Helicobacter-induced Intestinal Metaplasia in the Stomach Correlates with Elk-1 and Serum Response Factor Induction of Villin*

Received for publication, November 29, 2004
Published, JBC Papers in Press, December 2, 2004, DOI 10.1074/jbc.M413399200

Gabriele Rieder‡§, Arthur J. Tessier‡§, Xiaotan T. Qiao¶, Blair Madison¶, Deborah L. Gumucio¶, and Juanita L. Merchant¶†**

From the Departments of Internal Medicine, Molecular and Integrative Physiology, and Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109-0682

Chronic Helicobacter pylori infection results in serious sequelae, including atrophy, intestinal metaplasia, and gastric cancer. Intestinal metaplasia in the stomach is defined by the presence of intestine-like cells expressing enterocyte-specific markers, such as villin. In this study, we demonstrate that villin is expressed in intestine-like cells that develop after chronic infection with H. pylori in both human stomach and in a mouse model. Transfection studies were used to identify specific regions of the villin promoter that are inducible by exposure of the cells to H. pylori. We demonstrated that induction of the villin promoter by H. pylori in a human gastric adenocarcinoma cell line (AGS) required activation of the Erk pathway. Elk-1 and the serum response factor (SRF) are downstream transcriptional targets of the Erk pathway. We observed inducible binding of Elk-1 and the SRF after 3 and 24 h of treatment with H. pylori, suggesting that the bacteria alone are sufficient to initiate a cascade of signaling events responsible for villin expression. Thus, H. pylori induction of villin in the stomach correlates with activation and cooperative binding of Elk-1 and the SRF to the proximal promoter of villin.

Chronic inflammation of the gastric mucosa (chronic gastritis) develops in response to Helicobacter pylori infection or bacterial overgrowth in the hypochlorhydric stomach (1, 2). Over time, the inflammatory process progresses, and alteration of the epithelial cell population occurs, which includes gradual loss of parietal cells coinciding with an increase in the number of mucous cells. Proliferation of mucous cell types with evidence of an intestinal phenotype (intestinal metaplasia) is a major precursor lesion in gastric cancer (3). Moreover, intestinal metaplasia is a lesion that develops in a variety of cancers derived from organs of the foregut. Villin is expressed in intestinal metaplasia observed in Barrett’s esophagus and in chronic atrophic gastritis (19). Therefore, villin is an important marker of the pre-neoplastic cell type that forms in the gut in response to chronic injury (20).

Whether true villin-positive intestinal metaplasia is a feature of the altered pattern of differentiation observed with Helicobacter colonization and inflammation has not been examined. In this report, we established that villin expression emerges in the infected stomachs of human subjects and a mouse model of Helicobacter infection. Further, we showed that the first 554 bp of the villin promoter contain elements capable of responding to H. pylori in culture. Within this proximal promoter region, we found that a serum response element (SRE)1 confers inducible regulation of the villin promoter by H. pylori and that Elk-1 and the serum response factor (SRF) form a ternary complex at this element.

MATERIALS AND METHODS

Bacteria—The mouse-adapted H. pylori SS1 strain was used to inoculate mice for up to 14 months and was a gift from Dr. K. Eaton, University of Michigan. The H. pylori J99 strain (American Type Culture Collection 700824) is a human isolate that was used in the cell culture experiments because of its ability to strongly induce human cell lines. The bacteria were cultivated on blood agar plates containing Campylobacter base agar (Difco) supplemented with 5% horse blood (Colorado Serum, Denver, CO), 5 μg/ml vancomycin, 10 μg/ml trimethoprim lactate, and 2 μg/ml nystatin (Sigma). The plates were

---

* This work was supported in part by Public Health Service Grants R01DK61410 (to J. L. M.) and P01DK62041 (to J. L. M. and D. L. G.) and the Roger McDermitt Research Fund and Cancer Innovation Grant from the University of Michigan Comprehensive Cancer Center CA46952 (to D. L. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

† These authors contributed equally to this work.

** To whom correspondence should be addressed: Medical Science Research Building I, 1150 W. Medical Center Dr., Rm. 3510, Ann Arbor, MI 48109-0682. Tel.: 734-647-2944; Fax: 734-763-4686.

1 The abbreviations used are: SRE, serum response element; SRF, serum response factor; EBS, Ets binding site; YY1, YingYang1; DAPI, 4',6-diamidino-2-phenylindole; m.o.i., multiplicity of infection.

4906 This paper is available online at http://www.jbc.org

Printed in U.S.A.
incubated under microaerophilic conditions (CampyPak Plus, BBL; BD Biosciences) at 37 °C for 1–2 days.

