eradication, without altering acid output, will become the mainstay of duodenal ulcer treatment because it cures the disease," (252). This has lead to acceptance of a combination of triple-antibiotic therapy and bismuth supplementation as the appropriate treatment for individuals seeking relief from *H. pylori*-induced gastric disease. Marshall and Warren ultimately received the Nobel Prize in Medicine in 2005 for their discovery of *H. pylori* and its role in gastritis and peptic ulcer disease.

H. pylori **epidemiology**

H. pylori colonizes the stomachs of approximately half of the world's population (36, 202), and, according to a number of studies over the course of 2007-08, overall rates of infection vary from a low of 11% to a high of 69% **(Figure 2)** (37). Infection of such a large percentage of the population throughout the world suggests that *H. pylori* has developed a very robust ability to colonize its host, though our understanding of its mechanism of transmission remains rather limited. However, prevalence appears to vary widely by geography, age, race, and socioeconomic status. Rates of colonization are higher in developing countries than those in developed countries (247). This observation is consistent with epidemiological studies suggesting that the strongest risk factors for *H. pylori* infection include inadequate sanitation practices, socioeconomic status, and

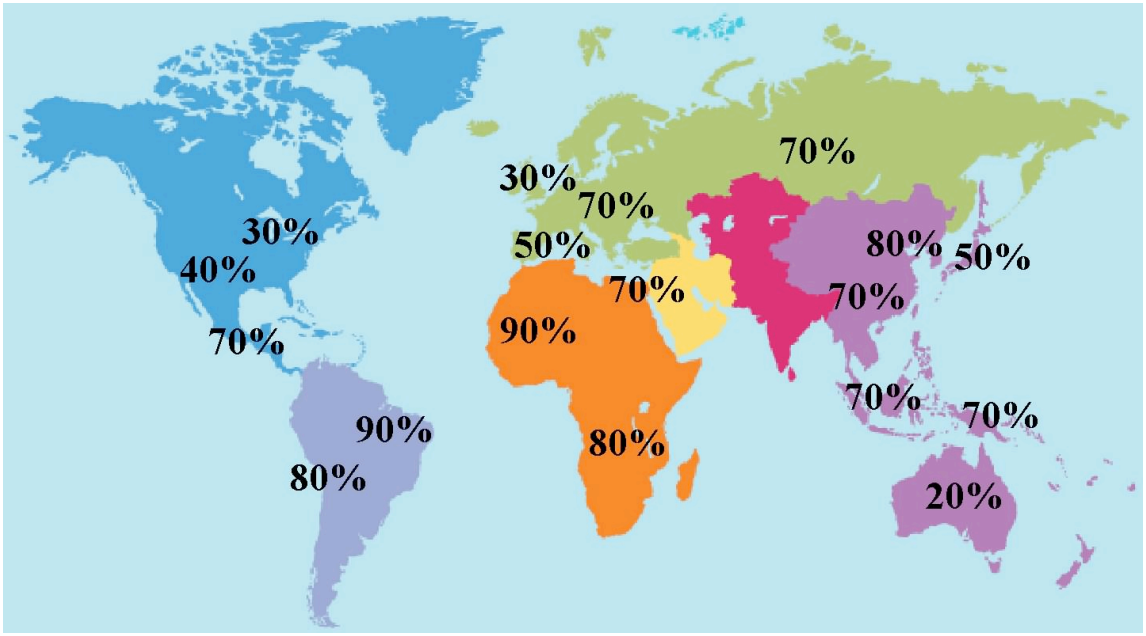

Figure 2. The prevalence of *H. pylori* infection varies widely by geography. Colonization rates are typically higher in developing countries than those in developed countries. Reproduced from Marshall (184).

crowded or high-density living conditions (36, 107, 166, 180, 196). Epidemiologic data suggests that most transmission tends to occur through close contact between individuals within households (9, 154, 155, 192, 335). Multiple studies have demonstrated intra-institutional and intra- familial clustering of *H. pylori* infection, further suggesting that poor hygiene and crowded living conditions may facilitate transmission within these groups (36, 107, 180, 196). Fortunately, many studies have also noted an overall decline over time in prevalence both in developing and developed countries (37) that will likely continue as standards of living improve around the world.

Colonization by *H. pylori* induces chronic inflammation

Although the majority of people colonized by *H. pylori* (80%-90%) do not display any symptoms of disease (28), most, if not all, develop gastritis in response to the presence of this pathogen. Chronic active gastritis is typically characterized by diffuse infiltration of the gastric mucosa by white blood cells that represent chronic inflammation, including lymphocytes, plasma cells, and macrophages. Many times, scattered eosinophils and mast cells are also observed. The term "active" is applied to gastritis when polymorphonuclear neutrophils are observed, which typically represents acute inflammation (56). This neutrophilic component appears to persist in *H. pylori*-induced chronic inflammation and is thought to significantly contribute to pathogenesis through the release of a number of damaging inflammatory mediators like reactive oxygen species (261).

The severity of gastritis is influenced by both the local and systemic immune response in addition to local pro-inflammatory effects directly mediated by *H. pylori*.

Recognition of *H. pylori* by monocytes and macrophages occurs via activation of toll-like receptors (TLR)s and leads to induction of an adaptive immune response, typically polarized toward a Th1-cytokine pattern (144, 181, 287). Polarization toward a T helper 1 (Th1) response leads to increased atrophy and metaplasia (294); however, it is the pattern of inflammation within the stomach, rather than the degree of inflammation, which is thought to promote one of two mutually exclusive disease outcomes (261). Chronic antral-predominant inflammation is associated with hyperchlorhydria and a predisposition for duodenal ulceration, whereas corpus-predominant or pan-gastritis is associated with hypochlorhydria and a predisposition for gastric ulceration and adenocarcinoma **(Figure 3)** (17).

Almost 150 years ago, Virchow noted that many tumors arise in settings of chronic inflammation (23, 58), and, later, Dvorak put forth a description of cancer as "wounds that do not heal" (79), suggesting a direct link between chronic inflammation and cancer. There are a number of mechanisms by which *H. pylori*-induced chronic inflammation might promote carcinogenesis. For example, chronic inflammation is known to induce increased tissue turnover, leading to hyperproliferation, which can result in more frequent mitotic errors and increased rates of mutagenesis (300). This has led to investigation of the role of *H. pylori*-induced chronic inflammation in the development of gastric adenocarcinoma.

H. pylori is a risk factor for development of gastric adenocarcinoma

Gastric adenocarcinoma is the second leading cause of cancer-related death in the world (241). Approximately 867,000 persons are diagnosed with the disease and 649,000

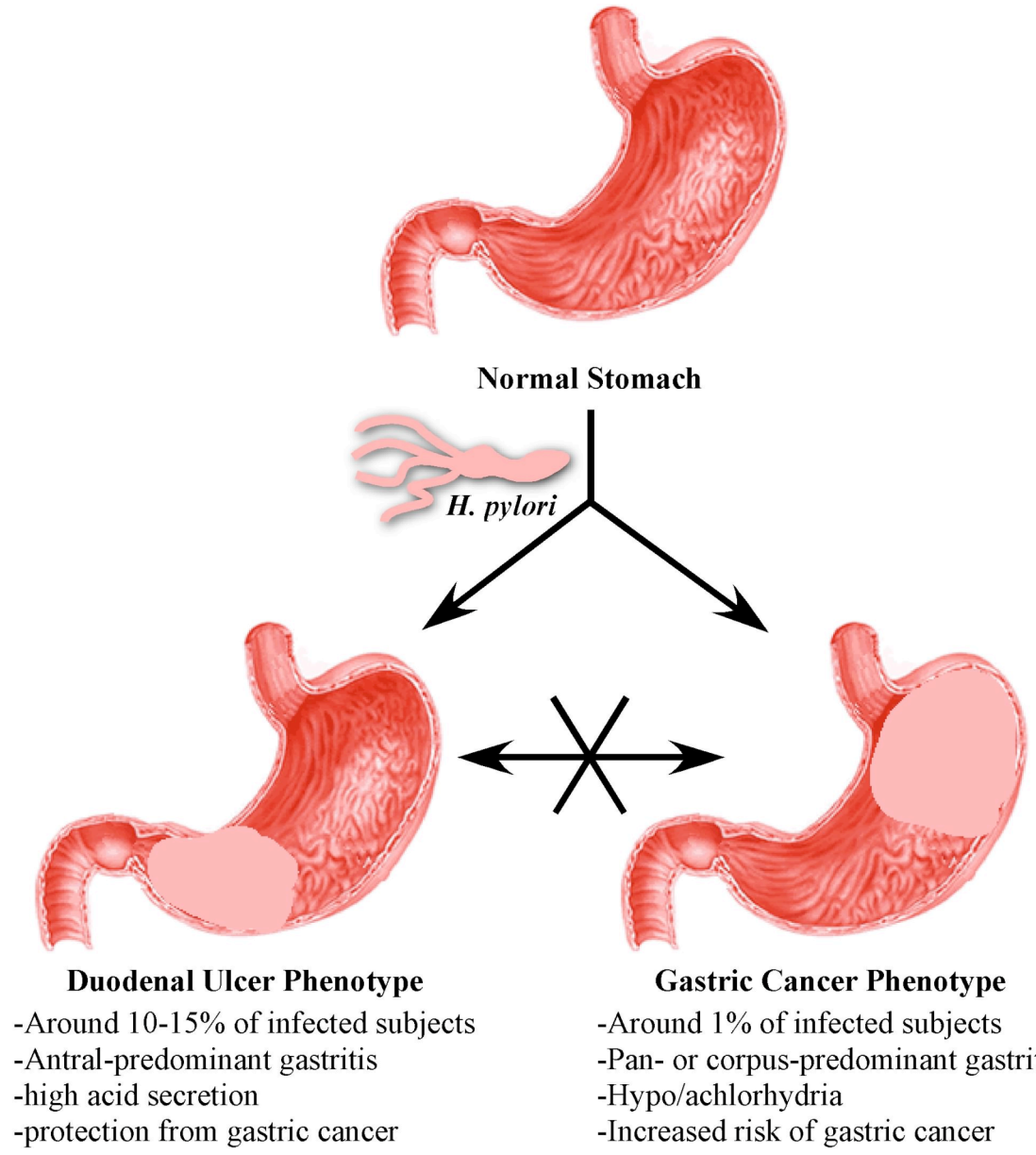

Figure 3. The pattern of *H. pylori*-induced inflammation promotes one of two mutually exclusive pathophysiologic outcomes. Adapted from Amieva and El-Omar (9).

persons die from this malignancy each year, with five-year survival rates in the United States of less than 15% (53). Noncardia gastric cancer is typically classified as one of two histologically distinct variants as first described by Lauren in 1965 (164). The first, diffuse-type gastric adenocarcinoma, commonly affects younger persons, affects men and women equally, and consists of individually infiltrating neoplastic cells that do not form glandular structures and are not associated with intestinal metaplasia (286). The second, intestinal-type adenocarcinoma, so-named for its origination from islands of intestinal epithelium found in the gastric mucosa, occurs at a much later age, predominates in men, and progresses through a well-defined series of preneoplastic histologic steps. These steps, as proposed by Dr. Pelayo Correa in 1975 (55), begin with initiation by chronic active nonatrophic gastritis and proceed through a continuum of stages consisting of multifocal atrophy, intestinal metaplasia, dysplasia, and finally invasive carcinoma (**Figure 4)** (51, 53, 56, 241, 286).

Colonization of the human stomach by *H. pylori* persists for decades, and epidemiological studies in humans and experimental infections in rodents have clearly demonstrated that long-term interactions between *H. pylori* and the host significantly increase the risk for peptic ulcers, Non-Hodgkin's lymphoma of the stomach, gastric atrophy, and distal gastric adenocarcinoma (199, 240, 241, 246, 286, 314). Based upon these data, the International Agency for Research on Cancer, a branch of the World Health Organization, stated that *H. pylori* is a primary risk factor for gastric adenocarcinoma, and, accordingly, classified *H. pylori* as a class I carcinogen in 1994. In recent years, a number of randomized prospective studies have demonstrated that the eradication of *H. pylori* in patient populations with a high-risk for the development of gastric cancer results

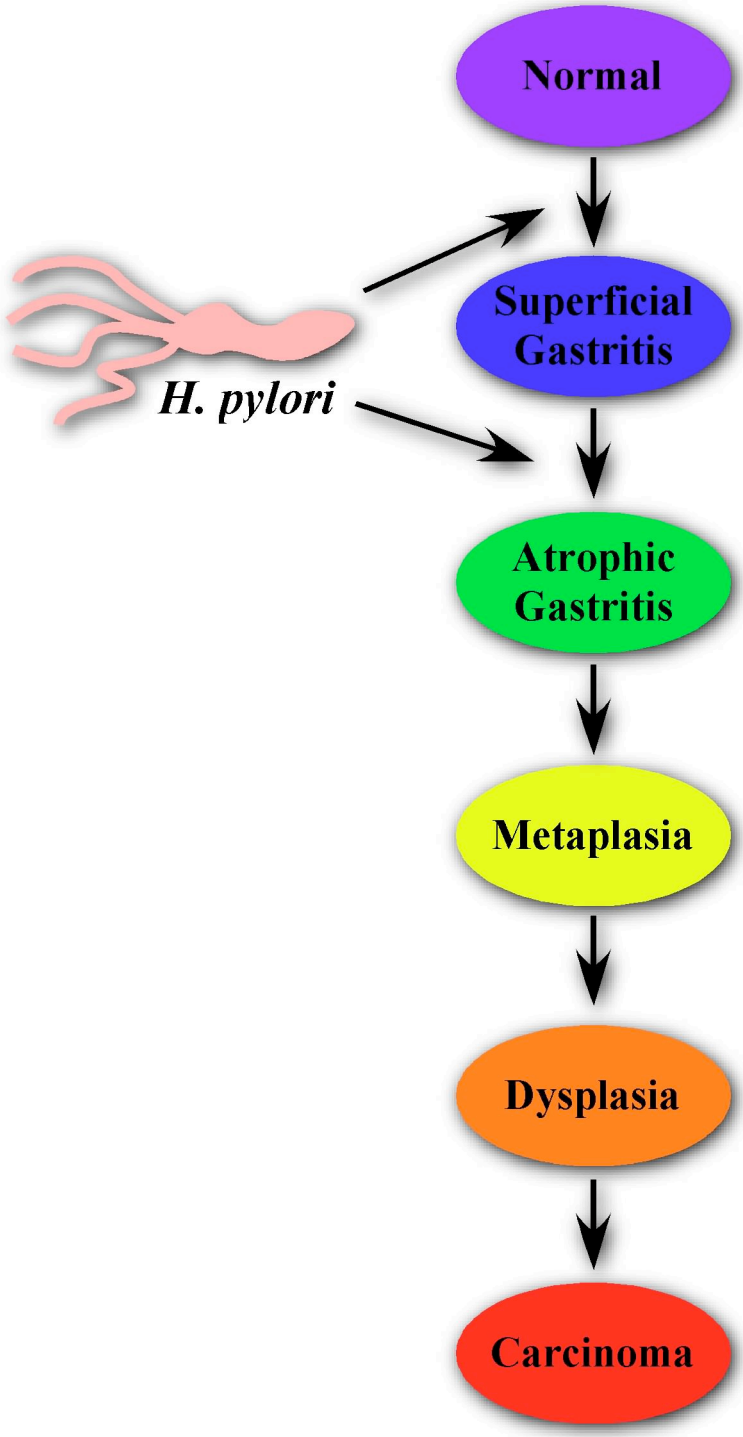

Figure 4. The development of *H. pylori*-induced gastric adenocarcinoma progresses through a well-defined series of histologic steps (55).

in the regression of pre-neoplastic lesions (54, 167, 169, 197, 362) and significantly reduces the incidence of gastric cancer in persons who have yet to develop precancerous lesions (343). In addition, eradication of *H. pylori* in experimental Mongolian gerbil infection models results in significant reduction in gastric cancer development (221, 263). Taken together, these studies indicate that anti-*Helicobacter* therapy may be effective in the prevention of gastric cancer development, further supporting a role for *H. pylori* in gastric carcinogenesis.

Since virtually all persons colonized with *H. pylori* develop superficial gastritis, it is likely that the organism plays a causative role early in disease progression. However, only a small percentage of colonized persons ever develop neoplasia, raising the hypothesis that enhanced cancer risk may be related to *H. pylori* strain differences, inflammatory responses governed by host genetic diversity [e.g. interleukin (IL) -1β) polymorphisms] (81, 179), and/or specific interactions between host and microbial determinants. Further, some studies have indicated that colonization with *H. pylori* displays an inverse relationship with the development of gastroesophageal diseases including gastroesophageal reflux disease, Barrett's esophagus and esophageal adenocarcinoma (50, 75, 175, 316, 322), as well as certain atopic diseases including hay fever, asthma, and eczema (29, 47, 48). These observations underscore the importance of identifying mechanisms that regulate the biological interactions of these organisms with their hosts during carcinogenesis in order to determine the proper line of treatment on an individual basis.

Gastric adenocarcinoma may be derived from a specific stem cell population

Since the 19th century, epithelial cancers have been thought to arise from resident epithelial cells (129). More recently, this epithelial theory of cancer has been expanded to suggest that tumors arise not differentiated cells of the epithelium, but rather from a tissue-specific population of stem cells (110). A tumor mass itself is similar to an abnormal organ in that it is made up of a heterogeneous mixture of cell types possessing a broad range of proliferative capacities and levels of differentiation. The cancer stem cell theory proposes that, within the cellular milieu of the tumor, there exist resident progenitor or stem cells that, in the context of chronic inflammation, are forced into multiple rounds of cell division in an environment conducive to the accumulation of mutations (110). This population of cells, capable of extensive proliferation and metastatic spread, gives rise to the heterogeneous mixture of cell types that make up the tumor. A landmark paper published by Houghton *et al.* in 2004 demonstrated that, in a mouse model of *Helicobacter*-induced gastric carcinogenesis, bone marrow-derived stem cell (BMDC) engraftment within the gastric mucosa occurs in response to chronic inflammation (128). Ultimately, all intraepithelial cancer cells observed in *Helicobacter*-infected mice originated from BMDCs, suggesting an innate susceptibility of BMDCs to neoplastic transformation. These experiments led to a new paradigm for gastric cancer—chronic inflammation promotes tissue injury; resulting tissue stem cell failure leads to recruitment and engraftment of BMDCs into the tissue stem cell niche; infiltrating BMDCs initiate differentiation, but in doing so fail to regulate growth programs, ultimately resulting in the development of cancer (171). Identification of bone marrow-derived stem cells within the context of *Helicobacter*-induced gastric cancer has provided

a new and exciting avenue of investigation that will hopefully allow anti-cancer therapies to become more specifically directed and effective.

H. pylori virulence factors

H. pylori strains exhibit extreme genetic diversity from patient to patient (8, 106, 270, 309), and studies have demonstrated that the genetic composition of isolates can change over time (136). Although this extraordinary diversity has hindered the search for bacterial determinants associated with cancer, several genetic loci have been identified that augment risk for carcinogenesis.

The cytotoxin associated gene (*cag*) pathogenicity island is a 40 kB locus present in approximately 60% of U.S. *H. pylori* strains (7, 8, 43, 309). Although all *H. pylori* strains induce gastritis, strains harboring the *cag* island (cag^+) augment the risk for severe gastritis, atrophic gastritis, gastric ulcer disease, and distal gastric cancer compared to cag^- strains (30, 59, 63, 64, 160, 239, 243, 244, 251, 265, 281, 311, 324). *Cag* genotype also influences the topography of colonization in the human stomach, as *H. pylori* cag^- strains are located predominately within the mucus gel layer, while disease-associated cag^+ strains are found immediately adjacent and frequently adherent to epithelial cells (40). Several *cag* genes encode products that form a type IV secretion system that acts as a molecular syringe to inject bacterial products into host cells **(Figure 5)**. One of these proteins, CagE, is required for translocation of bacterial gene products into host cells. Another component of the secretion system, CagL, serves as a specialized bacterial adhesin that binds to and activates the integrin $\alpha_5\beta_1$ receptor, triggering the delivery of

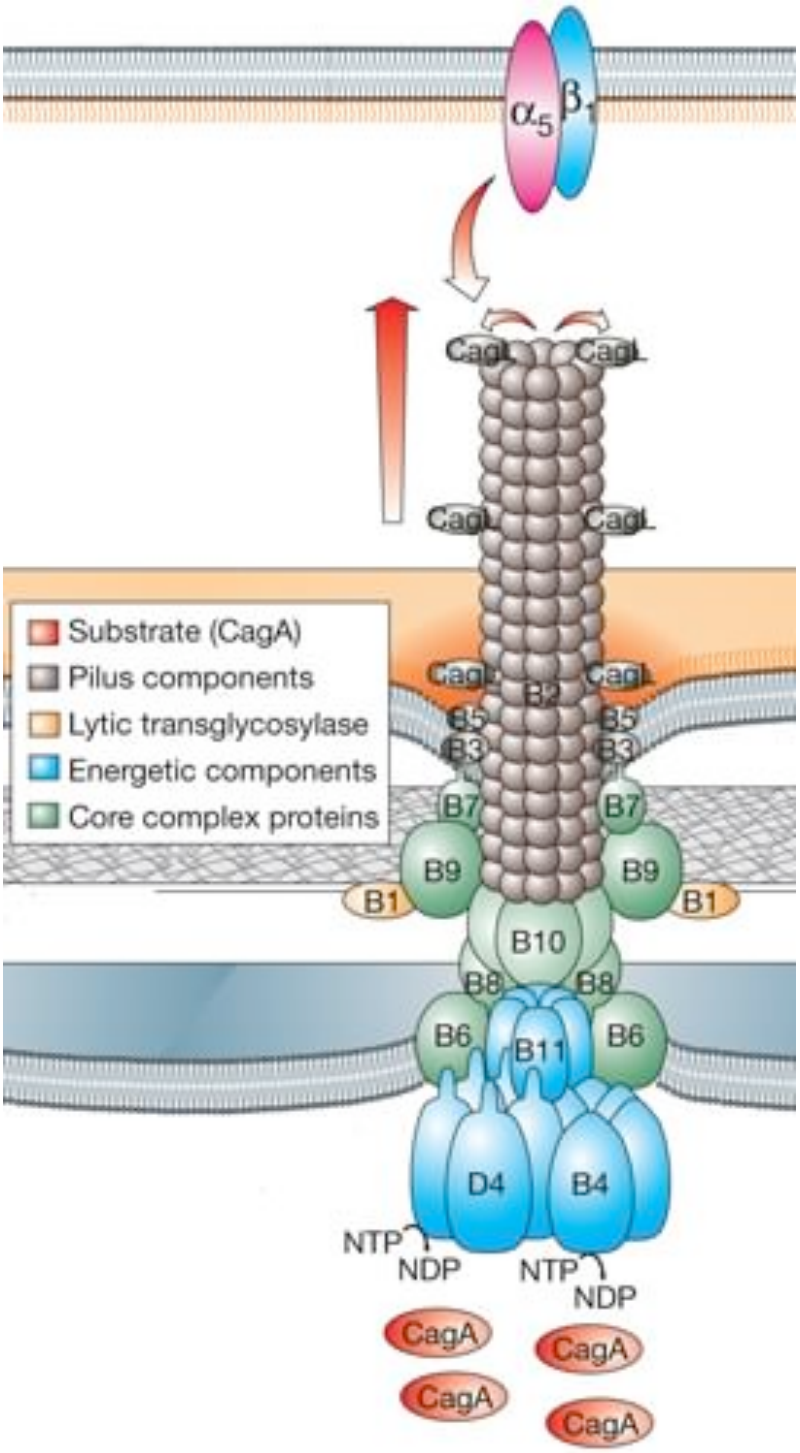

Figure 5. The *H. pylori* type IV secretion system translocates bacterial effector molecules into host cells. The image represents the proposed structure of the type IV secretion system. Reproduced with permission from Steffen Backert (163).

bacterial molecules into the cytoplasm of host cells (163).The product of the terminal gene of the *cag* island, CagA, is translocated into epithelial cells via this secretion system, where it induces a number of host-cell alterations **(Figure 6)**. CagA can be phosphorylated by members of Src family of kinases at its tyrosine phosphorylation motifs (glutamate-proline-isoleucine-tyrosine-alanine, EPIYA) found within the carboxy-terminal variable region of the protein (16, 22, 225, 274, 275, 291, 292). Phospho-CagA subsequently binds and activates three eukaryotic Src Homology 2 (SH2) domain-containing proteins: the protein tyrosine phosphatase SHP-2, carboxy-terminal Src kinase (Csk), and the adaptor protein Crk (119, 297, 303, 313). Phosphorylated and non-phosphorylated CagA can also activate ERK 1/2, a member of the mitogen-activated protein kinase (MAPK) family, leading to an intracellular signaling cascade that induces morphological changes with resemblance to unrestrained stimulation by growth factors (16, 22, 118, 119, 200, 225, 274, 275, 290, 292, 313). Non-phosphorylated CagA interacts with Par1, a MARK kinase that phosphorylates microtubule-associated proteins (268), leading to disruption of apical-junctional complexes in polarized MDCK epithelial cells (10). CagA clearly induces the activation of several transcription factors, including nuclear factor κB (NF-κB), activator protein 1 (AP-1), cAMP response element (CRE), serum response element (SRE), and serum response factor (SRF), and β-catenin (33, 45, 77, 96, 123, 153, 198, 214, 352), though the role of CagA phosphorylation in regulating these molecules remains unclear.

In addition to CagA, *H. pylori* translocates components of bacterial peptidoglycan (PG) into host cells via its type IV secretion system (321). The bacterial enzyme, soluble lytic transglyocosylase (Slt), encoded by the gene HP0645, functions in

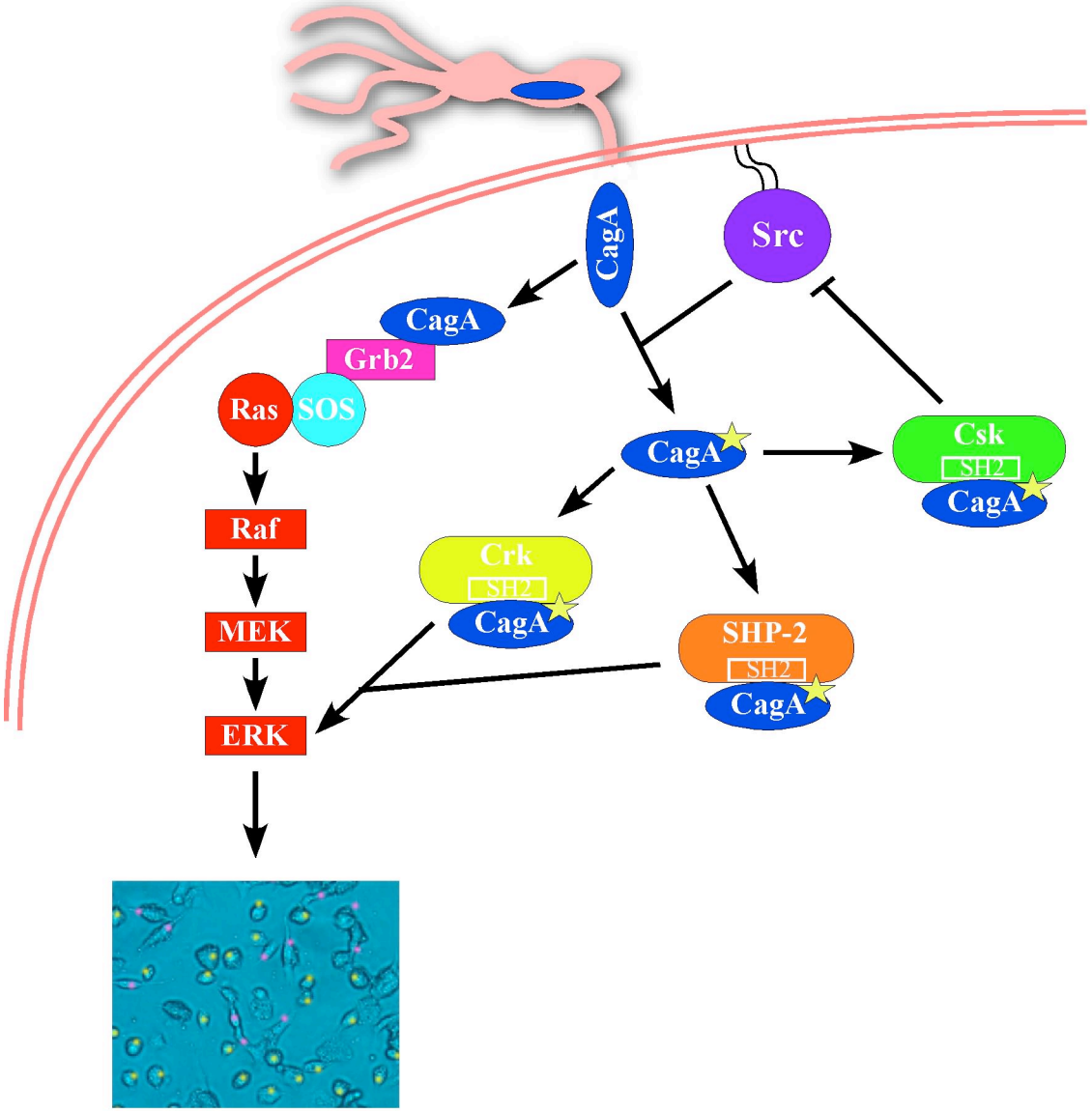

Figure 6. Translocation of CagA results in a number of host cell alterations, exemplified by induction of the hummingbird phenotype. The yellow star represents phosphorylation. Micrograph provided by and reprinted with the permission of Natasha Schneider.

anhydromuropeptide release during PG turnover **(Figure 7)** (46). Mutation of *H. pylori slt* results in an accumulation of G-M-tripeptide in the bacterial peptidoglycan layer (46) and an approximately 40% reduction in the amount of disaccharide tripeptide liberated by the bacteria compared to wild type (321). The host intracellular protein, nucleotide-binding oligomerization domain containing 1 (Nod1), also known as caspase recruitment domain 4 (CARD4), is a major pathogen-recognition protein that has been shown to respond to intracellular peptidoglycan components, with the minimal moiety required for recognition being γ -D-glutamyl-meso-DAP, a dipeptide primarily found in PG from Gram-negative bacteria **(Figure 7)** (44). Activation of Nod1 initiates a signaling cascade through the CARD domain-containing kinase, RIP-like interacting CLARP kinase (RICK), which ultimately results in increased NF-κB activity (44, 104, 105, 134, 321). Accordingly, co-culture of an *H. pylori slt*$^-$ mutant with cells expressing exogenous Nod1 results in attenuated NF-κB activity and IL-8 synthesis when compared to co-culture with the parental wild type *H. pylori* (321), suggesting that *H. pylori*-mediated transcription of NF-κB target genes is dependent on this host cell-signaling pathway.

H. pylori possesses another genetic locus linked to gastric cancer, *vacA*, which encodes the bacterial toxin known as vacuolating cytotoxin A (VacA). Cover *et al.* first described the ability of *H. pylori* broth culture supernatants to induce vacuolation of cells *in vitro* in 1990 (59), and subsequently the toxin responsible for this phenomenon was purified, cloned, and designated VacA. Unlike the *cag* island, virtually all *H. pylori* strains examined possess *vacA* (18, 60), although cytotoxic activity is highly dependent on *vacA* gene structure. Extensive study has demonstrated that the ability to induce vacuolation is dependent on the formation of oligomeric VacA structures in the host

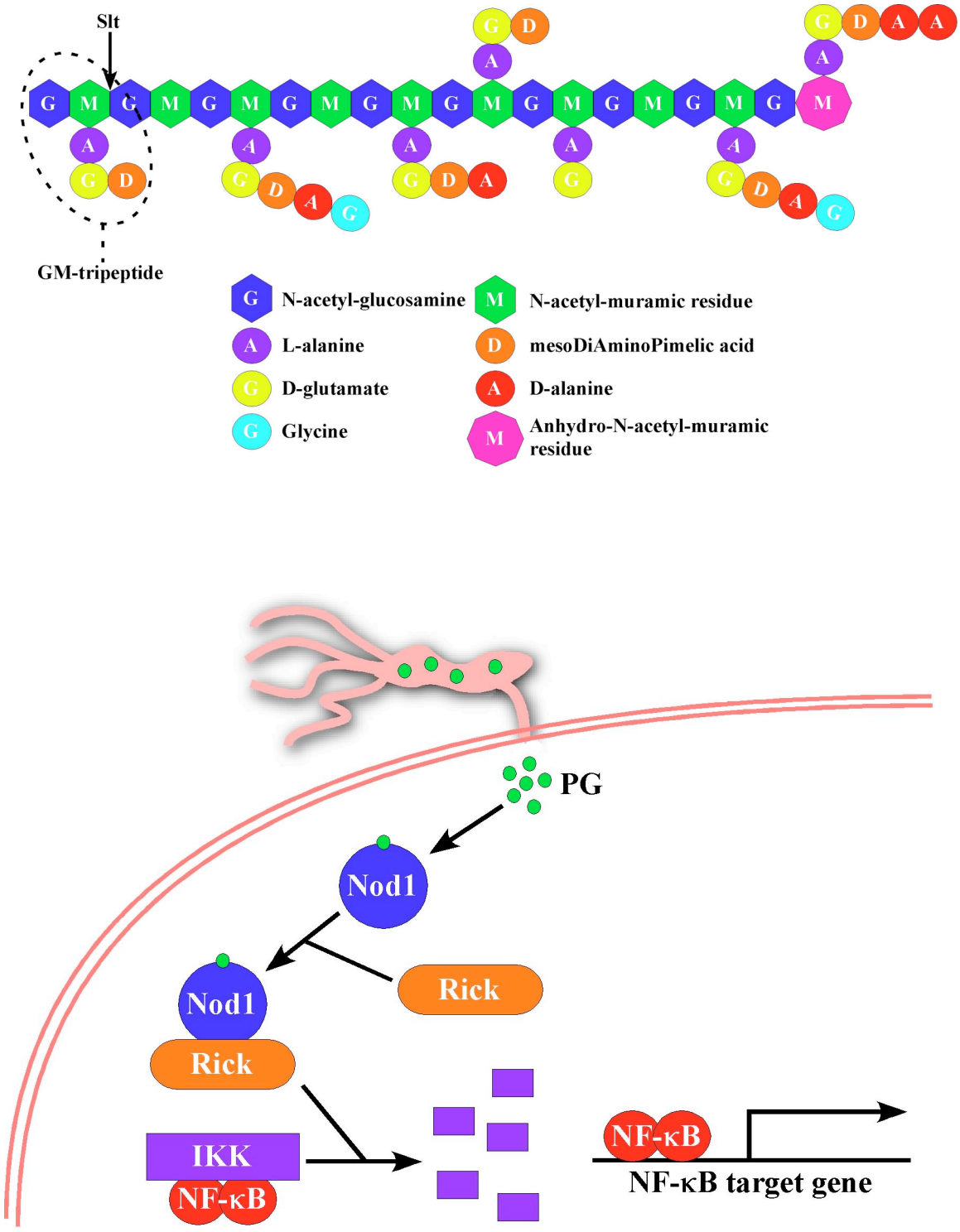

Figure 7. *H. pylori* translocates components of bacterial PG into host cells via its type IV secretion system. Upper panel: Soluble lytic transglyocosylase (slt) cleaves PG leading to the release of GM-tripeptides during PG turnover. Adapted from Chaput *et al*. (46). Lower panel: PG translocated into the host cell is recognized by the Nod1 receptor, resulting in activation of the transcription factor, NF-κB.

cell membrane that exhibit anion-selective channel activity (70, 137, 299, 310). In additon to vacuolation and membrane channel formation, the activities of VacA include disruption of endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of apoptosis, and immune modulation (162). Specifically, VacA has been shown to suppress T and B cell activation-induced proliferation *in vitro* (31, 103, 295, 312), which may contribute to *H. pylori* persistence through dysregulation of the adaptive immune response.

Rodent models of *H. pylori* infection

Since the discovery of *H. pylori* as an etiologic agent for gastric disease, a number of animal models of experimental *Helicobacter* infection have been tested, including mice, rats, cats, guinea pigs, ferrets, pigs, and macaques (129). The first studies to suggest a carcinogenic role for *Helicobacter* species in the gut involved ferrets infected with *Helicobacter mustelae*. Initially, the formation of gastric adenocarcinoma included oral administration of a known carcinogen, *N*-methyl-*N*-nitro-*N*'-nitrosoguanidine (MNNG), to previously infected ferrets (95). Later reports by the same group demonstrated the spontaneous development of gastric adenocarcinoma in naturally infected ferrets over longer periods of time without exposure to MNNG (91).

Although ferrets provided the first model of *Helicobacter*-induced carcinogenesis, this system does not allow for analysis of host genetics that may play a role in progression through the carcinogenic cascade. For this purpose, infection of several inbred mouse strains with either *H. felis* or *H. pylori* have been utilized, although progression to high-grade dysplasia and invasive gastric carcinoma appears to be more

consistent with *H. felis* infection (129). The ability of *Helicobacter* to colonize a given mouse line is strain-dependent, and the susceptibility of the C57BL/6 mouse to infection has made it the preferred model for the study of metaplasia and gastric cancer formation. Initial infection leads to infiltration of the gastric mucosa by both acute and chronic inflammatory cell types and an increase in apoptosis of cells within the glandular epithelium is observed. This is followed by an increase in proliferation that directly results in expansion of the size and location of the proliferative zone, leading to atrophy and metaplasia (129, 329). The lesions in mice infected with *H. pylori* do not typically progress beyond this point; however, mice infected with *H. felis* consistently progress to dysplasia and invasive cancer within 12 to 16 months post-infection (39, 93, 129).

The importance of developing a model in which infection with *H. pylori*, rather than *H. felis*, results in the development of gastric carcinoma led to the development of the insulin-gastrin (INS-GAS) mouse model by the laboratory of Dr. James Fox (328). This mouse possesses a transgene within which the gastrin gene has been placed under the control of a rat insulin promoter. A state of hypergastrinemia results from increased serum gastrin levels and exhibits a synergistic effect with *H. pylori* infection leading to a uniformly developed atrophy, intestinal metaplasia, and dysplasia by 6 weeks and carcinoma by 24 weeks post-infection in male mice (92, 94). The INS-GAS mouse has provided an effective model for investigating discrete host-microbial interactions that culminate in gastric cancer within the context of biologic conditions induced by *H. pylori*.

The INS-GAS mouse will certainly be a useful tool for the study of host genetics in *H. pylori*-induced gastric cancer. Nonetheless, the most robust and useful model for

studying the role of microbial factors in *H. pylori*-induced progression through the carcinogenic cascade is the Mongolian gerbil. The experimental Mongolian gerbil model was developed by Yokota *et al.* (359), after which multiple groups have reported the development of poorly differentiated gastric adenocarcinoma in as high as forty percent of male Mongolian gerbils with long-term *H. pylori* infection (124, 126, 227, 331, 368). Further, infection results in sequential histopathological changes leading to gastric cancer that closely resemble the carcinogenic cascade induced by *H. pylori* in humans. Recently, our group has demonstrated that infection of male Mongolian gerbils with a rodent-adapted strain of *H. pylori* rapidly and reproducibly induces the development of gastric cancer by eight weeks post-infection, development of cancer is dependent on specific bacterial virulence factors, and eradication by anti-*Helicobacter* therapy decreased the incidence and severity of malignant lesions (97, 263), providing further support for the Mongolian gerbil as a suitable model of experimental *H. pylori* infection.

p120^{ctn} regulates intercellular adhesion

p120^{ctn}, originally identified as a substrate for Src- and receptor-tyrosine kinases (254, 257, 259), is the prototypic member of the catenin (ctn) family, an Armadillo domain protein subfamily whose members interact with the cadherin cytoplasmic tail and modulate cadherin function (254, 255, 279, 289). Cadherins are a family of cell-cell adhesion receptors that are important in several physiological processes, including development, morphogenesis, and cancer (301, 357). E-cadherin is the most well-defined cell-cell adhesion molecule in epithelial tissues, and its turnover is regulated by binding of p120^{ctn} to the cadherin juxtamembrane domain **(Figure 8)** (12, 13, 74, 108, 135, 220,

254, 307, 358). p120ctn is not required for proper trafficking of nascent E-cadherin, but associates with E-cadherin once it has reached the cell surface (74, 201, 258). If $p120^{ctn}$ is absent, E-cadherin is immediately turned over and either recycled or degraded (74, 258). This mechanism is postulated to be dependent on signaling to the regulatory domain of $p120^{ctn}$ (258), likely through phosphorylation. These signaling events may modulate affinity for E-cadherin and involve competition with other proteins for E-cadherin binding, although the exact mechanism remains undefined (258). Regardless, the core function of $p120^{ctn}$ is to control the amount of E-cadherin available at the cell surface (74, 135, 258, 347), thereby regulating cell-cell adhesive strength.

p120ctn contains an amino-terminal regulatory domain

Multiple isoforms of $p120^{ctn}$ arise from alternative N-terminal splicing events resulting in differential usage of four distinct start codons **(Figure 9)** (149, 204). All contain a central Armadillo Repeat Domain and a carboxy-terminal tail with unknown function, and isoforms 1-3 contain an amino-terminal regulatory domain. Isoforms are co-expressed at different levels depending on the cell type, with epithelial cells normally expressing isoforms 3 and 4, whereas mesenchymal cells express full-length isoform 1 (4, 14, 149, 204, 258). In addition, isoform 1 possesses a 100 amino-acid N-terminal coiled-coil domain that appears to be important in inhibition of Ras homolog gene family, member A, (RhoA) activity (355). This isoform is preferentially expressed in motile cell types such as fibroblasts and macrophages, and its expression promotes increased cell migration and invasion (355). Isoform 3 lacks this domain, and is expressed in sessile cells such as epithelial cells. Isoform 4 lacks both the coiled-coil and regulatory domains,

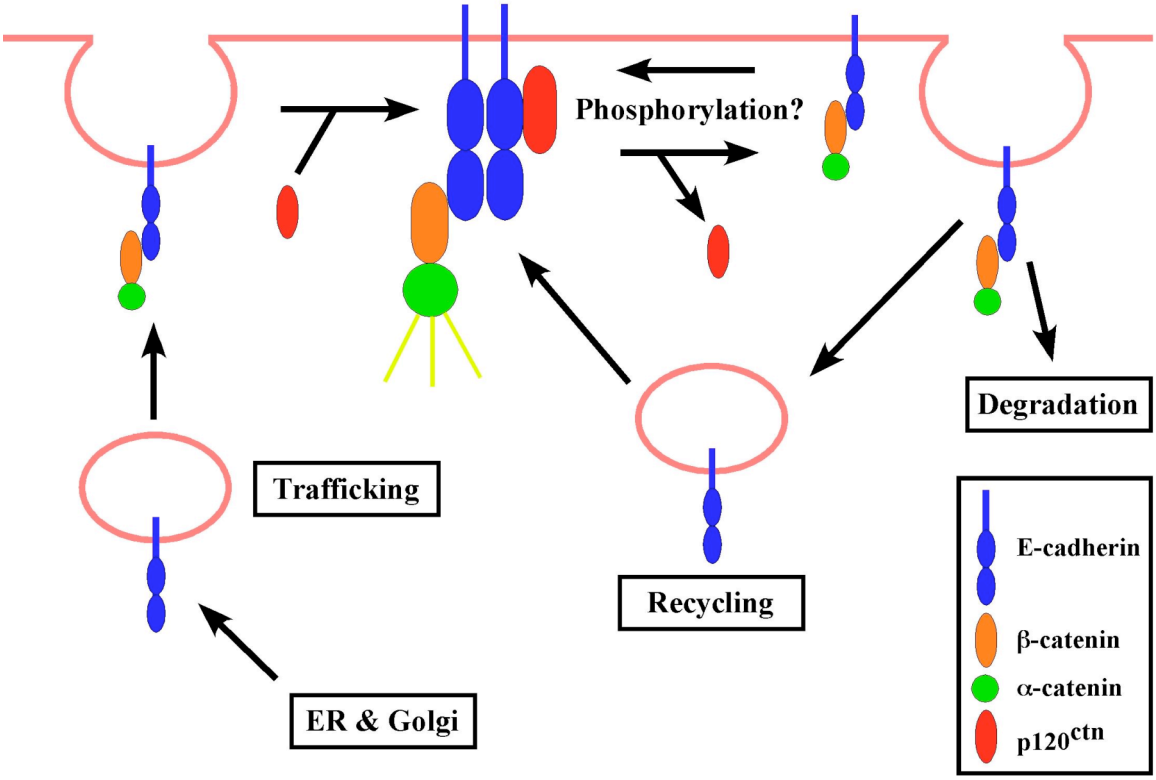

Figure 8. p120^{ctn} binding of the juxtamembrane domain of E-cadherin targets E-cadherin to the plasma membrane and regulates its turnover. Adapted from Reynolds *et al*. (258).