**Human Tissue**—Institutional Review Board approval from the University of Michigan was obtained prior to the acquisition of human tissue by Dr. Nguyen T. Vinh and Dr. Nguyen N. Thanh during endoscopy at Friendship Hospital and Tran Hung Dao Central Hospital, Hanoi, Vietnam. The biopsies were fixed in formalin and then paraffin-embedded.

**Plasmids**—The ~554 villin P/Intron reporter construct was prepared as described previously (21) and consists of 554 bp of the mouse 5′-flanking sequence, the first exon, and the entire first intron. Dominant-negative Elk-1 (E-20) and Erk-2 (C-14) antibodies were gifts from Dr. Melanie Cobb (University of Texas Southwestern, Dallas, TX) (22). Mutagenesis of the Ets and SRE sites within the villin reporter construct was accomplished using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s protocol using the following primer: 5′-GGGGCATATCTAATCCTTATAGT-GAGGAAGAT-3′. Deletion of the SRE/EBS binding site was created the same way using the following QuikChange primer: 5′-CTATGATTCTGGAATATGGAATCTCAG-3′.

**Animals and Helicobacter Challenge**—C57BL/6 mice were purchased from Charles River (Bar Harbor, ME) and maintained in individual sterile microisolators for up to 14 months. Five days before infection, all mice were treated for 3 days with streptomycin (5 mg/kg) in their drinking water to reduce the natural bacterial flora. SS1 bacteria were harvested in *Brucella* broth (BBL), the concentration was adjusted according to the OD at 550 nm, and a 0.2-ml suspension containing 10^9 viable bacteria was used for oral infection. Each animal was challenged with bacteria three times over 5 days. All mice were fasted overnight with access to water and *libitum* before analysis. The study protocol used was approved by the University of Michigan Animal Care and Use Committee, which maintains an American Association of Assessment and Accreditation of Laboratory Animal Care facility. Infection by *H. pylori* was verified by testing re-isolates for urease (using a drop of urea broth containing 10 g of urea, 0.5% w/v phenol red, 0.22 g IgG antibody (2B6, Medical & Biological Laboratories Co., Ltd., containing 10^8 viable bacteria was used for oral infection. Each animal adjusted according to the OD at 550 nm, and a 0.2-ml suspension in their drinking water to reduce the natural bacterial flora. SS1 bacteria of Michigan was obtained prior to the acquisition of human tissue by Dr. H.9262

**Histology**—Paraffin sections of the human biopsies or mouse stomach were stained with hematoxylin and eosin for grading the intensity of inflammation and metaplasia. The presence of metaplasia was confirmed by a periodic acid-Schiff procedure/Alcian blue stain.

**Immunohistochemistry**—Paraffin-embedded tissue sections of *H. pylori* SS1-infected and -non-infected mice were used. Deparaffinized sections were used for citrate buffer retrieval (10 mM citric acid for 10 min at 95–95 °C) and blocked with 15% donkey serum. To document villin expression in the stomach mucosa, primary mouse anti-proton pump IgG antibody (2B6, Medical & Biological Laboratories Co., Ltd., Naka-ku Nagoya, Japan), detecting the β subunit of the mouse H+K-ATPase, was allowed to react with the sections at room temperature for 1 h. After rinsing the slides in phosphate-buffered saline, secondary antibodies were applied using Texas Red-conjugated anti-mouse IgG from donkey and fluorescein isothiocyanate-conjugated anti-goat IgG from donkey (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in a dilution of 1:75 at room temperature for 1 h. DAPI (5 ng/ml/slide, Sigma) was added to counterstain the nuclei and mounted with an anti-fade aqueous mount (Biomeda Corp, Foster City, CA). The cells were visualized with an Olympus BX60 fluorescence microscope and photographed with the digital SPOT camera (Diagnostic Instruments).

**H. pylori Co-culture with Human Gastric Cell Lines**—NCI-N87 cells were cultured to 60% confluency in Dulbecco’s modified Eagle’s medium, supplemented with 5% fetal bovine serum, 5% horse serum, 100 μg/ml streptomycin, 100 μg/ml penicillin, and 50% 95% air-humidified atmosphere. The cells were then starved for 48 h in F-12 middle supplemented with 100 μg/ml penicillin and 100 μg/ml streptomycin. Unlike AGS cells, the NCI-N87 cell line expresses a variety of gastric peptides (26). Endogenous regulation of villin protein was examined in NCI-N87 cells using 1:100 m.o.i. (ratio of 100 bacteria/eukaryotic cell) of either Campylobacter jejuni, SS1, or J99 *H. pylori* strains for 24 h. Due to the higher efficiency of transfection, all transfections, co-culture studies, and nuclear extract preparation were performed using AGS cells.