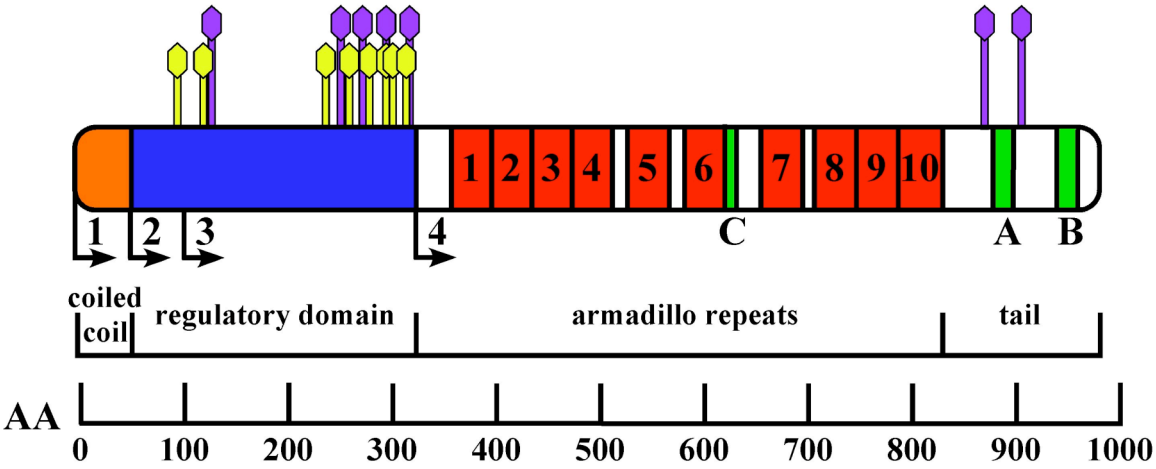

Figure 9. Multiple isoforms of p120^{ctn} arise from alternative N-terminal splicing events resulting from differential usage of four distinct start codons. All contain a central Armadillo Repeat Domain and a carboxy-terminal tail with unknown function, and isoforms 1-3 contain an amino-terminal regulatory domain. The protein also contains three internal alternatively spliced sequences known as exons A, B, and C. It can be phosphorylated at a number of tyrosine (yellow) and serine/threonine residues (purple). Adapted from Reynolds and Ferguson (258).

is rarely observed at the protein level, and is of unknown physiological significance. The protein also contains three internal alternatively spliced sequences known as exons A, B, and C. Exon B contains a functional nuclear export signal (317), while the role of Exon A is unknown and Exon C is rarely present.

p120ctn is phosphorylated at a number of sites by assorted Src- and receptor-tyrosine kinases

Multiple ligand-receptor pathways have been implicated in signaling to $p120^{ctn}$ through phosphorylation events, including protein kinase C (PKC)- and epidermal growth factor receptor (EGFR)- dependent pathways (183, 345), both of which are activated by *H. pylori* (147, 222). The majority of these sites have been mapped to the regulatory domain of $p120^{ctn}$ **(Figure 9)**, indicating a likely role in the regulation of $p120^{ctn}$ function, but the consequences of phosphorylation at individual serine/threonine and tyrosine residues has only recently begun to be investigated. Observations made in cells overexpressing isoform 4, which lacks the regulatory domain and, thus, most phosphorylation sites, have revealed likely roles for phosphorylation events in 1) positive and negative regulation of cell-cell adhesion via direct interaction with Src family members, 2) motility and scattering activities induced by receptor-tyrosine kinase signaling, and 3) promotion of protein-protein interactions by acting as a scaffold for protein tyrosine phosphatases (PTP)s (61, 182, 258). Accordingly, association of $p120^{ctn}$ with E-cadherin at the plasma membrane leads to phosphorylation at several serine and threonine residues independent of cadherin-cadherin dimerization, and mutation of these residues reduces p120-dependent stabilization of stabilization of E-cadherin (100). Fyn-dependent phosphorylation of $p120^{ctn}$ at Tyr112 prevents interaction with RhoA, whereas

Fyn/Src-dependent phosphorylation at Tyr217 promotes this interaction, thereby modulating cellular motility and scattering (42). The PTP Src homology phosphatase-1 (SHP-1), which is activated by binding to tyrosine-phosphorylated proteins, binds to $p120^{ctn}$ in a manner dependent on EGF-stimulated tyrosine phosphorylation of $p120^{ctn}$ (148). Dysregulation of all of these cellular processes has been associated with carcinogenesis, implicating differential phosphorylation of $p120^{ctn}$ in the development of cancer.

$p120^{ctn}$ is mislocalized in several cancer types

Reduced expression or aberrant redistribution of $p120^{ctn}$ has been observed in a number of different epithelial malignancies, including gastric, colorectal, bladder, breast, prostate, lung, pancreatic, melanoma, and endometrial tumors (138, 142, 190, 308). Three of these studies examined $p120^{ctn}$ in sections of human gastric tumors by immunohistochemistry, and demonstrated reduced overall expression as well as strong cytoplasmic staining within the same tumor sections (138, 141, 142). This altered subcellular localization is common throughout many additional types of cancer and has been suggested to contribute to a metastatic phenotype, though its consequences remains undefined.

In cancer cell lines, loss of E-cadherin or overexpression of $p120^{ctn}$ have been shown to result in mislocalization of $p120^{ctn}$ to the cytoplasm (254), due to lack of cadherin binding sites in the former or saturation of cadherin binding sites in the latter. These cytoplasmic pools of $p120^{ctn}$ induce a range of cell-type dependent morphological changes related to the promotion of cell motility and metastasis. A striking branching

phenotype has been observed in fibroblasts (13, 256), while lesser effects, such as abnormal lamellipodia formation, have been noted in epithelial cells (12, 13). These alterations in cell morphology are the result of $p120^{ctn}$ interactions with Rho GTPases (13). Cytoplasmic $p120^{ctn}$ potently inhibits RhoA, likely through a direct guanine nucleotide dissociation inhibitor (GDI)-like manner (12, 13, 220), and activates Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42, GTP binding protein (Cdc42), through a proposed indirect interaction with Rac guanine exchange factors (GEF)s (13, 108, 220), leading to increased cell motility and scattering, a hallmark of cancer.

$p120^{ctn}$ relieves transcriptional repression by Kaiso

In addition to aberrant cytoplasmic localization, $p120^{ctn}$, found at low levels in the nucleus of normal cells, is present at increased levels within the nucleus of cadherin-deficient cells (258, 262, 317, 356) as well as some tumors (190, 272, 337). The mechanism resulting in its nuclear translocation remains unknown, though it is not dependent on conventional nuclear localization sequences (NLS)s (262). $p120^{ctn}$ has been shown to colocalize with microtubules at the nuclear periphery in E-cadherin deficient cell lines (262), suggesting that interactions with the cytoskeleton may be required for transport to the nucleus.

Recent evidence has revealed that nuclear $p120^{ctn}$ acts to relieve transcriptional repression mediated by Kaiso **(Figure 10)**, a member of the broad complex, tramtrak, bric a brac/pox virus and zinc finger (BTB/POZ) family (72). Kaiso acts as a dual specificity repressor that recognizes both sequence-specific consensus sites (CTGCNA)

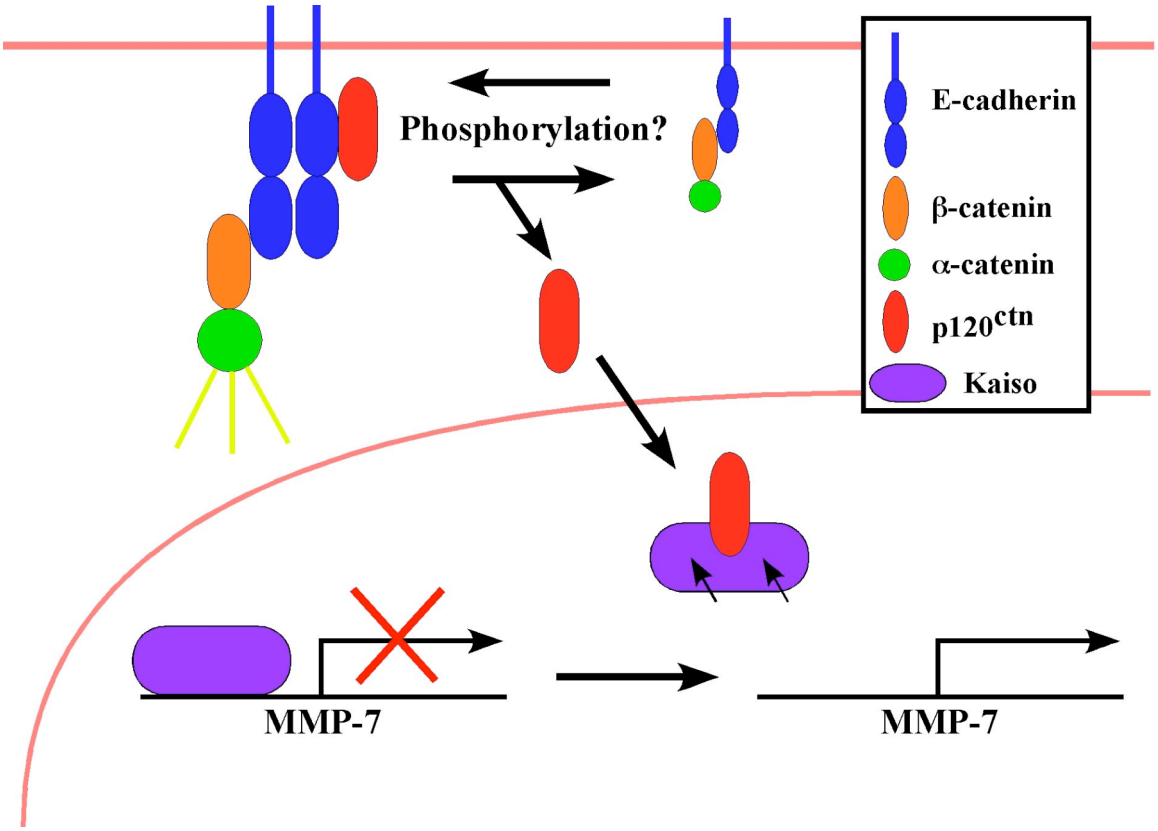

Figure 10. Nuclear p120^{ctn} relieves Kaiso-mediated transcriptional repression of *mmp-7*. Activation of p120^{ctn} is hypothesized to occur via alterations in the phosphorylation state of specific residues. Relief of Kaiso-mediated repression may occur through steric hindrance of Kaiso:DNA interactions.

and methylated CpG nucleotides (73, 237, 250). With regard to sequence-specific binding, the Kaiso/p120^{ctn} complex has been shown to modulate non-canonical Wnt signals (152) and, along with T cell factor (TCF)/β-catenin complexes, coordinately regulate canonical Wnt gene targets such as *peroxisome proliferators-activated receptor-γ* (*PPAR-γ*), *c-Myc*, *c-Fos*, *Cyclin D1*, and *mmp-7* (237, 288), all of which are activated by infection with *H. pylori*. The mechanism of Kaiso-mediated repression involves both histone modifications via recruitment of macromolecular histone deacetylase corepressor complexes (72), as well as interfering with β- catenin:TCF complex formation (237). Further, translocation of p120^{ctn} to the nucleus is required to relieve Kaiso-mediated repression, which may occur through steric disruption of Kaiso:DNA interactions (150, 237). In at least three human cell lines, exogenous p120^{ctn} overexpression resulting in nuclear localization of p120^{ctn} inhibits Kaiso mediated transcriptional repression of *mmp-7* as evidenced by chromatin immunoprecipitation analysis (288), suggesting a coordinated regulation of Wnt gene target transcription that involves p120^{ctn}.

Matrix metalloproteinases

The matrix metalloproteinases (MMP)s are made up of a family of at least twenty-five enzymes that are thought to play a role in tissue breakdown and remodeling during both normal and pathological processes (132, 339). MMPs are primarily distinguished from other classes of proteases by their dependence on metal ions (specifically zinc) and neutral pH for activity (132, 336, 339). Almost all MMPs share homologous protein sequences and contain four basic domains: 1) a pre-domain/signal sequence that directs secretion from the cell, 2) a latency/pro-domain that must be

cleaved to render an active protein, 3) a zinc-binding catalytic domain, and 4) a hinge region followed by a hemopexin-like domain that confers much of the substrate specificity to the individual MMP **(Figure 11)** (339). However, MMP-7 along with MMP-23 and MMP-26, lacks the C-terminal hemopexin domain common to the other MMP members (132), meaning that it contains only the minimal number of domains, (pre-, pro-, and catalytic) required for secretion and activity.

MMPs are synthesized as latent enzymes (zymogens) that can be stored in inflammatory cell granules but are usually secreted or membrane-associated via direct anchoring within the membrane or tethering to other cell surface-associated proteins, including integrins, CD44, and cell-surface-associated heparan sulphate proteoglycans (35, 57, 80, 363, 364). These molecules are kept inactive by an interaction between a cysteine-sulphydryl group in the propeptide domain with the zinc ion bound by the catalytic domain (293). Activation within the local microenvironment via proteolytic processing results in digestion of structural proteins of the extracellular matrix (ECM) leading to discrete modifications of tissue architecture (132, 282, 339). In addition to ECM substrates, MMPs cleave a number of cell surface molecules and other non-matrix proteins, including proteinase inhibitors, clotting factors, chemotactic molecules, latent growth factors, growth factor binding proteins, cell surface receptors, and cell-cell and cell-matrix adhesion molecules (57, 193, 249). This wide range of substrates affords these molecules influence over a number of physiological and pathological processes, such as embryonic development, tissue morphogenesis, wound repair, cancer, and inflammatory diseases (80, 132, 282, 293, 336). The activity of MMPs is not only regulated at the level of proteolytic processing, but is also strictly controlled by a group

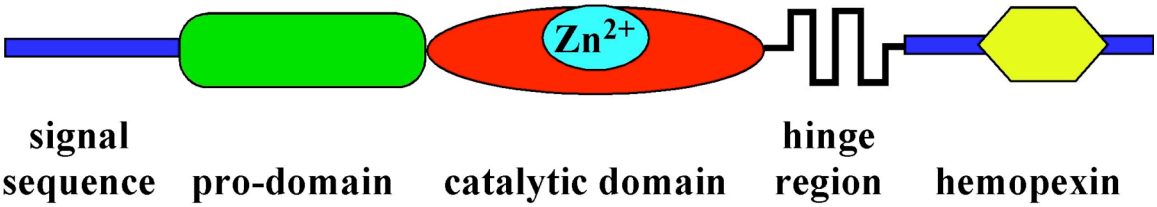

Figure 11. MMPs share homologous protein sequences. They typically contain four basic domains: 1) a pre-domain/signal sequence that directs secretion from the cell, 2) a latency/pro-domain that must be cleaved to render an active protein, 3) a zinc-binding catalytic domain, and 4) a hinge region followed by a hemopexin-like domain that confers much of the substrate specificity to the individual MMP. MMP-7 lacks the C-terminal hemopexin domain.

of common inhibitors, the tissue inhibitors of metalloproteinases (TIMP)s, as well as by α2-macroglobulin (57, 282). The balance between the various MMPs and their inhibitors is thought to be a major determinant of their function within the tissue microenvironment.

Matrix metalloproteinase-7

MMP-7 is secreted as a 28-kDa proenzyme that is activated by removal of a 9-kDa prodomain from the N-terminus by a variety of proteins including endoproteinases, plasmin, and trypsin (132, 215, 282, 339). MMP-7 is one of the few MMPs that is expressed in polarized glandular epithelium, and constitutive expression has been observed in the ductal and glandular epithelium of normal mammary and parotid glands, liver, pancreas, prostate, and the peribroncial glands and conducting airways in the lung (57, 215, 282, 293). Its function is restricted by its release into either apical or basolateral compartments or both, although immunohistochemical analyses have demonstrated MMP-7 staining in the glandular epithelium to be primarily apical and lumenal (68, 112).

MMP-7 has broad substrate specificity against components of the ECM and is known to cleave elastin, type IV collagen, fibronectin, vitronectin, aggrecan, and proteoglycans (132, 215, 282, 339). Like other members of the MMP family, MMP-7 can regulate numerous non-ECM bioactive molecules, mainly by facilitating the ectodomain shedding of cell-surface molecules like tumor necrosis factor-α (TNF-α) precursor (102, 206), Fas ligand (FasL) (88, 248, 318), heparin-binding epidermal growth factor (HB-EGF) (49, 364), E-cadherin (218), and β4-integrin (323), many of which are likely involved in carcinogenesis. Additional biological roles for MMP-7 in the immune

system are only now being elucidated. In the mouse small intestine, MMP-7 is constitutively co-expressed with pro-α-defensins, leading to their activation and enhancement of bacterial killing (19, 283, 340). MMP-7 also functions during acute inflammation and wound repair, as evidenced by a recent report demonstrating that, in a mouse model of acute lung injury, MMP-7 is required for formation of chemotactic gradients that direct neutrophil migration (172).

MMP-7 plays a role in carcinogenesis

Although MMP-7 is associated with a number of physiological and pathological remodeling processes, the most important is probably carcinogenesis. Over-expression of MMP-7 occurs within the tumor cells themselves, as opposed to tumor-associated stromal or endothelial cells, in epithelial-derived malignancies of many organs (132), including the lung (132), skin (143, 145, 156), breast (117), prostate (41, 113, 234), uterus (69, 173, 315), head and neck (133, 233, 333), colon (2, 3, 194, 207, 231, 349, 360), liver (101, 232, 350), pancreas (69, 139, 173, 351), esophagus (1, 228, 271, 306, 348, 354), and the stomach (1, 5, 6, 121, 125, 194, 277, 353). Microarray-based expression analyses have demonstrated that MMP-7 is expressed >6-fold in human gastric adenocarcinoma specimens compared to non-neoplastic tissue (121). In mice, over-expression via a transgene that expresses human MMP-7 under the control of the mouse mammary tumor virus (MMTV)-long terminal repeat promoter/enhancer leads to hyperproliferation and increased cancer susceptibility of the mammary epithelium (267). Human carcinoma cell lines transfected with wild type or activated MMP-7 possess enhanced tumorigenic potential as opposed to vector control cells in an orthotopic injection model (342).

Conversely, mice with a genetic predisposition for intestinal adenocarcinoma [multiple intestinal neoplasia (*Min*) mice] that are then bred onto a background of MMP-7 deficiency develop fewer cancers than wild-type mice (338). This evidence, along with the observations of increased MMP-7 expression seen in a high proportion of premalignant lesions in the colon (polyps) (302), pancreas (metaplastic duct lesions) (69), and stomach (gastric ulcers) (69, 269), suggests that this enzyme may play an important role early in carcinogenesis.

There are a number of mechanisms through which it has been suggested that MMP-7 promotes carcinogenesis. Apoptosis or programmed cell death is the physiological process by which unwanted cells are eliminated and can be triggered by a wide range of stimuli. One of the major inducers of apoptosis is FasL, a transmembrane stimulator of the death receptor Fas. MMP-7 has been shown to shed the ectodomain of membrane-bound FasL, potentiating the induction or inhibition of apoptosis, depending on the duration of exposure (88, 248). Constitutive expression can result in chronic solubilization of FasL, and it has been suggested that this deregulated expression somehow selects for a population of cells with reduced sensitivity to apoptotic stimuli (88). Additionally, MMP-7 cleavage of cell surface-associated TNF-α to produce soluble TNF-α may increase apoptosis via binding to the TNF-receptor 1 (206).

MMP-7 cleavage of non-ECM proteins can also promote tumorigenesis by augmenting tumor growth. MMP-7 increases cellular proliferation by cleaving pro-HB-EGF to yield mature HB-EGF, which promotes cell survival by stimulating the ErbB4 EGF receptor and inhibiting apoptosis (364), a signaling pathway that we have previously demonstrated to be activated by *H. pylori* (147). MMP-7 is also known to regulate the

insulin-like growth factor (IGF) axis through its proteolytic action on IGF binding proteins (IGFBP)s (203). The bioavailability of IGFs is regulated by interactions with the IGFBPs, of which MMP-7 is able to degrade all six family members, thereby favoring cancer cell growth and survival (213). Further, MMP-7 can proteolytically activate the secreted form of ADAM28, a member of the A Disintegrin and Metalloproteinase (ADAM) family, which can, in turn digest IGFBP-3 (205).

Angiogenesis is the physiological process of growth and development of new blood vessels, a process that is essential for tumor growth and invasion into surrounding tissues. MMP-7 expression has been detected within vascular endothelial cells found near tumor cells in a variety of human cancers (211, 285). In primary gastric cancer biopsies, immunohistochemical staining demonstrated that increased MMP-7 expression correlates with greater microvessel density (367). Subcutaneous injection of human colon cancer cells that endogenously express high levels of MMP-7 into nude mice resulted in increased blood vessel formation that was abrogated by injection of an MMP-7-specific antisense oligonucleotide (216). These results, coupled with evidence that MMP-7 accelerates the proliferation of human umbilical vein endothelial cells *in vitro* (131, 216), suggest that MMP-7 may directly induce angiogenesis through the induction of increased vascular endothelial cell proliferation.

MMP-7 can facilitate cancer invasion through direct cleavage of ECM components or indirectly by proteolytic activation of other MMPs, including MMP-2 and MMP-9 (326, 327). Accordingly, studies have demonstrated that MMP-7 promotes the *in vitro* invasiveness of cancer cell lines originating from a number of organs including the stomach, and that expression levels correlate *in vivo* with tumor invasiveness (2, 5,

132, 139, 282, 304, 348-351). MMP-7 has also been shown to cleave E-cadherin from the cell surface, generating an 80 kDa ectodomain fragment that inhibits E-cadherin in a paracrine manner, thus limiting cell-cell adhesion and promoting invasion (165, 218). Additionally, MMP-7 is responsible for shedding of E-cadherin *in vivo*; in an experimental model of acute lung injury, soluble E-cadherin fragments were detected in the bronchio-alveolar lavage fluid of wild type mice, but not from *mmp-7*$^{-/-}$ mice (195).

MMP-7 expression is regulated at the level of transcription

The promoter region of the human *mmp-7* gene contains several consensus binding sites for known activators of transcription, including two functional Tcf binding sites, an AP-1 binding site, and at least two Ets binding sites (66, 288), as well as a binding site for the transcriptional repressor, Kaiso, overlapping one of the ETS binding sites **(Figure 12)**, suggesting that transcriptional regulation of *mmp-7* is mediated by multiple intracellular signaling pathways. *Mmp-7* transcripts are found in the tumor epithelium of approximately 90% of intestinal adenomas resulting from germ line-inactivating mutations in the adenomatosis polyposis coli (APC) tumor suppressor gene (302, 338). Under normal conditions, free cytoplasmic β-catenin, a proto-oncoprotein involved in intestinal tumorigenesis, rapidly associates with a complex that includes APC, axin, and glycogen synthase kinase 3-β (GSK3-β), which results in phosphorylation of β-catenin by GSK3-β, polyubiquitination, and degradation by the 26S proteosome (209, 230). A loss-of-function mutation in APC leads to the stabilization and accumulation of cytoplasmic β-catenin, resulting in efficient transport to the nucleus (85, 116) , where it interacts with members of the Tcf/lymphoid enhancer factor-1 (LEF-1)

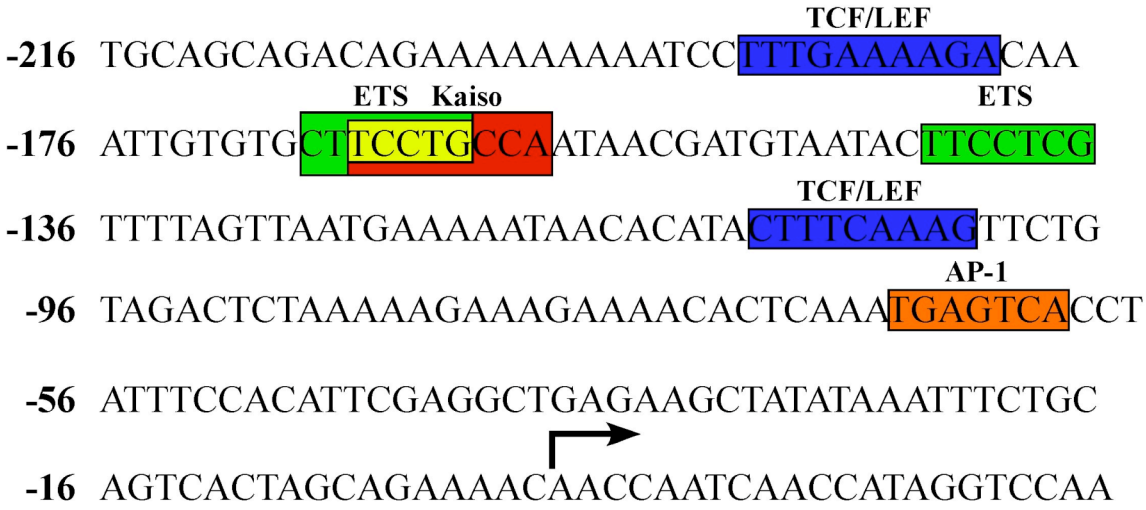

Figure 12. The human *mmp-7* promoter region contains consensus binding sites for TCF/LEF, ETS, Kaiso, and AP-1 (highlighted). The nucleotide sequence from -216 to +22 bp relative to the transcription initiation site at +1, indicated by the arrow, is shown. (288)

DNA binding protein family to transactivate transcription of target genes (25, 130). In murine intestinal tumors, immunohistochemical staining combined with *in situ* hybridization reveals an overlap of nuclear β-catenin accumulation with *mmp-7* transcripts (67). Overexpression of a stable mutant form of β-catenin induces endogenous MMP-7 expression in primary hepatocytes (168). Further, cotransfection of colon cancer cell lines with a mouse matrilysin promoter-luciferase reporter and a stable mutant form of β-catenin upregulates luciferase expression in a Tcf-dependent manner. This induction can be abrogated by co-expression of the cytoplasmic domain of E-cadherin, which blocks association of β-catenin with Tcf factors by sequestering cytoplasmic β-catenin (67). Together, these data suggest that β-catenin transactivation may be required for transcriptional upregulation of MMP-7.

Although β-catenin clearly transactivates the mouse MMP-7 promoter *in vitro*, its accumulation is not sufficient to induce human MMP-7 promoter activity (66). The abundance of β-catenin/Tcf complex does not consistently correlate directly with MMP-7 levels in tumor cell lines. Rare dysplastic glandular structures within mouse intestinal tumors have also been shown to display accumulation of β-catenin within the nucleus, however they do not express similarly high levels of *mmp-7* transcripts (67). Further, the sequence and position of Tcf binding sites within the promoter of β-catenin target genes determines the extent of their β-catenin responsiveness (109). Therefore, nuclear β-catenin accumulation in conjunction with increased activity of other transcription factors appears to be required for activation of *mmp-7* transcription.

As mentioned previously, the human *mmp-7* promoter shares in common with other MMP family members a consensus binding sequence for members of the AP-1

family of transcription factors. Basal promoter activity appears to be highly dependent on an AP-1 site that is usually located at approximately 70 bp upstream of the transcription start site (26). Alterations in AP-1 activity in response to a variety of stimuli, particularly those mediated by the Ras family of small G proteins, regulate the promoter of a number of target genes, including *mmp-7* (26, 76, 341). More recently, it was demonstrated that upregulation of *mmp-7* transcription requires a functional AP-1 binding site and is specifically mediated by the AP-1 family member, c-jun (361, 365), indicating that coordination between AP-1 and β-catenin are involved in transcriptional regulation of *mmp-7*.

Collaboration between Ets and AP-1 transcription factors has been shown to regulate the promoters of several genes associated with tumorigenesis, including MMP family members (76). Accordingly, co-expression of members of the polyomavirus enhancer activator 3 (PEA3) subfamily of Ets transcription factors and c-jun or co-expression of PEA3 and β-catenin synergistically activate the human MMP-7 promoter in human embryonic kidney 293 (HEK293) cells. Co-transfection of vectors encoding all three transcriptions factors, PEA3 family members, β-catenin, and c-jun further augments MMP-7 promoter activity, and the synergistic effect of β-catenin/PEA3 on MMP-7 promoter activity is enhanced by ectopic expression of the Tcf family member, Lef-1. Co-expression of all four transcription factors, β-catenin, PEA3, c-jun, and Lef-1 induces the highest level of *mmp-7* promoter activity and is sufficient to upregulate endogenous *mmp-7* expression, as evidenced by reverse trancription polymerase chain reaction (RT-PCR) (66, 109), indicating that coordinated regulation of *mmp-7* expression is orchestrated by the interaction of several known transcriptional activators.

H. pylori infection results in increased MMP-7 expression

MMP-7 can be induced by a variety of stimuli, and bacterial contact is among the most potent. Co-culture of epithelial cell lines, including colon, lung, and bladder, with a variety of Gram-negative mucosal pathogens leads to sustained production of proteolytically active MMP-7 to levels 50-fold over those in unexposed cells (176). Analysis of gastric biopsies from patients infected with *H. pylori* revealed significantly increased MMP-7 expression when compared with uninfected patients or patients infected with *cag*$^-$ strains of *H. pylori* (24, 68, 344). In addition, it has been shown that co-culture of gastric epithelial cells with *H. pylori* results in a marked upregulation of MMP-7 expression in a *cag*-dependent manner **(Figure 13)** (24, 68, 344). Immunostaining of human primary gastric glands co-cultured with *H. pylori ex vivo* revealed that MMP-7 was localized to the advancing edge of migrating gastric epithelial cell colonies, including lamellipodia, and plays a role in increased gastric gland spreading due to increased epithelial cell migration (344). Co-culture experiments using pharmacologic inhibitors indicate that stimulation of the MAPK signaling pathway, specifically extracellular regulated kinase 1/2 (ERK1/2), is required for *H. pylori*-mediated upregulation of MMP-7 expression (68). Pharmacologic inhibition of the Rho family of small GTPases, specifically RhoA via activation of NF-κB and AP-1 transcription factors and Rac via activation of NF-κB, attenuates *H. pylori*-induced *mmp-7* promoter activity (344). Although *mmp-7* is a known target gene of the Wnt signaling pathway, which, as mentioned previously, is activated in response to *H. pylori*, the role of this signaling pathway in MMP-7 upregulation remains undefined.

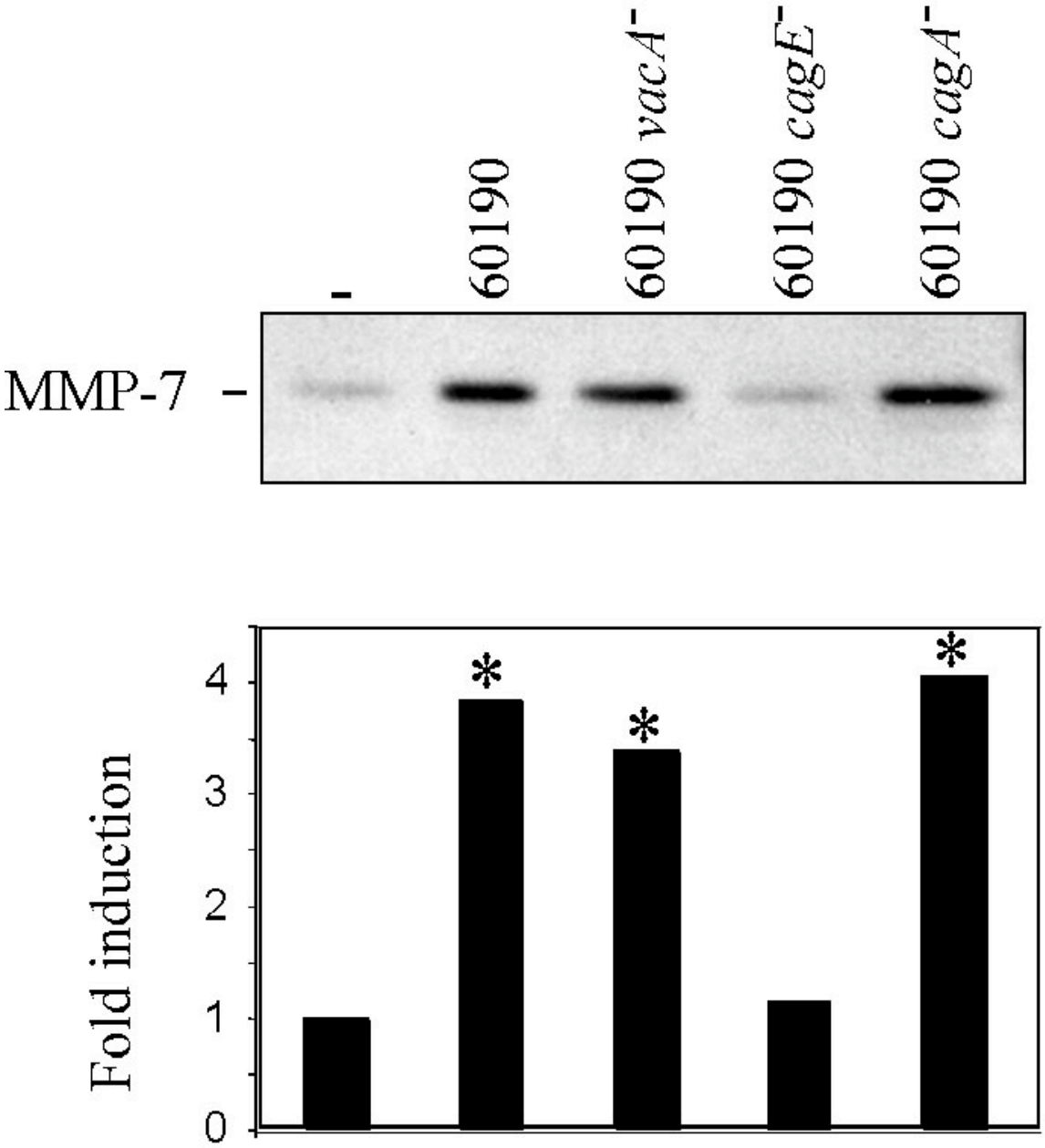

Figure 13. ***H. pylori*** **stimulates increased MMP-7 expression in a** ***cag*****-dependent manner.** (A) AGS cells were cultured in the absence or presence of the *H. pylori* cag^+ strain 60190 or its isogenic $cagA^-$, $cagE^-$, or $vacA^-$ null mutant derivatives at an MOI of 100. Concentrated 24 hour co-culture supernatants were subjected to Western blot analysis. (-), cells incubated with medium alone. A representative blot is shown. Anti-actin blots served as normalization controls for AGS cell viability under different experimental conditions. (B) Densitometric analysis of multiple Western blot repetitions performed on at least 3 occasions. *$p<0.05$ vs. AGS cells alone.

H. pylori induces alterations in p120^{ctn} phosphorylation and localization

The ability of *H. pylori* to stimulate changes in the phosphorylation state and localization of p120^{ctn} has only recently been described (158). In biopsies positive for *H. pylori* infection, phosphorylation of p120^{ctn} at Tyr228 was weakly increased and showed a membranous localization different from that of non-phosphorylated p120^{ctn}, whereas in *H. pylori*-negative biopsies, phosphorylated p120^{ctn} was coexpressed with the non-phosphorylated form and also observed diffusely within the cytoplasm. Infection of primary gastric cell cultures resulted in increased recruitment of non-phosphorylated p120^{ctn} to perinuclear vesicles and an increase in phosphorylated p120^{ctn} expression at the cell membrane and the leading edge of migrating cells. Further, phosphorylated p120^{ctn} was detected within the nucleus by six hours post-infection. These results suggest that *H. pylori* may be able to mediate alterations in p120^{ctn} that could be involved in increased MMP-7 expression and the development of gastric cancer.

Summary and dissertation goals

Gastric adenocarcinoma is strongly associated with the presence of *H. pylori*, and both microbial and host factors influence the risk for carcinogenesis. In Chapter II, *H. pylori*-mediated induction of increased MMP-7 expression in gastric epithelial cells was examined *in vitro*. A novel role for *H. pylori* in the stimulation of the p120^{ctn}/Kaiso signaling axis leading to relief of Kaiso-mediated repression of *mmp-7* transcription was described. These experiments demonstrated p120^{ctn} may be involved in the development of gastric injury through *H. pylori*-mediated alterations in its subcellular localization and phosphorylation state and through interactions with Kaiso that lead to increased

transcription of target genes. Increased expression of MMP-7 in response to *H. pylori* infection may alter a number of processes involved in carcinogenesis, including the inflammatory response, proliferation, and apoptosis. In Chapter III, the role of MMP-7 in an experimental mouse model of *H. pylori*-induced gastric injury was investigated. Bacterial challenge of mice deficient in MMP-7 resulted in increased inflammation and cellular turnover when compared to wild type mice, suggesting that MMP-7 may serve a protective role within *H. pylori*-infected gastric mucosa. Taken together, these data indicate that *H. pylori* stimulates increased gene transcription through dysregulation of a number of signaling pathways and that upregulation of these host effectors mediates the development of malignant lesions. Molecular delineation of signaling events activated by such host-microbial interactions will not only improve our understanding of *H. pylori*-induced carcinogenesis, but may also provide mechanistic insights into other malignancies.

CHAPTER II

p120^{ctn} AND KAISO REGULATE *HELICOBACTER PYLORI*-INDUCED EXPRESSION OF MATRIX METALLOPROTEINASE-7

Summary

H. pylori is the strongest known risk factor for gastric adenocarcinoma, yet only a fraction of infected persons develop cancer. One *H. pylori* constituent that augments disease risk is the *cag* pathogenicity island, which encodes a secretion system that translocates bacterial effector molecules into host cells. MMP-7, a member of a family of enzymes with tumor-initiating properties, is over-expressed in pre-malignant and malignant gastric lesions, and *H. pylori cag*$^{+}$ strains selectively increase MMP-7 protein levels in gastric epithelial cells *in vitro* and *in vivo*. We now report that *H. pylori*-mediated *mmp-7* induction is transcriptionally regulated via aberrant activation of p120^{ctn}, a component of adherens junctions. *H. pylori* increases *mmp-7* mRNA levels in a *cag*- and p120^{ctn}-dependent manner and induces translocation of p120^{ctn} to the nucleus *in vitro* and in a novel *ex vivo* gastric gland culture system. Nuclear translocation of p120^{ctn} in response to *H. pylori* relieves Kaiso-mediated transcriptional repression of *mmp-7*, which is implicated in tumorigenesis. These results indicate that selective and coordinated induction of *mmp-7* expression by *H. pylori cag*$^{+}$ isolates may explain in part the augmentation in gastric cancer risk associated with these strains.

Introduction

Helicobacter pylori induces an inflammatory response in the stomach that persists for decades, and biological costs incurred by this pathogen include an increased risk for gastric adenocarcinoma and non-Hodgkins lymphoma of the stomach (52, 111, 208, 219, 240, 241, 246, 314). However, only a fraction of colonized persons ever develop neoplasia, and enhanced cancer risk is related to strain-specific differences, aberrant host responses, and/or specific interactions between microbial and host determinants.

H. pylori strains that possess the *cag* pathogenicity island increase the risk for cancer compared to strains that lack this genetic locus (241). The *cag* island encodes proteins, such as CagE, that form a type IV secretion system which translocates components of bacterial peptidoglycan and CagA, the product of the terminal gene of the island, into host cells (15, 21, 225, 276, 292, 321). Following translocation, peptidoglycan initiates innate immune signaling via activation of the intracellular pattern recognition receptor, Nod-1, and the transcriptional activator NF-κB (321). Intracellular CagA undergoes Src-dependent tyrosine phosphorylation and activates a eukaryotic phosphatase, SHP-2, leading to dephosphorylation of host cell proteins and cellular morphological changes (21, 120, 276, 291). Recently, CagA has been shown to activate β-catenin and induce NF-κB-mediated IL-8 release from gastric epithelial cells (33, 96). The presence of the *cag* island also influences the topography of colonization in the stomach, as *H. pylori* cag^- strains predominate within the mucus gel layer, while cag^+ strains are found immediately adjacent to epithelial cells (40).

Matrix metalloproteinase (MMP) -7 is a member of a family of zinc-dependent proteolytic enzymes with tumor-initiating properties and is expressed and secreted by

epithelial cells (57, 80). We and others have previously demonstrated that *H. pylori cag*$^{+}$ strains selectively upregulate MMP-7 protein levels in gastric epithelial cells (68, 344). Over-expression of MMP-7 occurs in pre-malignant and malignant gastric lesions (1, 6, 121, 125, 194, 269, 277, 353), and genetic polymorphisms linked to increased MMP-7 expression are associated with *H. pylori* infection status, gastric ulceration (a precursor for gastric cancer), and tumor-related survival among gastric cancer patients (115, 159). In mice, over-expression of MMP-7 leads to hyperproliferation and increased cancer susceptibility (267), and cell lines that over-express MMP-7 develop enhanced tumorigenic potential (342). Conversely, mice with a genetic predisposition for intestinal adenocarcinoma that are then bred onto a background of MMP-7 deficiency develop fewer cancers than wild-type mice (338). Taken together, these data suggest that MMP-7 may play an important role early in gastric carcinogenesis.

A host molecule that has been implicated in regulation of MMP-7 expression is p120^{ctn}. p120^{ctn} was originally identified as a substrate for Src- and receptor-tyrosine kinases (254, 257, 259) and is a member of the catenin (ctn) family, an Armadillo domain protein subfamily whose members interact with the cadherin cytoplasmic tail and modulate cadherin function (254, 255, 279, 289). Aberrant redistribution of p120^{ctn} has been observed in a number of epithelial malignancies, including gastric cancer (138, 141, 142, 190, 308). Typically found at low levels in the nuclei of normal cells, increased levels of p120^{ctn} have been observed in nuclei of tumor cells (190, 272, 337), and recent evidence has revealed that nuclear p120^{ctn} acts to relieve transcriptional repression mediated by Kaiso, a member of the BTB/POZ (72). The Kaiso/p120^{ctn} complex can modulate non-canonical Wnt signaling (152) and, along with TCF/β-catenin complexes,

coordinately regulate canonical Wnt gene targets such as *cyclin D1* and *mmp-7* (237, 288), both of which are up-regulated by *H. pylori* (24, 45, 68, 122, 344). Since MMP-7 exerts cancer-initiating properties and is specifically induced by contact with *H. pylori in vitro* and *in vivo*, we sought to define the molecular pathways underpinning increased MMP-7 expression in order to define a potential tumor-promoting response to this pathogen.

Experimental procedures

H. pylori strains: The *H. pylori* strains 7.13 or SS1 were grown in *Brucella* broth with 5% fetal bovine serum (FBS) for 18 hours with shaking in an atmosphere of 5% CO_2 at 37°C, harvested by centrifugation, and added to gastric epithelial cells at a multiplicity of infection (MOI) of 100. The 7.13 isogenic *cagA*$^-$ and *cagE*$^-$ null mutant strains were constructed by insertional mutagenesis using *aphA* (conferring kanamycin resistance) (96) and were selected on *Brucella* agar containing kanamycin (25 μg/ml). For luciferase assays, *H. pylori* strains were cultured on agar plates containing 10% horse serum in an atmosphere of 5% CO_2 at 37°C for 48 hours. Bacteria were harvested in phosphate buffered saline (PBS), pH 7.4, added to host cells at an MOI of 100 and routinely monitored using an inverted microscope (model TS 100, INTAS).

Cell culture and reagents: MKN28 human gastric epithelial cells (kindly provided by Dr. Robert Coffey, Vanderbilt University) were grown in Royal Park Memorial Institute (RPMI) medium 1640 (Mediatech, Inc.) supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, and 20 μg/ml gentamicin in an atmosphere of 5% CO_2 at 37°C.

Phoenix 293 cells (generously provided by Dr. Todd Graham, Vanderbilt University) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 50 units of penicillin/ml, and 50 μg of streptomycin/ml in an atmosphere of 5% CO_2 at 37°C.

Primary gastric cell extraction and culture: All animal studies were approved by the Vanderbilt Institutional Animal Care and Usage Committee. Stomachs were removed from euthanized 8-week old male FVB/n mice (Harlan), ligated at the pylorus and esophagus, inverted, and injected with 1 ml of 0.5 mg/ml collagenase A as previously described (344). Stomachs were then washed in Hank's balanced salt solution (HBSS) 3 times at 37°C. Tissue was incubated in 10 ml of 1 mM dithiothreitol (DTT) for 15 minutes at 37°C with shaking, washed in HBSS 3 times at 37°C, and incubated in 0.37 mg/ml collagenase for 30 minutes at 37°C. Following the first collagenase digestion, samples were washed again in HBSS (3 times, 37°C) and incubated for a further 30 minutes in collagenase (0.37 mg/ml, 37°C). Tissue was triturated using a wide mouthed pipette and larger fragments of tissue were allowed to settle under gravity for 45 seconds. The supernatant containing isolated gastric cell colonies was removed and transferred to a clean 50 ml conical tube, shaken vigorously to release additional cell colonies, and left on ice to sediment for 45 minutes. The supernatant was then carefully removed and discarded, and isolated cell colonies were plated in chamber slides. Colonies of gastric epithelial cells were cultured in DMEM NUT Mix F-12 (HAM) supplemented with 10% FBS and 1% antibiotic-antimycotic solution and incubated in a humidified incubator at

37°C under an atmosphere of 5% CO_2. Cell colonies were cultured for up to 72 hours and the medium was changed every 24 hours.

siRNA constructs: pSUPER.retro.puro (Oligoengine) plasmid containing a human-specific targeting sequence directed towards $p120^{ctn}$ was kindly provided by Dr. Albert Reynolds, Vanderbilt University (74, 183). pSUPER.retro.puro (Oligoengine) plasmid containing a scrambled non-targeting sequence was kindly provided by Dr. Howard Crawford, SUNY-Stonybrook. Non-targeting siRNA oligos (D-001210-01) or ON-TARGETplus SMARTpool siRNA oligos directed towards Kaiso (ZBTB33; L-019982-00) were purchased from Dharmacon.

Viral production and retroviral transduction: Phoenix 293 packaging cell lines at 50% confluence were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Fresh medium was added 24 hours post-transfection, and tissue culture medium was collected and filtered through a 0.45 μm filter 72 hours post-transfection. For retroviral transduction, MKN28 cells at 50% confluence were incubated overnight with freshly harvested virus containing 4 μg/ml of polybrene. To generate stable cell lines, cells transduced with the pSUPER.retro.puro virus were selected with puromycin (1.5 μg/ml) for 48 hours. Clonal populations were selected using cloning rings and limiting dilution techniques.

Luciferase assay: The plasmids 4xKBS-pGL3 and p120-pCMV were kindly provided by Dr. Juliet M. Daniel, McMaster University (150). To monitor Kaiso-dependent gene

expression, MKN28 cells were cultured in 24-well plates and co-transfected with 100 ng 4xKBS-pGL3, 4 ng phRL Null, and 100 ng $p120^{ctn}$-pCMV or 100 ng of the empty vector pCMV for 12 hours using Genejuice (Novagene) as recommended by the manufacturer's instructions. Transfection efficiency ranged from 10 to 15%. Eighteen hours post-transfection, cells were co-cultured with *H. pylori* or medium alone. After 48 hours, cells were harvested in 100 μl reporter lysis buffer (Promega) and luciferase activity was determined in a dual channel luminometer. Results were normalized for transfection efficiency by co-transfection with the Renilla luciferase plasmid (phRL Null).

Transient transfection of siRNA: MKN28 cells (1.5×10^5) in 12-well plates were transiently transfected using DharmaFECT 2 transfection reagent (Dharmacon) according to the manufacturer's instructions. Briefly, transfection reagent (1.0 μl/well) was mixed with siRNA oligos (2.5 μl of 20 μm solution/well) in 100 μl Opti-MEM (Life Technologies). Cells were incubated with the transfection mixture for 24 hours, fresh medium was added, and bacterial co-cultures were performed 24 hours later.

Real time RT-PCR: MKN28 gastric epithelial cells were grown to confluence and then co-cultured with *H. pylori* or medium alone for 2, 4, 8, or 12 hours. RNA was prepared from co-cultures using TRIzol Reagent following the manufacturer's instructions (Invitrogen). RT-PCR was performed using TaqMan reverse transcription reagents (Applied Biosystems), which was followed by real-time quantitative PCR using the TaqMan gene expression assay and a 7300 real-time PCR system (Applied Biosystems). *mmp-7, $p120^{ctn}$,* and *Kaiso* cDNA were quantified using the human-specific *mmp-7*

TaqMan gene expression primer set (Hs00159163_m1), *CTNND1* TaqMan gene expression primer set (Hs00609741_m1), and *ZBTB33* TaqMan gene expression primer set (Hs00406811_m1), respectively, and expression levels were normalized to levels of 18S rRNA.

Immunofluorescence: Gastric cells were cultured in glass chamber slides and subsequently co-cultured with *H. pylori* or medium alone. Six hours post-infection, cells were washed twice with Dulbecco's phosphate buffered saline (DPBS) containing calcium chloride, and fixed in 4.7% paraformaldehyde in DPBS for 15 minutes at room temperature. Cells were then subjected to antigen retrieval by immersion in diH_20, followed by immersion in citrate buffer (pH=6.0, 8 mM citric acid, 2 mM sodium citrate tribasic dihydrate) and heating in a 1200-watt microwave for 15 minutes at 20% power. After cooling for 30 minutes at room temperature, cells were rinsed in diH_20, incubated with DPBS containing 3% bovine serum albumin (BSA; Sigma) and 0.1% Triton X-100 for 20 minutes, followed by incubation in 3% BSA for 1 hour at room temperature. Slides were immunostained with mouse monoclonal anti-$p120^{ctn}$ antibody (pp120, BD Bioscience), rabbit anti-$p120^{ctn}$ antibody (F1αSH), mouse monoclonal anti-Kaiso antibody (6F/6F8, BD Bioscience), mouse monoclonal anti-Kaiso antibody (11D), rabbit polyclonal anti-Kaiso antibody, or rabbit anti-*H. pylori* antibody (DakoCytomation) at a concentration of 1:100 overnight at 4°C. Washed slides were incubated with goat anti-mouse AlexaFluor 488-conjugated antibody (Molecular Probes), goat anti-mouse AlexaFluor 546-conjugated antibody (Molecular Probes), or goat anti-rabbit AlexaFluor 488-conjugated antibody (Molecular Probes) at a concentration of 1:100 for 2 hours at

room temperature. Washed slides were then incubated with TOTO-3 dimeric cyanine nucleic acid dye at a concentration of 1:100 for 20 minutes at room temperature (Molecular Probes). Slides were mounted using ProLong Gold antifade reagent (Invitrogen). Imaging was performed on a Zeiss LSM 510 Confocal Microscope using a 63X/1.40 Pan-APOCHROMAT oil objective at room temperature and acquisition was performed with the manufacturer's proprietary software. All 3D reconstructions and fluorescence profile analyses were performed using the Zeiss LSM Image Examiner 3.2 software.

Chromatin immunoprecipitation: Chromatin immunoprecipitation (ChIP) experiments were performed as previously described (114). Briefly, MKN28 cells were grown to confluence in 10 cm dishes, and then incubated with 1.6% formaldehyde (Sigma) in PBS for 10 minutes at room temperature. The crosslinking reaction was stopped by adding glycine to a final concentration of 0.14 M. Cells were washed twice with PBS and harvested in 250μl ChIP radio immunoprecipitation assay (RIPA) buffer [50mM Tris, pH 8.0, 150mM NaCl, 1% NP-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 5mM EDTA] containing protease inhibitor cocktail (P2714; Sigma). Cells were subjected to sonication consisting of eight 10-second pulses at 50% amplitude and centrifuged at 14,000 x g at 4°C for 15 minutes. For each immunoprecipitation, supernatant containing 1 mg total protein as quantified by the Bradford assay (Pierce) was pre-cleared by the addition of 10 μg mouse IgG conjugated to protein A sepharose beads (PAS; Zymogen) with rocking at 4°C for 1 hour. PAS beads were removed by centrifugation. Two μg of antibody and 30 μl PAS beads were added for each immunoprecipitation with rocking at

4°C overnight. PAS beads were concentrated by centrifugation and washed twice with 1 mL of ChIP RIPA buffer, four times with 1 mL of IP Wash buffer (100mM Tris, pH 8.5, 500mM LiCl, 1% NP-40,1% deoxycholate), followed by 2 washes with ChIP RIPA buffer. During each wash, samples were rotated for 5 minutes at 4°C. Two hundred μl of Crosslink Reversal Buffer (125 mM Tris, pH 6.8, 10% β-mercaptoethanol, 4% SDS) was added directly to PAS beads from each sample and boiled for 30 minutes. DNA was purified by phenol-chloroform extraction and precipitated with ethanol. DNA pellets were resuspended in 20 μl PCR-grade water. Five μl of resuspended DNA was subjected to PCR to amplify the *mmp-7* promoter region (forward primer: TAGAGGCAGTGTTCCCCATT, reverse primer: CCAAATCCTGTGGTTCTCC) as previously described (288). PCR products were electrophoresed on a 1.5% agarose gel at 100 V for 30 minutes.

Western analysis: Cells were lysed in RIPA buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1% SDS) containing protease inhibitor cocktail, and protein concentrations were quantified by the Bradford assay. Proteins (30 μg) were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (PVDF, Pall Corporation, Ann Arbor, MI). Protein levels were assessed by Western blotting using mouse monoclonal anti-p120^{ctn} antibody (1:1000, pp120), anti-E-cadherin antibody (1:1000, BD Bioscience), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:2000, Chemicon), or goat polyclonal anti-actin antibody (1:2000, C-11, Santa Cruz Biotechnology). Primary antibodies were detected using goat anti-mouse or donkey anti-goat (1:2500, Santa Cruz Biotechnology) horseradish peroxidase-cojugated

secondary antibodies and visualized by Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences) according to the manufacturer's instructions on a Syngene Chemigenius system. For analysis of exogenous $p120^{ctn}$ expression, transfected cells were harvested in lysis buffer (20 mM Tris-HCl pH 7.5, 1 mM ETDA, 100 mM NaCl, 1% Triton X-100, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 x complete protease inhibitors [Roche], 1 mM Na_3VO_4, 1 mM sodium molybdate, 20 mM NaF, 10 mM sodium pyrophosphate, 20 mM β-glycerophosphate), separated by SDS PAGE, and transferred to PVDF membranes. Protein levels were assessed by Western blotting using anti-$p120^{ctn}$ antibody (pp120) and anti-GAPDH antibody (Abcam).

Subcellular fractionation: Fractionation was performed as described by Frey *et al.* (99). Briefly, cells were lysed in low-detergent buffer (20 mM HEPES, 2 mM EDTA, 2 mM EGTA, 0.5 mg/ml digitonin) containing protease inhibitors, centrifuged for 40 minutes at 100,000 x g, and the soluble fraction was collected. The residual pellet was resuspended in high detergent buffer (10 mM Tris pH 7.4, 2 mM EDTA, 2 mM EGTA, 1% Triton, 0.2% deoxycholate, 0.1% SDS) containing protease inhibitors, centrifuged for 5 minutes at 20,000 x g, and the membranous fraction was collected.

Immunoprecipitation: Prior to collection, MKN28 cells were treated with sodium pervanadate to inhibit endogenous phosphatase activity as previously described (183). Cells were then lysed in IP Lysis Buffer (50 mM Tris, pH 7.2, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.2% sodium deoxycholate) containing protease inhibitor cocktail, phosphatase inhibitor cocktails 1 and 2 (Sigma), and 150 mM vanadate/ 300 mM H_2O_2,

harvested, and protein concentrations were quantified by the Bradford assay. One mg total protein was isolated from each sample, and total volumes were equilibrated with IP Lysis Buffer. Twenty μL of mouse IgG (Sigma) was added, followed by 30 minutes of incubation at 4°C with rolling. Then, 20 μL of protein A/G PLUS-agarose immunoprecipitation reagent (Santa Cruz Biotechnology) was added, followed by 30 minutes of incubation at 4°C with rolling. Samples were centrifuged at 4°C for 10 minutes at 10,000 x g. The supernatants were then isolated and incubated overnight with 1 μg of the appropriate IP antibody (pp120, BD Bioscience; or c-myc, 9E10, Santa Cruz Biotechnology) at 4°C with rolling. Thirty-five μl of protein A/G PLUS-agarose immunoprecipitation reagent was added to each sample, followed by 1 hour of incubation at 4°C with rolling. Samples were centrifuged at 10,000 x g at 4°C for 2 minutes, and the supernatant discarded. The remaining protein A/G reagent was washed 6 times with 500 μL cold PBS containing 150 mM vanadate/ 300 mM H_2O_2. After the last wash, 75 μl of Laemmli buffer was added to each sample and boiled for 5 minutes. The samples were centrifuged at room temperature at 10,000 x g for 5 minutes, and the supernatants were analyzed by Western blot analysis. Immunoblotting was performed using a mouse monoclonal anti-phospho-tyrosine antibody (1:200, PY99, Santa Cruz Biotechnology) and anti-phospho-p120 antibodies (1:1000; pY96, pY228, pY291; BD Bioscience).

Quantitative culture of adherent bacteria: *H. pylori*:MKN28 cell co-cultures were washed twice after 24 hours with PBS, pH 7.6 to remove non-adherent bacteria, and total cell extracts were harvested as described (68). Serial 10-fold dilutions of 1-ml aliquots of cell extracts were cultured on 5% sheep blood agar plates, and incubated for 3 to 5 days

under microaerobic conditions before *H. pylori* colonies were counted. Results are expressed as colony forming units (cfu) per ml.

Statistical analysis: The student's *t* test was used to evaluate the data, and significance was defined as $P<0.05$. Fluorescence intensity profiles were generated by creating Lowess spline curves of the XY data output from the Zeiss LSM image examiner software.

Results

H. pylori-mediated mmp-7 expression is transcriptionally regulated and is dependent on a functional cag pathogenicity island.

We and others previously demonstrated that MMP-7 protein levels are increased in gastric cells following co-culture with *H. pylori* (68, 344), and that this is dependent on specific genes within the *cag* island (*e.g. cagE* but not *cagA*) (68). *cagE* encodes a homolog of VirB4, a protein involved in toxin export in *Bordetella pertussis*. Based on homology, CagE is postulated to function as a NTPase, which is required for energy-dependent delivery of CagA and other *cag* island substrates from the bacterial cytoplasm directly into host cells (20). Inactivation of *cagE*, therefore, results in a non-functional type IV *cag* secretion system. To determine whether increased MMP-7 protein expression was transcriptionally mediated, MKN28 human gastric epithelial cells were co-cultured with the *H. pylori* wild-type cag^+ strain 7.13, a 7.13 $cagA^-$ mutant, a 7.13 $cagE^-$ mutant, or medium alone. At defined time points, steady-state *mmp-7* mRNA levels were assessed by real time RT-PCR. Levels of *mmp-7* mRNA began to increase 4 hours post-infection with the wild-type strain 7.13 and peaked by 8 hours at levels approximately 12-fold

above control **(Figure 14)**. Inactivation of *cagA* had no significant effect on *H. pylori*-mediated *mmp*-7 induction **(Figure 14)**, confirming our previous results for MMP-7 protein expression (68). However, inactivation of *cagE* significantly attenuated *mmp-7* expression **(Figure 14)**, indicating that *H. pylori* strain 7.13 induces *cag*-dependent transcriptional up-regulation of *mmp-7* in human gastric epithelial cells.

Kaiso binds the mmp-7 promoter region in gastric epithelial cells.

Recent data have shown that Kaiso mediates transcriptional repression of *mmp-7* in colonic epithelial cells (288); therefore, we used laser scanning immunofluorescent microscopy to determine whether gastric epithelial cells that produce MMP-7 in response to *H. pylori* **(Figure 14)** also express Kaiso. As shown in **Figure 15A,** Kaiso is expressed throughout the cell, including the nuclei, in MKN28 cells. These results were confirmed using additional monoclonal and polyclonal anti-Kaiso antibodies **(Figure 16)**.

The human *mmp-7* promoter contains two conserved copies of the consensus Kaiso site, one of which overlaps an Ets transcription factor consensus site and is in close proximity to AP-1 and TCF/Lef transcription factor binding sites (288). Kaiso has previously been shown to bind this region of the *mmp-7* promoter in at least three human epithelial cell lines by chromatin immunoprecipitation assay (288). Although the immunofluorescence data in **Figure 15A** indicate that Kaiso is present in the requisite subcellular locale to function as a transcriptional repressor, it is not known whether Kaiso binds to the *mmp-7* promoter in MKN28 cells. Therefore, primers specific for this region of the *mmp-7* promoter were used for ChIP to confirm that Kaiso can bind and regulate *mmp-7* in gastric epithelial cells. *mmp-7* promoter fragments were specifically detected

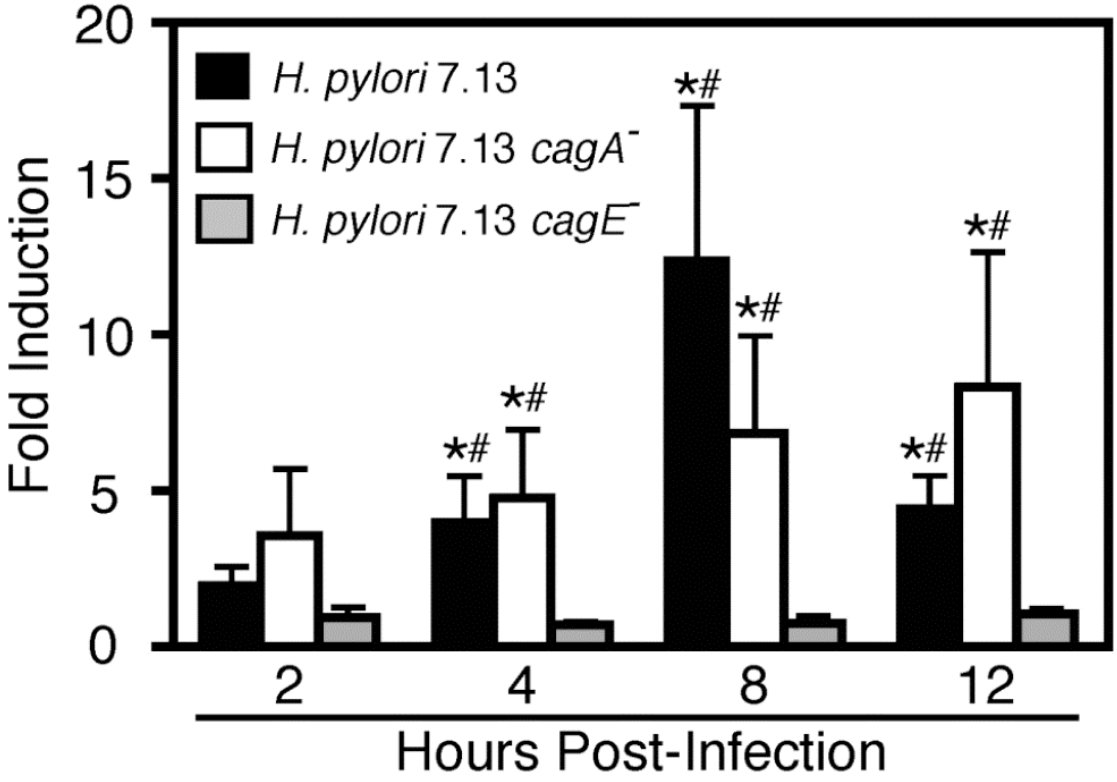

Figure 14. *H. pylori* infection of MKN28 cells results in an increase in *mmp-7* steady-state mRNA levels in a *cagE*-dependent manner. MKN28 cells were co-cultured with the *H. pylori cag*$^{+}$ strain 7.13 (MOI=100), the 7.13 *cagA*$^{-}$ or *cagE*$^{-}$ isogenic mutants, or medium alone. At defined time points, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold induction of *mmp-7* mRNA in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 4 occasions. Error bars, standard error of the mean (SEM). *, $P<0.05$ versus medium alone. $^{\#}$, $P<0.05$ versus *cagE*$^{-}$.

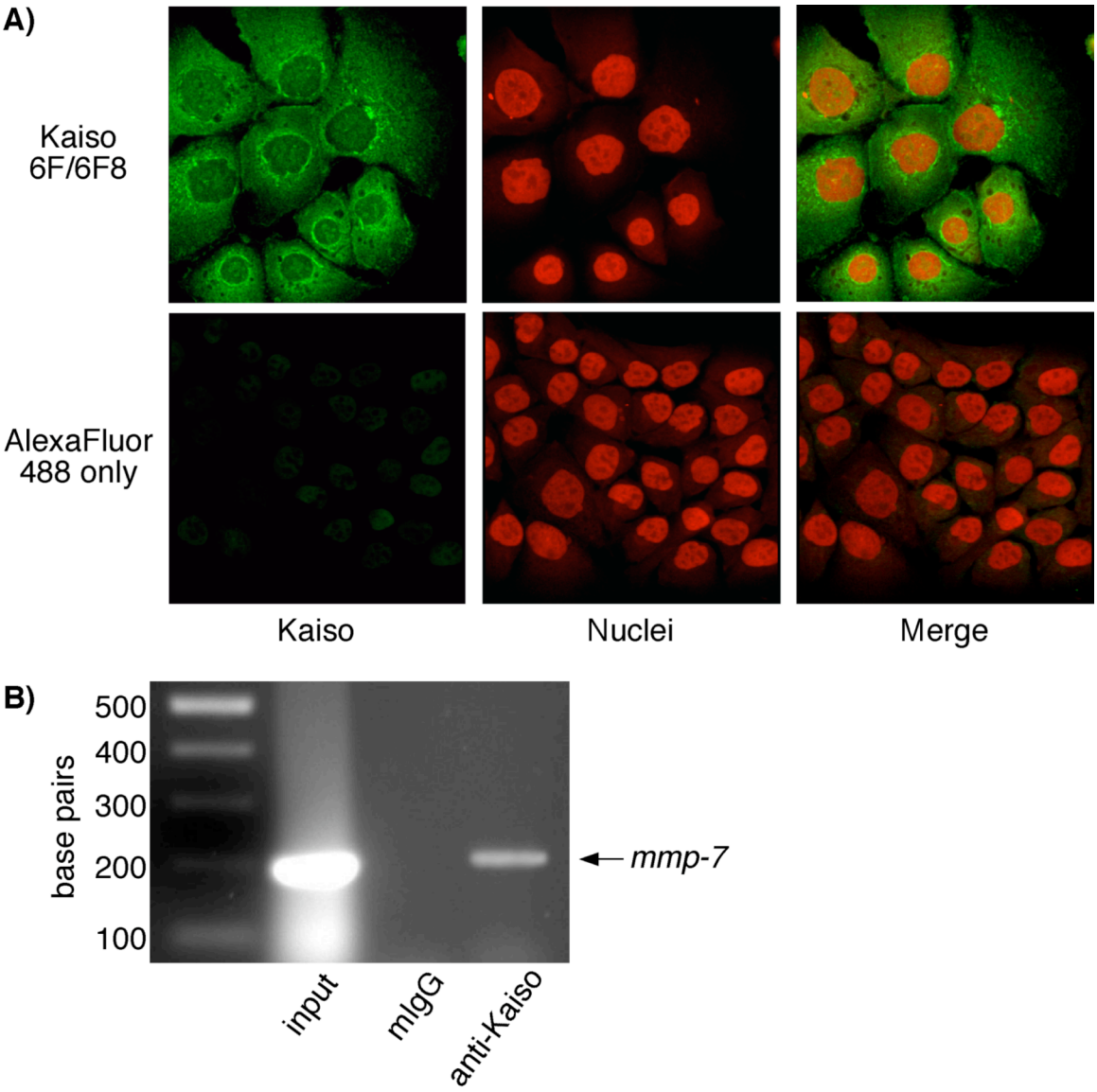

Figure 15. MKN28 cells express the transcriptional repressor, Kaiso, which binds the promoter region of *mmp-7*. (A) MKN28 cells were fixed and incubated with amonoclonal anti-Kaiso antibody (6F/6F8) or PBS alone, followed by incubation with an anti-mouse AlexaFluor-488 antibody and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. Kaiso, green; nuclei, red; 630X magnification. (B) MKN28 cells were subjected to chromatin immunoprecipitation analysis using a monoclonal anti-Kaiso antibody (6F/6F8), mouse IgG (negative control), or input DNA alone (positive control) followed by PCR amplification using human *mmp-7* promoter-specific primers. Experiments were performed on at least 2 occasions. Products were visualized by agarose gel electrophoresis and a representative gel is shown.

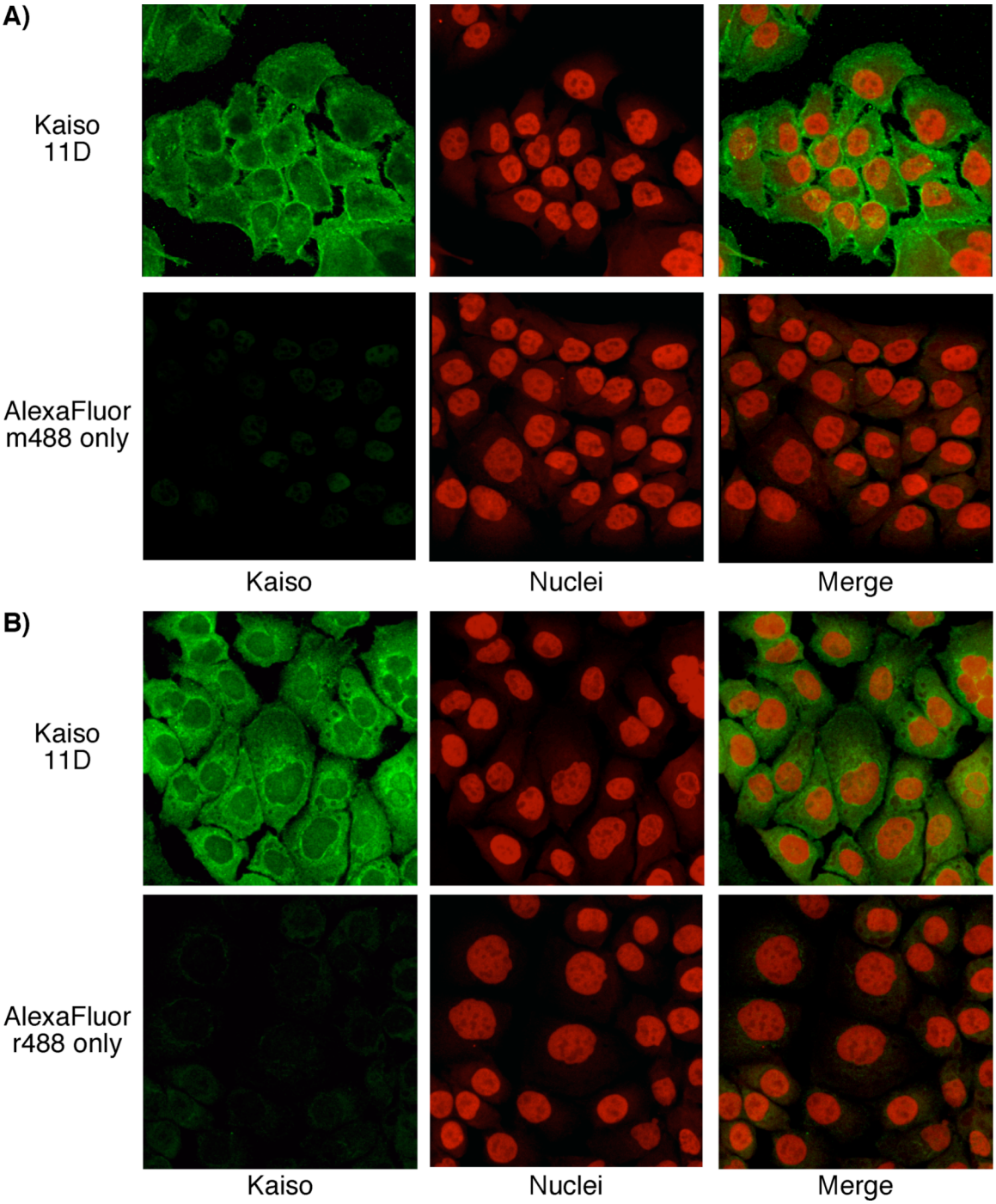

Figure 16. MKN28 cells express the transcriptional repressor, Kaiso. MKN28 cells were fixed and incubated with an anti-Kaiso antibody or PBS alone, followed by incubation with an AlexaFluor-488 antibody and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. Kaiso, green; nuclei, red. 630X magnification. (A) Staining with a mouse monoclonal anti-Kaiso antibody (11D) and anti-mouse AlexaFluor-488 secondary antibody. (B) Staining with a rabbit polyclonal anti-Kaiso antibody and anti-rabbit AlexaFluor-488 secondary antibody.

in input fractions and Kaiso immunocomplexes, but not in isotype control immunocomplexes **(Figure 15B)**, indicating that Kaiso binds specifically to the promoter region of *mmp-7* in MKN28 cells.

H. pylori strain 7.13 alters subcellular distribution of p120^{ctn} and induces aberrant localization of p120^{ctn} to the nucleus.

Interactions between p120^{ctn} and Kaiso can coordinately regulate genes implicated in carcinogenesis (72, 73, 150, 152, 237, 288), and translocation of p120^{ctn} to the nucleus relieves Kaiso-mediated transcriptional repression of *mmp-7* (150, 237, 288). To determine whether *H. pylori* infection alters total p120^{ctn} levels, MKN28 cells were co-cultured with *H. pylori* strain 7.13 (MOI=100) or medium alone and subjected to Western blot analysis **(Figure 17A)**. Densitometric analysis of multiple immunoblot experiments indicated that total p120^{ctn} levels were not significantly altered following infection when compared to uninfected controls (data not shown). Similarly, mRNA levels of p120^{ctn}, as measured by real time RT-PCR, remained unchanged by infection with *H. pylori* **(Figure 18A)**.

We next sought to determine whether *H. pylori* may induce alterations in subcellular localization of p120^{ctn}. MKN28 cells were co-cultured with *H. pylori* strain 7.13 and, at defined time points, total cell lysates were fractionated into a soluble fraction containing cytosolic components and an insoluble fraction containing nuclear and membranous components. Immunoblot analysis of these fractions revealed that *H. pylori* significantly decreased soluble, and concomitantly increased insoluble levels of p120^{ctn} over 24 hours of infection **(Figure 17B)**. Immunoblot analysis performed on cells co-

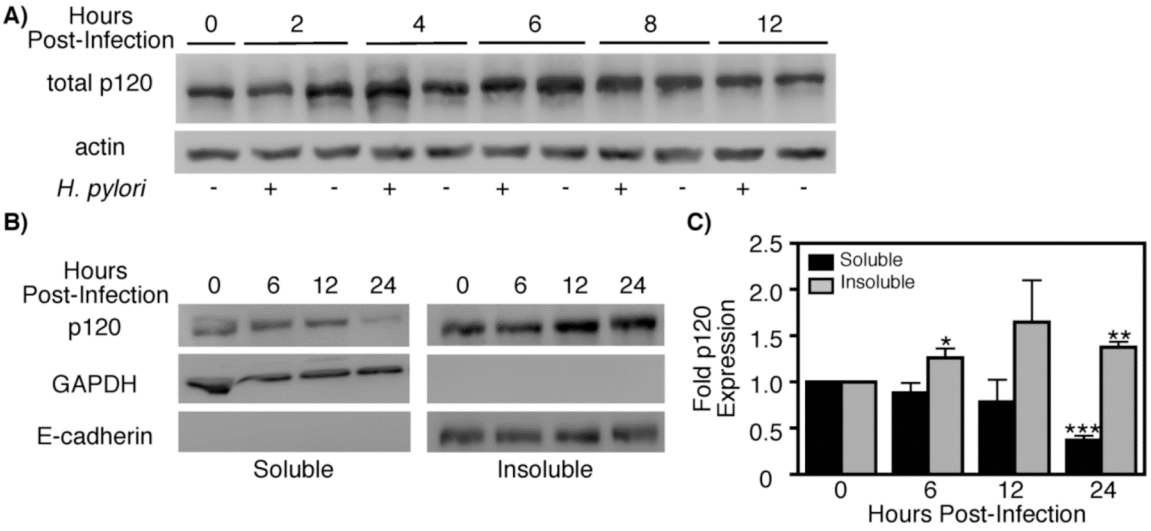

Figure 17. *H. pylori* alters subcellular localization of p120^{ctn} and induces aberrant localization of p120^{ctn} to the nucleus. (A) MKN28 cells were co-cultured with *H. pylori* strain 7.13 (MOI=100) or medium alone. At defined time points, total protein was extracted and analyzed by Western blot using a monoclonal anti-p120^{ctn} antibody (pp120). Experiments were performed on at least 3 occasions. A representative blot is shown. Anti-actin blots served as normalization controls for MKN28 viability under different experimental conditions. (B) MKN28 cells were co-cultured with *H. pylori* strain 7.13 (MOI=100). At defined time points, total protein was extracted, subjected to subcellular fractionation, and analyzed by Western blot using an anti-p120^{ctn} antibody. Representative blots are shown. Anti-GAPDH and anti-E-cadherin antibodies served as normalization controls for purification of soluble and insoluble subcellular fractions, respectively. (C) Densitometric analysis of multiple Western blot repetitions performed on at least 3 occasions. Graph represents fold p120 expression in infected versus uninfected cells. Error bars = SEM. *, P<0.05. **, P<0.01. ***, P<0.001. vs. time 0.

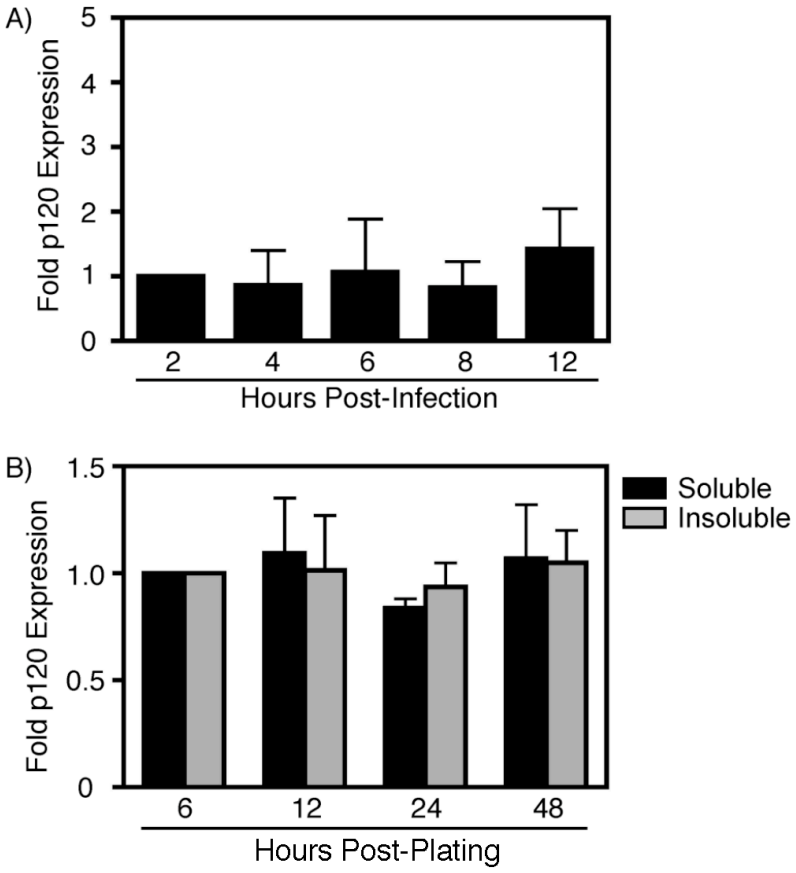

Figure 18. *H. pylori* infection or increases in MKN28 cell confluence over time did not contribute to changes in total or subcellular p120^{ctn} levels. (A) MKN28 cells were co-cultured with the *H. pylori cag*$^{+}$ strain 7.13 (MOI=100) or medium alone. At defined time points, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold *p120*^{ctn} mRNA expression in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 3 occasions. Error bars, SEM. (B) MKN28 cells were cultured with medium alone. At defined time points, total protein was extracted, subjected to subcellular fractionation, and analyzed by Western blot using an anti-p120^{ctn} antibody. The graph represents densitometric analysis of multiple Western blot repetitions performed on at least 3 occasions. Error bars, SEM.

cultured with medium alone showed no alteration in soluble or insoluble p120^{ctn} levels **(Figure 18B)**, indicating that increases in cell confluence over time did not contribute to changes in subcellular p120^{ctn} levels. These results demonstrate that *H. pylori* alters the distribution, but not the total amount, of the cellular p120^{ctn} pool.

Having identified alterations in the subcellular localization of p120^{ctn}, we next determined if *H. pylori* strain 7.13 could induce nuclear translocation of p120^{ctn} in MKN28 cells. Cells were co-cultured with *H. pylori* strain 7.13 or medium alone for 6 hours, and p120^{ctn} localization was assessed by laser scanning immunofluorescent microscopy. Nuclear and perinuclear aggregations of p120^{ctn} were observed in cells co-cultured with *H. pylori* when compared with medium alone **(Figure 19A)**. As shown in **Figure 19B**, p120^{ctn} specifically co-localized with Kaiso in the nuclei of infected but not uninfected cells, placing these molecules in position to potentially regulate genes implicated in carcinogenesis.

H. pylori strain 7.13 alters the phosphorylation state of p120^{ctn}.

Having demonstrated that infection with *H. pylori* strain 7.13 induces nuclear translocation of p120^{ctn}, we next sought to identify the mechanism underlying *H. pylori*-induced aberrant subcellular distribution of p120^{ctn}. Previous studies have suggested that phosphorylation of a number of tyrosine residues within the p120^{ctn} regulatory domain may play a role in p120^{ctn} signaling (42, 183, 253, 280). Therefore, we investigated whether *H. pylori* infection similarly altered levels of p120^{ctn} tyrosine phosphorylation. MKN28 cells were co-cultured with *H. pylori* strain 7.13 and, at defined time points, cells were treated with phosphatase inhibitors, harvested, and subjected to

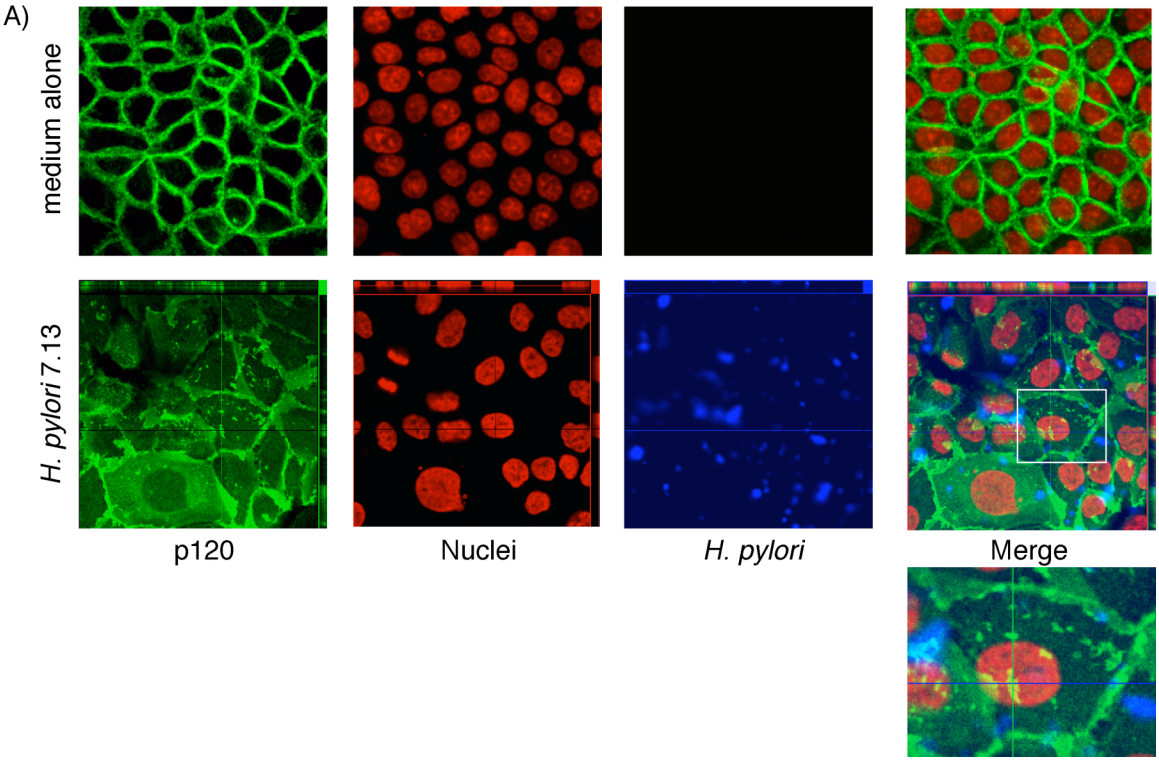

Figure 19. *H. pylori* induces aberrant localization of p120^{ctn} to the nucleus in MKN28 cells. MKN28 gastric epithelial cells were co-cultured with *H. pylori* strain 7.13 or medium alone for 6 hours. (A) Cells were fixed and incubated with a monoclonal anti-p120^{ctn} antibody (pp120) and a polyclonal anti-*H. pylori* antibody, followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. p120^{ctn}, green; nuclei, red; *H. pylori*, blue. 630X magnification. Localization of p120^{ctn} in 3-dimensions is represented by orthogonal views of infected cells. (B) Cells were fixed and incubated with a rabbit polyclonal anti-p120^{ctn} antibody (F1αSH) and a monoclonal anti-Kaiso antibody (6F/6F8), followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as a single transverse image through the middle of the cell. p120^{ctn}, green; Kaiso, red; nuclei, blue. Uninfected, 630X magnification. Infected, 1260X magnification. Graph indicates fluorescence intensity as measured linearly through the nucleus demonstrating co-localization of Kaiso and p120^{ctn} within the nuclei of infected cells.

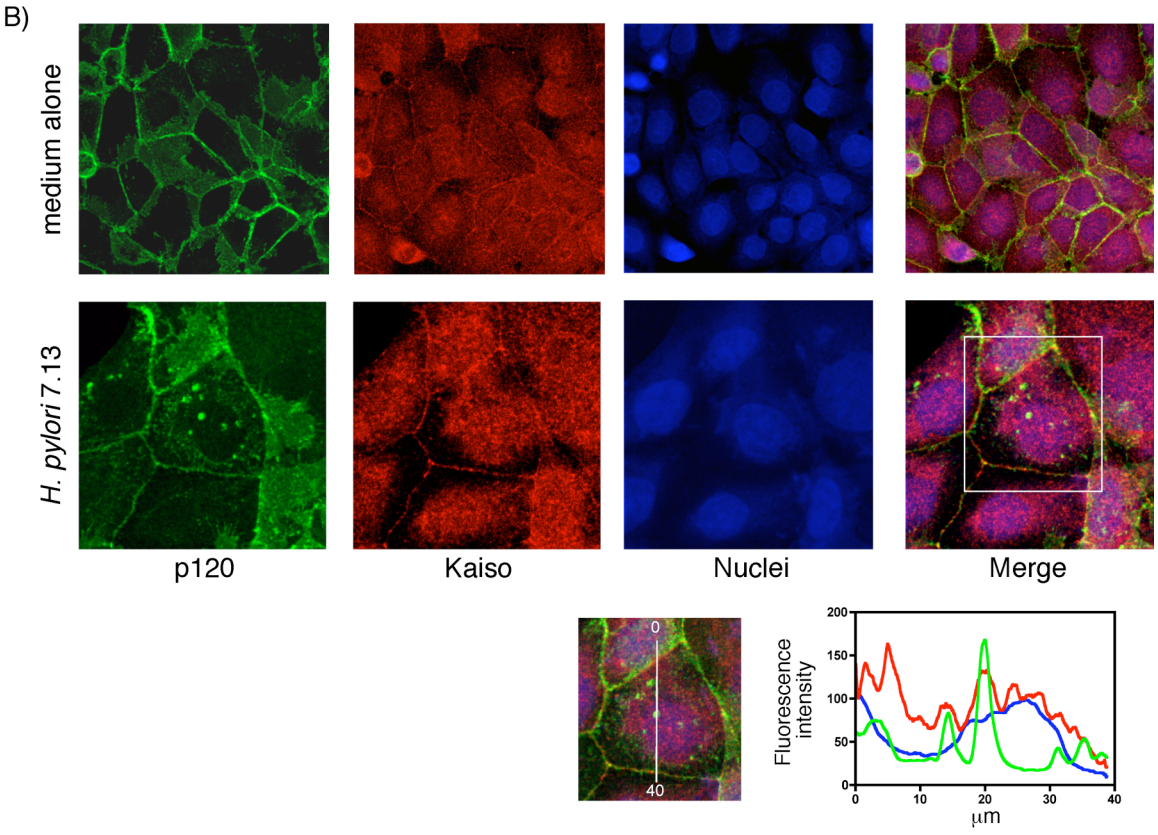

Figure 19, continued.

immunoprecipitation using an anti-p120^{ctn} antibody. Of interest, immunoblot analysis of p120^{ctn} immunocomplexes using a general anti-phospho-tyrosine antibody revealed that *H. pylori* significantly decreased total tyrosine phosphorylation when compared to uninfected controls **(Figure 20)**, suggesting that dephosphorylation of p120^{ctn} tyrosine residues may contribute to alterations in subcellular localization. To define which specific residues might be dephosphorylated, immunoblot analysis was performed using anti-phospho antibodies targeting three specific p120^{ctn} residues: Y228, Y291, and Y96. However, levels of phosphorylation at each of these residues were not significantly altered by infection **(Figure 20A)**, indicating that other, as of yet uncharacterized, tyrosine phosphorylation sites represent targets for dephosphorylation by *H. pylori*.