**Immunoblots**—Whole cell extracts were prepared, lysed in 10 mM HEPES, 0.4% Triton X-100, 30 mM NaCl, 0.3 mM MgCl2, 0.2 mM EDTA, 2% glycerol, 0.2 mM NaVio, 2 mM sodium fluoride, 2 mM sodium pyrophosphate, 10 μg/ml leupeptin, 20 μg/ml aprotinin, and 1 mM dithiothreitol. The extracts were heat-denatured in Laemmli sample buffer and resolved on a 10% SDS-polyacrylamide gel. The lysate was electrophoretically transferred onto nitrocellulose membrane (Bio-Rad), blocked with 5% non-fat dry milk in NaF-supplemented TTBS (100 mM Tris-HCl, pH 7.5, 0.9% NaCl, 0.1% Tween 20) for 1 h, transferred to the membrane, and incubated with the sections at room temperature for 1 h. After rinsing in TTBS, horseradish peroxidase-coupled antibody (1:2000 dilution) was applied for 1 h. The protein-antibody complexes were detected by chemiluminescence (SuperSignal, Pierce Biochemicals). Erk-2 (C-14) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); phospho-Erk (p44/42 MAPK) antibody and phospho-38 antibody were from New England Biolabs (Bedford, MA).

To detect phospho-Erk-1, nuclear protein extracts were prepared by a two-buffer detergent method (28) supplemented with 50 μM NaF to inhibit phosphatase activity. The extracts were heat-denatured in Laemmli sample buffer and resolved on a 4%–20% SDS-polyacrylamide gel. The proteins were electrophoretically transferred onto a Hybond-C Extra nitrocellulose membrane (Amersham Biosciences). The membrane was washed three times with NaF-supplemented TTBS (add 50 mM NaF), blocked for 1 h in 5% nonfat dry milk in NaF-supplemented TTBS, and then incubated with phospho-Erk-1 antibody (Santa Cruz) for 2 days at 4 °C. After three rinses with NaF-supplemented TTBS, the membrane was incubated with a secondary antibody (peroxo-oxidase conjugated antibody; Cell Signaling Technology, Beverly, MA) at 1:1000 dilution for 1 h at room temperature. Protein-antibody complexes were detected with the LumiGlo reagent and peroxide chemiluminescent detection kit (Cell Signaling Technology).

**Gel Shift Assays**—For nuclear extract preparation, the cells were co-cultured with the bacteria for either 3 or 24 h. They were then washed twice with cold Tris-buffered saline, harvested, and placed on ice for 10 min. All nuclei were prepared for 48 h but *H. pylori*. Nuclear protein extracts were prepared by a two-buffer detergent method (28). A double-stranded 28-bp oligonucleotide probe 5′-TTCCCT-TATATGTTGAAGGAGTCCTCGG-3′ (sense strand) was hybridized and then end-labeled with [γ-32P]dATP using polynucleotide kinase. Gel shift reactions were carried out at 25 °C in a total volume of 20 μl containing 10 mM Tris-HCl (pH 7.9), 100 mM KCl, 5 mM MgCl2, 0.5 mM dithiothreitol, 10% glycerol, 600 ng of poly[d(I-D)] 6 μg of nuclear extract, and the corresponding antibody or unlabeled competing oligonucleotides (10× the molar concentration of the probe). After a 10-min preincubation, 30,000/cycles/min of labeled probe (10,000–15,000 cycles/min/0.1 ng) was added to each reaction followed by the loading buffer containing bromphenol blue. Supershifts were performed by adding 2 μg of anti-Erk-1, anti-SRF, anti-Y1, or anti-GATA-4 antibodies (Santa Cruz Biotechnology) 20 min before adding the probe. The reactions were run on a 4%–20% acrylamide gel containing 45 mM Tris, 45 mM boric acid, and 1 mM EDTA. The gels were dried and then used to expose x-ray film.

**Statistical Analysis**—The results were statistically tested by unpaired t test or one-way analysis of variance as appropriate, using commercially available software (GraphPad Prism, GraphPad Software, San Diego, CA). P < 0.05 was considered significant.