H. pylori relieves Kaiso-mediated transcriptional repression in a cag pathogenicity island-dependent manner.

H. pylori induces an increase in transcription of *mmp-7* in MKN28 gastric epithelial cells. Having also shown that Kaiso binds the promoter region of *mmp-7* and that *H. pylori* induces aberrant localization of p120^{ctn} to the nucleus, we next sought to determine if inhibition of Kaiso-mediated transcriptional repression was required for *mmp-7* transcriptional upregulation. We utilized the pGL3-4xKBS plasmid (4xKBS), which carries four tandem copies of the consensus Kaiso binding site upstream of the *luciferase* reporter gene (150). Previous studies suggest that the ability to inhibit Kaiso-mediated transcriptional repression of an artificial promoter such as 4xKBS requires p120^{ctn} levels greater than those endogenously present within cells (150). Therefore, we co-transfected MKN28 cells with 4xKBS and a p120^{ctn} expression vector prior to *H.*

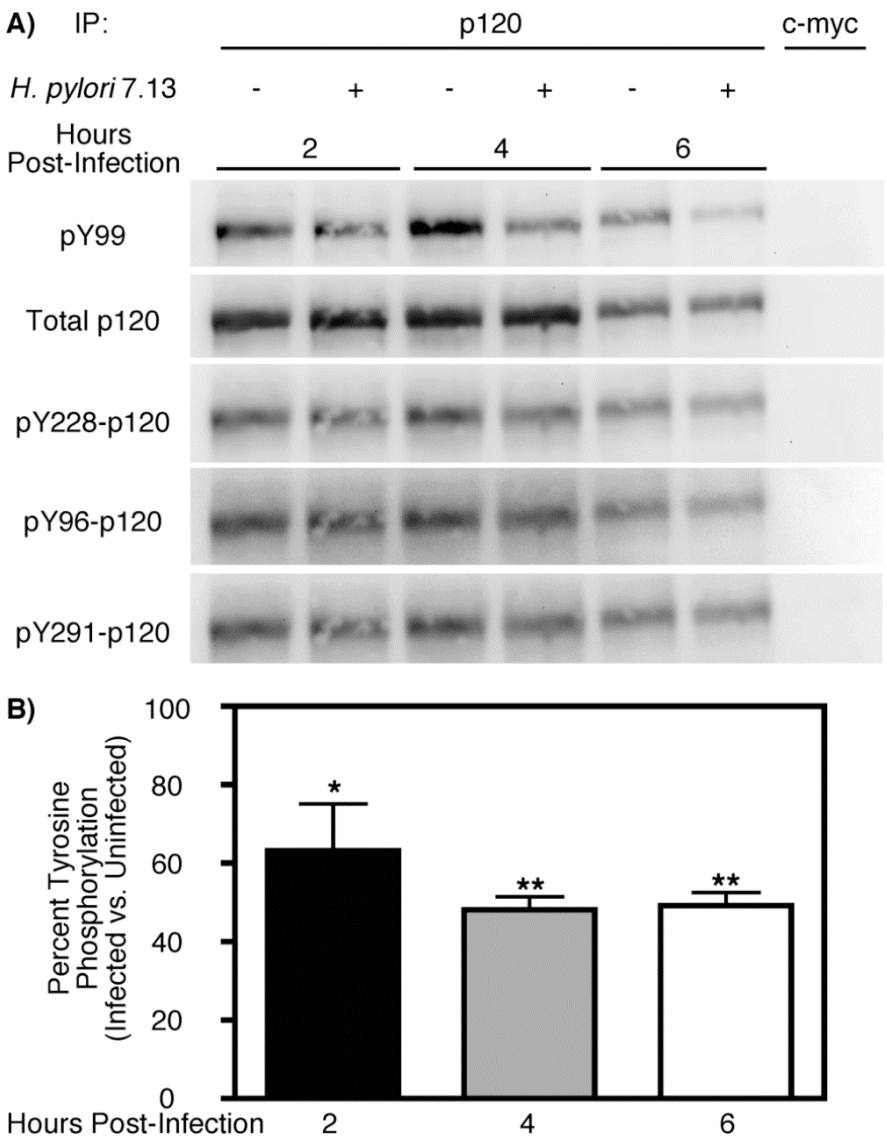

Figure 20. *H. pylori* infection of MKN28 cells results in reduced levels of p120^{ctn} tyrosine phosphorylation. (A) MKN28 cells were co-cultured with *H. pylori* strain 7.13 (MOI=100) or medium alone. At defined time points, total protein was extracted, subjected to immunoprecipitation with an anti-p120^{ctn} antibody (pp120), and analyzed by Western blot using a total anti-phospho-tyrosine antibody (pY99) or anti-phospho-antibodies specific to the p120^{ctn} tyrosine residues 228, 291, or 96. Experiments were performed on at least 3 occasions. A representative blot is shown. Anti-p120^{ctn} blots (pp120) served as normalization controls for MKN28 viability under different experimental conditions. As a negative control, 500 μg total protein from uninfected and infected samples taken 6 hours post-infection was subjected to immunoprecipitation with an anti-c-myc antibody (9E10). (B) Densitometric analysis of multiple Western blot repetitions performed on at least 3 occasions. Graph represents percent total tyrosine phosphorylation as determined by Western blotting with pY99 antibody in infected versus uninfected cells. Error bars = SEM. *, $P<0.05$, **, $P<0.001$ vs. uninfected cells treated with medium alone.

pylori co-culture **(Figure 21A)**. Expression of exogenous p120^{ctn} did not significantly relieve Kaiso-mediated transcriptional repression in uninfected cells **(Figure 22)**. However, co-culture with *H. pylori* strain 7.13 resulted in a significant inhibition (~2.5-fold) of Kaiso-mediated repression of luciferase expression when compared to medium alone **(Figure 21B)**. Inactivation of *cagE*, but not *cagA*, significantly attenuated the ability of *H. pylori* strain 7.13 to relieve Kaiso-mediated transcriptional repression **(Figure 21B)**. These results demonstrate that *H. pylori* relieves Kaiso-mediated transcriptional repression in a *cagE*-dependent manner and that p120^{ctn} is likely required for this inhibition.

p120^{ctn} is required for H. pylori-mediated upregulation of mmp-7 expression.

To demonstrate coordinated regulation of *H. pylori*-induced *mmp-7* expression by p120^{ctn} and Kaiso, siRNA was used to suppress levels of endogenous p120^{ctn}. MKN28 cells were retrovirally transduced with pRetroSuper vector containing sequences encoding either scrambled siRNA or human-specific p120^{ctn} siRNA. Clonal populations of transduced cells were isolated and expanded and, as shown in **Figure 23A**, a >75% reduction in levels of p120^{ctn} was achieved. Since *H. pylori* has previously been shown to bind to components of epithelial tight junctions, we established that p120^{ctn} deficiency did not alter bacterial binding to MKN28 cells. As assessed by quantitative culture, *H. pylori* strain 7.13 bound to cells transduced with p120^{ctn}-specific siRNA as avidly as cells transduced with scrambled siRNA, thereby eliminating the possibility that differences in bacterial adherence mediate p120^{ctn}-dependent responses **(Figure 23B)**.

MKN28 cells stably transduced with control or p120^{ctn}-specific siRNA were then

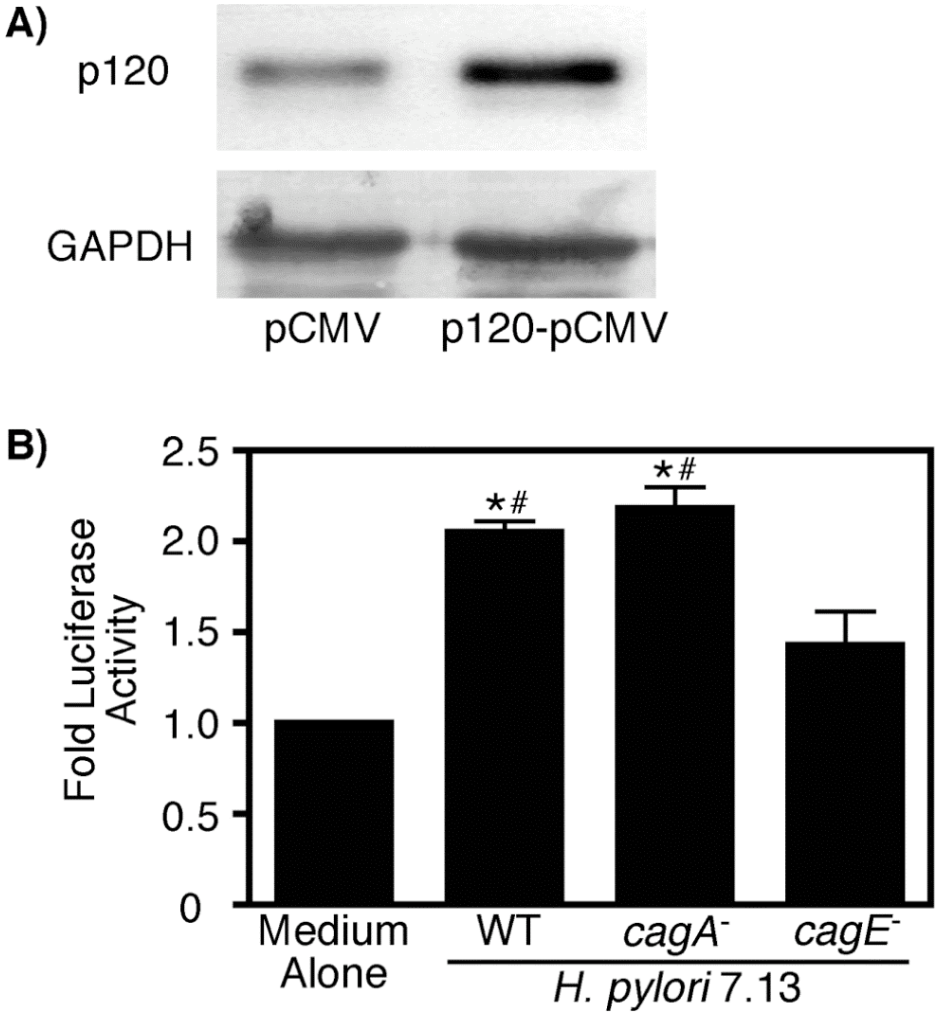

Figure 21. *H. pylori* infection of MKN28 cells mediates a release of Kaiso from its putative binding site on a luciferase reporter plasmid. MKN28 cells were co-transfected with 100 ng 4xKBS-pGL3, 4 ng phRL Null, and 100 ng of the empty vector pCMV or 100 ng p120^{ctn}-pCMV for 12 hours. (A) Eighteen hours post-transfection, total protein was extracted and analyzed by Western blot using a monoclonal anti-p120^{ctn} antibody (pp120). A representative blot is shown. Anti-GAPDH blots served as normalization controls for MKN28 viability under different experimental conditions. (B) Eighteen hours post-transfection with p120^{ctn}-pCMV, cells were co-cultured with *H. pylori* strain 7.13 or its *cagA*$^-$ or *cagE*$^-$ isogenic mutants. After 48 hours, cells were harvested and luciferase activity was determined in a dual channel luminometer. Data are represented as fold luciferase activity for experiments performed on at least three occasions. Error bars, SEM. *, P<0.01 versus medium alone. $^{\#}$, P<0.05 vs. *cagE*$^-$.

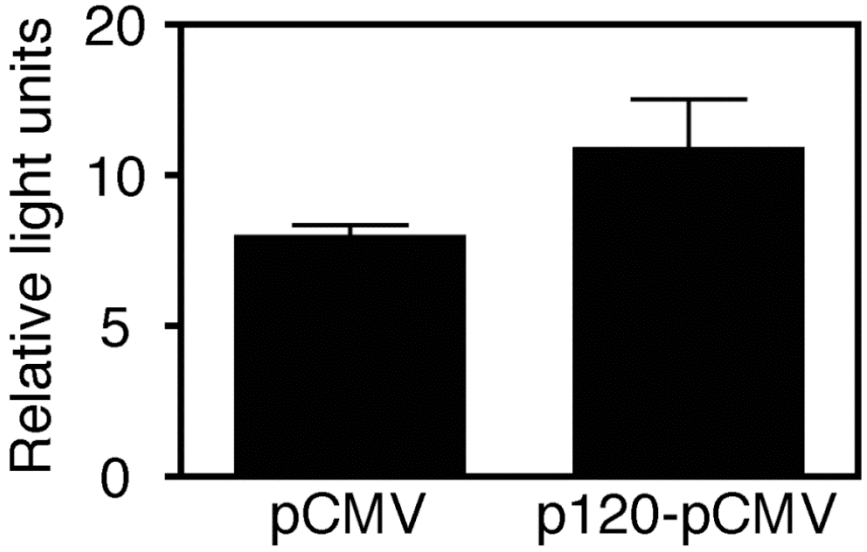

Figure 22. Exogenous expression of p120^{ctn} does not significantly relieve Kaiso-mediated transcriptional repression in uninfected cells. MKN28 cells were cotransfected with 100 ng 4xKBS-pGL3, 4 ng phRL Null, and 100 ng of the empty vector pCMV or 100 ng p120^{ctn}-pCMV for 12 hours, and the medium subsequently replaced. After 48 hours, cells were harvested and luciferase activity was determined in a dual channel luminometer. Data are represented as relative light units (firefly luciferase vs. renilla luciferase) for experiments performed on at least three occasions. Error bars, SEM.

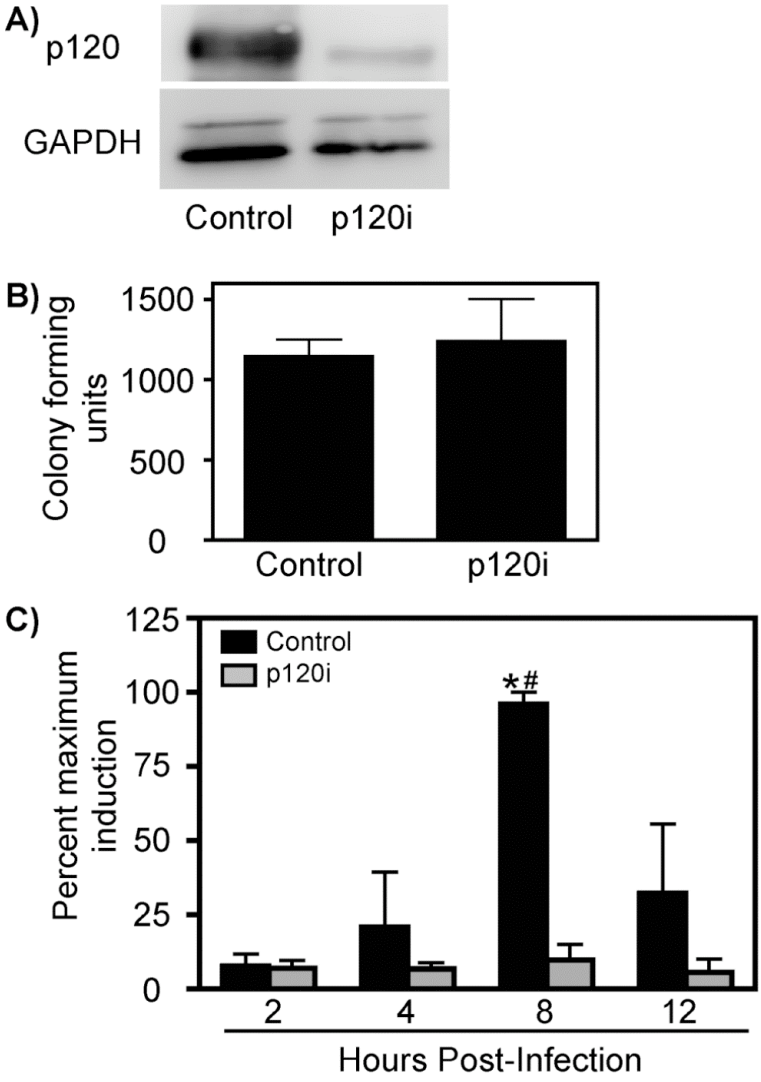

Figure 23. p120^{ctn} is required for the *H. pylori*-mediated increase in steady-state *mmp-7* mRNA levels. (A) MKN28 cells were retrovirally transduced with either scrambled (control) or human p120^{ctn}-specific siRNA (p120i), and clonal populations were selected. Total protein was extracted from control or p120i cells and analyzed by Western blot using a monoclonal anti-p120^{ctn} antibody (pp120). GAPDH Western blots served as normalization controls for cell viability under different experimental conditions. (B) Control or p120i cells were incubated with *H. pylori* strain 7.13 (MOI=100) for 24 hours. Cells were washed to remove non-adherent bacteria, harvested, and plated in serial dilutions on blood agar plates. Data represent the number of colony forming units for experiments performed on at least 3 occasions. (C) Vector control or p120i cells were co-cultured with *H. pylori* strain 7.13 (MOI=100) or medium alone. At defined time points, total RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data are represented as the percent maximum induction of *mmp-7* expression in infected versus uninfected cells for experiments performed on at least 3 occasions. Error bars, SEM. *, $P<0.05$ versus medium alone. $^{\#}$, $P<0.05$ versus p120i cells.

co-cultured with *H. pylori* strain 7.13 or medium alone. At defined time points, steady-state *mmp-7* mRNA levels were assessed by real time RT-PCR. As expected, levels of *mmp-7* mRNA were significantly increased by 8 hours in control MKN28 cells infected with *H. pylori*, but this increase was attenuated in infected $p120^{ctn}$-deficient cells **(Figure 23C)**, indicating that $p120^{ctn}$ is required for *H. pylori* to induce transcription of *mmp-7* in this system. Results were confirmed in two other clonal cell populations transduced with $p120^{ctn}$-specific siRNA (data not shown).

Suppression of Kaiso in $p120^{ctn}$-deficient cells restores the ability of H. pylori to induce mmp-7.

To more robustly demonstrate that $p120^{ctn}$ and Kaiso coordinately regulate *H. pylori*-induced *mmp-7* expression, we transiently transfected control or $p120^{ctn}$-deficient MKN28 cells with scrambled or Kaiso-specific siRNA. Real time RT-PCR analysis indicated that Kaiso expression was significantly reduced using Kaiso-specific, but not scrambled, siRNA **(Figure 24A)**. *H. pylori* strain 7.13 was then co-cultured with $p120^{ctn}$-deficient/Kaiso-deficient, $p120^{ctn}$-deficient/Kaiso-wild-type, or wild-type control MKN28 cells and *mmp-7* mRNA expression was quantified by real-time RT-PCR. Inhibition of Kaiso in $p120^{ctn}$-deficient cells restored the ability of *H. pylori* to induce expression of *mmp-7* **(Figure 24B)**, indicating a role for both of these transcriptional elements in *H. pylori*-mediated up-regulation of MMP-7.

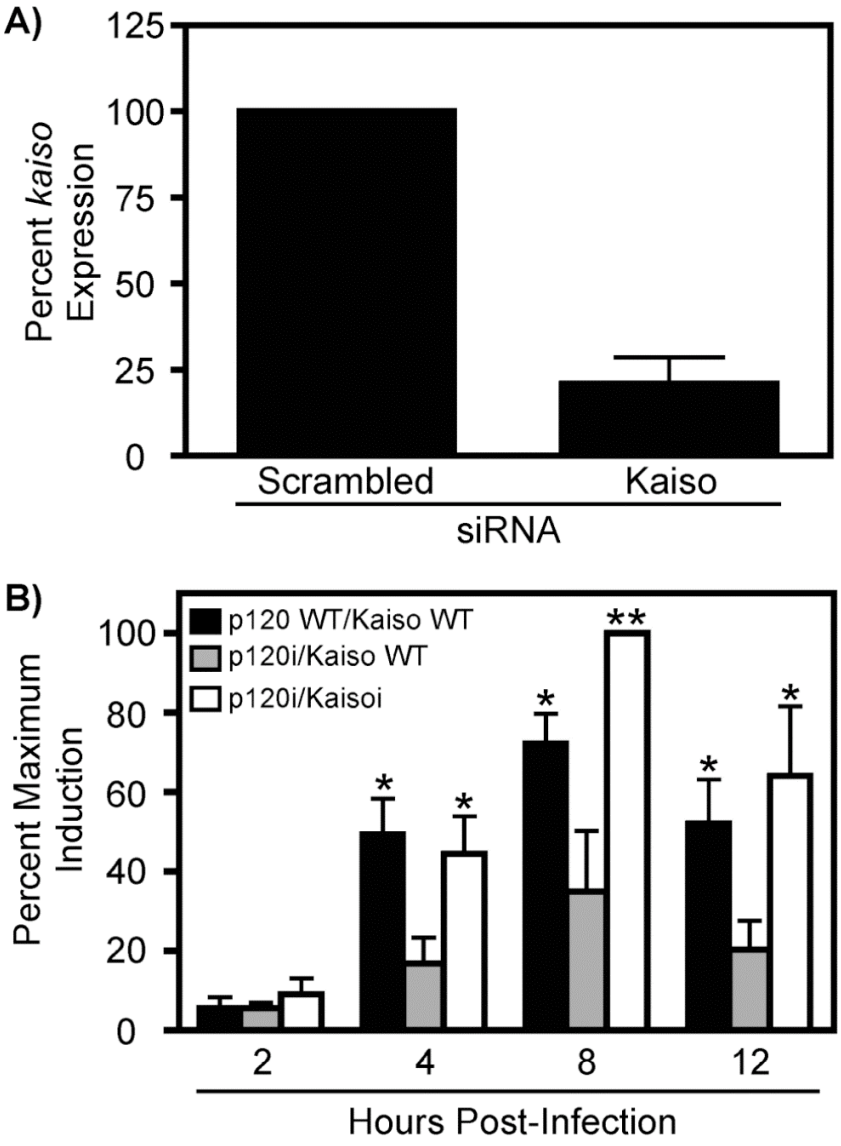

Figure 24. Suppression of Kaiso protein expression in p120i cells restores *H. pylori*-mediated increases in *mmp-7* mRNA levels. Control or p120i cells transiently transfected with scrambled siRNA, and p120i cells transiently transfected with Kaiso-specific siRNA were co-cultured with *H. pylori* strain 7.13 (MOI=100) or medium alone. At defined time points, total RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. (A) Data are represented as an average percentage of *kaiso* expression in uninfected p120i cells at each time point for experiments performed on at least 3 occasions. (B) Data are represented as the percent maximum induction of *mmp-7* expression in infected versus uninfected cells for experiments performed on at least 3 occasions. Error bars, SEM. *, $P<0.05$ and **, $P<0.01$ versus p120i cells.

H. pylori induces aberrant localization of p120^{ctn} to the nucleus in ex vivo gastric cell colonies.

Our current data demonstrate that *H. pylori* induces increased transcription of *mmp-7* through a p120^{ctn}-dependent mechanism involving inhibition of Kaiso-mediated transcriptional repression. We next extended these results using a model of *H. pylori* infection that more closely recapitulates cellular organization in the stomach. Gastric cell colonies were isolated from 8-week-old male FVB/n mice, co-cultured with the mouse-adapted *H. pylori* strain SS1 or medium alone for 6 hours, and p120^{ctn} localization was assessed by laser scanning immunofluorescent microscopy. Mouse-adapted *H. pylori* strain SS1 was used for these studies since gastric cell colonies were harvested from mice. Consistent with our results in MKN28 cells, nuclear aggregations of p120^{ctn} were observed to specifically co-localize with Kaiso in cells co-cultured with *H. pylori*, but not in cells incubated with medium alone **(Figure 25)**. These results indicate that, similar to cultured gastric epithelial cells, *H. pylori* can alter p120^{ctn} localization in a model system that approximates events within colonized gastric mucosa.

Discussion

MMP-7 is a host effector that mediates carcinogenesis and is induced by *H. pylori* within infected human gastric mucosa. Our current experiments identified the p120^{ctn}/Kaiso signaling pathway as a regulator of microbially-induced expression of this carcinogenic factor by 1) demonstrating that *H. pylori* can alter the topography of p120^{ctn} localization within gastric epithelial cells *in vitro* and in a physiologically relevant *ex vivo* primary gastric cell culture system, 2) establishing that *H. pylori* can inhibit Kaiso-mediated transcriptional repression of an artificial luciferase promoter, 3) capitalizing on

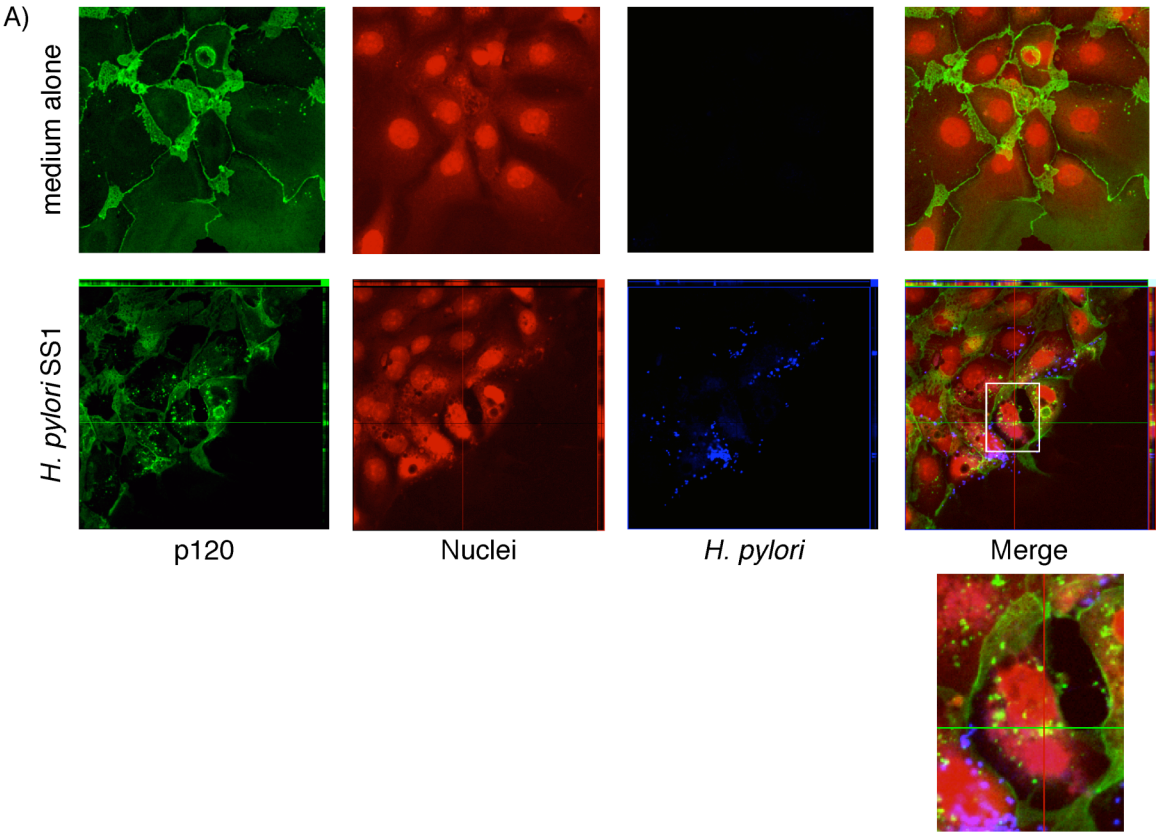

Figure 25. *H. pylori* induces aberrant localization of p120^{ctn} to the nucleus in *ex vivo* gastric cell colonies. (A) Primary murine gastric epithelial cell colonies were co-cultured with *H. pylori* strain SS1 or medium alone for 6 hours. Cells were fixed and incubated with a monoclonal anti-p120^{ctn} antibody (pp120) and a polyclonal anti-*H. pylori* antibody, followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as projection images of a Z-stack. p120^{ctn}, green; *H. pylori*, blue; nuclei, red. 630X magnification. Localization of p120^{ctn} in 3-dimensions is represented by orthogonal views of infected cells. (B) Primary murine gastric epithelial cell colonies were co-cultured with *H. pylori* strain SS1 or medium alone for 6 hours. Cells were fixed and incubated with a rabbit polyclonal anti-p120^{ctn} antibody (F1αSH) and a monoclonal anti-Kaiso antibody (6F/6F8), followed by incubation with an anti-mouse AlexaFluor-546 antibody, anti-rabbit AlexaFluor-488 antibody, and TOTO-3 nucleic acid dye. Cells were visualized by laser scanning immunofluorescent microscopy and are represented as a single transverse image through the middle of the cell. p120^{ctn}, green; Kaiso, red; nuclei, blue. Uninfected, 630X magnification. Infected, 1260X magnification. Graph indicates fluorescence intensity as measured linearly through the nucleus demonstrating co-localization of Kaiso and p120^{ctn} within the nuclei of infected cells.

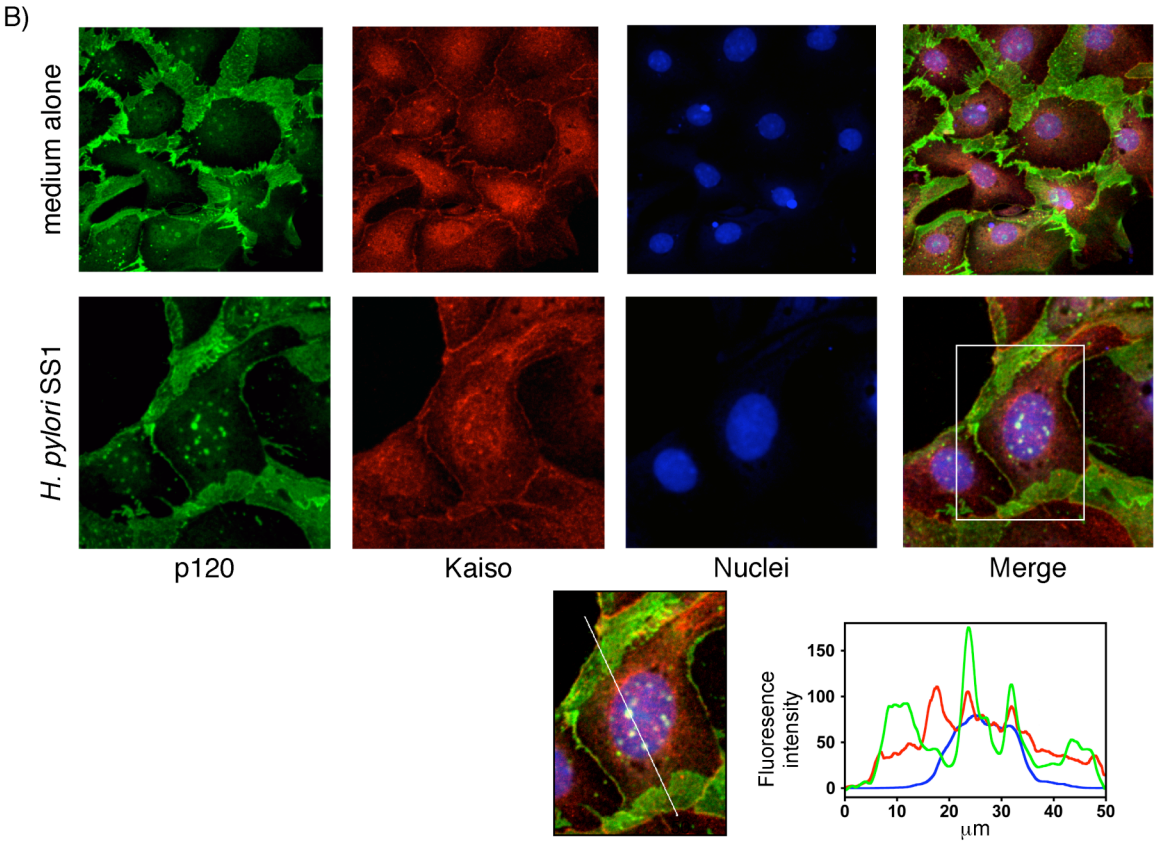

Figure 25, continued.

a gastric cell model of p120^{ctn}-deficiency to demonstrate a requirement for p120^{ctn} in the transcriptional upregulation of *mmp-7*, and 4) combining transient gene silencing techniques with a p120^{ctn}-deficient model system to delineate an interaction between p120^{ctn} and Kaiso. Collectively, these studies indicate that *H. pylori* co-opts p120^{ctn} as a signaling molecule to relieve Kaiso-mediated repression of *mmp-7*.

p120^{ctn} is a multidimensional protein that performs several distinct functions within host cells. p120^{ctn} can regulate E-cadherin, a cell-cell adhesion molecule that functions as a component of the adherens junction of epithelial tissues, and turnover of E-cadherin is regulated by binding of p120^{ctn} to the cadherin juxtamembrane domain (12, 13, 74, 135, 254, 255, 257, 259, 279, 289, 307). Studies have demonstrated that loss of E-cadherin or overexpression of p120^{ctn} results in mislocalization of p120^{ctn} to the cytoplasm (254), where it induces a range of morphological changes that promote cell motility and metastasis. The effects of p120^{ctn} appear to involve physical and functional interactions with Rho GTPases and their regulators, GAPs and GEFs (13, 108, 220). Based on our current data, a role for p120^{ctn} in altering cell morphology and motility induced by *H. pylori* warrants further investigation, and such studies are ongoing in our laboratory.

Nuclear p120^{ctn} relieves transcriptional repression exerted by Kaiso, which acts as a dual specificity repressor that recognizes both sequence-specific consensus sites (CTGCNA) and methylated CpG nucleotides (73, 237, 250). Since *H. pylori* infection has been associated with gastric cancer and nuclear mislocalization of p120^{ctn} in human gastric epithelium (158), we investigated mislocalization of p120^{ctn} in conjunction with altered expression of the oncogenic molecule MMP-7. Infection of p120^{ctn}-deficient

gastric epithelial cells clearly demonstrated that p120^{ctn} is required for *H. pylori*-mediated increases in *mmp-7* transcription. Further, silencing Kaiso expression in p120^{ctn}-deficient cells restored the ability of *H. pylori* to induce *mmp-7* transcription, indicating that an interaction between p120^{ctn} and Kaiso, whether direct or indirect, is also required.

To date, the signals that induce nuclear translocation of p120^{ctn} remain undefined (71), and delineation of these pathways is critical for understanding the relevance of p120^{ctn}/Kaiso-mediated transcriptional regulation in the context of carcinogenesis. For example, it will be important to determine which subcellular pool of p120^{ctn} is responsible for relief of Kaiso-mediated transcriptional repression. Our subcellular fractionation data suggest that p120^{ctn} from a free cytoplasmic pool translocates to the nucleus. However, work from Weydig *et al.* demonstrated that *H. pylori* mediates internalization of p120^{ctn} found in adherens junctions at the plasma membrane (334), which may then be translocated to the nucleus. p120^{ctn} is also phosphorylated at a number of sites by Src- and receptor tyrosine kinases (253), and multiple ligand-receptor pathways have been implicated in signaling to p120^{ctn} through phosphorylation, including PKC and EGFR -dependent pathways (183, 346), both of which are activated by *H. pylori* (146, 222). However, our data demonstrate that *H. pylori* mediates a decrease in total p120^{ctn} tyrosine phosphorylation, indicating a previously undescribed role for protein tyrosine phosphatases in *H. pylori*-mediated signaling to p120^{ctn}. It remains undefined whether signaling to p120^{ctn} by *H. pylori* occurs directly or through another cellular intermediary.

Our current results have also provided insights into the augmentation in cancer risk exerted by *cag*$^{+}$ strains and indicate that oncogenic epithelial responses such as

MMP-7 expression may be regulated by different microbial effectors. Previous studies from our laboratory and others have demonstrated that intracellular events with carcinogenic potential, such as ß-catenin nuclear translocation, are dependent upon the presence of CagA within host cells, which, in turn, is mediated by a functional *cag* secretion system (96, 210, 297). Our new data indicate that aberrant activation of $p120^{ctn}$ is dependent on a functional *cag* secretion system, but not CagA *per se*, suggesting that other substrates translocated by the *cag* island may mediate $p120^{ctn}$ signaling. One candidate is *H. pylori* peptidoglycan, which is translocated into host cells by the *cag* type IV secretion system and sensed by intracellular Nod1, which then activates NF-κB-dependent responses such as secretion of IL-8 and ß-defensin 2 (32, 321). Another possibility based on a recent investigation (163) is that binding of CagL to cell surface $\alpha_5ß_1$ integrins can alter local membrane dynamics and eventuate in the assembly of focal adhesions that trigger integrin signaling cascades (20), which may aberrantly activate $p120^{ctn}$.

It is of interest that the *H. pylori* strain we used to infect murine gastric colonies (SS1) has been reported to contain a non-functional *cag* island. We specifically chose a mouse-adapted *H. pylori* strain for these studies since we were examining murine cells; hence the choice of strain SS1. However, results from a recent paper (Ferrero *et al*., 2008) have suggested that *H. pylori* can activate NF-κB signaling in mouse gastric epithelial cells via a *cag* pathogenicity island-independent pathway, suggesting that signaling pathways in murine cells may be activated by different microbial components than corresponding pathways in human cells. We also did not analyze the functional effects of $p120^{ctn}$/Kaiso interactions in murine gastric glands, only differences in

localization. Differences in model systems may have contributed to these results. In contrast to MKN28 human gastric epithelial cell monolayers, our gastric gland culture model uses tissues isolated from mice that contain not only epithelial cells but also stromal and lamina propria cells. Thus, there are several potential reasons that may explain the ability of *H. pylori* strain SS1 to induce nuclear localization of $p120^{ctn}$ in murine gastric epithelial cells. Collectively, these data indicate that multiple *H. pylori* disease-related virulence constituents may be required for collaborative functional interactions between Kaiso, $p120^{ctn}$, and ß-catenin in the nucleus in order to mediate expression of certain Wnt target genes such as *mmp-7*.

Gastric adenocarcinoma is strongly associated with the presence of *H. pylori*, and both microbial and host factors influence the risk for carcinogenesis. Interactions between *H. pylori* and epithelial cells play an important role in the development of gastric injury. $p120^{ctn}$ is a multifunctional host protein that orchestrates epithelial responses with carcinogenic potential. Our results indicate that $p120^{ctn}$ is aberrantly activated by *H. pylori* and regulates expression of the carcinogenic effector, *mmp-7*. Molecular delineation of pathways activated by host-microbial interactions will not only improve our understanding of *H. pylori*-induced carcinogenesis, but may also provide mechanistic insights into other malignancies that arise within the context of inflammatory states (e.g. ulcerative colitis and colon cancer).

CHAPTER III

HELICOBACTER PYLORI INFECTION RESULTS IN ENHANCED INFLAMMATION AND CELLULAR TURNOVER IN MMP-7-DEFICIENT MICE

Summary

Approximately half of the world's population is infected with *H. pylori*, the strongest known risk factor for gastric adenocarcinoma. This organism employs a number of strategies that facilitate persistence within the gastric microenvironment, one of which is co-opting host cell signaling pathways, leading to enhanced virulence and development of gastric disease. MMP-7, a member of a family of enzymes with tumor-initiating properties, is over-expressed in pre-malignant and malignant gastric lesions, and *H. pylori* cag^+ strains selectively increase MMP-7 protein levels in gastric epithelial cells *in vitro* and *in vivo*. Our experiments now demonstrate that, in a murine model of *H. pylori*-mediated injury, inflammation is increased in the absence of MMP-7. MMP-7-deficiency also leads to increased epithelial cell turnover within the gastric mucosa, and is not dependent on MMP-7-mediated bacterial killing. Collectively, these studies indicate that *H. pylori*-mediated induction of MMP-7 may serve to protect the gastric mucosa from pathophysiological processes that promote carcinogenesis.

Introduction

Infection with the gastric pathogen *Helicobacter pylori* induces an inflammatory response that persists for decades resulting in an increased risk for peptic ulceration,

gastric adenocarcinoma, and non-Hodgkins lymphoma of the stomach. *H. pylori* has specifically adapted to survive within the gastric niche, and a strategy employed by this organism that facilitates persistence is co-opting host cell signaling pathways, which can result in enhanced virulence.

One host molecule that may influence disease outcome is matrix metalloproteinase-7, a member of a family of proteolytic enzymes that play important roles in tissue destruction and remodeling during both normal and pathological processes (132, 339). MMP-7 is one of a small number of MMPs that are expressed in polarized glandular epithelium, and over-expression is present in epithelial-derived malignancies of a number of organs, including the stomach (1, 5, 6, 121, 125, 194, 277, 353). Microarray-based expression analyses have demonstrated that MMP-7 is expressed >6-fold in human gastric adenocarcinoma specimens compared to non-neoplastic tissue (121). In mice, over-expression of MMP-7 leads to hyperproliferation and increased cancer susceptibility of the mammary epithelium (267). Conversely, mice with a genetic predisposition for intestinal adenocarcinoma (*Min* mice) that are then bred onto a background of MMP-7 deficiency develop fewer cancers than wild type mice (338). These data, along with reports of increased MMP-7 expression in a high proportion of premalignant lesions in the colon (polyps) (302), pancreas (metaplastic duct lesions) (69), and stomach (gastric ulcers) (69, 269), suggest that this enzyme plays an important role early in the progression to gastric cancer.

There are several putative mechanisms whereby MMP-7 may promote carcinogenesis. MMP-7 cleaves the ectodomain of membrane-bound FasL, potentiating the induction or inhibition of apoptosis, depending on the duration of exposure (88, 248,

330). In addition, MMP-7 is known to increase cellular proliferation by activation of HB-EGF, which promotes cell survival by stimulating the ErbB4 EGF receptor (364), a signaling pathway that is activated by *H. pylori* (147).

Our group and others have demonstrated that *H. pylori* infection increases expression of MMP-7 *in vitro* and *in vivo* (24, 68, 226, 344). Because MMP-7 influences cellular turnover and is specifically induced by contact with *H. pylori*, we sought to more clearly define the role of MMP-7 in *H. pylori*-induced inflammation *in vivo* by experimental infection of wild type and MMP-7-deficient C57BL/6 mice. Here we demonstrate that infection of *mmp-7*$^{-/-}$ mice with a rodent-adapted strain of *H. pylori* results in an augmented inflammatory response and increased levels of apoptosis and proliferation compared to wild type mice, suggesting that MMP-7 may mediate the host response to this pathogen.

Experimental procedures

H. pylori strains: The mouse-adapted *H. pylori* strain SS1 was grown in *Brucella* broth with 5% FBS (Gibco) for 18 hours with shaking in an atmosphere of 5% CO_2 at 37°C, and then harvested by centrifugation, as described previously (226).

Mice and experimental infections: MMP-7-null mice developed as previously described (338), were obtained from Dr. Lynn Matrisian. Briefly, a 6.5 kb fragment spanning the *mmp-7* coding region was cloned from a 129/Sv library. A 1.6 kb phosphoglycerate kinase neomycin cassette was then inserted within the region spanning exons 3 and 4

(266), and this construct was electroporated into R1 embryonic stem (ES) cells. Three targeted clones were microinjected into C57BL/6 blastocysts after karyotype analysis. *mmp-7*$^{+/-}$ heterozygous F_1 mice were backcrossed to C57BL/6 mice beyond the N_{12} generation. For experimental infections, 6- to 8-week old wild type male and *mmp-7*$^{-/-}$ C57BL/6 mice were used. All experiments were approved by the Vanderbilt Institutional Animal Care and Use Committtee. Five hundred μl *Brucella* broth containing 5.0 x 10^9 CFU *H. pylori* or 500 μl *Brucella* broth alone were used as inocula and were delivered to fasted mice via orogastric gavage every 48 hours for a total of three inoculations, as described previously (96).

Mice were euthanized at 12 or 36 weeks post-challenge. These time-points were selected based on previous data demonstrating that inflammation (12 weeks) and pre-malignant lesions (e.g. hyperplasia, 36 weeks) develop by these intervals. At necropsy, linear strips extending from the squamocolumnar junction through the proximal duodenum were fixed in 10% neutral buffered formalin, and paraffin-embedded by the Vanderbilt Human Tissue Acquisition and Pathology Shared Resource. Approximately one-fourth of the stomach from each mouse was homogenized, plated, and incubated under microaerobic conditions at 37°C for 5-6 days, as described previously (96). *H. pylori* was verified by Gram's stain, urease, catalase, and oxidase reactions.

Tissue staining and immunohistochemical analysis: Five μm paraffin-embedded sections were cut and placed on slides for analysis. For scoring of inflammation, samples were stained with hematoxylin and eosin. Indices of inflammation and injury in the gastric corpus and antrum were scored on an ordinal scale from 0 to 3 in increments of 1 by a

single pathologist blinded to treatment groups as described previously (96). Individual component scores were combined to yield a single score of inflammation for each mouse.

Immunohistochemical analysis of MMP-7 expression was performed using a rat anti-MMP7 monoclonal antibody (87). Briefly, sections were deparaffinized in xylene, rehydrated using a graded series of ethanol, and microwaved in 10 mM citrate buffer for 10 minutes to allow antigen retrieval. Sections were then blocked for 1 hour with 10% goat serum and incubated overnight at 4^{o}C with anti-MMP-7 antibody diluted 1:500 or the corresponding isotype control (rat IgG2a, BD Biosciences, San Jose, CA). The sections were then incubated with an alkaline-phosphatase conjugated goat anti-rat antibody (Abcam, Cambridge, MA) for 1 hr at room temperature. Next, sections were incubated with Vector Red substrate using the alkaline phosphatase substrate kit 1 (Vector Labs, Burlingame, CA) according to the manufacturer's directions. Slides were then rinsed with distilled water and counterstained with Mayer's hematoxylin (Sigma, St Louis, MO). Slides were mounted using an aqueous mounting medium ("Fluoro-Gel", Electron Microscopy Sciences, Hatfield, PA). All other immunohistochemical analyses were performed by the Vanderbilt Immunohistochemistry Core. Briefly, sections were rehydrated and placed in heated Target Retrieval Solution (Labvision, Fremont, CA). For anti-*H. pylori*, anti-CD3, and anti-CD20 staining, antigen retrieval was performed by incubation with citrate buffer (pH=6.0) in a pressure cooker for 15 minutes followed by cooling at room temperature for 10 minutes. For all slides, endogenous peroxidase was then neutralized with 0.03% hydrogen peroxide containing sodium azide for 5 minutes, followed by a casein-based protein block (DakoCytomation, Carpinteria, CA) to minimize nonspecific staining. For anti-myeloperoxidase (MPO) and anti-CD20

staining, sections were incubated with mouse Ig blocking reagent (MKB-2213; Vector) for 60 minutes. Next, sections were incubated with rabbit polyclonal anti-*H. pylori* (B0471; Dako; 1:100) for 30 minutes, rat polyclonal anti-MPO (MCA771G; Serotec; 1:400) for 60 minutes, rabbit polyclonal anti-CD3 (A0452; Dako; 1:50) for 30 minutes, mouse monoclonal anti-CD20 (Dako; M0755; 1:200) for 30 minutes, rabbit polyclonal anti-Ki-67 (VP-K451; Vector Laboratories, Burlingame, CA; 1:2000) for 60 minutes, or rabbit anti-human cleaved caspase-3 (Promega, Madison, WI; 1:600) for 60 minutes. For anti-MPO staining, sections were incubated with rabbit polyclonal anti-rat IgG (Dako; Z0494; 1:100) for 30 minutes. Slides were then subjected to the Dako Envision+ HRP/DAB System (DakoCytomation). Slides were counterstained with Mayer's hematoxylin, dehydrated, and coverslipped.

Statistical analysis: The Student's *t* test was used for statistical analyses of intergroup comparisons. Significance was defined as $p \leq 0.05$.

Results

H. pylori infection results in increased expression of MMP-7 in the stomachs of wild type C57BL/6 mice.

We previously demonstrated that gastric biopsies from human patients infected with *H. pylori* exhibit elevated levels of MMP-7 expression in a focal pattern (68). McCaig *et al*. determined that experimental infection of INS-GAS mice with another *Helicobacter* species, *H. felis*, results in increased MMP-7 expression in protein extracts harvested from mouse corpus as early as 3 months, which persisted to 9 months post-infection (191). In order to validate our mouse model infected with *H. pylori*, we first

established that challenge of wild type C57BL/6 mice with *H. pylori* strain SS1 results in increased MMP-7 expression. Wild type mice were challenged with SS1 or *Brucella* broth alone and levels of MMP-7 expression were determined by immunohistochemistry. All mice challenged with *H. pylori* were successfully infected. Twelve weeks post-challenge, there was no evidence of MMP-7 expression in the stomachs of control mice inoculated with broth alone. In contrast, MMP-7 was detected in the stomachs of 58% (7 of 12) of mice challenged with *H. pylori*. Immunolabeling was focal and localized exclusively to gastric epithelial cells **(Figure 26)**, consistent with its epithelial-cell derivation (57, 80), which mirrors the expression pattern previously observed in human subjects (68). These results indicate that experimental infection of C57BL/6 mice with *H. pylori* strain SS1 induces gastric epithelial MMP-7 expression.

H. pylori-induced gastric inflammation is augmented in the absence of MMP-7.

To determine whether induction of MMP-7 is physiologically relevant within the context of *H. pylori*-induced inflammation *in vivo*, we next infected wild type and *mmp-7*$^{-/-}$ mice in three independent experiments. All mice challenged with *H. pylori* were successfully infected. By 12 and 36 weeks post-challenge, all *H. pylori*-infected wild type and *mmp-7*$^{-/-}$ mice had developed gastritis. The most extensive inflammation was observed at the transition zones between the antrum or cardia and the corpus. Inflammatory cells, consisting of polymorphonuclear cells and large mononuclear cells, were observed within the lamina propria, and the infiltrate separated and displaced the glands. Gastric pits were lengthened and lined by less mature flattened epithelial cells with basophilic cytoplasm; mitotic figures were frequently identified. Within the

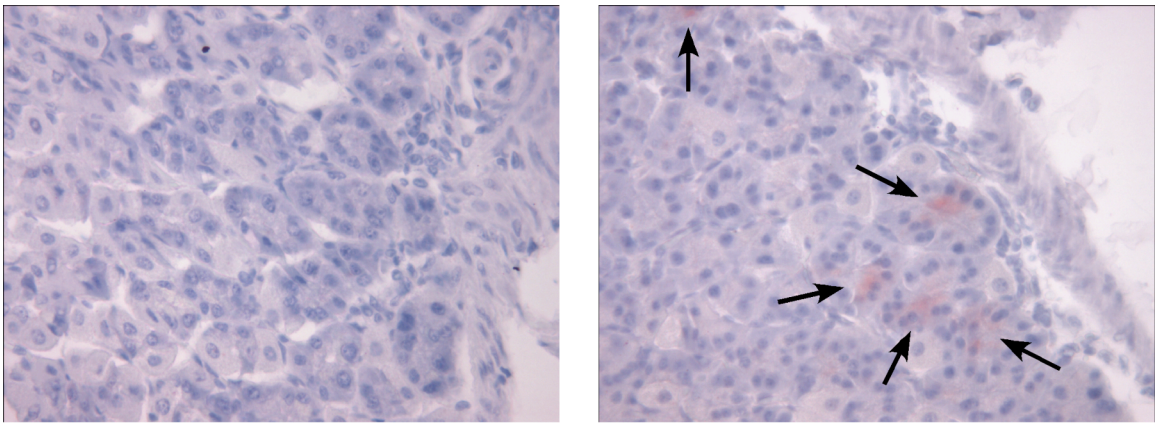

Figure 26. Infection of wild type C57BL/6 mice with *H. pylori* results in increased MMP-7 expression. Wild type mice were inoculated with either *Brucella* broth (negative control) or *H. pylori*. Twelve weeks post-infection, mice were sacrificed and stomachs were analyzed for MMP-7 expression by immunohistochemistry. Arrows indicate positive staining for MMP-7. 100X magnification.

submucosa, edema often accompanied the cellular infiltrate.

In contrast to infected wild-type mice, *H. pylori*-colonized *mmp-7*$^{-/-}$ mice developed significantly enhanced inflammation **(Figure 27)**. Acute inflammatory scores were significantly higher in infected *mmp-7*$^{-/-}$ mice when compared with wild type mice at 12 weeks (P=0.0006) and scores exhibited a similar trend at 36 weeks (P=0.0823) **(Figure 28A)**. Analysis of inflammation within specific regions of the stomach revealed significantly increased levels of acute inflammation in both the antrum (P=0.0012) and corpus (P=0.0212) at 12 weeks post-infection and in the antrum (P=0.004) at 36 weeks post-infection **(Figure 28B)**. Chronic inflammatory scores were increased in infected *mmp-7*$^{-/-}$ compared to wild type mice at 12 weeks post-infection (P=0.0731) and this difference was statistically significant at 36 weeks post-infection (P=0.0014) **(Figure 28C)**. Similar to acute inflammation, chronic inflammatory scores were significantly higher in the corpus (P=0.0254) at 12 weeks post-infection and in both the antrum (P=0.0009) and corpus (P=0.0063) at 36 weeks post-infection **(Figure 28D)**.

Immunohistochemical analysis of immune cell infiltrate correlates with scoring of inflammation.

Based on the difference in global inflammatory scores induced by MMP-7 deficiency, we next sought to immunophenotype the inflammatory infiltrate. Immunohistochemical analysis was performed using antibodies to MPO, CD3, and CD20 in order to identify neutrophils, T cells, and B cells, respectively. For each sample, the number of specific immune cells per high-powered field of view was quantified for the maximum number of random fields of view available per sample. Staining for specific immune cell types correlated well with overall histopathological scoring. At 36 weeks

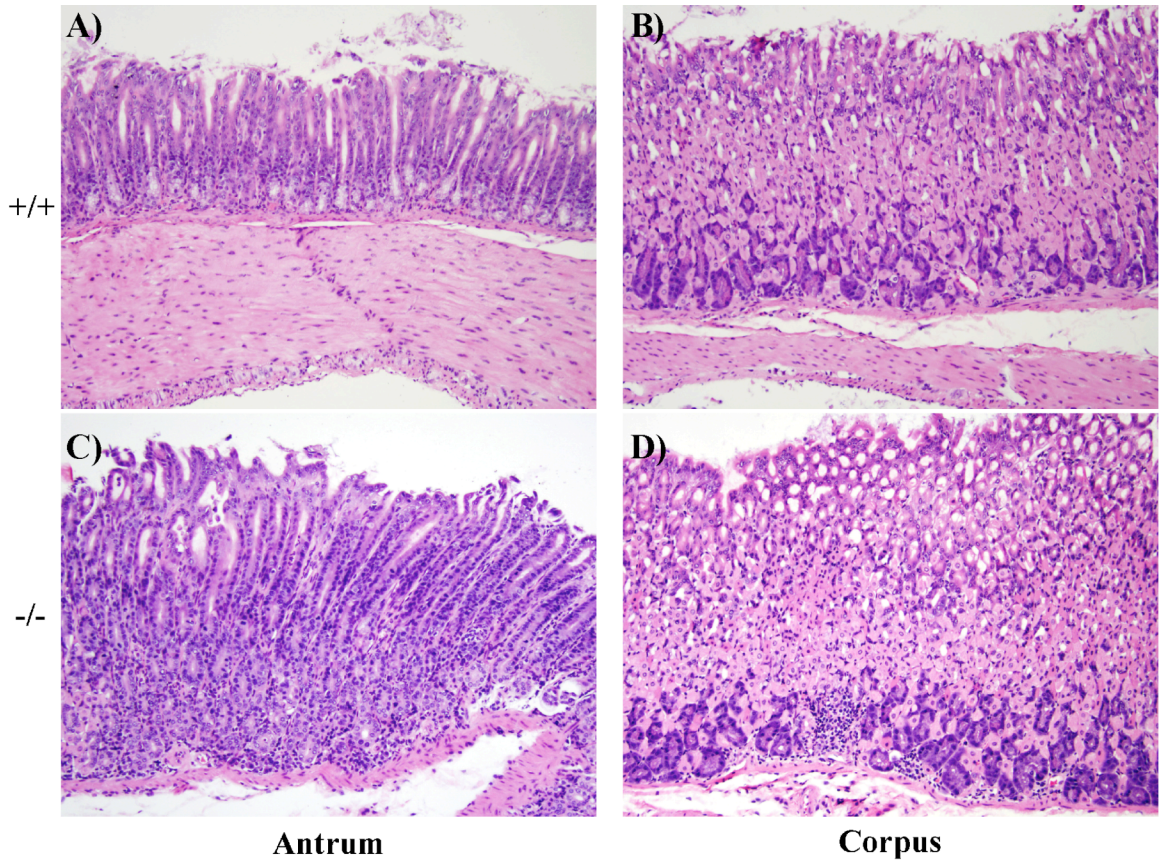

Figure 27. *H. pylori* challenge of *mmp-7*$^{-/-}$ mice results in significantly enhanced inflammation compared to wild type mice. Representative images of H&E stained sections from the antrum (A & C) and corpus (B & D) of wild type mice (A & B) and *mmp-7*$^{-/-}$ mice (C & D) infected for 12 weeks. +/+, Wild Type; -/-, *mmp-7*$^{-/-}$. 100X magnification.

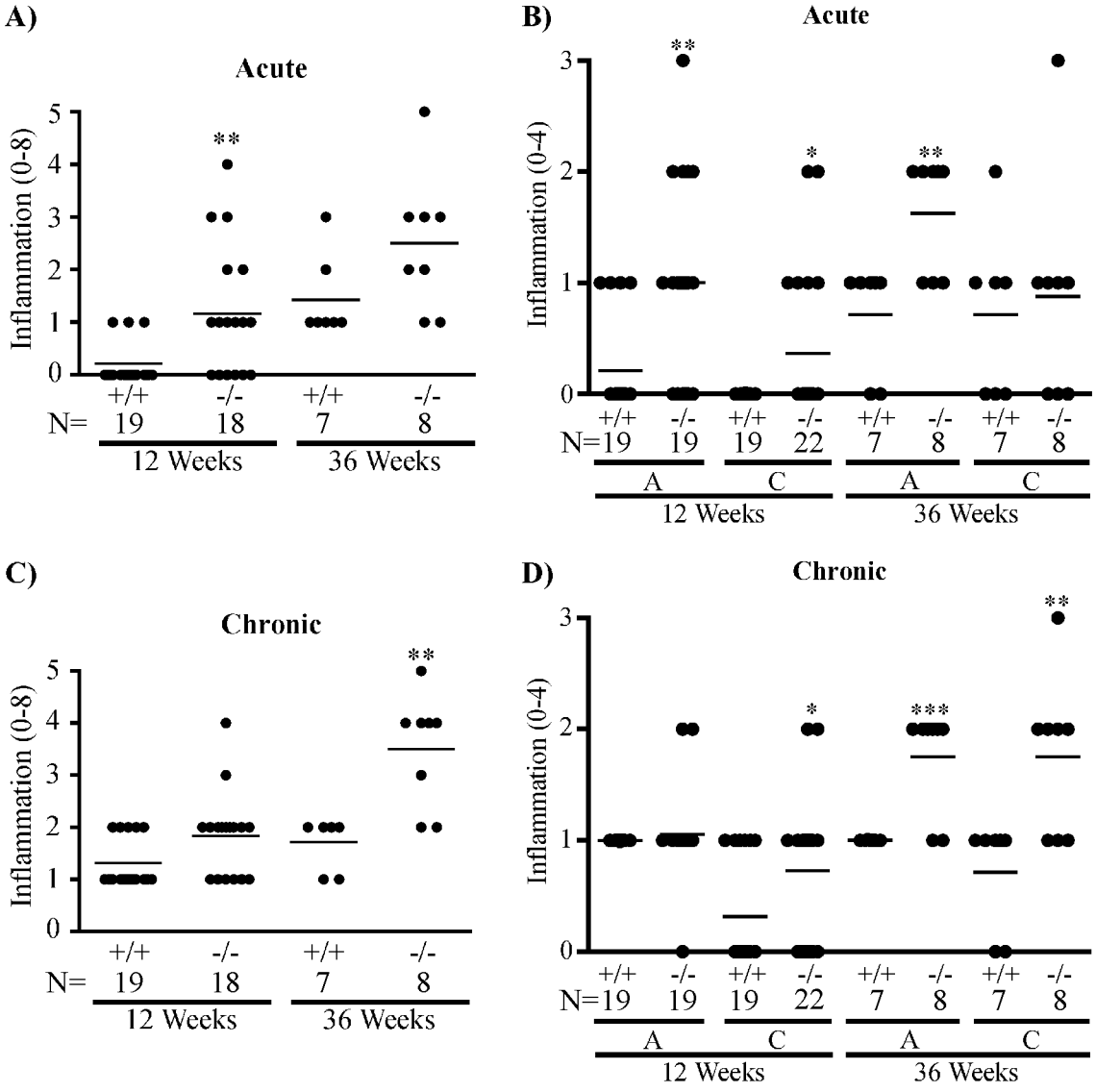

Figure 28. MMP-7 deficiency augments acute and chronic inflammatory scores in the presence of *H. pylori* infection. A & B) Acute inflammation. C & D) Chronic inflammation. Panels A and C represent combined antral and corpus inflammatory scores. Data are shown as vertical scatterplots with mean values. +/+, Wild Type; -/-, *mmp-7*$^{-/-}$. In panels B and D, A=antrum, C=corpus. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; vs. wild type.

post-infection, *mmp-7*$^{-/-}$ mice had significantly more neutrophils (P=0.0390) and T cells (P=0.003) when compared with wild type mice **(Figure 29)**. Infected *mmp-7*$^{-/-}$ mice also exhibited increased numbers of B cells compared to wild type mice, however, the difference was not statistically significant.

The ability of H. pylori to colonize the stomachs of mmp-7$^{-/-}$ *mice does not differ compared to wild type mice.*

MMP-7-mediated cleavage of α-defensins in the murine small intestine has been shown to be required for bactericidal activity (19, 340). When compared to wild type mice, *mmp-7*$^{-/-}$ mice are more susceptible to infection of the lower GI tract with both *Escherichia coli* and *Chlamydia trachomatis*, indicating that MMP-7 has important functions in intestinal mucosal defense in the mouse (235, 340). Since *H. pylori*-induced inflammation was more intense in *mmp-7*$^{-/-}$ mice, we next determined if MMP-7 might play a role in gastric mucosal defense, which could explain our observed differences in levels of inflammation. Immunohistochemical analysis for *H. pylori* was performed on tissue samples obtained from mice infected with *H. pylori* for 12 weeks. There were no differences in colonization density on either the mucosal surface or within the lumen in wild type versus *mmp-7*$^{-/-}$ mice **(Figure 30)**, indicating that MMP-7 likely does not play a role in the initial host response to *H. pylori* challenge.

Cellular turnover is increased in H. pylori-infected mmp-7$^{-/-}$ *mice compared to wild type mice.*

H. pylori infection results in increased rates of cellular turnover (34, 178, 245, 284, 325), and elevated levels of MMP-7 have previously been demonstrated to augment levels of proliferation and apoptosis (88, 206, 248, 364). Therefore, we next sought to

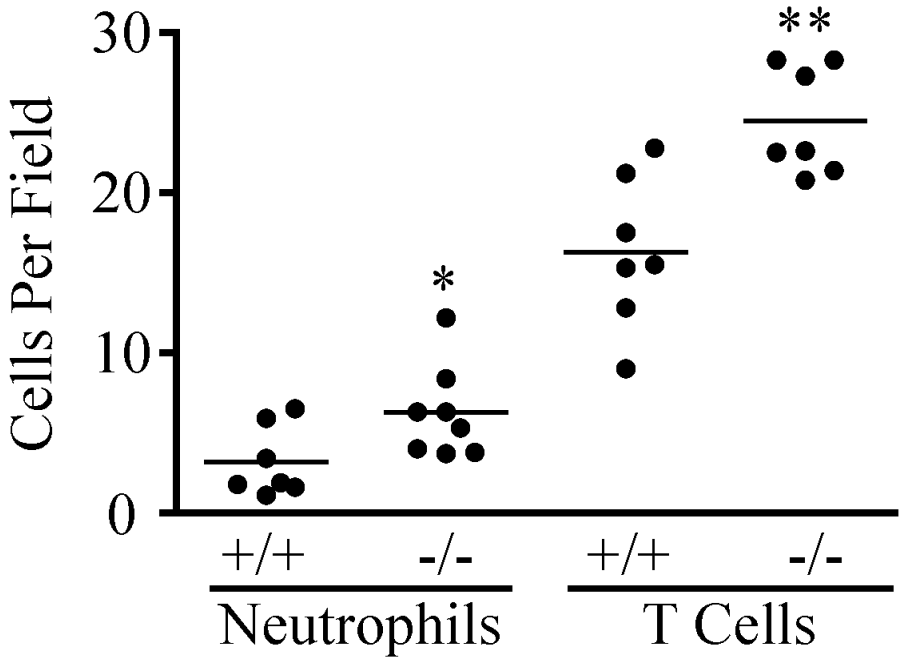

Figure 29. MMP-7 deficiency augments neutrophil and T cell infiltration in response to *H. pylori* infection. Immunohistochemical analysis for neutrophils (MPO) and T cells (CD3) was performed on sections from mice infected for 36 weeks. Data are shown as vertical scatterplots with mean values. Points represent the average number of cells per high-power field for each mouse. +/+, Wild Type; -/-, *mmp-7*$^{-/-}$. *, $p<0.05$; **, $p<0.01$; vs. wild type.

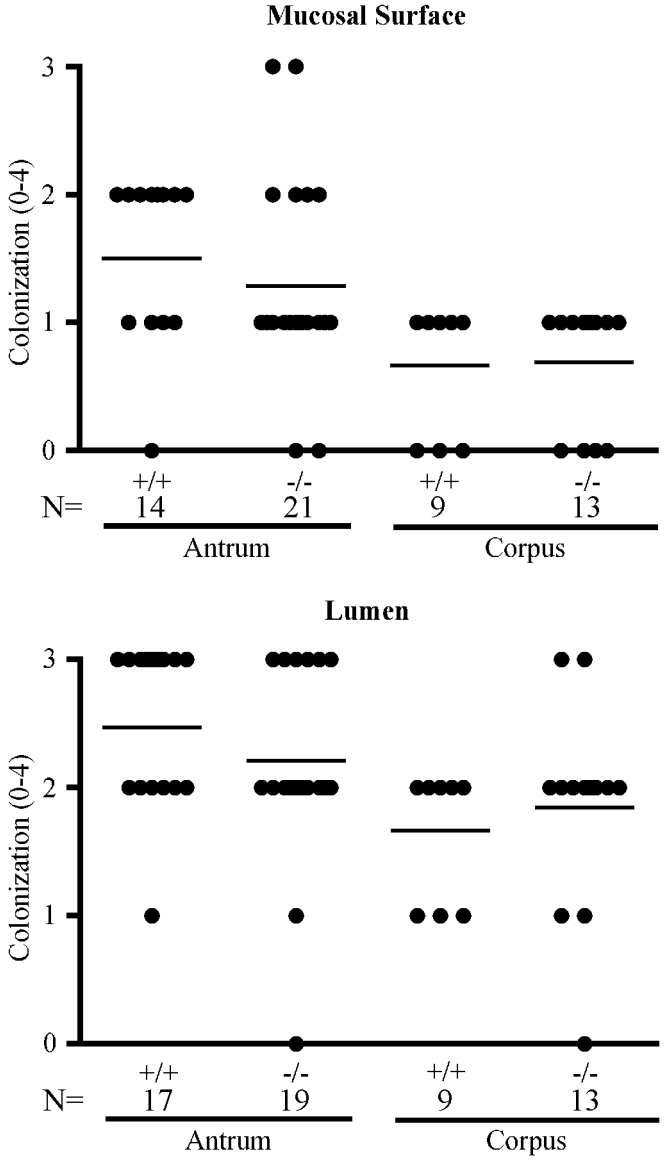

Figure 30. ***H. pylori*** **colonization density does not differ between wild type and** ***mmp-7*** $^{-/-}$ **mice.** Immunohistochemical analysis for *H. pylori* was performed on sections from mice infected for 12 weeks and colonization density was scored on an ordinal scale from 0 to 4. Colonization densities at both the mucosal surface (top panel) and within the gastric lumen (bottom panel) did not differ between wild type and *mmp-7* $^{-/-}$ mice for either the antrum or corpus. Data are shown as vertical scatterplots with mean values. +/+, Wild Type; -/-, *mmp-7* $^{-/-}$.

determine whether proliferation and apoptosis were increased in wild type mice infected with *H. pylori* compared to infected *mmp-7*$^{-/-}$ mice. Immunohistochemical analysis for Ki67, a marker of actively dividing cells, and active caspase-3, a marker for cells undergoing apoptosis, was performed on a subset of samples obtained from experiments in which mice were infected with *H. pylori* for 12 or 36 weeks. For each sample, the number of positive cells and the total number of cells were quantified in at least ten high-powered fields. Of interest, *mmp-7*$^{-/-}$ mice exhibited significantly higher levels of proliferation at both 12 (P=0.003) and 36 (P=0.0177) weeks post-infection **(Figure 31)**. In wild type mice, virtually all epithelial cells that stained positively for Ki67 were located within the neck region of the gastric glands. In contrast, staining in *mmp-7*$^{-/-}$ mice extended bidirectionally from the isthmus. A similar trend was observed for apoptosis at both 12 (P=0.0007) and 36 (P=0.0192) weeks **(Figure 32)**. In contrast to proliferation, apoptotic cells were present among superficial epithelial cells and were primarily localized to the upper one third of the gastric foveolae. These results suggest that increased levels of inflammation present in MMP-7-deficient mice may result in enhanced rates of cellular turnover, likely influenced by paracrine signaling from infiltrating immune cells (82, 86, 229, 296).

Discussion

MMP-7 is a host effector that is induced by *H. pylori* in epithelial cells within infected gastric mucosa. Our experiments now demonstrate that, in a murine model of *H. pylori*-mediated injury, inflammation is increased in the absence of MMP-7. MMP-7-deficiency also leads to increased epithelial cell turnover within the gastric mucosa, and

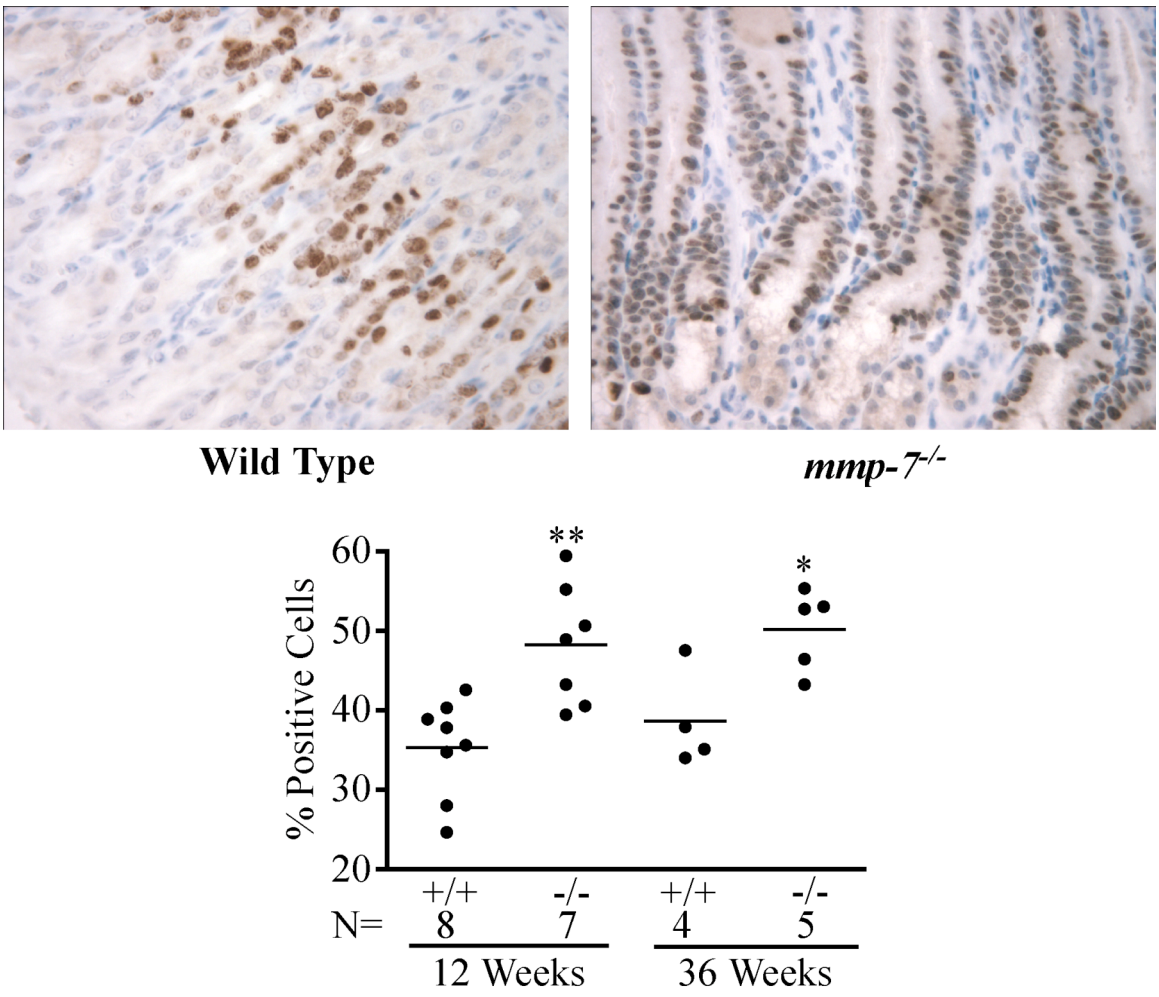

Figure 31. *H. pylori* challenge of *mmp-7*$^{-/-}$ mice results in significantly increased levels of proliferation compared to wild type mice. Representative images of anti-Ki67 staining (top panels). The percentage of cells positive for Ki67, a marker of proliferation, was calculated for 10 high-powered fields for each mouse. The average of these 10 fields is represented by a single dot in the vertical scatterplot. +/+, Wild Type; -/-, *mmp-7*$^{-/-}$. *, $p < 0.05$; **, $p < 0.01$; versus wild type.

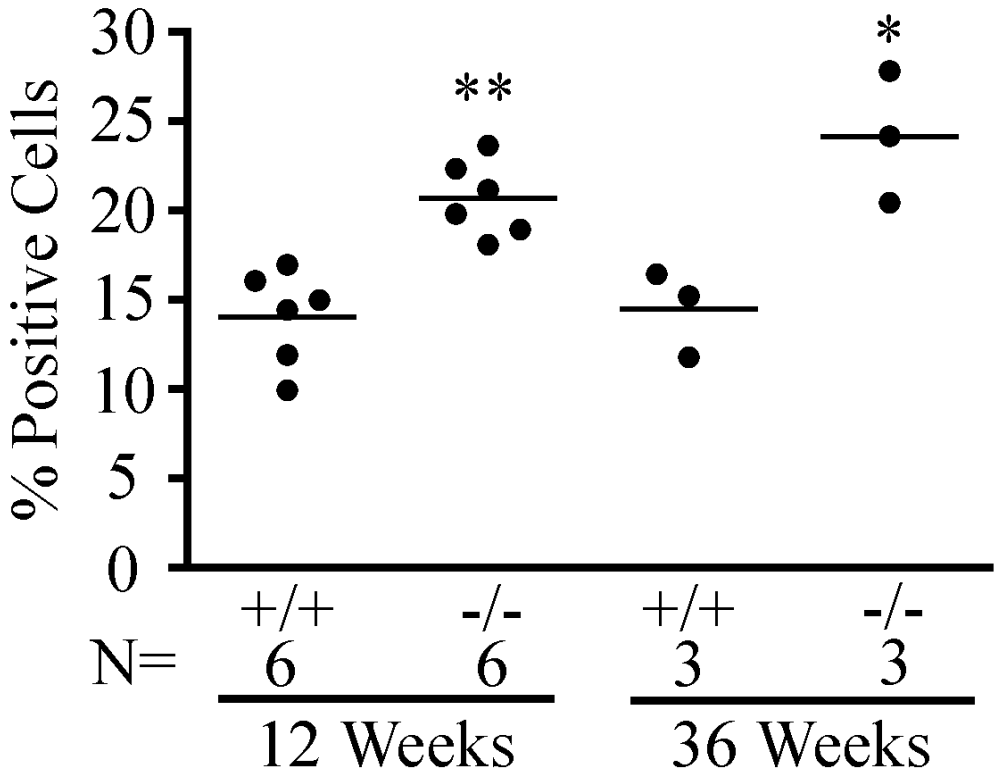

Figure 32. *H. pylori* challenge of *mmp-7*$^{-/-}$ mice results in significantly increased levels of apoptosis compared to wild type mice. The percentage of cells positive for active caspase-3, a marker of apoptosis, was calculated for 10 high-powered fields for each mouse. The average of these 10 fields is represented by a single dot in the vertical scatterplot. +/+, Wild Type; -/-, *mmp-7*$^{-/-}$. *, $p < 0.05$; **, $p < 0.01$; versus wild type.

is not dependent on MMP-7-mediated bacterial killing. Collectively, these studies indicate that *H. pylori*-mediated induction of MMP-7 may serve to protect the gastric mucosa from pathophysiological processes that promote carcinogenesis.

Numerous studies suggest that MMP-7 plays a role in carcinogenesis (1, 3, 5, 6, 69, 125, 267, 277, 330, 338, 353). As persistent inflammation induced by *H. pylori* promotes the development of gastric adenocarcinoma, our results demonstrating that levels of *H. pylori*-induced inflammation were increased within the stomachs of *mmp-7*$^{-/-}$ mice when compared to wild type mice were somewhat unexpected. However, one potential mechanism through which an augmentation of acute and chronic inflammation observed in *mmp-7*$^{-/-}$ mice may occur is dysregulation of epithelial-derived chemoattractant production. Gastric epithelial cells secrete IL-8, a lymphocyte- and neutrophil-activating chemotactic cytokine, in response to *H. pylori* infection (33, 43, 62, 65, 89, 140, 260, 278), which establishes a haptotactic gradient towards the epithelial surface. Li *et al.* have demonstrated that, in mouse model of acute lung injury, MMP-7 promotes a transepithelial gradient of KC, the murine functional homolog of human IL-8, by ectodomain shedding of syndecan-1, a heparan sulfate proteoglycan found on epithelial cell surfaces (172). KC associates with shed syndecan-1, establishing a gradient that drives neutrophils toward the alveolar lumen. In *mmp-7*$^{-/-}$ mice, neutrophils exhibit an impaired ability to migrate from the interstitium into the alveolar luminal compartment due to a lack of this gradient, trapping the neutrophils at the epithelial-matrix interface. We speculate that *H. pylori*-infected *mmp-7*$^{-/-}$ mice may similarly lack the ability to establish proper chemoattractant gradients within the stomach, preventing transepithelial migration of neutrophils and other immune cells into the lumen of gastric

glands where they would normally respond to microbial infection. In turn, retention of these immune cells in the interstitium may manifest as increased levels of inflammation. Future studies will be required to definitively define the mechanisms underpinning these observations.

H. pylori infection results in increased levels of cellular turnover within the gastric epithelia through a number of mechanisms (229, 325), and both apoptosis and cell proliferation are increased in precancerous lesions in the context of *H. pylori* infection (38, 178, 245). Many functions of MMP-7 that have been described to date are thought to promote apoptosis and proliferation (88, 206, 248, 330, 364). Our results, however, indicate that both of these cellular phenotypes are augmented in *H. pylori*-infected mice lacking MMP-7 when compared to wild type mice, in a manner that is directly related to levels of *H. pylori*-induced inflammation. The importance of the microenvironment in the regulation of apoptosis and proliferation is firmly established and can be influenced by complex interactions between factors derived from both epithelial and non-epithelial compartments (319). *H. pylori* infection induces an influx of a number of immune cells, including neutrophils, that can augment cell damage via increases in oxidative stress, leading to increased apoptosis (82, 212, 298). Therefore, in the context of chronic *H. pylori* infection, recruitment of leukocytes into the gastric mucosa results in the activation of signaling cascades that can activate apoptosis. In turn, epithelial cell loss reciprocally stimulates the proliferative response of epithelial cell precursors, in order to compensate for reduced cell mass. We speculate that, in *mmp-7*$^{-/-}$ mice, increased numbers of infiltrating immune cells further augment this process, accounting for the differences observed when compared to infected wild type mice.

Infection with *H. pylori* is strongly associated with the development of gastric adenocarcinoma, and both microbial and host factors influence the risk for carcinogenesis. Interactions between *H. pylori*, epithelial cells, and other cells found within the gastric microenvironment play an important role in the development of gastric injury. MMP-7 is a multifunctional host protease that mediates a number of pathophysiological processes associated with the promotion of tumor development. Our results indicate that MMP-7 expression is increased by *H. pylori* and regulates levels of inflammation and epithelial cell turnover. Molecular delineation of pathways activated by host-microbial interactions will not only improve our understanding of *H. pylori*-induced carcinogenesis and persistence, but may also provide mechanistic insights into other malignancies that arise within the context of inflammatory states.

CHAPTER IV

CONCLUSION

Summary

The discovery of *H. pylori* within the stomach of its human host over 25 years ago has been one of the most important findings within the field of cancer biology to date, leading to *H. pylori*'s identification as an etiologic agent responsible for the development of gastric adenocarcinoma and the award of the 2005 Nobel Prize for Medicine and Physiology to Dr. Barry Marshall and Dr. Robin Warren. For centuries, the mysterious cause of a variety of gastric ailments, including gastritis, ulceration, and cancer, remained unknown. Physicians could treat the symptoms of gastric disease through the use of bismuth compounds and, more recently, acid-reducing pharmaceutical agents such as ranitidine, cemetidine, and proton pump inhibitors, but were unable to attack the underlying cause, leaving their patients on treatment regimens for life. Marshall and Warren's discovery led to simple treatments involving triple-antibiotic therapy combined with bismuth supplementation that have improved the quality of life for thousands suffering from gastritis and ulceration. With gastric cancer being the second leading cause of cancer-related death world-wide, eradication of *H. pylori* has already resulted in reduction in rates of gastric cancer in most developed nations and will hopefully provide a reduction in mortality rates worldwide as detection and treatment becomes more widespread in developing countries.

All people colonized by *H. pylori* develop some level of superficial gastritis, and the chronicity of this inflammation is thought to lead to disease. The ubiquitous nature of *H. pylori*-associated inflammation itself suggests that inflammation likely plays an important role in the bacterium's life cycle, possibly by disrupting glandular architecture in order to promote nutrient release or provide access to particular compartments within the cellular milieu, although, the advantage provided this pathogen by a hyperinflammatory microenvironment remains unclear. It is clear, however, that the survival mechanisms employed by *H. pylori*, facilitating its persistence within the gastric niche and avoidance of detection by the immune system, trigger the dysregulation of a number of host-cell signaling pathways, many of which play a role in the initiation and promotion of malignant disease. Therefore, in addition to its importance in the field of gastroenterology, the discovery of *H. pylori* as a causative agent of gastric cancer has provided an exceptionally valuable model of inflammation-associated cancer that continues to provide insight into other malignancies.

Transcriptional upregulation of host effector molecules is a common theme both in the response to infection by pathogens and within transformed cells or tissues exhibiting premalignant lesions. This dissertation work was designed to address the mechanism by which *H. pylori* infection increases transcription of MMP-7 in human gastric epithelial cells, with specific respect to both the bacterial side (i.e. virulence factors) and the host side (i.e. signaling cascades and transcription factors). We also sought to determine the functional role of MMP-7 subsequent to transcriptional upregulation as it relates to the development of gastric disease through the use of a mouse model of experimental *H. pylori* infection. At the time this project was undertaken, only three manuscripts had been

published on the topic of *H. pylori* and MMP-7. Our group and others had demonstrated that infection of gastric cell lines with *H. pylori* increased MMP-7 expression in a *cag*-dependent manner, and patients colonized with *H. pylori* had elevated levels of MMP-7 expression when compared to uninfected patients (24, 68, 344). Further, a number of cell-signaling molecules were implicated in this upregulation, including ERK1/2, RhoA, and Rac, as well as at least two transcription factors, NF-κB and AP-1 (68, 344). Taken together, these data suggested that a complex interplay of bacterial and host effector molecules potentiates in increased expression of MMP-7, a molecule known to be upregulated in a number of epithelial malignancies.

In Chapter II, the $p120^{ctn}$/Kaiso signaling axis was identified as a critical mediator of *H. pylori*-associated MMP-7 induction. Previously, exogenous overexpression of $p120^{ctn}$ was shown to result in translocation of $p120^{ctn}$ to the nucleus and relief of transcriptional repression of Wnt/β-catenin target genes by the host molecule, Kaiso (288). However, a role for this signaling cascade in normal physiological processes had yet to be described. We demonstrated that, in gastric epithelial cells, Kaiso binds the promoter region of *mmp-7* **(Figure 16)** and infection with *H. pylori* results in the release of Kaiso-mediated repression of an artificial construct in a *cag*-dependent manner **(Figure 21)**. Furthermore, we demonstrated that *H. pylori* infection alters $p120^{ctn}$ phosphorylation and results in its translocation to the nucleus **(Figures 19 & 20)**, and that $p120^{ctn}$ is required for *H. pylori*-mediated increases in *mmp-7* expression **(Figure 23)**, likely due to an interaction with Kaiso **(Figure 24)**. These data suggest a novel mechanism of pathogenesis utilized by cag^{+} *H. pylori* strains by which intracellular signaling pathways involving $p120^{ctn}$ are aberrantly activated, resulting in a release of Kaiso-mediated

repression and increased transcription of the Wnt/β-catenin target gene, MMP-7 **(Figure 33)**.

A role for MMP-7 in the promotion of tumorigenesis has clearly been established in the literature. In Chapter III, we examined the role of *H. pylori*-induced increases in MMP-7 expression in a mouse model of *H. pylori*-mediated gastric carcinogenesis. We demonstrated that chronic infection of *mmp-7*$^{-/-}$ C57BL/6 mice results in increased levels of inflammation, including increased numbers of neutrophils, T cells, and B cells when compared to wild type mice **(Figures 26-28)**. As persistent inflammation induced by *H. pylori* promotes the development of gastric adenocarcinoma, these results were somewhat unexpected. We speculate that one mechanism through which inflammation is augmented in *mmp-7*$^{-/-}$ mice is dysregulation of epithelial-derived chemoattractant production. In addition, MMP-7 typically functions to enhance cellular turnover. However, increased rates of proliferation and apoptosis were observed in *mmp-7*$^{-/-}$ mice when compared with wild type mice **(Figures 31 & 32)**. Rates of cellular turnover are influenced by complex interactions of factors derived from both epithelial and non-epithelial compartments. In the context of chronic *H. pylori* infection, recruitment of leukocytes into the gastric mucosa results in the activation of signaling cascades that drive apoptosis. In turn, epithelial cell death stimulates the proliferative response of epithelial cell precursors, in order to compensate for lost cells. We hypothesize that, in *mmp-7*$^{-/-}$ mice, increased numbers of infiltrating immune cells further augment this process, accounting for the differences observed when compared with wild type mice.

Taken together, these data demonstrate that *H. pylori* dysregulates host cell-signaling mechanisms leading to specific increases in transcription of *mmp-7*. Interaction

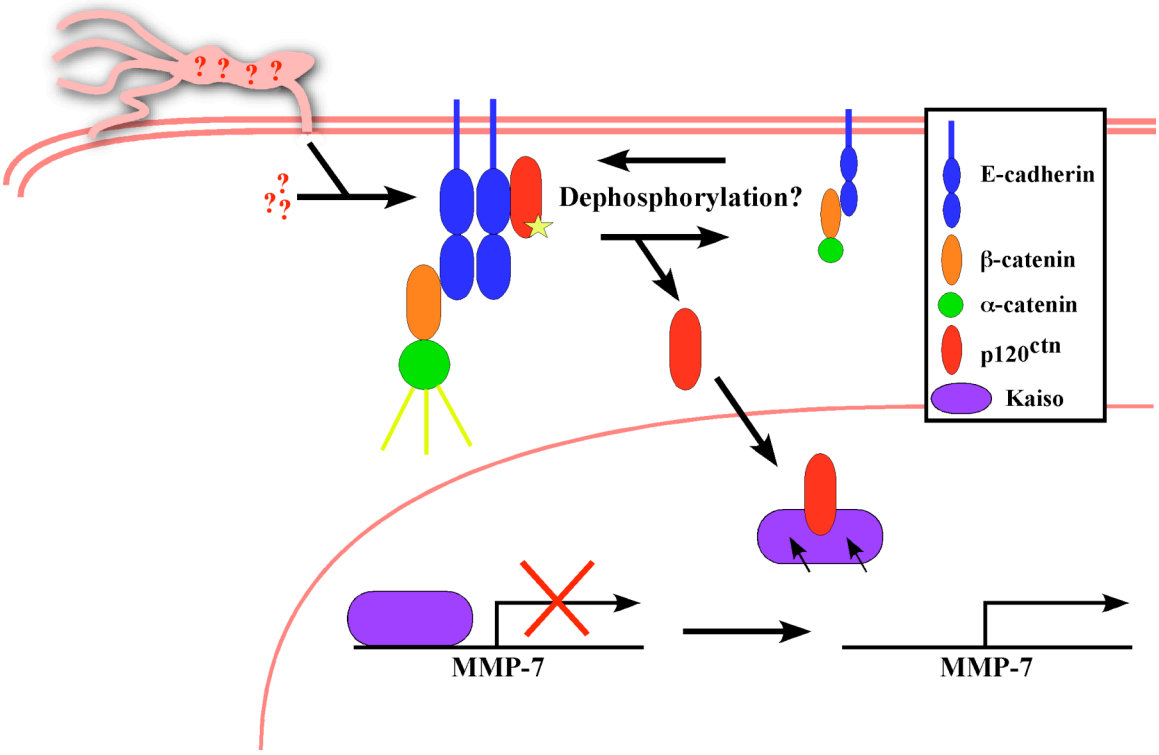

Figure 33. *H. pylori* infection results in increased MMP-7 expression through transcriptional regulation of *mmp-7* via aberrant activation of p120^{ctn}. This is *cag*-dependent, indicating a role for either a direct physical interaction between the type IV secretion system and the host cell or injection of a yet unidentified bacterial effector molecule. Aberrant activation of p120^{ctn} may occur through alterations in phosphorylation at specific residues, as *H. pylori* infection results in decreased levels of p120^{ctn} tyrosine phosphorylation.

of *H. pylori* with gastric epithelial cells leads to aberrant activation of $p120^{ctn}$ in a *cag*-dependent manner. Signaling to $p120^{ctn}$, likely through alterations in phosphorylation, induces alterations in its subcellular localization, including its translocation to the nucleus, where it can interact with Kaiso to inhibit Kaiso-mediated transcriptional repression. Increased *mmp-7* transcription follows, resulting in increased MMP-7 protein expression and secretion by gastric epithelial cells. In mice, infection with *H. pylori* results in focal increases in MMP-7 protein expression, localized specifically to epithelial cells. Increased levels of MMP-7 have a protective effect within the gastric epithelium, as infection of mice deficient in MMP-7 results in enhanced levels of inflammation and cellular turnover compared to wild type mice. Although MMP-7 plays a protective role in this experimental model of *H. pylori* infection, MMP-7 is a multifunctional molecule that has been demonstrated to enhance the progression of several epithelial-derived malignancies other than gastric cancer, making it an ideal target for therapeutic intervention. Therefore, it will be important to delineate more specific functional roles of MMP-7 within the context of *H. pylori* infection in order to reveal how MMP-7 may serve to limit disease progression in this and other models of gastric cancer.

Preliminary data and future directions

Kaiso binds the promoter region of several Wnt/β-catenin target genes implicated in carcinogenesis.

Kaiso not only represses transcription of *mmp-7*, but is also known to bind the promoter region of several other Wnt/β-catenin target genes in a sequence-specific manner, including *PPAR-γ*, *c-Myc*, *c-Fos*, *Cyclin D1* (237, 288), all of which are activated

by infection with *H. pylori*. Further, Kaiso can bind to methylated CpG nucleotides (73, 250), implicating it in the transcriptional regulation of a number of yet unidentified target genes. Therefore, it will be important to identify other carcinogenesis-related genes that are transcriptionally regulated via this *H. pylori*/p120^{ctn}/Kaiso signaling axis. Toni Nagy, a doctoral candidate in this lab, has already identified at least three more genes that appear to be regulated by this mechanism. She co-cultured MKN28 gastric epithelial cells that had been stably transfected with either scrambled or p120^{ctn}-specifc siRNA with *H. pylori* 7.13 or medium alone, extracted total RNA, and subjected the RNA to real time RT-PCR to measure steady-state levels of *c-Myc* or *cyclin D1*mRNA. mRNA levels of both genes were increased in infected scrambled control cells when compared to uninfected **(Figure 34)**. However, increases were attenuated in p120^{ctn} siRNA-transfected cells when compared to scrambled controls, demonstrating that p120^{ctn} is required for *H. pylori*-mediated transcriptional upregulation of these genes. In the future, RNA microarray technology could be utilized in combination with these cell lines in order to identify a panel of genes that are dependent on p120^{ctn} for *H. pylori*-mediated transcriptional upregulation. Once confirmed by real time RT-PCR, it would be important to determine if the increased expression of candidate genes is dependent not only on p120^{ctn}, but also on Kaiso. This could be accomplished through experiments similar to those in **Figure 24**, in which cells transfected with p120^{ctn}-specific siRNA alone or cotransfected with p120^{ctn}- and Kaiso-specific siRNA are infected with *H. pylori*. Real time RT-PCR analysis could confirm whether co-transfection restores *H. pylori*-mediated increases in steady-state levels of mRNA corresponding to candidate genes compared to transfection of p120^{ctn}-specific siRNA alone.

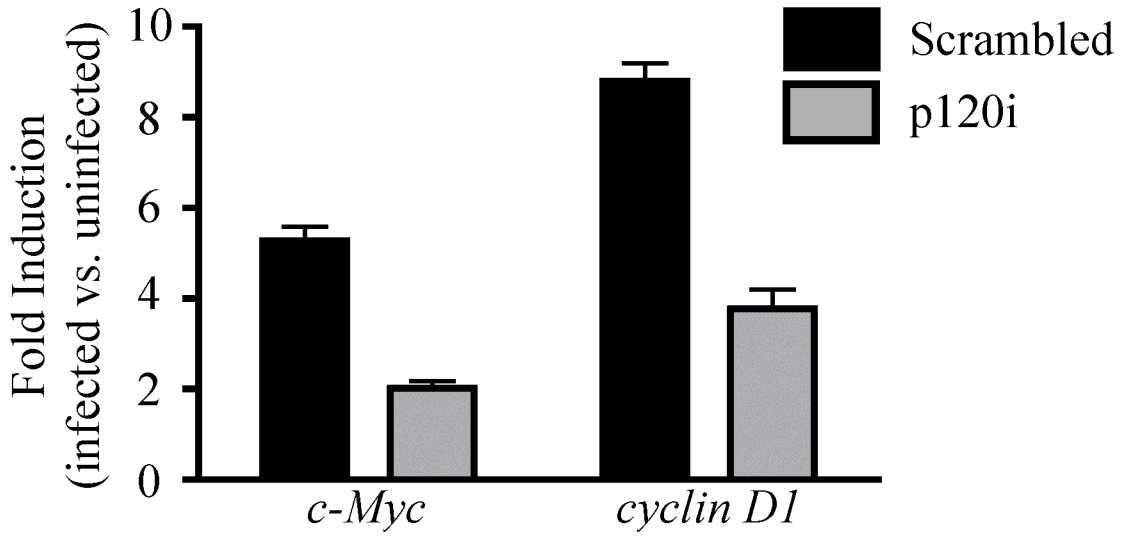

Figure 34. *H. pylori* infection of MKN28 cells results in an increase in *c-Myc* and *cyclin D1* steady-state mRNA levels in a p120^{ctn}-dependent manner. MKN28 cells transfected with scrambled control siRNA (scrambled) or human p120^{ctn}-specific siRNA (p120i) were co-cultured with the *H. pylori cag*$^{+}$ strain 7.13 (MOI=100) or medium alone for 24 hours. RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold induction of mRNA in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 2 occasions. Error bars, SEM.

The function of p120^{ctn} is mediated by alterations in its phosphorylation state.

p120^{ctn} is phosphorylated at a number of sites, the majority of which have been mapped to its regulatory domain, indicating a likely role in the regulation of p120^{ctn} function. However, the consequences of phosphorylation at individual serine/threonine and tyrosine residues have only recently begun to be investigated. Multiple ligand-receptor pathways have been implicated in signaling to p120^{ctn} through phosphorylation events, PKC and EGFR -dependent pathways (183, 345), both of which are activated by *H. pylori* (147, 222). Our immunoblot analyses using a general anti-phospho-tyrosine antibody demonstrated that *H. pylori* infection significantly decreases total tyrosine phosphorylation of p120^{ctn}, but we were unable to identify the specific residues which might be involved **(Figure 19)**. This observation, in combination with results indicating *H. pylori*-mediated alterations in p120^{ctn} subcellular localization at corresponding time points, supports a suggested role for dephosphorylation in dissociation of p120^{ctn} from E-cadherin and the adherens junction complex (258), which may facilitate its nuclear translocation. As more antibodies directed towards specific phosphorylated p120^{ctn} residues become available, it will be important to test them in similar experiments to identify other, as of yet uncharacterized, residues that represent targets for both phosphorylation and dephosphorylation by *H. pylori*. In addition, the use of pharmacological inhibitors directed towards signaling molecules activated by *H. pylori*, such as Src, PKC, and EGFR, could be utilized to identify what upstream pathways may be required for alterations in the phosphorylation state of p120^{ctn}.

Rho family GTPases are regulated by p120^{ctn}.

Rho family GTPases are critical mediators of cytoskeletal dynamics and cell-cell adhesion (11). Several groups have demonstrated that p120^{ctn} can inhibit RhoA (12, 220), and activate Rac and Cdc42 (108, 220). Cytoplasmic p120^{ctn} promotes cell motility via activation of Rac1 and Cdc42 resulting in actin polymerization and formation of lamellipodia and filopodia at the leading edge of the cell. RhoA inhibition via p120^{ctn} can also promote the reorganization of actin structures in the leading edge of cells and a reduction in adhesion at the cell's trailing end, essentially increasing contractility across the cell body (217, 264). Taken together, these data suggest that coordinated regulation of these Rho GTPases by p120^{ctn} could exert profound effects on cell motility and adhesion. *H. pylori* infection of gastric epithelial cells *in vitro* leads to dramatic cell spreading and increased cell motility, as evidenced by induction of the hummingbird phenotype in AGS cells **(Figure 6)**. The results described in Chapter II clearly demonstrate that *H. pylori* induces alterations in the subcellular localization and phosphorylation state of p120^{ctn} **(Figures 17 & 20)**, likely leading to specific signaling events. Therefore, future studies should be directed towards elucidating a role for p120^{ctn} regulation of Rho GTPases in *H. pylori*-mediated alterations in cellular morphology and motility.

Rho GTPases also mediate signaling cascades, such as MAP kinase pathways and NF-κB, that could extend the effects of p120^{ctn} to additional signaling events induced by *H. pylori* (11). Wroblewski *et al.* demonstrated a requirement for RhoA and, to a lesser extent, Rac in *H. pylori*-mediated induction of an artificial *mmp-7* promoter construct (344), suggesting that Rho GTPase signaling is required for increases in MMP-7 expression in response to *H. pylori.* Future studies should examine the role of p120^{ctn} in

regulation of Rho GTPases in response to *H. pylori*. Cells transfected with scrambled or $p120^{ctn}$-specific siRNA could be infected with *H. pylori* and then subjected to assays of GTPase activation, such as the rhotekin pull-down assay, to ascertain whether $p120^{ctn}$ is required for activation fo Rho family GTPases. As suggested in Chapter II, it will also be important to determine which specific molecules (i.e. RhoA, Rac, and Cdc42) fall within the $p120^{ctn}$ signaling cascade that leads to increased MMP-7 expression. In addition, further study of this pathway may also reveal a requirement for $p120^{ctn}$ and Rho GTPases in the increased transcription of a number of other genes related to carcinogenesis.

Increases in mmp-7 expression mediated by H. pylori are dependent on activation of host cell signaling pathways by specific bacterial virulence factors.

In Chapter II, we demonstrated that *H. pylori*-mediated induction of *mmp-7* is dependent on a functional *cag* pathogenicity island but not on *cagA*, as an *H. pylori* 7.13 *cagE*$^-$ isogenic mutant had an attenuated ability to increase steady-state levels of *mmp-7* mRNA, while a *cagA*$^-$ mutant induced increases similar to wild type bacteria **(Figure 14)**. *Mmp-7* is considered a prototypical target gene of β-catenin. However, Dr. Aime Franco, a previous graduate student in our lab, demonstrated that CagA is required for the activation of β-catenin in AGS gastric epithelial cells (96). The disparity between these results is apparent—how does an *H. pylori cagA*$^-$ isogenic mutant activate transcription of *mmp-7* if it does not activate β-catenin? One possibility is that CagA is not required for β-catenin activation in MKN28 cells. Transfection of a β-catenin-responsive luciferase construct into these cells followed by infection with wild type and *cagA*$^-$ strains of *H. pylori* could be used to determine if the isogenic *cagA*$^-$ mutant can activate β-catenin in these cells. Another possibility is that other activators of transcription, such as the PEA3

and AP-1 family of transcription factors, are sufficient to activate *mmp-7* transcription once Kaiso-mediated repression has been relieved. ChIP analysis could be used to convincingly determine which transcription factors bind to the *mmp-7* promoter in response to *H. pylori*, including β-catenin. Pharmacological inhibition or siRNA towards individual transcription factors found to bind could then be utilized to discern the contribution of each molecule to the total increase in *mmp-7* mRNA.

There are a number of other bacterial virulence factors specific to *H. pylori* that may be involved in the upregulation of *mmp-7*. We created isogenic mutants of two genes in *H. pylori* 7.13: *vacA* and *slt*. Infection of gastric epithelial cells with the *vacA*$^-$ mutant, which lacks functional vacuolating cytotoxin, led to increases in *mmp-7* steady-state mRNA levels similar to that of wild type **(Figure 35A)**. Infection with the *slt*$^-$ mutant, which lacks soluble lytic transglycosylase, resulting in an approximate 75% ($P<0.0001$) reduction in the amount of bacterial PG translocated into the host cell, showed an approximately 75% reduction in *mmp-7* expression when compared to wild type **(Figure 35B)**, indicating that PG is required for upregulation of *mmp-7*. Intracellular bacterial PG is recognized by Nod1, which in turn activates a signaling cascade that culminates in activation NF-κB, a transcription factor required for *H. pylori*-mediated increases in *mmp-7* expression in AGS cells (344). To confirm the role of NF-κB in our system, we transfected MKN28 gastric epithelial cells with dominant negative IκBα or dominant negative IKKβ, inhibitors of NF-κB activation, or sonicated salmon sperm DNA, and infected them with *H. pylori* 7.13. *H. pylori*-mediated increases in *mmp-7* expression were significantly attenuated in cells transfected with either of the dominant negative constructs compared to cells transfected with salmon sperm DNA **(Figure 36)**, confirming

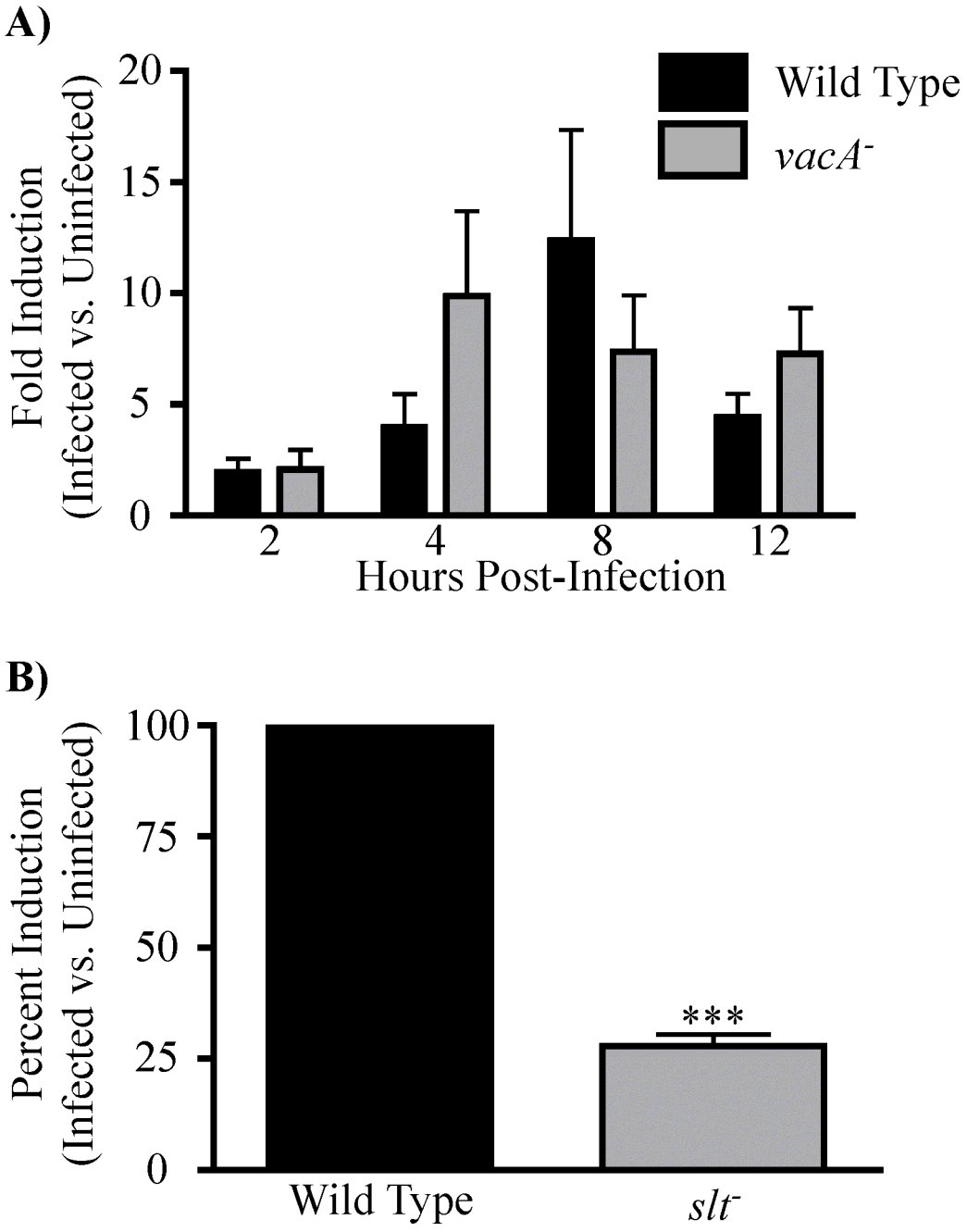

Figure 35. *H. pylori*-induced increases in *mmp-7* steady-state mRNA levels are dependent on *slt*, but not *vacA*. MKN28 cells were co-cultured with the *H. pylori cag*$^{+}$ strain 7.13 (MOI=100) or medium alone. At specified time points, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold induction of mRNA in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 3 occasions. A) The *H. pylori vacA*$^{-}$ mutant induces *mmp-7* expression to the same levels as wild type. B) At 8 hours post-infection, the *H. pylori slt*$^{-}$ mutant's induction of *mmp-7* is attenuated compared to wild type. Error bars, SEM. ***, $p < 0.001$ vs. wild type.

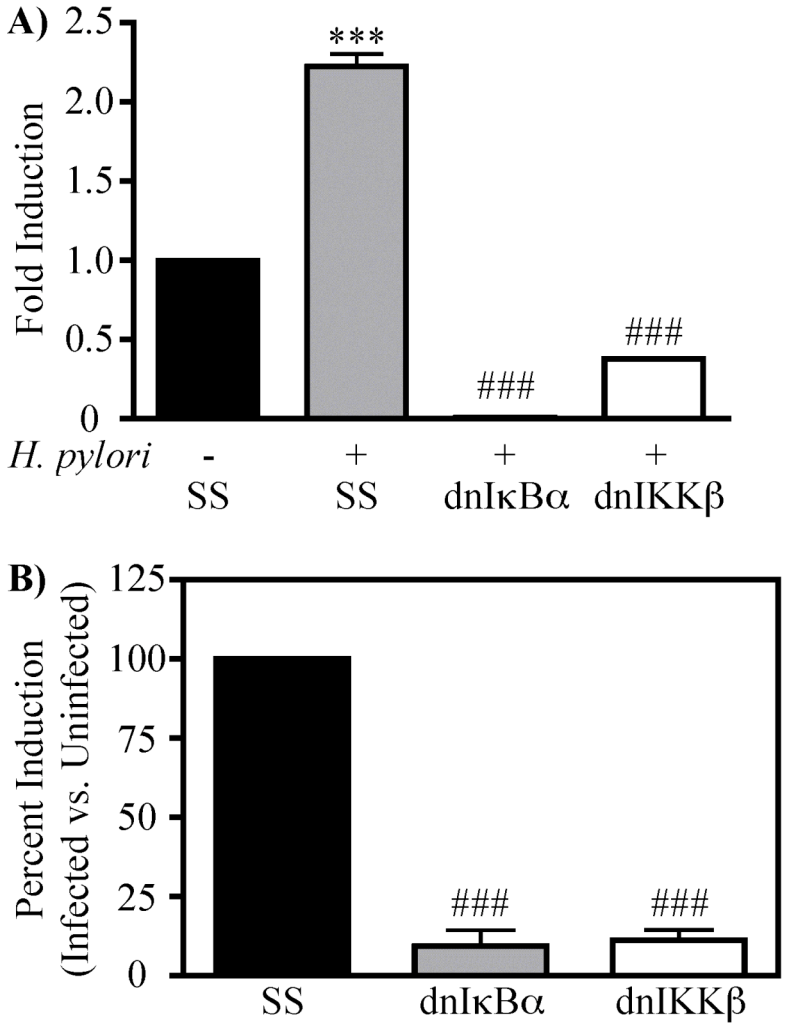

Figure 36. *H. pylori*-induced increases in *mmp-7* steady-state mRNA levels are dependent on NF-κB. (A) MKN28 gastric epithelial cells were cotransfected with an NF-κB responsive luciferase reporter, *Renilla* luciferase construct (control for transfection efficiency) and either sonicated salmon sperm DNA (SS), a dominant negative IκBα construct (dnIκBα), or a dominant negative IKKβ construct (dnIKKβ) using Fugene 6 (Roche, Indianapolis, IN) according to the manufacturer's instructions. Cells were co-cultured with *H. pylori* 7.13 (MOI=100) or medium alone. Twenty-four hours post-infection, cell lysates were analyzed for relative luciferase assay, confirming that the dominant negative constructs significantly attenuated NF-κB activity. (B) MKN28 gastric epithelial cells were transfected with either SS, dnIκBα, or dnIKKβ construct using Fugene 6. Cells were co-cultured with *H. pylori* 7.13 (MOI=100) or medium alone. Eight hours post-infection, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent fold induction of mRNA in cells co-cultured with *H. pylori* versus medium alone from experiments performed on at least 3 occasions. Error bars, SEM. ***, $p < 0.001$ vs. uninfected. ###, $p < 0.001$ vs. SS.

the requirement for functional NF-κB in *H. pylori*-induced *mmp-7* expression in MKN28 gastric epithelial cells.

Taken together, these data indicate that PG and NF-κB, a bacterial molecule and host effector linked indirectly through a signaling pathway involving Nod1 and the kinase RICK (134), are required for *H. pylori*-mediated increases in *mmp-7* expression. A role for Nod1 in the activation of NF-κB specifically in response to *H. pylori* PG has only been demonstrated for an artificial NF-κB-responsive luciferase construct in HEK293 cells expressing exogenous Nod1 (321). Therefore, the physiologic role of Nod1 and RICK in the recognition of PG as intermediaries in NF-κB and activation and subsequent *mmp-7* transcription must to be defined in a gastric epithelial cell system. siRNA or dominant negative constructs directed towards Nod1 and RICK could be utilized prior to infection with *H. pylori* to determine the requirement of these molecules for NF-κB activation and increased *mmp-7* expression.

MMP-7 regulates turnover of glandular epithelial cells.

Hyperproliferation induced by *H. pylori* can promote tumorigenesis. MMP-7 increases cellular proliferation by cleaving pro-HB-EGF to yield mature HB-EGF. Soluble HB-EGF can bind ErbB4 EGF receptor leading to activation and stimulation of cellular proliferation (364), a receptor that we have previously demonstrated to be activated by *H. pylori* in a metalloproteinase-dependent manner (147). Although proliferation was increased in *mmp-7*$^{-/-}$ mice when compared to wild type mice in our experimental animal model of *H. pylori* infection **(Figure 31)**, we attribute this more to paracrine signaling by increased numbers of inflammatory cells in the *mmp-7*$^{-/-}$ mice than

lack of MMP-7, as the presence of functional MMP-7 typically enhances proliferation *in vitro* (112, 213). Further, infection of MKN28 cells stably transfected with scrambled control siRNA led to significantly increased doubling times when grown in 3D culture compared to uninfected cells or infected cells stably transfected with *mmp-7*-specific siRNA **(Figure 37)**, suggesting that *H. pylori*-mediated increases in MMP-7 expression also contribute to hyperproliferation *in vitro*. Additional work should include a determination of the molecules activated by MMP-7 that lead to increased proliferation *in vitro*, and, taken together, the data above indicate that HB-EGF is the most likely candidate. To determine whether HB-EGF activates ErbB4 in response to *H. pylori*, cells could be pre-treated with neutralizing antibodies targeted towards HB-EGF, infected with *H. pylori*, and the phosphorylation state of the ErbB4 receptor analyzed by immunoprecipitation and immunoblot with a phospho-tyrosine antibody. To determine if MMP-7 plays a role in activation of ErbB4 in response to *H. pylori*, a similar experiment could be performed in which cells stably transfected with control scrambled siRNA or *mmp-7*-specific siRNA could be infected with *H. pylori* and the phosphorylation state of the ErbB4 receptor analyzed. It will also be important to utilize residue-specific anti-phospho antibodies to determine which residues of ErbB4 are involved in the cell signaling response.

H. pylori infection leads to significantly enhanced levels of inflammation in mmp-7$^{-/-}$ mice when compared to wild type mice.

As increased expression of MMP-7 typically correlates with enhanced carcinogenesis (1, 5, 6, 121, 125, 194, 277, 338, 353), we anticipated that *mmp-7$^{-/-}$* mice would exhibit lower levels of *H. pylori*-induced inflammation, the precursor to the

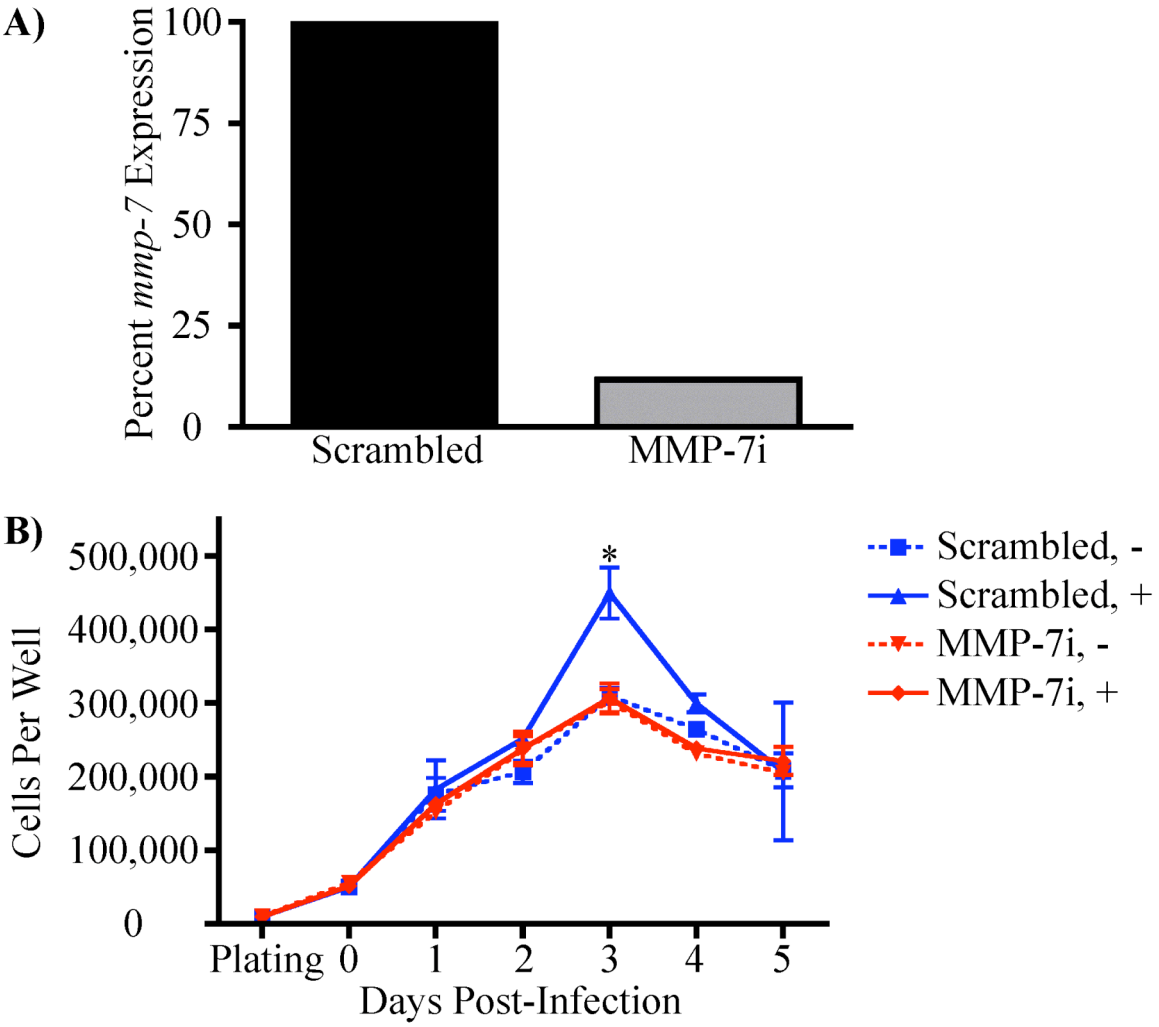

Figure 37. *H. pylori* infection of wild type MKN28 cells stably led to significantly increased doubling times when grown in 3D culture compared to MMP-7-deficient MKN28 cells or uninfected cells. (A) MKN28 cells were stably transfected with either a scrambled control siRNA construct (Scrambled) or a human *mmp-7*-specific siRNA construct (MMP-7i; kindly provided by Dr. Howard Crawford, SUNY-Stoneybrook) as described in Chapter II. To confirm successful knock down of *mmp-7* expression, RNA was extracted, subjected to reverse transcription, and analyzed in triplicate by real time PCR. Data represent percent *mmp-7* mRNA expression. (B) Cell proliferation assays in three dimensions were carried out as described in Lynch *et al* (177). Briefly, 2 x 10^4 cells were plated into 48-well plates that were coated with 100 µl of a basement membrane matrix (Matrigel, BD Biosciences) and 100 µl. Cells were co-cultured with *H. pylori* 7.13 (MOI=100) or medium alone beginning 2 days after plating. Cells were retrieved from the three-dimensional matrix by treating with dispase (BD Biosciences), and viable cells were counted using a trypan blue exclusion assay. Data represent the number of cells per well from experiments performed in duplicate on at least 3 occasions. -, Medium Alone. +, *H. pylori* 7.13. *, $p < 0.05$ vs. MMP-7i or uninfected cells.

development of gastric cancer. Unexpectedly, infection of *mmp-7*$^{-/-}$ mice resulted in significantly higher levels of inflammation when compared to wild type mice **(Figure 26 & 27)**. Increased expression of MMP-7 is also thought to result in increased rates of cellular turnover (88, 206, 248, 330, 364). However, we found that infection of *mmp-7*$^{-/-}$ mice resulted in significantly higher levels of proliferation and apoptosis when compared to wild type mice **(Figures 31 & 32)**. Taken together, these results indicate that levels of inflammation in these mice correspond to levels of proliferation and apoptosis, suggesting that one may influence the other. Only one study in the literature has demonstrated enhanced inflammation and disease in animals deficient in MMP-7 relative to wild type animals. Li *et al.* showed that, in mouse model of acute lung injury, MMP-7 generates a transepithelial gradient of KC by ectodomain shedding of syndecan-1 (172). KC associates with shed syndecan-1, providing a chemotactic gradient that drives neutrophils toward the alveolar lumen. As a consequence of *mmp-7* deficiency, neutrophils exhibited an impaired ability to advance from the interstitium into the alveolar luminal compartment due to a lack of this gradient. We suggest that *H. pylori*-infected *mmp-7*$^{-/-}$ mice may lack the ability to produce proper chemoattractant gradients, preventing transepithelial migration of neutrophils and other immune cells into the lumen of gastric glands where they would normally respond to microbial infection. In turn, retention of these immune cells in the interstitium manifests itself as increased levels of inflammation.

Future experiments should be designed to address whether MMP-7 regulates the activity of an epithelial-derived neutrophil chemotactic factor within the gastric mucosa. As *H. pylori* infection induces significantly increased levels of IL-8 (33, 43, 62, 65, 89, 140, 260, 278), KC, the same murine functional homolog of IL-8 found to be important

in the bleomycin-induced lung injury model, may also be important in the stomach. Wild type and *mmp-7*$^{-/-}$ mice could be challenged with *H. pylori*, sacrificed, and protein levels of KC could be measured in sera, gastric mucosal scrapings, and gastric tissue homogenates. If KC does play a role in neutrophil egress, serum levels of KC should be similar between wild type and *mmp-7*$^{-/-}$ mice. However, levels in tissue homogenates would be expected to be higher in *mmp-7*$^{-/-}$ mice compared with wild type, as KC would not track along its gradient nor be taken up by advancing neutrophils, leading to its accumulation. Levels in mucosal scrapings may be higher in wild type mice with KC being driven towards the glandular compartments and translocating into the gastric lumen.

Li *et al.* used several key pieces of information to determine that syndecan-1 is the molecule responsible for correct distribution and effective chemoattraction by KC, including the fact that IL-8 must be associated with heparan sulfate to have neutrophil chemotactic activity (332) and to bind to its receptor, CXCR2 (127), and that syndecan proteoglycans, a source of heparan sulfate on the cell surface, are shed in response to inflammation by a metalloproteinase (27, 90). Mammals express four different syndecans, type I transmembrane proteoglycans found on most all adherent cells (238). Although syndecan-1 is important for neutrophil efflux in the lungs, expression of all four syndecans in *H. pylori*-infected gastric tissue from wild type and *mmp-7*$^{-/-}$ mice should be examined by immunohistochemistry. If one of these molecules associates with KC to form a chemotactic gradient, expression will likely be reduced or absent in gastric glands from wild type mice when compared to *mmp-7*$^{-/-}$ mice due to clearance upon endocytosis by infiltrating neutrophils and localization to the glandular lumen. Further, KC could be

immunoprecipitated from mucosal scrapings followed by immunoblot for each syndecan to determine if shed syndecans are able to associate with KC in wild type mice but not in *mmp-7*$^{-/-}$ mice.

It is possible that expression of a number of other pro-inflammatory chemokines, such as macrophage-inflammatory protein (MIP) -2 (223, 224, 273), IL-1β (94, 151, 161, 366), TNF-α (151, 157, 161, 170, 305), known to be upregulated in response to *H. pylori*, may be dysregulated in *mmp-7*$^{-/-}$ mice. These molecules may include not only those that influence neutrophil efflux, but also those that regulate infiltration by T and B cells. In addition, it is possible that MMP-7 may function in the activation of anti-inflammatory chemokines, such as IL-10 and IL-4, which are known to antagonize immune cell tissue infiltration. Dysregulation of such anti-inflammatory signals could account for the increased levels of inflammation in *mmp-7*$^{-/-}$ mice compared to wild type mice. Therefore, expression of a number of chemokines should be examined in *H. pylori*-challenged wild type and *mmp-7*$^{-/-}$ mice. Ultimately, MMP-7 may function directly in the regulation of chemokine expression or indirectly through mechanisms that facilitate the formation of chemotactic gradients similar to that seen with KC and syndecan-1.

It is also possible that the pattern of *H. pylori*-induced inflammation may be very different in an acute model of experimental infection as compared to our chronic infection model. Increased levels of MMP-7 may result in increased proliferation and apoptosis at these early time points in wild type mice when compared with *mmp-7*$^{-/-}$ mice, similar to the increased proliferation observed *in vitro* **(Figure 37)**. Because these effects are exerted prior to chronic infiltration of immune cells into the gastric mucosa, they may not be observed in infections lasting 12 weeks or longer. Experiments are currently

underway to infect wild type and *mmp-7*$^{-/-}$ mice for only 24 and 48 hours. We will again compare patterns of inflammation and rates of apoptosis and proliferation between wild type and *mmp-7*$^{-/-}$ mice at these early time points.

Although the experiments in Chapter III have shed some light on the role of MMP-7 in *H. pylori*-induced inflammation *in vivo*, the requirement for infections lasting over a year in order to induce carcinoma makes it difficult to asses the role of MMP-7 in carcinogenesis in this system. INS-GAS mice develop hypergastrinemia due to increased serum gastrin levels that exhibits a synergistic effect with *H. pylori* infection leading to a uniformly developed atrophy, intestinal metaplasia, and dysplasia by 6 weeks and carcinoma by 24 weeks post-infection in male mice (92, 94). To define the role of MMP-7 in an *in vivo* model of *H. pylori*-induced cancer, we are in the process of backcrossing C57/BL6 *mmp-7*$^{-/-}$ mice onto the FVB/N INS-GAS background. Once crossed onto this background, the INS-GAS *mmp-7*$^{-/-}$ mice will be infected with the carcinogenic *H. pylori* strain 7.13 and monitored for the development of premalignant lesions as well as gastric cancer. According to the data from C57BL/6 mice, we expect that, in the absence of *mmp-7*$^{-/-}$, the development of gastric cancer would be enhanced, as inflammation is a key mediator of *H. pylori*-induced cancer. However, it is possible that MMP-7 may function directly in yet undefined signaling pathways that promote carcinogenesis, outweighing the parcrine influence of increased infiltration by immune cells. The results of these experiments will add to our understanding of the function of MMP-7 *in vivo*, possibly demonstrating a protective role for MMP-7 that has yet to be described.

Final Remarks

The discovery of *H. pylori* and its subsequent identification as an etiologic agent in the development of gastric disease has resulted in a paradigm shift in the way that physicians think about and treat gastric ailments. Prior to Marshall and Warren's presentation of their discovery to the medical community, gastroenterologists sought to treat the symptoms of disease, unknowingly allowing the underlying cause to persist. Upon widespread acceptance of their findings, progress in the field of *H. pylori* pathogenesis was rapid and far-reaching, leading to the development of antibiotic therapies that could simply and effectively cure painful gastritis and ulceration through the eradication of *H. pylori*. Furthermore, when viewed in the context of Dr. Pelayo Correa's established model of the histopathologic progression of gastric disease to gastric cancer **(Figure 4)**, scientists soon began to direct their attention towards the role of *H. pylori* in the development of gastric adenocarcinoma. Resulting discoveries have had extensive impact in the field of cancer biology and produced new models of inflammation as an inducer and promoter of carcinogenesis.

Although over half of the world's population is infected with *H. pylori*, only a fraction of colonized persons ever develop neoplasia, and enhanced cancer risk is related to strain-specific differences, aberrant host responses, and/or specific interactions between microbial and host determinants—a theme repeated time and time again throughout the literature. The work described in Chapter II has identified a host cell-signaling pathway, the $p120^{ctn}$/Kaiso axis, that is co-opted by *H. pylori* during the course of infection. Aberrant activation of this signaling pathway is dependent on specific microbial factors

(i.e. a functional *cag* pathogenicity island) and leads to increased transcription of *mmp-7*, a select target gene of Wnt/β-catenin signaling. $p120^{ctn}$ is a multidimensional protein that performs several distinct functions within host cells related to cell-cell adhesion, morphology, motility, and gene transcription. Molecular delineation of signaling pathways and gene transcription regulated by *H. pylori*-mediated $p120^{ctn}$ signaling will not only improve our understanding of *H. pylori*-induced carcinogenesis, but may also provide mechanistic insights into other malignancies that arise within the context of inflammatory states.

Extensive literature exists describing the role of the matrix metalloproteinases in host cells. Yet, when it comes to determining the full range of their myriad functions, we have only begun to scratch the surface. The development of research tools allowing analysis of the specific roles of individual members of the MMP family, such as pharmacologic inhibitors and fluorescent signaling beacons, will likely bear out numerous unidentified functions of individual MMPs and, in doing so, reshape our over-arching view of MMP function within the context of disease. Although MMP-7 function was initially associated with normal physiologic processes such as wound healing and repair, the majority of recent research has focused on the role of MMP-7 in the promotion of carcinogenesis. However, the work described in Chapter III suggests a novel role for MMP-7 in protection from inflammation-induced disease, emphasizing the importance of the dichotomous nature of MMP-7 function in determining disease outcome. The success of future research into this molecule's role in carcinogenesis necessitates an investigation of both its numerous beneficial and adverse functions, as well as how the balance among them becomes dysregulated, resulting in the development of disease.

The pathogenesis of *H. pylori* infection is a dynamic process influenced by the bacterium, the host, and interactions between the two over the host's lifetime. Effective prevention of gastric cancer will require not only the development of new methods of therapeutic intervention, but also the ability to determine which patients are truly at risk for cancer development and for whom treatment will be an effective course of action. This can only be accomplished through relentless pursuit of experimental data that serves to refine our overview of the processes underlying *H. pylori* pathogenesis. The work described in this dissertation provides important additions to the contextual framework surrounding the mechanisms regulating *H. pylori*-mediated development of gastric cancer.

BIBLIOGRAPHY

1. **Adachi, Y., F. Itoh, H. Yamamoto, K. Matsuno, Y. Arimura, M. Kusano, T. Endoh, Y. Hinoda, M. Oohara, M. Hosokawa, and K. Imai.** 1998. Matrix metalloproteinase matrilysin (MMP-7) participates in the progression of human gastric and esophageal cancers. Int J Oncol **13:**1031-1035.

2. **Adachi, Y., H. Yamamoto, F. Itoh, Y. Arimura, M. Nishi, T. Endo, and K. Imai.** 2001. Clinicopathologic and prognostic significance of matrilysin expression at the invasive front in human colorectal cancers. Int J Cancer **95:**290-294.

3. **Adachi, Y., H. Yamamoto, F. Itoh, Y. Hinoda, Y. Okada, and K. Imai.** 1999. Contribution of matrilysin (MMP-7) to the metastatic pathway of human colorectal cancers. Gut **45:**252-258.

4. **Aho, S., L. Levansuo, O. Montonen, C. Kari, U. Rodeck, and J. Uitto.** 2002. Specific sequences in p120ctn determine subcellular distribution of its multiple isoforms involved in cellular adhesion of normal and malignant epithelial cells. J Cell Sci **115:**1391-1402.

5. **Aihara, R., E. Mochiki, T. Nakabayashi, K. Akazawa, T. Asao, and H. Kuwano.** 2005. Clinical significance of mucin phenotype, beta-catenin and matrix metalloproteinase 7 in early undifferentiated gastric carcinoma. Br J Surg **92:**454-462.

6. **Ajisaka, H., S. Fushida, Y. Yonemura, and K. Miwa.** 2001. Expression of insulin-like growth factor-2, c-MET, matrix metalloproteinase-7 and MUC-1 in primary lesions and lymph node metastatic lesions of gastric cancer. Hepatogastroenterology **48:**1788-1792.

7. **Akopyants, N. S., S. W. Clifton, D. Kersulyte, J. E. Crabtree, B. E. Youree, C. A. Reece, N. O. Bukanov, E. S. Drazek, B. A. Roe, and D. E. Berg.** 1998. Analyses of the *cag* pathogenicity island of *Helicobacter pylori*. Mol Microbiol **28:**37-53.

8. **Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **397:**176-180.

9. **Amieva, M. R., and E. M. El-Omar.** 2008. Host-bacterial interactions in Helicobacter pylori infection. Gastroenterology **134:**306-323.

10. **Amieva, M. R., R. Vogelmann, A. Covacci, L. S. Tompkins, W. J. Nelson, and S. Falkow.** 2003. Disruption of the epithelial apical-junctional complex by Helicobacter pylori CagA. Science **300:**1430-1434.

11. **Anastasiadis, P. Z.** 2007. p120-ctn: A nexus for contextual signaling via Rho GTPases. Biochim Biophys Acta **1773:**34-46.

12. **Anastasiadis, P. Z., S. Y. Moon, M. A. Thoreson, D. J. Mariner, H. C. Crawford, Y. Zheng, and A. B. Reynolds.** 2000. Inhibition of RhoA by p120 catenin. Nat Cell Biol **2:**637-644.

13. **Anastasiadis, P. Z., and A. B. Reynolds.** 2001. Regulation of Rho GTPases by p120-catenin. Curr Opin Cell Biol **13:**604-610.

14. **Anastasiadis, P. Z., and A. B. Reynolds.** 2000. The p120 catenin family: complex roles in adhesion, signaling and cancer. J Cell Sci **113 (Pt 8):**1319-1334.

15. **Asahi, M., T. Azuma, S. Ito, Y. Ito, H. Suto, Y. Nagai, M. Tsubokawa, Y. Tohyama, S. Maeda, M. Omata, T. Suzuki, and C. Sasakawa.** 2000. *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J Exp Med **191:**593-602.

16. **Asahi, M., T. Azuma, S. Ito, Y. Ito, H. Suto, Y. Nagai, M. Tsubokawa, Y. Tohyama, S. Maeda, M. Omata, T. Suzuki, and C. Sasakawa.** 2000. Helicobacter pylori CagA protein can be tyrosine phosphorylated in gastric epithelial cells [see comments]. J Exp Med **191:**593-602.

17. **Atherton, J. C.** 2006. The pathogenesis of Helicobacter pylori-induced gastro-duodenal diseases. Annu Rev Pathol **1:**63-96.

18. **Atherton, J. C., P. Cao, R. M. Peek, Jr., M. K. Tummuru, M. J. Blaser, and T. L. Cover.** 1995. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. J Biol Chem **270:**17771-17777.

19. **Ayabe, T., D. P. Satchell, P. Pesendorfer, H. Tanabe, C. L. Wilson, S. J. Hagen, and A. J. Ouellette.** 2002. Activation of Paneth cell alpha-defensins in mouse small intestine. J Biol Chem **277:**5219-5228.

20. **Backert, S., and M. Selbach.** 2008. Role of type IV secretion in *Helicobacter pylori* pathogenesis. Cell Microbiol.

21. **Backert, S., E. Ziska, V. Brinkmann, U. Zimny-Arndt, A. Fauconnier, P. R. Jungblut, M. Naumann, and T. F. Meyer.** 2000. Translocation of the *Helicobacter pylori* CagA protein in gastric epithelial cells by a type IV secretion apparatus. Cell Microbiol **2:**155-164.

22. **Backert, S., E. Ziska, V. Brinkmann, U. Zimny-Arndt, A. Fauconnier, P. R. Jungblut, M. Naumann, and T. F. Meyer.** 2000. Translocation of the Helicobacter pylori CagA protein in gastric epithelial cells by a type IV secretion apparatus. Cell Microbiol **2:**155-164.

23. **Balkwill, F., and A. Mantovani.** 2001. Inflammation and cancer: back to Virchow? Lancet **357:**539-545.

24. **Bebb, J. R., D. P. Letley, R. J. Thomas, F. Aviles, H. M. Collins, S. A. Watson, N. M. Hand, A. Zaitoun, and J. C. Atherton.** 2003. *Helicobacter pylori* upregulates matrilysin (MMP-7) in epithelial cells *in vivo* and *in vitro* in a Cag dependent manner. Gut **52:**1408-1413.

25. **Behrens, J., J. P. von Kries, M. Kuhl, L. Bruhn, D. Wedlich, R. Grosschedl, and W. Birchmeier.** 1996. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature **382:**638-642.

26. **Benbow, U., and C. E. Brinckerhoff.** 1997. The AP-1 site and MMP gene regulation: what is all the fuss about? Matrix Biol **15:**519-526.

27. **Bernfield, M., M. Gotte, P. W. Park, O. Reizes, M. L. Fitzgerald, J. Lincecum, and M. Zako.** 1999. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem **68:**729-777.

28. **Blaser, M. J.** 1997. The versatility of Helicobacter pylori in the adaptation to the human stomach. J Physiol Pharmacol **48:**307-314.

29. **Blaser, M. J., Y. Chen, and J. Reibman.** 2008. Does Helicobacter pylori protect against asthma and allergy? Gut **57:**561-567.

30. **Blaser, M. J., G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, and A. Nomura.** 1995. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res **55:**2111-2115.

31. **Boncristiano, M., S. R. Paccani, S. Barone, C. Ulivieri, L. Patrussi, D. Ilver, A. Amedei, M. M. D'Elios, J. L. Telford, and C. T. Baldari.** 2003. The Helicobacter pylori vacuolating toxin inhibits T cell activation by two independent mechanisms. J Exp Med **198:**1887-1897.

32. **Boughan, P. K., R. H. Argent, M. Body-Malapel, J. H. Park, K. E. Ewings, A. G. Bowie, S. J. Ong, S. J. Cook, O. E. Sorensen, B. A. Manzo, N. Inohara, N. J. Klein, G. Nunez, J. C. Atherton, and M. Bajaj-Elliott.** 2006. Nucleotide-binding oligomerization domain-1 and epidermal growth factor receptor: critical regulators of beta-defensins during *Helicobacter pylori* infection. J Biol Chem **281:**11637-11648.

33. **Brandt, S., T. Kwok, R. Hartig, W. Konig, and S. Backert.** 2005. NF-kappaB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. Proc Natl Acad Sci U S A **102:**9300-9305.

34. **Brenes, F., B. Ruiz, P. Correa, F. Hunter, T. Rhamakrishnan, E. Fontham, and T. Y. Shi.** 1993. Helicobacter pylori causes hyperproliferation of the gastric epithelium: pre- and post-eradication indices of proliferating cell nuclear antigen. Am J Gastroenterol **88:**1870-1875.

35. **Brooks, P. C., S. Stromblad, L. C. Sanders, T. L. von Schalscha, R. T. Aimes, W. G. Stetler-Stevenson, J. P. Quigley, and D. A. Cheresh.** 1996. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell **85:**683-693.

36. **Brown, L. M.** 2000. Helicobacter pylori: epidemiology and routes of transmission. Epidemiol Rev **22:**283-297.

37. **Bruce, M. G., and H. I. Maaroos.** 2008. Epidemiology of Helicobacter pylori infection. Helicobacter **13 Suppl 1:**1-6.

38. **Cahill, R. J., C. Kilgallen, S. Beattie, H. Hamilton, and C. O'Morain.** 1996. Gastric epithelial cell kinetics in the progression from normal mucosa to gastric carcinoma. Gut **38:**177-181.

39. **Cai, X., J. Carlson, C. Stoicov, H. Li, T. C. Wang, and J. Houghton.** 2005. Helicobacter felis eradication restores normal architecture and inhibits gastric cancer progression in C57BL/6 mice. Gastroenterology **128:**1937-1952.

40. **Camorlinga-Ponce, M., C. Romo, G. Gonzalez-Valencia, O. Munoz, and J. Torres.** 2004. Topographical localisation of *cagA* positive and *cagA* negative *Helicobacter pylori* strains in the gastric mucosa; an *in situ* hybridisation study. J Clin Pathol **57:**822-828.

41. **Cardillo, M. R., F. Di Silverio, and V. Gentile.** 2006. Quantitative immunohistochemical and in situ hybridization analysis of metalloproteinases in prostate cancer. Anticancer Res **26:**973-982.

42. **Castano, J., G. Solanas, D. Casagolda, I. Raurell, P. Villagrasa, X. R. Bustelo, A. Garcia de Herreros, and M. Dunach.** 2007. Specific phosphorylation of p120-catenin regulatory domain differently modulates its binding to RhoA. Mol Cell Biol **27:**1745-1757.

43. **Censini, S., C. Lange, Z. Xiang, J. E. Crabtree, P. Ghiara, M. Borodovsky, R. Rappuoli, and A. Covacci.** 1996. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci U S A **93:**14648-14653.

44. **Chamaillard, M., M. Hashimoto, Y. Horie, J. Masumoto, S. Qiu, L. Saab, Y. Ogura, A. Kawasaki, K. Fukase, S. Kusumoto, M. A. Valvano, S. J. Foster, T. W. Mak, G. Nunez, and N. Inohara.** 2003. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat Immunol **4:**702-707.

45. **Chang, Y. J., M. S. Wu, J. T. Lin, R. G. Pestell, M. J. Blaser, and C. C. Chen.** 2006. Mechanisms for Helicobacter pylori CagA-induced cyclin D1 expression that affect cell cycle. Cell Microbiol **8:**1740-1752.

46. **Chaput, C., A. Labigne, and I. G. Boneca.** 2007. Characterization of Helicobacter pylori lytic transglycosylases Slt and MltD. J Bacteriol **189:**422-429.

47. **Chen, Y., and M. J. Blaser.** 2008. Helicobacter pylori colonization is inversely associated with childhood asthma. J Infect Dis **198:**553-560.

48. **Chen, Y., and M. J. Blaser.** 2007. Inverse associations of Helicobacter pylori with asthma and allergy. Arch Intern Med **167:**821-827.

49. **Cheng, K., G. Xie, and J. P. Raufman.** 2007. Matrix metalloproteinase-7-catalyzed release of HB-EGF mediates deoxycholyltaurine-induced proliferation of a human colon cancer cell line. Biochem Pharmacol **73:**1001-1012.

50. **Chow, W. H., M. J. Blaser, W. J. Blot, M. D. Gammon, T. L. Vaughan, H. A. Risch, G. I. Perez-Perez, J. B. Schoenberg, J. L. Stanford, H. Rotterdam, A. B. West, and J. F. Fraumeni, Jr.** 1998. An inverse relation between *cagA+* strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. Cancer Res **58:**588-590.

51. **Correa, P.** 1988. A human model of gastric carcinogenesis. Cancer Res **48:**3554-3560.

52. **Correa, P.** 1996. *Helicobacter pylori* and gastric cancer: state of the art. Cancer Epidemiol Biomarkers Prev **5:**477-481.

53. **Correa, P.** 2004. Is gastric cancer preventable? Gut **53:**1217-1219.

54. **Correa, P., E. T. Fontham, J. C. Bravo, L. E. Bravo, B. Ruiz, G. Zarama, J. L. Realpe, G. T. Malcom, D. Li, W. D. Johnson, and R. Mera.** 2000. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-helicobacter pylori therapy. J Natl Cancer Inst **92:**1881-1888.

55. **Correa, P., W. Haenszel, C. Cuello, S. Tannenbaum, and M. Archer.** 1975. A model for gastric cancer epidemiology. Lancet **2:**58-60.

56. **Correa, P., and J. Houghton.** 2007. Carcinogenesis of Helicobacter pylori. Gastroenterology **133:**659-672.

57. **Coussens, L. M., B. Fingleton, and L. M. Matrisian.** 2002. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science **295:**2387-2392.

58. **Coussens, L. M., and Z. Werb.** 2002. Inflammation and cancer. Nature **420:**860-867.

59. **Cover, T. L., C. P. Dooley, and M. J. Blaser.** 1990. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect Immun **58:**603-610.

60. **Cover, T. L., M. K. Tummuru, P. Cao, S. A. Thompson, and M. J. Blaser.** 1994. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. J Biol Chem **269:**10566-10573.

61. **Cozzolino, M., V. Stagni, L. Spinardi, N. Campioni, C. Fiorentini, E. Salvati, S. Alema, and A. M. Salvatore.** 2003. p120 Catenin is required for growth factor-dependent cell motility and scattering in epithelial cells. Mol Biol Cell **14:**1964-1977.

62. **Crabtree, J. E., A. Covacci, S. M. Farmery, Z. Xiang, D. S. Tompkins, S. Perry, I. J. Lindley, and R. Rappuoli.** 1995. Helicobacter pylori induced interleukin-8 expression in gastric epithelial cells is associated with CagA positive phenotype. J Clin Pathol **48:**41-45.

63. **Crabtree, J. E., J. D. Taylor, J. I. Wyatt, R. V. Heatley, T. M. Shallcross, D. S. Tompkins, and B. J. Rathbone.** 1991. Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration, and gastric pathology. Lancet **338:**332-335.

64. **Crabtree, J. E., J. I. Wyatt, G. M. Sobala, G. Miller, D. S. Tompkins, J. N. Primrose, and A. G. Morgan.** 1993. Systemic and mucosal humoral responses to *Helicobacter pylori* in gastric cancer. Gut **34:**1339-1343.

65. **Crabtree, J. E., Z. Xiang, I. J. Lindley, D. S. Tompkins, R. Rappuoli, and A. Covacci.** 1995. Induction of interleukin-8 secretion from gastric epithelial cells by a cagA negative isogenic mutant of Helicobacter pylori. J Clin Pathol **48:**967-969.

66. **Crawford, H. C., B. Fingleton, M. D. Gustavson, N. Kurpios, R. A. Wagenaar, J. A. Hassell, and L. M. Matrisian.** 2001. The PEA3 subfamily of Ets transcription factors synergizes with beta-catenin-LEF-1 to activate matrilysin transcription in intestinal tumors. Mol Cell Biol **21:**1370-1383.

67. **Crawford, H. C., B. M. Fingleton, L. A. Rudolph-Owen, K. J. Goss, B. Rubinfeld, P. Polakis, and L. M. Matrisian.** 1999. The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. Oncogene **18:**2883-2891.

68. **Crawford, H. C., U. S. Krishna, D. A. Israel, L. M. Matrisian, M. K. Washington, and R. M. Peek, Jr.** 2003. *Helicobacter pylori* strain-selective induction of matrix metalloproteinase-7 *in vitro* and within gastric mucosa. Gastroenterology **125:**1125-1136.

69. **Crawford, H. C., C. R. Scoggins, M. K. Washington, L. M. Matrisian, and S. D. Leach.** 2002. Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. J Clin Invest **109:**1437-1444.

70. **Czajkowsky, D. M., H. Iwamoto, T. L. Cover, and Z. Shao.** 1999. The vacuolating toxin from helicobacter pylori forms hexameric pores in lipid bilayers at low pH [In Process Citation]. Proc Natl Acad Sci U S A **96:**2001-2006.

71. **Daniel, J. M.** 2007. Dancing in and out of the nucleus: p120(ctn) and the transcription factor Kaiso. Biochim Biophys Acta **1773:**59-68.

72. **Daniel, J. M., and A. B. Reynolds.** 1999. The catenin p120(ctn) interacts with Kaiso, a novel BTB/POZ domain zinc finger transcription factor. Mol Cell Biol **19:**3614-3623.

73. **Daniel, J. M., C. M. Spring, H. C. Crawford, A. B. Reynolds, and A. Baig.** 2002. The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. Nucleic Acids Res **30:**2911-2919.

74. **Davis, M. A., R. C. Ireton, and A. B. Reynolds.** 2003. A core function for p120-catenin in cadherin turnover. J Cell Biol **163:**525-534.

75. **de Martel, C., A. E. Llosa, S. M. Farr, G. D. Friedman, J. H. Vogelman, N. Orentreich, D. A. Corley, and J. Parsonnet.** 2005. Helicobacter pylori infection and the risk of development of esophageal adenocarcinoma. J Infect Dis **191:**761-767.

76. **Denhardt, D. T.** 1996. Oncogene-initiated aberrant signaling engenders the metastatic phenotype: synergistic transcription factor interactions are targets for cancer therapy. Crit Rev Oncog **7:**261-291.

77. **Ding, S. Z., I. N. Olekhnovich, T. L. Cover, R. M. Peek, Jr., M. F. Smith, Jr., and J. B. Goldberg.** 2008. Helicobacter pylori and mitogen-activated protein kinases mediate activator protein-1 (AP-1) subcomponent protein expression and DNA-binding activity in gastric epithelial cells. FEMS Immunol Med Microbiol **53:**385-394.

78. **Doenges, J. L.** 1938. Spirochaetes in the gastric glands of *Macacus rhesus* and humans without definite history of related disease. Proc Soc Exp Med Biol **38:**536-538.

79. **Dvorak, H. F.** 1986. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med **315:**1650-1659.

80. **Egeblad, M., and Z. Werb.** 2002. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer **2:**161-174.

81. **El-Omar, E. M., M. Carrington, W. H. Chow, K. E. McColl, J. H. Bream, H. A. Young, J. Herrera, J. Lissowska, C. C. Yuan, N. Rothman, G. Lanyon, M. Martin, J. F. Fraumeni, Jr., and C. S. Rabkin.** 2000. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature **404:**398-402.

82. **Ernst, P.** 1999. Review article: the role of inflammation in the pathogenesis of gastric cancer. Aliment Pharmacol Ther **13 Suppl 1:**13-18.

83. **Ernst, P. B., and B. D. Gold.** 2000. THE DISEASE SPECTRUM OF HELICOBACTER PYLORI: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. Annu Rev Microbiol **54:**615-640.

84. **Everhart, J. E.** 2000. Recent developments in the epidemiology of Helicobacter pylori. Gastroenterol Clin North Am **29:**559-578.

85. **Fagotto, F., U. Gluck, and B. M. Gumbiner.** 1998. Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin. Curr Biol **8:**181-190.

86. **Fan, X., S. E. Crowe, S. Behar, H. Gunasena, G. Ye, H. Haeberle, N. Van Houten, W. K. Gourley, P. B. Ernst, and V. E. Reyes.** 1998. The effect of class II major histocompatibility complex expression on adherence of Helicobacter pylori and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1-mediated damage. J Exp Med **187:**1659-1669.

87. **Fingleton, B., W. C. Powell, H. C. Crawford, J. R. Couchman, and L. M. Matrisian.** 2007. A rat monoclonal antibody that recognizes pro- and active MMP-7 indicates polarized expression in vivo. Hybridoma (Larchmt) **26:**22-27.

88. **Fingleton, B., T. Vargo-Gogola, H. C. Crawford, and L. M. Matrisian.** 2001. Matrilysin [MMP-7] expression selects for cells with reduced sensitivity to apoptosis. Neoplasia **3:**459-468.

89. **Fischer, W., J. Puls, R. Buhrdorf, B. Gebert, S. Odenbreit, and R. Haas.** 2001. Systematic mutagenesis of the Helicobacter pylori cag pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. Mol Microbiol **42:**1337-1348.

90. **Fitzgerald, M. L., Z. Wang, P. W. Park, G. Murphy, and M. Bernfield.** 2000. Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling

pathways and mediated by a TIMP-3-sensitive metalloproteinase. J Cell Biol **148:**811-824.

91. **Fox, J. G., C. A. Dangler, W. Sager, R. Borkowski, and J. M. Gliatto.** 1997. Helicobacter mustelae-associated gastric adenocarcinoma in ferrets (Mustela putorius furo). Vet Pathol **34:**225-229.

92. **Fox, J. G., A. B. Rogers, M. Ihrig, N. S. Taylor, M. T. Whary, G. Dockray, A. Varro, and T. C. Wang.** 2003. Helicobacter pylori-associated gastric cancer in INS-GAS mice is gender specific. Cancer Res **63:**942-950.

93. **Fox, J. G., B. J. Sheppard, C. A. Dangler, M. T. Whary, M. Ihrig, and T. C. Wang.** 2002. Germ-line p53-targeted disruption inhibits helicobacter-induced premalignant lesions and invasive gastric carcinoma through down-regulation of Th1 proinflammatory responses. Cancer Res **62:**696-702.

94. **Fox, J. G., T. C. Wang, A. B. Rogers, T. Poutahidis, Z. Ge, N. Taylor, C. A. Dangler, D. A. Israel, U. Krishna, K. Gaus, and R. M. Peek, Jr.** 2003. Host and microbial constituents influence *Helicobacter pylori*-induced cancer in a murine model of hypergastrinemia. Gastroenterology **124:**1879-1890.

95. **Fox, J. G., J. S. Wishnok, J. C. Murphy, S. R. Tannenbaum, and P. Correa.** 1993. MNNG-induced gastric carcinoma in ferrets infected with Helicobacter mustelae. Carcinogenesis **14:**1957-1961.

96. **Franco, A. T., D. A. Israel, M. K. Washington, U. Krishna, J. G. Fox, A. B. Rogers, A. S. Neish, L. Collier-Hyams, G. I. Perez-Perez, M. Hatakeyama, R. Whitehead, K. Gaus, D. P. O'Brien, J. Romero-Gallo, and R. M. Peek, Jr.** 2005. Activation of beta-catenin by carcinogenic *Helicobacter pylori*. Proc Natl Acad Sci U S A **102:**10646-10651.

97. **Franco, A. T., E. Johnston, U. Krishna, Y. Yamaoka, D. A. Israel, T. A. Nagy, L. E. Wroblewski, M. B. Piazuelo, P. Correa, and R. M. Peek, Jr.** 2008. Regulation of gastric carcinogenesis by Helicobacter pylori virulence factors. Cancer Res **68:**379-387.

98. **Freedburg AS, B. L.** 1940. The presence of spirochaetes in human gastric mucosa. Am J Dig Dis **7:**443-445.

99. **Frey, M. R., M. L. Saxon, X. Zhao, A. Rollins, S. S. Evans, and J. D. Black.** 1997. Protein kinase C isozyme-mediated cell cycle arrest involves induction of p21(waf1/cip1) and p27(kip1) and hypophosphorylation of the retinoblastoma protein in intestinal epithelial cells. J Biol Chem **272:**9424-9435.

100. **Fukumoto, Y., Y. Shintani, A. B. Reynolds, K. R. Johnson, and M. J. Wheelock.** 2008. The regulatory or phosphorylation domain of p120 catenin controls E-cadherin dynamics at the plasma membrane. Exp Cell Res **314:**52-67.

101. **Gao, Z. H., M. S. Tretiakova, W. H. Liu, C. Gong, P. D. Farris, and J. Hart.** 2006. Association of E-cadherin, matrix metalloproteinases, and tissue inhibitors of metalloproteinases with the progression and metastasis of hepatocellular carcinoma. Mod Pathol **19:**533-540.

102. **Gearing, A. J., P. Beckett, M. Christodoulou, M. Churchill, J. Clements, A. H. Davidson, A. H. Drummond, W. A. Galloway, R. Gilbert, J. L. Gordon, and et al.** 1994. Processing of tumour necrosis factor-alpha precursor by metalloproteinases. Nature **370:**555-557.

103. **Gebert, B., W. Fischer, E. Weiss, R. Hoffmann, and R. Haas.** 2003. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. Science **301:**1099-1102.

104. **Girardin, S. E., I. G. Boneca, L. A. Carneiro, A. Antignac, M. Jehanno, J. Viala, K. Tedin, M. K. Taha, A. Labigne, U. Zahringer, A. J. Coyle, P. S. DiStefano, J. Bertin, P. J. Sansonetti, and D. J. Philpott.** 2003. Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan. Science **300:**1584-1587.

105. **Girardin, S. E., I. G. Boneca, J. Viala, M. Chamaillard, A. Labigne, G. Thomas, D. J. Philpott, and P. J. Sansonetti.** 2003. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem **278:**8869-8872.

106. **Go, M. F., V. Kapur, D. Y. Graham, and J. M. Musser.** 1996. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. J Bacteriol **178:**3934-3938.

107. **Goodman, K. J., P. Correa, H. J. Tengana Aux, H. Ramirez, J. P. DeLany, O. Guerrero Pepinosa, M. Lopez Quinones, and T. Collazos Parra.** 1996. Helicobacter pylori infection in the Colombian Andes: a population-based study of transmission pathways. Am J Epidemiol **144:**290-299.

108. **Grosheva, I., M. Shtutman, M. Elbaum, and A. D. Bershadsky.** 2001. p120 catenin affects cell motility via modulation of activity of Rho-family GTPases: a link between cell-cell contact formation and regulation of cell locomotion. J Cell Sci **114:**695-707.

109. **Gustavson, M. D., H. C. Crawford, B. Fingleton, and L. M. Matrisian.** 2004. Tcf binding sequence and position determines beta-catenin and Lef-1 responsiveness of MMP-7 promoters. Mol Carcinog **41:**125-139.

110. **Hamburger, A. W., and S. E. Salmon.** 1977. Primary bioassay of human tumor stem cells. Science **197:**461-463.

111. **Hansson, L. E., L. Engstrand, O. Nyren, D. J. Evans, Jr., A. Lindgren, R. Bergstrom, B. Andersson, L. Athlin, O. Bendtsen, and P. Tracz.** 1993. *Helicobacter pylori* infection: independent risk indicator of gastric adenocarcinoma. Gastroenterology **105:**1098-1103.

112. **Harrell, P. C., L. J. McCawley, B. Fingleton, J. O. McIntyre, and L. M. Matrisian.** 2005. Proliferative effects of apical, but not basal, matrix metalloproteinase-7 activity in polarized MDCK cells. Exp Cell Res **303:**308-320.

113. **Hashimoto, K., Y. Kihira, Y. Matuo, and T. Usui.** 1998. Expression of matrix metalloproteinase-7 and tissue inhibitor of metalloproteinase-1 in human prostate. J Urol **160:**1872-1876.

114. **Hearnes, J. M., D. J. Mays, K. L. Schavolt, L. Tang, X. Jiang, and J. A. Pietenpol.** 2005. Chromatin immunoprecipitation-based screen to identify functional genomic binding sites for sequence-specific transactivators. Mol Cell Biol **25:**10148-10158.

115. **Hellmig, S., S. Ott, P. Rosenstiel, U. Robert Folsch, J. Hampe, and S. Schreiber.** 2006. Genetic variants in matrix metalloproteinase genes are associated with development of gastric ulcer in H. Pylori infection. Am J Gastroenterol **101:**29-35.

116. **Henderson, B. R.** 2000. Nuclear-cytoplasmic shuttling of APC regulates beta-catenin subcellular localization and turnover. Nat Cell Biol **2:**653-660.

117. **Heppner, K. J., L. M. Matrisian, R. A. Jensen, and W. H. Rodgers.** 1996. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol **149:**273-282.

118. **Higashi, H., R. Tsutsumi, A. Fujita, S. Yamazaki, M. Asaka, T. Azuma, and M. Hatakeyama.** 2002. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. Proc Natl Acad Sci U S A **99:**14428-14433.

119. **Higashi, H., R. Tsutsumi, S. Muto, T. Sugiyama, T. Azuma, M. Asaka, and M. Hatakeyama.** 2002. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. Science **295:**683-686.

120. **Higashi, H., R. Tsutsumi, S. Muto, T. Sugiyama, T. Azuma, M. Asaka, and M. Hatakeyama.** 2002. SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science **295:**683-686.

121. **Hippo, Y., H. Taniguchi, S. Tsutsumi, N. Machida, J. M. Chong, M. Fukayama, T. Kodama, and H. Aburatani.** 2002. Global gene expression analysis of gastric cancer by oligonucleotide microarrays. Cancer Res **62:**233-240.

122. **Hirata, Y., S. Maeda, Y. Mitsuno, M. Akanuma, Y. Yamaji, K. Ogura, H. Yoshida, Y. Shiratori, and M. Omata.** 2001. *Helicobacter pylori* activates the *cyclin D1* gene through mitogen-activated protein kinase pathway in gastric cancer cells. Infect Immun **69:**3965-3971.

123. **Hirata, Y., S. Maeda, Y. Mitsuno, K. Tateishi, A. Yanai, M. Akanuma, H. Yoshida, T. Kawabe, Y. Shiratori, and M. Omata.** 2002. Helicobacter pylori CagA protein activates serum response element-driven transcription independently of tyrosine phosphorylation. Gastroenterology **123:**1962-1971.

124. **Hirayama, F., S. Takagi, E. Iwao, Y. Yokoyama, K. Haga, and S. Hanada.** 1999. Development of poorly differentiated adenocarcinoma and carcinoid due to long-term Helicobacter pylori colonization in Mongolian gerbils. J Gastroenterol **34:**450-454.

125. **Honda, M., M. Mori, H. Ueo, K. Sugimachi, and T. Akiyoshi.** 1996. Matrix metalloproteinase-7 expression in gastric carcinoma. Gut **39:**444-448.

126. **Honda, S., T. Fujioka, M. Tokieda, R. Satoh, A. Nishizono, and M. Nasu.** 1998. Development of Helicobacter pylori-induced gastric carcinoma in Mongolian gerbils. Cancer Res **58:**4255-4259.

127. **Hoogewerf, A. J., G. S. Kuschert, A. E. Proudfoot, F. Borlat, I. Clark-Lewis, C. A. Power, and T. N. Wells.** 1997. Glycosaminoglycans mediate cell surface oligomerization of chemokines. Biochemistry **36:**13570-13578.

128. **Houghton, J., C. Stoicov, S. Nomura, A. B. Rogers, J. Carlson, H. Li, X. Cai, J. G. Fox, J. R. Goldenring, and T. C. Wang.** 2004. Gastric cancer originating from bone marrow-derived cells. Science **306:**1568-1571.

129. **Houghton, J., and T. C. Wang.** 2005. Helicobacter pylori and gastric cancer: a new paradigm for inflammation-associated epithelial cancers. Gastroenterology **128:**1567-1578.

130. **Huber, O., R. Korn, J. McLaughlin, M. Ohsugi, B. G. Herrmann, and R. Kemler.** 1996. Nuclear localization of beta-catenin by interaction with transcription factor LEF-1. Mech Dev **59:**3-10.

131. **Huo, N., Y. Ichikawa, M. Kamiyama, T. Ishikawa, Y. Hamaguchi, S. Hasegawa, Y. Nagashima, K. Miyazaki, and H. Shimada.** 2002. MMP-7 (matrilysin) accelerated growth of human umbilical vein endothelial cells. Cancer Lett **177:**95-100.

132. **Ii, M., H. Yamamoto, Y. Adachi, Y. Maruyama, and Y. Shinomura.** 2006. Role of matrix metalloproteinase-7 (matrilysin) in human cancer invasion, apoptosis, growth, and angiogenesis. Exp Biol Med (Maywood) **231:**20-27.

133. **Impola, U., V. J. Uitto, J. Hietanen, L. Hakkinen, L. Zhang, H. Larjava, K. Isaka, and U. Saarialho-Kere.** 2004. Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. J Pathol **202:**14-22.

134. **Inohara, N., and G. Nunez.** 2003. NODs: intracellular proteins involved in inflammation and apoptosis. Nat Rev Immunol **3:**371-382.

135. **Ireton, R. C., M. A. Davis, J. van Hengel, D. J. Mariner, K. Barnes, M. A. Thoreson, P. Z. Anastasiadis, L. Matrisian, L. M. Bundy, L. Sealy, B. Gilbert, F. van Roy, and A. B. Reynolds.** 2002. A novel role for p120 catenin in E-cadherin function. J Cell Biol **159:**465-476.

136. **Israel, D. A., N. Salama, U. Krishna, U. M. Rieger, J. C. Atherton, S. Falkow, and R. M. Peek, Jr.** 2001. Helicobacter pylori genetic diversity within the gastric niche of a single human host. Proc Natl Acad Sci U S A **98:**14625-14630.

137. **Iwamoto, H., D. M. Czajkowsky, T. L. Cover, G. Szabo, and Z. Shao.** 1999. VacA from Helicobacter pylori: a hexameric chloride channel. FEBS Lett **450:**101-104.

138. **Jawhari, A. U., M. Noda, M. Pignatelli, and M. Farthing.** 1999. Up-regulated cytoplasmic expression, with reduced membranous distribution, of the src substrate p120(ctn) in gastric carcinoma. J Pathol **189:**180-185.

139. **Jones, L. E., M. J. Humphreys, F. Campbell, J. P. Neoptolemos, and M. T. Boyd.** 2004. Comprehensive analysis of matrix metalloproteinase and tissue inhibitor expression in pancreatic cancer: increased expression of matrix metalloproteinase-7 predicts poor survival. Clin Cancer Res **10:**2832-2845.

140. **Jung, H. C., J. M. Kim, I. S. Song, and C. Y. Kim.** 1997. Helicobacter pylori induces an array of pro-inflammatory cytokines in human gastric epithelial cells: quantification of mRNA for interleukin-8, -1 alpha/beta, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1 and tumour necrosis factor-alpha. J Gastroenterol Hepatol **12:**473-480.

141. **Karatzas, G., A. J. Karayiannakis, K. N. Syrigos, E. Chatzigianni, S. Papanikolaou, F. Riza, and D. Papanikolaou.** 1999. E-cadherin expression correlates with tumor differentiation in colorectal cancer. Hepatogastroenterology **46:**232-235.

142. **Karayiannakis, A. J., K. N. Syrigos, D. Alexiou, N. Kalahanis, T. Rosenberg, E. Bastounis, and M. Pignatelli.** 1999. Expression patterns of the novel catenin p120cas in gastrointestinal cancers. Anticancer Res **19:**4401-4405.

143. **Karelina, T. V., G. I. Goldberg, and A. Z. Eisen.** 1994. Matrilysin (PUMP) correlates with dermal invasion during appendageal development and cutaneous neoplasia. J Invest Dermatol **103:**482-487.

144. **Kawahara, T., S. Teshima, A. Oka, T. Sugiyama, K. Kishi, and K. Rokutan.** 2001. Type I Helicobacter pylori lipopolysaccharide stimulates toll-like receptor 4 and activates mitogen oxidase 1 in gastric pit cells. Infect Immun **69:**4382-4389.

145. **Kawasaki, K., T. Kawakami, H. Watabe, F. Itoh, M. Mizoguchi, and Y. Soma.** 2007. Expression of matrilysin (matrix metalloproteinase-7) in primary cutaneous and metastatic melanoma. Br J Dermatol **156:**613-619.

146. **Keates, S., S. Sougioultzis, A. C. Keates, D. Zhao, R. M. Peek, Jr., L. M. Shaw, and C. P. Kelly.** 2001. cag+ Helicobacter pylori induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem **276:**48127-48134.

147. **Keates, S., S. Sougioultzis, A. C. Keates, D. Zhao, R. M. Peek, Jr., L. M. Shaw, and C. P. Kelly.** 2001. *cag+ Helicobacter pylori* induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem **276:**48127-48134.

148. **Keilhack, H., U. Hellman, J. van Hengel, F. van Roy, J. Godovac-Zimmermann, and F. D. Bohmer.** 2000. The protein-tyrosine phosphatase SHP-1 binds to and dephosphorylates p120 catenin. J Biol Chem **275:**26376-26384.

149. **Keirsebilck, A., S. Bonne, K. Staes, J. van Hengel, F. Nollet, A. Reynolds, and F. van Roy.** 1998. Molecular cloning of the human p120ctn catenin gene (CTNND1): expression of multiple alternatively spliced isoforms. Genomics **50:**129-146.

150. **Kelly, K. F., C. M. Spring, A. A. Otchere, and J. M. Daniel.** 2004. NLS-dependent nuclear localization of p120ctn is necessary to relieve Kaiso-mediated transcriptional repression. J Cell Sci **117:**2675-2686.

151. **Kim, S. G., J. S. Kim, J. M. Kim, H. Chae Jung, and I. Sung Song.** 2005. Inhibition of proinflammatory cytokine expression by NF-kappaB (p65) antisense oligonucleotide in Helicobacter pylori-infected mice. Helicobacter **10:**559-566.

152. **Kim, S. W., J. I. Park, C. M. Spring, A. K. Sater, H. Ji, A. A. Otchere, J. M. Daniel, and P. D. McCrea.** 2004. Non-canonical Wnt signals are modulated by the Kaiso transcriptional repressor and p120-catenin. Nat Cell Biol **6:**1212-1220.

153. **Kim, S. Y., Y. C. Lee, H. K. Kim, and M. J. Blaser.** 2006. Helicobacter pylori CagA transfection of gastric epithelial cells induces interleukin-8. Cell Microbiol **8:**97-106.

154. **Kivi, M., A. L. Johansson, M. Reilly, and Y. Tindberg.** 2005. Helicobacter pylori status in family members as risk factors for infection in children. Epidemiol Infect **133:**645-652.

155. **Kivi, M., and Y. Tindberg.** 2006. Helicobacter pylori occurrence and transmission: a family affair? Scand J Infect Dis **38:**407-417.

156. **Kivisaari, A. K., M. Kallajoki, T. Mirtti, J. A. McGrath, J. W. Bauer, F. Weber, R. Konigova, D. Sawamura, K. C. Sato-Matsumura, H. Shimizu, M. Csikos, K. Sinemus, W. Beckert, and V. M. Kahari.** 2008. Transformation-specific matrix metalloproteinases (MMP)-7 and MMP-13 are expressed by tumour cells in epidermolysis bullosa-associated squamous cell carcinomas. Br J Dermatol **158:**778-785.

157. **Klausz, G., E. Buzas, P. Scharek, L. Tiszlavicz, Z. Gyulai, A. K. Fulop, A. Falus, and Y. Mandi.** 2004. Effects of Helicobacter pylori infection on gastric inflammation and local cytokine production in histamine-deficient (histidine decarboxylase knock-out) mice. Immunol Lett **94:**223-228.

158. **Krueger, S., T. Hundertmark, D. Kuester, T. Kalinski, U. Peitz, and A. Roessner.** 2007. *Helicobacter pylori* alters the distribution of ZO-1 and p120ctn in primary human gastric epithelial cells. Pathol Res Pract **203:**433-444.

159. **Kubben, F. J., C. F. Sier, M. J. Meijer, M. van den Berg, J. J. van der Reijden, G. Griffioen, C. J. van de Velde, C. B. Lamers, and H. W. Verspaget.** 2006. Clinical impact of MMP and TIMP gene polymorphisms in gastric cancer. Br J Cancer **95:**744-751.

160. **Kuipers, E. J., G. I. Perez-Perez, S. G. Meuwissen, and M. J. Blaser.** 1995. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA* status. J Natl Cancer Inst **87:**1777-1780.

161. **Kundu, P., A. K. Mukhopadhyay, R. Patra, A. Banerjee, D. E. Berg, and S. Swarnakar.** 2006. Cag pathogenicity island-independent up-regulation of matrix metalloproteinases-9 and -2 secretion and expression in mice by Helicobacter pylori infection. J Biol Chem **281:**34651-34662.

162. **Kusters, J. G., A. H. van Vliet, and E. J. Kuipers.** 2006. Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev **19:**449-490.

163. **Kwok, T., D. Zabler, S. Urman, M. Rohde, R. Hartig, S. Wessler, R. Misselwitz, J. Berger, N. Sewald, W. Konig, and S. Backert.** 2007. *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature **449:**862-866.

164. **Lauren, P.** 1965. The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. An Attempt at a Histo-Clinical Classification. Acta Pathol Microbiol Scand **64:**31-49.

165. **Lee, K. H., E. Y. Choi, M. S. Hyun, B. I. Jang, T. N. Kim, S. W. Kim, S. K. Song, J. H. Kim, and J. R. Kim.** 2007. Association of extracellular cleavage of

E-cadherin mediated by MMP-7 with HGF-induced in vitro invasion in human stomach cancer cells. Eur Surg Res **39:**208-215.

166. **Lehours, P., and O. Yilmaz.** 2007. Epidemiology of Helicobacter pylori infection. Helicobacter **12 Suppl 1:**1-3.

167. **Leung, W. K., S. R. Lin, J. Y. Ching, K. F. To, E. K. Ng, F. K. Chan, J. Y. Lau, and J. J. Sung.** 2004. Factors predicting progression of gastric intestinal metaplasia: results of a randomised trial on Helicobacter pylori eradication. Gut **53:**1244-1249.

168. **Levy, L., C. Neuveut, C. A. Renard, P. Charneau, S. Branchereau, F. Gauthier, J. T. Van Nhieu, D. Cherqui, A. F. Petit-Bertron, D. Mathieu, and M. A. Buendia.** 2002. Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. J Biol Chem **277:**42386-42393.

169. **Ley, C., A. Mohar, J. Guarner, R. Herrera-Goepfert, L. S. Figueroa, D. Halperin, I. Johnstone, and J. Parsonnet.** 2004. Helicobacter pylori eradication and gastric preneoplastic conditions: a randomized, double-blind, placebo-controlled trial. Cancer Epidemiol Biomarkers Prev **13:**4-10.

170. **Li, G. Q., H. H. Xia, M. H. Chen, Q. Gu, J. D. Wang, J. Z. Peng, A. O. Chan, C. H. Cho, H. L. So, S. K. Lam, P. J. Hu, Y. J. Liang, H. L. Lin, D. E. Berg, Z. H. Feng, R. Langenbach, and B. C. Wong.** 2006. Effects of cyclooxygenase-1 and -2 gene disruption on Helicobacter pylori-induced gastric inflammation. J Infect Dis **193:**1037-1046.

171. **Li, H. C., C. Stoicov, A. B. Rogers, and J. Houghton.** 2006. Stem cells and cancer: evidence for bone marrow stem cells in epithelial cancers. World J Gastroenterol **12:**363-371.

172. **Li, Q., P. W. Park, C. L. Wilson, and W. C. Parks.** 2002. Matrilysin shedding of syndecan-1 regulates chemokine mobilization and transepithelial efflux of neutrophils in acute lung injury. Cell **111:**635-646.

173. **Li, Y. J., Z. M. Wei, Y. X. Meng, and X. R. Ji.** 2005. Beta-catenin up-regulates the expression of cyclinD1, c-myc and MMP-7 in human pancreatic cancer: relationships with carcinogenesis and metastasis. World J Gastroenterol **11:**2117-2123.

174. **Lockard, V. G., and R. K. Boler.** 1970. Ultrastructure of a spiraled microorganism in the gastric mucosa of dogs. Am J Vet Res **31:**1453-1462.

175. **Loffeld, R. J., B. F. Werdmuller, J. G. Kuster, G. I. Perez-Perez, M. J. Blaser, and E. J. Kuipers.** 2000. Colonization with *cagA*-positive *Helicobacter pylori* strains inversely associated with reflux esophagitis and Barrett's esophagus. Digestion **62:**95-99.

176. **Lopez-Boado, Y. S., C. L. Wilson, L. V. Hooper, J. I. Gordon, S. J. Hultgren, and W. C. Parks.** 2000. Bacterial exposure induces and activates matrilysin in mucosal epithelial cells. J Cell Biol **148:**1305-1315.

177. **Lynch, C. C., T. Vargo-Gogola, M. D. Martin, B. Fingleton, H. C. Crawford, and L. M. Matrisian.** 2007. Matrix metalloproteinase 7 mediates mammary epithelial cell tumorigenesis through the ErbB4 receptor. Cancer Res **67:**6760-6767.

178. **Lynch, D. A., N. P. Mapstone, A. M. Clarke, G. M. Sobala, P. Jackson, L. Morrison, M. F. Dixon, P. Quirke, and A. T. Axon.** 1995. Cell proliferation in Helicobacter pylori associated gastritis and the effect of eradication therapy. Gut **36:**346-350.

179. **Machado, J. C., P. Pharoah, S. Sousa, R. Carvalho, C. Oliveira, C. Figueiredo, A. Amorim, R. Seruca, C. Caldas, F. Carneiro, and M. Sobrinho-Simoes.** 2001. Interleukin 1B and interleukin 1RN polymorphisms are associated with increased risk of gastric carcinoma. Gastroenterology **121:**823-829.

180. **Malaty, H. M., and D. Y. Graham.** 1994. Importance of childhood socioeconomic status on the current prevalence of Helicobacter pylori infection. Gut **35:**742-745.

181. **Mandell, L., A. P. Moran, A. Cocchiarella, J. Houghton, N. Taylor, J. G. Fox, T. C. Wang, and E. A. Kurt-Jones.** 2004. Intact gram-negative Helicobacter pylori, Helicobacter felis, and Helicobacter hepaticus bacteria activate innate immunity via toll-like receptor 2 but not toll-like receptor 4. Infect Immun **72:**6446-6454.

182. **Mariner, D. J., P. Anastasiadis, H. Keilhack, F. D. Bohmer, J. Wang, and A. B. Reynolds.** 2001. Identification of Src phosphorylation sites in the catenin p120ctn. J Biol Chem **276:**28006-28013.

183. **Mariner, D. J., M. A. Davis, and A. B. Reynolds.** 2004. EGFR signaling to p120-catenin through phosphorylation at Y228. J Cell Sci **117:**1339-1350.

184. **Marshall, B.** 2007 1994, posting date. Epidemiology. The Helicobacter Foundation. [Online.]

185. **Marshall, B. J., J. A. Armstrong, D. B. McGechie, and R. J. Glancy.** 1985. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust **142:**436-439.

186. **Marshall, B. J., C. S. Goodwin, J. R. Warren, R. Murray, E. D. Blincow, S. J. Blackbourn, M. Phillips, T. E. Waters, and C. R. Sanderson.** 1988. Prospective double-blind trial of duodenal ulcer relapse after eradication of Campylobacter pylori. Lancet **2:**1437-1442.

187. **Marshall BJ, R. H., Annear DI, Goodwin CS, Pearman J, Warren JR, Armstrong J.** 1984. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. Microbios Letters **25:**83-88.

188. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **1:**1311-1315.

189. **Marshall, B. J., and J. R. Warren.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet **321:**1273-1275.

190. **Mayerle, J., H. Friess, M. W. Buchler, J. Schnekenburger, F. U. Weiss, K. P. Zimmer, W. Domschke, and M. M. Lerch.** 2003. Up-regulation, nuclear import, and tumor growth stimulation of the adhesion protein p120 in pancreatic cancer. Gastroenterology **124:**949-960.

191. **McCaig, C., C. Duval, E. Hemers, I. Steele, D. M. Pritchard, S. Przemeck, R. Dimaline, S. Ahmed, K. Bodger, D. D. Kerrigan, T. C. Wang, G. J. Dockray, and A. Varro.** 2006. The role of matrix metalloproteinase-7 in redefining the gastric microenvironment in response to *Helicobacter pylori*. Gastroenterology **130:**1754-1763.

192. **McCallion, W. A., L. J. Murray, A. G. Bailie, A. M. Dalzell, D. P. O'Reilly, and K. B. Bamford.** 1996. Helicobacter pylori infection in children: relation with current household living conditions. Gut **39:**18-21.

193. **McCawley, L. J., and L. M. Matrisian.** 2001. Matrix metalloproteinases: they're not just for matrix anymore! Curr Opin Cell Biol **13:**534-540.

194. **McDonnell, S., M. Navre, R. J. Coffey, Jr., and L. M. Matrisian.** 1991. Expression and localization of the matrix metalloproteinase pump-1 (MMP-7) in human gastric and colon carcinomas. Mol Carcinog **4:**527-533.

195. **McGuire, J. K., Q. Li, and W. C. Parks.** 2003. Matrilysin (matrix metalloproteinase-7) mediates E-cadherin ectodomain shedding in injured lung epithelium. Am J Pathol **162:**1831-1843.

196. **Mendall, M. A., P. M. Goggin, N. Molineaux, J. Levy, T. Toosy, D. Strachan, and T. C. Northfield.** 1992. Childhood living conditions and Helicobacter pylori seropositivity in adult life. Lancet **339:**896-897.

197. **Mera, R., E. T. Fontham, L. E. Bravo, J. C. Bravo, M. B. Piazuelo, M. C. Camargo, and P. Correa.** 2005. Long term follow up of patients treated for Helicobacter pylori infection. Gut **54:**1536-1540.

198. **Meyer-ter-Vehn, T., A. Covacci, M. Kist, and H. L. Pahl.** 2000. Helicobacter pylori activates mitogen-activated protein kinase cascades and induces expression of the proto-oncogenes c-fos and c-jun. J Biol Chem **275:**16064-16072.

199. **Miehlke, S., A. Hackelsberger, A. Meining, U. von Arnim, P. Muller, T. Ochsenkuhn, N. Lehn, P. Malfertheiner, M. Stolte, and E. Bayerdorffer.** 1997. Histological diagnosis of *Helicobacter pylori* gastritis is predictive of a high risk of gastric carcinoma. Int J Cancer **73:**837-839.

200. **Mimuro, H., T. Suzuki, J. Tanaka, M. Asahi, R. Haas, and C. Sasakawa.** 2002. Grb2 is a key mediator of helicobacter pylori CagA protein activities. Mol Cell **10:**745-755.

201. **Miranda, K. C., S. R. Joseph, A. S. Yap, R. D. Teasdale, and J. L. Stow.** 2003. Contextual binding of p120ctn to E-cadherin at the basolateral plasma membrane in polarized epithelia. J Biol Chem **278:**43480-43488.

202. **Mitchell, H. M.** 1999. The epidemiology of Helicobacter pylori. Curr Top Microbiol Immunol **241:**11-30.

203. **Miyamoto, S., K. Yano, S. Sugimoto, G. Ishii, T. Hasebe, Y. Endoh, K. Kodama, M. Goya, T. Chiba, and A. Ochiai.** 2004. Matrix metalloproteinase-7 facilitates insulin-like growth factor bioavailability through its proteinase activity on insulin-like growth factor binding protein 3. Cancer Res **64:**665-671.

204. **Mo, Y. Y., and A. B. Reynolds.** 1996. Identification of murine p120 isoforms and heterogeneous expression of p120cas isoforms in human tumor cell lines. Cancer Res **56:**2633-2640.

205. **Mochizuki, S., M. Shimoda, T. Shiomi, Y. Fujii, and Y. Okada.** 2004. ADAM28 is activated by MMP-7 (matrilysin-1) and cleaves insulin-like growth factor binding protein-3. Biochem Biophys Res Commun **315:**79-84.

206. **Mohan, M. J., T. Seaton, J. Mitchell, A. Howe, K. Blackburn, W. Burkhart, M. Moyer, I. Patel, G. M. Waitt, J. D. Becherer, M. L. Moss, and M. E. Milla.** 2002. The tumor necrosis factor-alpha converting enzyme (TACE): a unique metalloproteinase with highly defined substrate selectivity. Biochemistry **41:**9462-9469.

207. **Mori, M., G. F. Barnard, K. Mimori, H. Ueo, T. Akiyoshi, and K. Sugimachi.** 1995. Overexpression of matrix metalloproteinase-7 mRNA in human colon carcinomas. Cancer **75:**1516-1519.

208. **Moss, S. F., and S. Sood.** 2003. *Helicobacter pylori*. Curr Opin Infect Dis **16:**445-451.

209. **Munemitsu, S., I. Albert, B. Souza, B. Rubinfeld, and P. Polakis.** 1995. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A **92:**3046-3050.

210. **Murata-Kamiya, N., Y. Kurashima, Y. Teishikata, Y. Yamahashi, Y. Saito, H. Higashi, H. Aburatani, T. Akiyama, R. M. Peek, Jr., T. Azuma, and M.**

Hatakeyama. 2007. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene **26:**4617-4626.

211. **Nagashima, Y., S. Hasegawa, N. Koshikawa, A. Taki, Y. Ichikawa, H. Kitamura, K. Misugi, Y. Kihira, Y. Matuo, H. Yasumitsu, and K. Miyazaki.** 1997. Expression of matrilysin in vascular endothelial cells adjacent to matrilysin-producing tumors. Int J Cancer **72:**441-445.

212. **Nakajima, N., H. Kuwayama, Y. Ito, A. Iwasaki, and Y. Arakawa.** 1997. Helicobacter pylori, neutrophils, interleukins, and gastric epithelial proliferation. J Clin Gastroenterol **25 Suppl 1:**S198-202.

213. **Nakamura, M., S. Miyamoto, H. Maeda, G. Ishii, T. Hasebe, T. Chiba, M. Asaka, and A. Ochiai.** 2005. Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. Biochem Biophys Res Commun **333:**1011-1016.

214. **Naumann, M., S. Wessler, C. Bartsch, B. Wieland, A. Covacci, R. Haas, and T. F. Meyer.** 1999. Activation of activator protein 1 and stress response kinases in epithelial cells colonized by Helicobacter pylori encoding the cag pathogenicity island. J Biol Chem **274:**31655-31662.

215. **Nelson, A. R., B. Fingleton, M. L. Rothenberg, and L. M. Matrisian.** 2000. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol **18:**1135-1149.

216. **Nishizuka, I., Y. Ichikawa, T. Ishikawa, M. Kamiyama, S. Hasegawa, N. Momiyama, K. Miyazaki, and H. Shimada.** 2001. Matrilysin stimulates DNA synthesis of cultured vascular endothelial cells and induces angiogenesis in vivo. Cancer Lett **173:**175-182.

217. **Nobes, C. D., and A. Hall.** 1999. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol **144:**1235-1244.

218. **Noe, V., B. Fingleton, K. Jacobs, H. C. Crawford, S. Vermeulen, W. Steelant, E. Bruyneel, L. M. Matrisian, and M. Mareel.** 2001. Release of an invasion promoter E-cadherin fragment by matrilysin and stromelysin-1. J Cell Sci **114:**111-118.

219. **Nomura, A., G. N. Stemmermann, P. H. Chyou, I. Kato, G. I. Perez-Perez, and M. J. Blaser.** 1991. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med **325:**1132-1136.

220. **Noren, N. K., B. P. Liu, K. Burridge, and B. Kreft.** 2000. p120 catenin regulates the actin cytoskeleton via Rho family GTPases. J Cell Biol **150:**567-580.

221. **Nozaki, K., N. Shimizu, Y. Ikehara, M. Inoue, T. Tsukamoto, K. Inada, H. Tanaka, T. Kumagai, M. Kaminishi, and M. Tatematsu.** 2003. Effect of early eradication on Helicobacter pylori-related gastric carcinogenesis in Mongolian gerbils. Cancer Sci **94:**235-239.

222. **Nozawa, Y., K. Nishihara, Y. Akizawa, N. Orimoto, M. Nakano, T. Uji, H. Ajioka, A. Kanda, N. Matsuura, and M. Kiniwa.** 2004. Protein kinase C activation by Helicobacter pylori in human gastric epithelial cells limits interleukin-8 production through suppression of extracellular signal-regulated kinase. J Pharmacol Sci **94:**233-239.

223. **Obonyo, M., D. G. Guiney, J. Fierer, and S. P. Cole.** 2003. Interactions between inducible nitric oxide and other inflammatory mediators during Helicobacter pylori infection. Helicobacter **8:**495-502.

224. **Obonyo, M., D. G. Guiney, J. Harwood, J. Fierer, and S. P. Cole.** 2002. Role of gamma interferon in Helicobacter pylori induction of inflammatory mediators during murine infection. Infect Immun **70:**3295-3299.

225. **Odenbreit, S., J. Puls, B. Sedlmaier, E. Gerland, W. Fischer, and R. Haas.** 2000. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. Science **287:**1497-1500.

226. **Ogden, S. R., L. E. Wroblewski, C. Weydig, J. Romero-Gallo, D. P. O'Brien, D. A. Israel, U. S. Krishna, B. Fingleton, A. B. Reynolds, S. Wessler, and R. M. Peek, Jr.** 2008. p120 and Kaiso regulate Helicobacter pylori-induced expression of matrix metalloproteinase-7. Mol Biol Cell **19:**4110-4121.

227. **Ogura, K., S. Maeda, M. Nakao, T. Watanabe, M. Tada, T. Kyutoku, H. Yoshida, Y. Shiratori, and M. Omata.** 2000. Virulence factors of Helicobacter pylori responsible for gastric diseases in Mongolian gerbil. J Exp Med **192:**1601-1610.

228. **Ohashi, K., T. Nemoto, K. Nakamura, and R. Nemori.** 2000. Increased expression of matrix metalloproteinase 7 and 9 and membrane type 1-matrix metalloproteinase in esophageal squamous cell carcinomas. Cancer **88:**2201-2209.

229. **Olivares, D., J. P. Gisbert, and J. M. Pajares.** 2005. Helicobacter pylori infection and gastric mucosal epithelial cell apoptosis. Rev Esp Enferm Dig **97:**505-520.

230. **Orford, K., C. Crockett, J. P. Jensen, A. M. Weissman, and S. W. Byers.** 1997. Serine phosphorylation-regulated ubiquitination and degradation of beta-catenin. J Biol Chem **272:**24735-24738.

231. **Ougolkov, A. V., K. Yamashita, M. Mai, and T. Minamoto.** 2002. Oncogenic beta-catenin and MMP-7 (matrilysin) cosegregate in late-stage clinical colon cancer. Gastroenterology **122:**60-71.

232. **Ozaki, I., T. Mizuta, G. Zhao, H. Yotsumoto, T. Hara, S. Kajihara, A. Hisatomi, T. Sakai, and K. Yamamoto.** 2000. Involvement of the Ets-1 gene in overexpression of matrilysin in human hepatocellular carcinoma. Cancer Res **60:**6519-6525.

233. **Pacheco, M. M., L. P. Kowalski, I. N. Nishimoto, and M. M. Brentani.** 2002. Differential expression of c-jun and c-fos mRNAs in squamous cell carcinoma of the head and neck: associations with uPA, gelatinase B, and matrilysin mRNAs. Head Neck **24:**24-32.

234. **Pajouh, M. S., R. B. Nagle, R. Breathnach, J. S. Finch, M. K. Brawer, and G. T. Bowden.** 1991. Expression of metalloproteinase genes in human prostate cancer. J Cancer Res Clin Oncol **117:**144-150.

235. **Pal, S., A. P. Schmidt, E. M. Peterson, C. L. Wilson, and L. M. de la Maza.** 2006. Role of matrix metalloproteinase-7 in the modulation of a Chlamydia trachomatis infection. Immunology **117:**213-219.

236. **Palmer, E. D.** 1954. Investigation of the gastric mucosa spirochetes of the human. Gastroenterology **27:**218-220.

237. **Park, J. I., S. W. Kim, J. P. Lyons, H. Ji, T. T. Nguyen, K. Cho, M. C. Barton, T. Deroo, K. Vleminckx, R. T. Moon, and P. D. McCrea.** 2005. Kaiso/p120-catenin and TCF/beta-catenin complexes coordinately regulate canonical Wnt gene targets. Dev Cell **8:**843-854.

238. **Park, P. W., O. Reizes, and M. Bernfield.** 2000. Cell surface heparan sulfate proteoglycans: selective regulators of ligand-receptor encounters. J Biol Chem **275:**29923-29926.

239. **Parsonnet, J., G. D. Friedman, N. Orentreich, and H. Vogelman.** 1997. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut **40:**297-301.

240. **Parsonnet, J., G. D. Friedman, D. P. Vandersteen, Y. Chang, J. H. Vogelman, N. Orentreich, and R. K. Sibley.** 1991. *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med **325:**1127-1131.

241. **Peek, R. M., Jr., and M. J. Blaser.** 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. Nat Rev Cancer **2:**28-37.

242. **Peek, R. M., Jr., and M. J. Blaser.** 2002. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nature Rev Cancer **2:**28-37.

243. **Peek, R. M., Jr., G. G. Miller, K. T. Tham, G. I. Perez-Perez, T. L. Cover, J. C. Atherton, G. D. Dunn, and M. J. Blaser.** 1995. Detection of *Helicobacter pylori* gene expression in human gastric mucosa. J Clin Microbiol **33:**28-32.

244. **Peek, R. M., Jr., G. G. Miller, K. T. Tham, G. I. Perez-Perez, X. Zhao, J. C. Atherton, and M. J. Blaser.** 1995. Heightened inflammatory response and cytokine expression *in vivo* to *cagA+ Helicobacter pylori* strains. Lab Invest **73:**760-770.

245. **Peek, R. M., Jr., S. F. Moss, K. T. Tham, G. I. Perez-Perez, S. Wang, G. G. Miller, J. C. Atherton, P. R. Holt, and M. J. Blaser.** 1997. Helicobacter pylori cagA+ strains and dissociation of gastric epithelial cell proliferation from apoptosis. J Natl Cancer Inst **89:**863-868.

246. **Peterson, W. L.** 1991. *Helicobacter pylori* and peptic ulcer disease. N Engl J Med **324:**1043-1048.

247. **Pounder, R. E., and D. Ng.** 1995. The prevalence of Helicobacter pylori infection in different countries. Aliment Pharmacol Ther **9 Suppl 2:**33-39.

248. **Powell, W. C., B. Fingleton, C. L. Wilson, M. Boothby, and L. M. Matrisian.** 1999. The metalloproteinase matrilysin proteolytically generates active soluble Fas ligand and potentiates epithelial cell apoptosis. Curr Biol **9:**1441-1447.

249. **Prenzel, N., E. Zwick, H. Daub, M. Leserer, R. Abraham, C. Wallasch, and A. Ullrich.** 1999. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature **402:**884-888.

250. **Prokhortchouk, A., B. Hendrich, H. Jorgensen, A. Ruzov, M. Wilm, G. Georgiev, A. Bird, and E. Prokhortchouk.** 2001. The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. Genes Dev **15:**1613-1618.

251. **Queiroz, D. M., E. N. Mendes, G. A. Rocha, A. M. Oliveira, C. A. Oliveira, P. P. Magalhaes, S. B. Moura, M. M. Cabral, and A. M. Nogueira.** 1998. *cagA*-positive *Helicobacter pylori* and risk for developing gastric carcinoma in Brazil. Int J Cancer **78:**135-139.

252. **Rauws, E. A., and G. N. Tytgat.** 1990. Cure of duodenal ulcer associated with eradication of Helicobacter pylori. Lancet **335:**1233-1235.

253. **Reynolds, A. B.** 2007. p120-catenin: Past and present. Biochim Biophys Acta **1773:**2-7.

254. **Reynolds, A. B., and R. H. Carnahan.** 2004. Regulation of cadherin stability and turnover by p120ctn: implications in disease and cancer. Semin Cell Dev Biol **15:**657-663.

255. **Reynolds, A. B., J. Daniel, P. D. McCrea, M. J. Wheelock, J. Wu, and Z. Zhang.** 1994. Identification of a new catenin: the tyrosine kinase substrate p120cas associates with E-cadherin complexes. Mol Cell Biol **14:**8333-8342.

256. **Reynolds, A. B., J. M. Daniel, Y. Y. Mo, J. Wu, and Z. Zhang.** 1996. The novel catenin p120cas binds classical cadherins and induces an unusual morphological phenotype in NIH3T3 fibroblasts. Exp Cell Res **225:**328-337.

257. **Reynolds, A. B., L. Herbert, J. L. Cleveland, S. T. Berg, and J. R. Gaut.** 1992. p120, a novel substrate of protein tyrosine kinase receptors and of p60v-src, is related to cadherin-binding factors beta-catenin, plakoglobin and armadillo. Oncogene **7:**2439-2445.

258. **Reynolds, A. B., and A. Roczniak-Ferguson.** 2004. Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene **23:**7947-7956.

259. **Reynolds, A. B., D. J. Roesel, S. B. Kanner, and J. T. Parsons.** 1989. Transformation-specific tyrosine phosphorylation of a novel cellular protein in chicken cells expressing oncogenic variants of the avian cellular *src* gene. Mol Cell Biol **9:**629-638.

260. **Rieder, G., R. A. Hatz, A. P. Moran, A. Walz, M. Stolte, and G. Enders.** 1997. Role of adherence in interleukin-8 induction in Helicobacter pylori-associated gastritis. Infect Immun **65:**3622-3630.

261. **Robinson, K., R. H. Argent, and J. C. Atherton.** 2007. The inflammatory and immune response to Helicobacter pylori infection. Best Pract Res Clin Gastroenterol **21:**237-259.

262. **Roczniak-Ferguson, A., and A. B. Reynolds.** 2003. Regulation of p120-catenin nucleocytoplasmic shuttling activity. J Cell Sci **116:**4201-4212.

263. **Romero-Gallo, J., E. J. Harris, U. Krishna, M. K. Washington, G. I. Perez-Perez, and R. M. Peek, Jr.** 2008. Effect of Helicobacter pylori eradication on gastric carcinogenesis. Lab Invest **88:**328-336.

264. **Rottner, K., A. Hall, and J. V. Small.** 1999. Interplay between Rac and Rho in the control of substrate contact dynamics. Curr Biol **9:**640-648.

265. **Rudi, J., C. Kolb, M. Maiwald, I. Zuna, A. von Herbay, P. R. Galle, and W. Stremmel.** 1997. Serum antibodies against *Helicobacter pylori* proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. Dig Dis Sci **42:**1652-1659.

266. **Rudnicki, M. A., T. Braun, S. Hinuma, and R. Jaenisch.** 1992. Inactivation of MyoD in mice leads to up-regulation of the myogenic HLH gene Myf-5 and results in apparently normal muscle development. Cell **71:**383-390.

267. **Rudolph-Owen, L. A., R. Chan, W. J. Muller, and L. M. Matrisian.** 1998. The matrix metalloproteinase matrilysin influences early-stage mammary tumorigenesis. Cancer Res **58:**5500-5506.

268. **Saadat, I., H. Higashi, C. Obuse, M. Umeda, N. Murata-Kamiya, Y. Saito, H. Lu, N. Ohnishi, T. Azuma, A. Suzuki, S. Ohno, and M. Hatakeyama.** 2007. Helicobacter pylori CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature **447:**330-333.

269. **Saarialho-Kere, U. K., M. Vaalamo, P. Puolakkainen, K. Airola, W. C. Parks, and M. L. Karjalainen-Lindsberg.** 1996. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. Am J Pathol **148:**519-526.

270. **Salama, N., K. Guillemin, T. K. McDaniel, G. Sherlock, L. Tompkins, and S. Falkow.** 2000. A whole-genome microarray reveals genetic diversity among *Helicobacter pylori* strains. Proc Natl Acad Sci U S A **97:**14668-14673.

271. **Salmela, M. T., M. L. Karjalainen-Lindsberg, P. Puolakkainen, and U. Saarialho-Kere.** 2001. Upregulation and differential expression of matrilysin (MMP-7) and metalloelastase (MMP-12) and their inhibitors TIMP-1 and TIMP-3 in Barrett's oesophageal adenocarcinoma. Br J Cancer **85:**383-392.

272. **Sarrio, D., G. Moreno-Bueno, C. Sanchez-Estevez, I. Banon-Rodriguez, G. Hernandez-Cortes, D. Hardisson, and J. Palacios.** 2006. Expression of cadherins and catenins correlates with distinct histologic types of ovarian carcinomas. Hum Pathol **37:**1042-1049.

273. **Sato, D., A. Yanaka, T. Shibahara, H. Matsui, A. Nakahara, T. Yanagawa, E. Warabi, T. Ishii, and I. Hyodo.** 2008. Peroxiredoxin I protects gastric mucosa from oxidative injury induced by H. pylori infection. J Gastroenterol Hepatol **23:**652-659.

274. **Segal, E. D., J. Cha, J. Lo, S. Falkow, and L. S. Tompkins.** 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. Proc Natl Acad Sci U S A **96:**14559-14564.

275. **Selbach, M., S. Moese, C. R. Hauck, T. F. Meyer, and S. Backert.** 2002. Src is the kinase of the Helicobacter pylori CagA protein in vitro and in vivo. J Biol Chem **277:**6775-6778.

276. **Selbach, M., S. Moese, C. R. Hauck, T. F. Meyer, and S. Backert.** 2002. Src is the kinase of the *Helicobacter pylori* CagA protein *in vitro* and *in vivo*. J Biol Chem **277:**6775-6778.

277. **Senota, A., F. Itoh, H. Yamamoto, Y. Adachi, Y. Hinoda, and K. Imai.** 1998. Relation of *matrilysin* messenger RNA expression with invasive activity in human gastric cancer. Clin Exp Metastasis **16:**313-321.

278. **Sharma, S. A., M. K. Tummuru, M. J. Blaser, and L. D. Kerr.** 1998. Activation of IL-8 gene expression by Helicobacter pylori is regulated by transcription factor nuclear factor-kappa B in gastric epithelial cells. J Immunol **160:**2401-2407.

279. **Shibamoto, S., M. Hayakawa, K. Takeuchi, T. Hori, K. Miyazawa, N. Kitamura, K. R. Johnson, M. J. Wheelock, N. Matsuyoshi, M. Takeichi, and et al.** 1995. Association of p120, a tyrosine kinase substrate, with E-cadherin/catenin complexes. J Cell Biol **128:**949-957.

280. **Shibata, T., A. Kokubu, S. Sekine, Y. Kanai, and S. Hirohashi.** 2004. Cytoplasmic p120ctn regulates the invasive phenotypes of E-cadherin-deficient breast cancer. Am J Pathol **164:**2269-2278.

281. **Shimoyama, T., S. Fukuda, M. Tanaka, T. Mikami, A. Munakata, and J. E. Crabtree.** 1998. CagA seropositivity associated with development of gastric cancer in a Japanese population. J Clin Pathol **51:**225-228.

282. **Shiomi, T., and Y. Okada.** 2003. MT1-MMP and MMP-7 in invasion and metastasis of human cancers. Cancer Metastasis Rev **22:**145-152.

283. **Shirafuji, Y., H. Tanabe, D. P. Satchell, A. Henschen-Edman, C. L. Wilson, and A. J. Ouellette.** 2003. Structural determinants of procryptdin recognition and cleavage by matrix metalloproteinase-7. J Biol Chem **278:**7910-7919.

284. **Shirin, H., E. M. Sordillo, S. H. Oh, H. Yamamoto, T. Delohery, I. B. Weinstein, and S. F. Moss.** 1999. Helicobacter pylori inhibits the G1 to S transition in AGS gastric epithelial cells. Cancer Res **59:**2277-2281.

285. **Sier, C. F., L. J. Hawinkels, H. J. Zijlmans, K. Zuidwijk, E. S. de Jonge-Muller, V. Ferreira, R. Hanemaaijer, A. A. Mulder-Stapel, G. G. Kenter, H. W. Verspaget, and A. Gorter.** 2008. Endothelium specific matrilysin (MMP-7) expression in human cancers. Matrix Biol **27:**267-271.

286. **Sipponen, P., and B. J. Marshall.** 2000. Gastritis and gastric cancer. Western countries. Gastroenterol Clin North Am **29:**579-592, v-vi.

287. **Smith, M. F., Jr., A. Mitchell, G. Li, S. Ding, A. M. Fitzmaurice, K. Ryan, S. Crowe, and J. B. Goldberg.** 2003. Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. J Biol Chem **278:**32552-32560.

288. **Spring, C. M., K. F. Kelly, I. O'Kelly, M. Graham, H. C. Crawford, and J. M. Daniel.** 2005. The catenin p120ctn inhibits Kaiso-mediated transcriptional

repression of the beta-catenin/TCF target gene matrilysin. Exp Cell Res **305:**253-265.

289. **Staddon, J. M., C. Smales, C. Schulze, F. S. Esch, and L. L. Rubin.** 1995. p120, a p120-related protein (p100), and the cadherin/catenin complex. J Cell Biol **130:**369-381.

290. **Stein, M., F. Bagnoli, R. Halenbeck, R. Rappuoli, W. J. Fantl, and A. Covacci.** 2002. c-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol **43:**971-980.

291. **Stein, M., F. Bagnoli, R. Halenbeck, R. Rappuoli, W. J. Fantl, and A. Covacci.** 2002. c-Src/Lyn kinases activate *Helicobacter pylori* CagA through tyrosine phosphorylation of the EPIYA motifs. Mol Microbiol **43:**971-980.

292. **Stein, M., R. Rappuoli, and A. Covacci.** 2000. Tyrosine phosphorylation of the Helicobacter pylori CagA antigen after cag-driven host cell translocation. Proc Natl Acad Sci U S A **97:**1263-1268.

293. **Sternlicht, M. D., and Z. Werb.** 2001. How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol **17:**463-516.

294. **Stoicov, C., M. Whary, A. B. Rogers, F. S. Lee, K. Klucevsek, H. Li, X. Cai, R. Saffari, Z. Ge, I. A. Khan, C. Combe, A. Luster, J. G. Fox, and J. Houghton.** 2004. Coinfection modulates inflammatory responses and clinical outcome of Helicobacter felis and Toxoplasma gondii infections. J Immunol **173:**3329-3336.

295. **Sundrud, M. S., V. J. Torres, D. Unutmaz, and T. L. Cover.** 2004. Inhibition of primary human T cell proliferation by Helicobacter pylori vacuolating toxin (VacA) is independent of VacA effects on IL-2 secretion. Proc Natl Acad Sci U S A **101:**7727-7732.

296. **Suzuki, H., M. Suzuki, M. Mori, T. Kitahora, H. Yokoyama, S. Miura, T. Hibi, and H. Ishii.** 1998. Augmented levels of gastric mucosal leucocyte activation by infection with cagA gene-positive Helicobacter pylori. J Gastroenterol Hepatol **13:**294-300.

297. **Suzuki, M., H. Mimuro, T. Suzuki, M. Park, T. Yamamoto, and C. Sasakawa.** 2005. Interaction of CagA with Crk plays an important role in *Helicobacter pylori*-induced loss of gastric epithelial cell adhesion. J Exp Med **202:**1235-1247.

298. **Suzuki, M., S. Miura, M. Mori, A. Kai, H. Suzuki, D. Fukumura, M. Suematsu, and M. Tsuchiya.** 1994. Rebamipide, a novel antiulcer agent, attenuates Helicobacter pylori induced gastric mucosal cell injury associated with neutrophil derived oxidants. Gut **35:**1375-1378.

299. **Szabo, I., S. Brutsche, F. Tombola, M. Moschioni, B. Satin, J. L. Telford, R. Rappuoli, C. Montecucco, E. Papini, and M. Zoratti.** 1999. Formation of anion-selective channels in the cell plasma membrane by the toxin VacA of Helicobacter pylori is required for its biological activity. Embo J **18:**5517-5527.

300. **Takaishi, S., T. Okumura, and T. C. Wang.** 2008. Gastric cancer stem cells. J Clin Oncol **26:**2876-2882.

301. **Takeichi, M.** 1995. Morphogenetic roles of classic cadherins. Curr Opin Cell Biol **7:**619-627.

302. **Takeuchi, N., Y. Ichikawa, T. Ishikawa, N. Momiyama, S. Hasegawa, Y. Nagashima, K. Miyazaki, N. Koshikawa, M. Mitsuhashi, and H. Shimada.** 1997. *Matrilysin* gene expression in sporadic and familial colorectal adenomas. Mol Carcinog **19:**225-229.

303. **Tammer, I., S. Brandt, R. Hartig, W. Konig, and S. Backert.** 2007. Activation of Abl by Helicobacter pylori: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology **132:**1309-1319.

304. **Tan, X., H. Egami, S. Ishikawa, H. Sugita, H. Kamohara, M. Nakagawa, F. Nozawa, M. Abe, and M. Ogawa.** 2005. Involvement of matrix metalloproteinase-7 in invasion-metastasis through induction of cell dissociation in pancreatic cancer. Int J Oncol **26:**1283-1289.

305. **Tanigawa, T., T. Watanabe, M. Hamaguchi, E. Sasaki, K. Tominaga, Y. Fujiwara, N. Oshitani, T. Matsumoto, K. Higuchi, and T. Arakawa.** 2004. Anti-inflammatory effect of two isoforms of COX in H. pylori-induced gastritis in mice: possible involvement of PGE2. Am J Physiol Gastrointest Liver Physiol **286:**G148-156.

306. **Tanioka, Y., T. Yoshida, T. Yagawa, Y. Saiki, S. Takeo, T. Harada, T. Okazawa, H. Yanai, and K. Okita.** 2003. Matrix metalloproteinase-7 and matrix metalloproteinase-9 are associated with unfavourable prognosis in superficial oesophageal cancer. Br J Cancer **89:**2116-2121.

307. **Thoreson, M. A., P. Z. Anastasiadis, J. M. Daniel, R. C. Ireton, M. J. Wheelock, K. R. Johnson, D. K. Hummingbird, and A. B. Reynolds.** 2000. Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. J Cell Biol **148:**189-202.

308. **Thoreson, M. A., and A. B. Reynolds.** 2002. Altered expression of the catenin p120 in human cancer: implications for tumor progression. Differentiation **70:**583-589.

309. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D.**

Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karp, H. O. Smith, C. M. Fraser, and J. C. Venter. 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539-547.

310. **Tombola, F., C. Carlesso, I. Szabo, M. de Bernard, J. M. Reyrat, J. L. Telford, R. Rappuoli, C. Montecucco, E. Papini, and M. Zoratti.** 1999. Helicobacter pylori vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. Biophys J **76:**1401-1409.

311. **Torres, J., G. I. Perez-Perez, Y. Leal-Herrera, and O. Munoz.** 1998. Infection with CagA+ *Helicobacter pylori* strains as a possible predictor of risk in the development of gastric adenocarcinoma in Mexico. Int J Cancer **78:**298-300.

312. **Torres, V. J., S. E. VanCompernolle, M. S. Sundrud, D. Unutmaz, and T. L. Cover.** 2007. Helicobacter pylori vacuolating cytotoxin inhibits activation-induced proliferation of human T and B lymphocyte subsets. J Immunol **179:**5433-5440.

313. **Tsutsumi, R., H. Higashi, M. Higuchi, M. Okada, and M. Hatakeyama.** 2002. Attenuation of helicobacter pylori CagA-SHP-2 signaling by interaction between CagA and C-terminal Src kinase. J Biol Chem **21:**21.

314. **Uemura, N., S. Okamoto, S. Yamamoto, N. Matsumura, S. Yamaguchi, M. Yamakido, K. Taniyama, N. Sasaki, and R. J. Schlemper.** 2001. *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med **345:**784-789.

315. **Ueno, H., K. Yamashita, I. Azumano, M. Inoue, and Y. Okada.** 1999. Enhanced production and activation of matrix metalloproteinase-7 (matrilysin) in human endometrial carcinomas. Int J Cancer **84:**470-477.

316. **Vaezi, M. F., G. W. Falk, R. M. Peek, J. J. Vicari, J. R. Goldblum, G. I. Perez-Perez, T. W. Rice, M. J. Blaser, and J. E. Richter.** 2000. CagA-positive strains of *Helicobacter pylori* may protect against Barrett's esophagus. Am J Gastroenterol **95:**2206-2211.

317. **van Hengel, J., P. Vanhoenacker, K. Staes, and F. van Roy.** 1999. Nuclear localization of the p120(ctn) Armadillo-like catenin is counteracted by a nuclear export signal and by E-cadherin expression. Proc Natl Acad Sci U S A **96:**7980-7985.

318. **Vargo-Gogola, T., H. C. Crawford, B. Fingleton, and L. M. Matrisian.** 2002. Identification of novel matrix metalloproteinase-7 (matrilysin) cleavage sites in murine and human Fas ligand. Arch Biochem Biophys **408:**155-161.

319. **Varro, A., S. Kenny, E. Hemers, C. McCaig, S. Przemeck, T. C. Wang, K. Bodger, and D. M. Pritchard.** 2007. Increased gastric expression of MMP-7 in hypergastrinemia and significance for epithelial-mesenchymal signaling. Am J Physiol Gastrointest Liver Physiol **292:**G1133-1140.

320. **Vial, J. D., and H. Orrego.** 1960. Electron microscope observations on the fine structure of parietal cells. J Biophys Biochem Cytol **7:**367-372.

321. **Viala, J., C. Chaput, I. G. Boneca, A. Cardona, S. E. Girardin, A. P. Moran, R. Athman, S. Memet, M. R. Huerre, A. J. Coyle, P. S. DiStefano, P. J. Sansonetti, A. Labigne, J. Bertin, D. J. Philpott, and R. L. Ferrero.** 2004. Nod1 responds to peptidoglycan delivered by the *Helicobacter pylori cag* pathogenicity island. Nat Immunol **5:**1166-1174.

322. **Vicari, J. J., R. M. Peek, G. W. Falk, J. R. Goldblum, K. A. Easley, J. Schnell, G. I. Perez-Perez, S. A. Halter, T. W. Rice, M. J. Blaser, and J. E. Richter.** 1998. The seroprevalence of *cagA*-positive *Helicobacter pylori* strains in the spectrum of gastroesophageal reflux disease. Gastroenterology **115:**50-57.

323. **von Bredow, D. C., R. B. Nagle, G. T. Bowden, and A. E. Cress.** 1997. Cleavage of beta 4 integrin by matrilysin. Exp Cell Res **236:**341-345.

324. **Vorobjova, T., I. Nilsson, K. Kull, H. I. Maaroos, A. Covacci, T. Wadstrom, and R. Uibo.** 1998. CagA protein seropositivity in a random sample of adult population and gastric cancer patients in Estonia. Eur J Gastroenterol Hepatol **10:**41-46.

325. **Wagner, S., W. Beil, J. Westermann, R. P. Logan, C. T. Bock, C. Trautwein, J. S. Bleck, and M. P. Manns.** 1997. Regulation of gastric epithelial cell growth by Helicobacter pylori: offdence for a major role of apoptosis. Gastroenterology **113:**1836-1847.

326. **Wang, F., S. Reierstad, and D. A. Fishman.** 2006. Matrilysin over-expression in MCF-7 cells enhances cellular invasiveness and pro-gelatinase activation. Cancer Lett **236:**292-301.

327. **Wang, F. Q., J. So, S. Reierstad, and D. A. Fishman.** 2005. Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase. Int J Cancer **114:**19-31.

328. **Wang, T. C., C. A. Dangler, D. Chen, J. R. Goldenring, T. Koh, R. Raychowdhury, R. J. Coffey, S. Ito, A. Varro, G. J. Dockray, and J. G. Fox.** 2000. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. Gastroenterology **118:**36-47.

329. **Wang, T. C., J. R. Goldenring, C. Dangler, S. Ito, A. Mueller, W. K. Jeon, T. J. Koh, and J. G. Fox.** 1998. Mice lacking secretory phospholipase A2 show altered apoptosis and differentiation with Helicobacter felis infection. Gastroenterology **114:**675-689.

330. **Wang, W. S., P. M. Chen, H. S. Wang, W. Y. Liang, and Y. Su.** 2006. Matrix metalloproteinase-7 increases resistance to Fas-mediated apoptosis and is a poor prognostic factor of patients with colorectal carcinoma. Carcinogenesis **27:**1113-1120.

331. **Watanabe, T., M. Tada, H. Nagai, S. Sasaki, and M. Nakao.** 1998. Helicobacter pylori infection induces gastric cancer in mongolian gerbils. Gastroenterology **115:**642-648.

332. **Webb, L. M., M. U. Ehrengruber, I. Clark-Lewis, M. Baggiolini, and A. Rot.** 1993. Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. Proc Natl Acad Sci U S A **90:**7158-7162.

333. **Werner, J. A., I. O. Rathcke, and R. Mandic.** 2002. The role of matrix metalloproteinases in squamous cell carcinomas of the head and neck. Clin Exp Metastasis **19:**275-282.

334. **Weydig, C., A. Starzinski-Powitz, G. Carra, J. Lower, and S. Wessler.** 2007. CagA-independent disruption of adherence junction complexes involves E-cadherin shedding and implies multiple steps in *Helicobacter pylori* pathogenicity. Exp Cell Res **313:**3459-3471.

335. **Weyermann, M., G. Adler, H. Brenner, and D. Rothenbacher.** 2006. The mother as source of Helicobacter pylori infection. Epidemiology **17:**332-334.

336. **Wielockx, B., C. Libert, and C. Wilson.** 2004. Matrilysin (matrix metalloproteinase-7): a new promising drug target in cancer and inflammation? Cytokine Growth Factor Rev **15:**111-115.

337. **Wijnhoven, B. P., M. Pignatelli, W. N. Dinjens, and H. W. Tilanus.** 2005. Reduced p120ctn expression correlates with poor survival in patients with adenocarcinoma of the gastroesophageal junction. J Surg Oncol **92:**116-123.

338. **Wilson, C. L., K. J. Heppner, P. A. Labosky, B. L. Hogan, and L. M. Matrisian.** 1997. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A **94:**1402-1407.

339. **Wilson, C. L., and L. M. Matrisian.** 1996. Matrilysin: an epithelial matrix metalloproteinase with potentially novel functions. Int J Biochem Cell Biol **28:**123-136.

340. **Wilson, C. L., A. J. Ouellette, D. P. Satchell, T. Ayabe, Y. S. Lopez-Boado, J. L. Stratman, S. J. Hultgren, L. M. Matrisian, and W. C. Parks.** 1999.

Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science **286:**113-117.

341. **Wisdom, R.** 1999. AP-1: one switch for many signals. Exp Cell Res **253:**180-185.

342. **Witty, J. P., S. McDonnell, K. J. Newell, P. Cannon, M. Navre, R. J. Tressler, and L. M. Matrisian.** 1994. Modulation of matrilysin levels in colon carcinoma cell lines affects tumorigenicity *in vivo*. Cancer Res **54:**4805-4812.

343. **Wong, B. C., S. K. Lam, W. M. Wong, J. S. Chen, T. T. Zheng, R. E. Feng, K. C. Lai, W. H. Hu, S. T. Yuen, S. Y. Leung, D. Y. Fong, J. Ho, and C. K. Ching.** 2004. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. JAMA **291:**187-194.

344. **Wroblewski, L. E., P. J. Noble, A. Pagliocca, D. M. Pritchard, C. A. Hart, F. Campbell, A. R. Dodson, G. J. Dockray, and A. Varro.** 2003. Stimulation of MMP-7 (matrilysin) by *Helicobacter pylori* in human gastric epithelial cells: role in epithelial cell migration. J Cell Sci **116:**3017-3026.

345. **Xia, X., R. H. Carnahan, M. H. Vaughan, G. A. Wildenberg, and A. B. Reynolds.** 2006. p120 serine and threonine phosphorylation is controlled by multiple ligand-receptor pathways but not cadherin ligation. Exp Cell Res.

346. **Xia, X., R. H. Carnahan, M. H. Vaughan, G. A. Wildenberg, and A. B. Reynolds.** 2006. p120 serine and threonine phosphorylation is controlled by multiple ligand-receptor pathways but not cadherin ligation. Exp Cell Res **312:**3336-3348.

347. **Xiao, K., D. F. Allison, K. M. Buckley, M. D. Kottke, P. A. Vincent, V. Faundez, and A. P. Kowalczyk.** 2003. Cellular levels of p120 catenin function as a set point for cadherin expression levels in microvascular endothelial cells. J Cell Biol **163:**535-545.

348. **Yamamoto, H., Y. Adachi, F. Itoh, S. Iku, K. Matsuno, M. Kusano, Y. Arimura, T. Endo, Y. Hinoda, M. Hosokawa, and K. Imai.** 1999. Association of matrilysin expression with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Cancer Res **59:**3313-3316.

349. **Yamamoto, H., S. Iku, Y. Adachi, A. Imsumran, H. Taniguchi, K. Nosho, Y. Min, S. Horiuchi, M. Yoshida, F. Itoh, and K. Imai.** 2003. Association of trypsin expression with tumour progression and matrilysin expression in human colorectal cancer. J Pathol **199:**176-184.

350. **Yamamoto, H., F. Itoh, Y. Adachi, H. Sakamoto, M. Adachi, Y. Hinoda, and K. Imai.** 1997. Relation of enhanced secretion of active matrix metalloproteinases with tumor spread in human hepatocellular carcinoma. Gastroenterology **112:**1290-1296.

351. **Yamamoto, H., F. Itoh, S. Iku, Y. Adachi, H. Fukushima, S. Sasaki, M. Mukaiya, K. Hirata, and K. Imai.** 2001. Expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human pancreatic adenocarcinomas: clinicopathologic and prognostic significance of matrilysin expression. J Clin Oncol **19:**1118-1127.

352. **Yamaoka, Y., T. Kudo, H. Lu, A. Casola, A. R. Brasier, and D. Y. Graham.** 2004. Role of interferon-stimulated responsive element-like element in interleukin-8 promoter in Helicobacter pylori infection. Gastroenterology **126:**1030-1043.

353. **Yamashita, K., I. Azumano, M. Mai, and Y. Okada.** 1998. Expression and tissue localization of matrix metalloproteinase 7 (matrilysin) in human gastric carcinomas. Implications for vessel invasion and metastasis. Int J Cancer **79:**187-194.

354. **Yamashita, K., M. Mori, T. Shiraishi, K. Shibuta, and K. Sugimachi.** 2000. Clinical significance of matrix metalloproteinase-7 expression in esophageal carcinoma. Clin Cancer Res **6:**1169-1174.

355. **Yanagisawa, M., D. Huveldt, P. Kreinest, C. M. Lohse, J. C. Cheville, A. S. Parker, J. A. Copland, and P. Z. Anastasiadis.** 2008. A p120 catenin isoform switch affects Rho activity, induces tumor cell invasion, and predicts metastatic disease. J Biol Chem **283:**18344-18354.

356. **Yanagisawa, M., I. N. Kaverina, A. Wang, Y. Fujita, A. B. Reynolds, and P. Z. Anastasiadis.** 2004. A novel interaction between kinesin and p120 modulates p120 localization and function. J Biol Chem **279:**9512-9521.

357. **Yap, A. S.** 1998. The morphogenetic role of cadherin cell adhesion molecules in human cancer: a thematic review. Cancer Invest **16:**252-261.

358. **Yap, A. S., C. M. Niessen, and B. M. Gumbiner.** 1998. The juxtamembrane region of the cadherin cytoplasmic tail supports lateral clustering, adhesive strengthening, and interaction with p120ctn. J Cell Biol **141:**779-789.

359. **Yokota, K., Y. Kurebayashi, Y. Takayama, S. Hayashi, H. Isogai, E. Isogai, K. Imai, T. Yabana, A. Yachi, and K. Oguma.** 1991. Colonization of Helicobacter pylori in the gastric mucosa of Mongolian gerbils. Microbiol Immunol **35:**475-480.

360. **Yoshimoto, M., F. Itoh, H. Yamamoto, Y. Hinoda, K. Imai, and A. Yachi.** 1993. Expression of MMP-7(PUMP-1) mRNA in human colorectal cancers. Int J Cancer **54:**614-618.

361. **Yoshinaga, K., K. Mimori, H. Inoue, Y. Kamohara, K. Yamashita, F. Tanaka, and M. Mori.** 2008. Activin A enhances MMP-7 activity via the

transcription factor AP-1 in an esophageal squamous cell carcinoma cell line. Int J Oncol **33:**453-459.

362. **You, W. C., L. M. Brown, L. Zhang, J. Y. Li, M. L. Jin, Y. S. Chang, J. L. Ma, K. F. Pan, W. D. Liu, Y. Hu, S. Crystal-Mansour, D. Pee, W. J. Blot, J. F. Fraumeni, Jr., G. W. Xu, and M. H. Gail.** 2006. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. J Natl Cancer Inst **98:**974-983.

363. **Yu, Q., and I. Stamenkovic.** 1999. Localization of matrix metalloproteinase 9 to the cell surface provides a mechanism for CD44-mediated tumor invasion. Genes Dev **13:**35-48.

364. **Yu, W. H., J. F. Woessner, Jr., J. D. McNeish, and I. Stamenkovic.** 2002. CD44 anchors the assembly of matrilysin/MMP-7 with heparin-binding epidermal growth factor precursor and ErbB4 and regulates female reproductive organ remodeling. Genes Dev **16:**307-323.

365. **Yuan, G., L. Qian, L. Song, M. Shi, D. Li, M. Yu, M. Hu, B. Shen, and N. Guo.** 2008. Heregulin-beta promotes matrix metalloproteinase-7 expression via HER2-mediated AP-1 activation in MCF-7 cells. Mol Cell Biochem **318:**73-79.

366. **Zhang, L., P. Su, A. Henriksson, J. O'Rourke, and H. Mitchell.** 2008. Investigation of the immunomodulatory effects of Lactobacillus casei and Bifidobacterium lactis on Helicobacter pylori infection. Helicobacter **13:**183-190.

367. **Zheng, H. C., J. M. Sun, X. H. Li, X. F. Yang, Y. C. Zhang, and Y. Xin.** 2003. Role of PTEN and MMP-7 expression in growth, invasion, metastasis and angiogenesis of gastric carcinoma. Pathol Int **53:**659-666.

368. **Zheng, Q., X. Y. Chen, Y. Shi, and S. D. Xiao.** 2004. Development of gastric adenocarcinoma in Mongolian gerbils after long-term infection with Helicobacter pylori. J Gastroenterol Hepatol **19:**1192-1198.