**RESULTS**

Chronic *Helicobacter* infection in both human subjects and mice induces gastritis, atrophy, and metaplasia. In human
stomach, intestinal metaplasia is correlated with the presence of Alcian blue-positive cells and an increase in intestine-specific markers, e.g., villin (29). The metaplasia appears prior to the development of gastric cancer (3). However, the expression of villin as a marker for intestinal metaplasia developing in response to Helicobacter infection has not been studied. Therefore, we examined biopsies from human subjects infected with H. pylori. The gastric mucosa of infected patients showed Alcian blue-positive cells consistent with the presence of intestinal-type acidic mucins and goblet cells (Fig. 1A). Using immunohistochemical staining, we found that these areas of intestinal metaplasia expressed villin protein (Fig. 1B). To examine whether villin also correlated with intestinal metaplastic changes in a H. pylori-infected animal model, mice were infected for 14 months with the mouse-adapted SS1 strain. Because the NCI-N87 cells transfect poorly, the AGS cell line was used in the subsequent transient transfection studies.

Prior studies have demonstrated that there are critical tissue-specific regulatory elements in the villin promoter between −1236 and −554 and between −554 and −446 (21). In addition, the first intron synergizes with the first 554 bp of the 5′-flanking sequence. We used the reporter construct containing 554 bp of the human villin promoter and the first intron to study regulation by H. pylori. We examined induction of the villin promoter for 24 h by three different bacterial species, H. pylori, Acinetobacter lwoffi, and C. jejuni (Fig. 4). The results demonstrated that villin protein increased with the J99 H. pylori strain but not with C. jejuni or the mouse-adapted SS1 strain. Because the NCI-N87 cells transfect poorly, the AGS cell line was used in the subsequent transient transfection studies.

We found that co-culturing the transient transfectants with H. pylori stimulated the villin reporter over 6-fold at a bacterial concentration of 200 m.o.i. C. jejuni is a luminal pathogen that does not cause gastritis, whereas, A. lwoffi does induce gastritis (30). We used the reporter construct containing 554 bp of the human villin promoter and the first intron to study regulation by H. pylori. We examined induction of the villin promoter for 24 h by three different bacterial species, H. pylori, Acinetobacter lwoffi, and C. jejuni (Fig. 4). The results showed greater induction with the H. pylori J99 strain compared with C. jejuni or A. lwoffi at 100 m.o.i. C. jejuni is a luminal pathogen that does not cause gastritis, whereas, A. lwoffi does induce gastritis (30). We found that co-culturing the transient transfectants with H. pylori stimulated the villin reporter over 6-fold at a bacterial concentration of 200 m.o.i. (Fig. 5A). Maximal stimulation occurred within 24–30 h (data not shown). To assess whether activation of the villin promoter was Mek-1-Erk-dependent, Erk kinase-deficient mutants and the Mek-1 inhibitor PD98059 were used to block villin induction by H. pylori. Dominant negative Erk-1 and -2 constructs were co-transfected with the villin reporter prior to co-culturing with H. pylori (Fig. 5B). Consistent with dominant negative inhibition, PD98059
also blocked villin induction by *H. pylori* (Fig. 5C). The p38 inhibitor SB203580 did not block villin induction (Fig. 5D). This indicated that the effect of *H. pylori* on the villin promoter specifically used the Erk pathway (Fig. 5D). To further document the role of the Mek-1-Erk pathway, an immunoblot was performed with phosphorylated Erk antibody and confirmed that co-culturing with *H. pylori* resulted in an increase in activated Erk protein (Fig. 6). We also found that *H. pylori* also stimulated p38 phosphorylation as reported previously (31). However, when coupled with the fact that SB203580 did not affect induction by *H. pylori*, we concluded that the p38 pathway was not upstream of villin activation. These results were consistent with prior studies implicating the Ras-Erk kinase pathway in *H. pylori* induction of the chemokine interleukin-8 (32). Moreover, it has recently been confirmed that *H. pylori* activates both the Erk-1 and p38 Map kinase pathways but that only the Erk-1 pathway regulates the transcription factor Egr-1 and its downstream targets, e.g. ICAM-1 (33).

We examined the villin promoter for likely targets of the Mek-1-Erk pathway and identified a cluster of Elk-1 sites adjacent to an SRE at -85 bp upstream from the cap site (Fig. 6A). Two adjacent elements consisting of the CArG box and an Ets binding site (EBS) form the SRE (34). The constitutive SRF forms a homodimer and binds the CArG box, which in turn stabilizes the binding of inducible Ets proteins, e.g. Elk-1, SAP-1, Net, collectively known as ternary complex factors. One of the Ets proteins will bind the short Ets site and make contact with the constitutively bound SRF homodimer to form the ternary complex (35, 36). To determine whether Elk-1 and SRF binding to the -85 villin SRE was required for *H. pylori* induction, we introduced point mutations into the element (Fig. 7A). Dominant negative Elk-1 was used to examine the contribution of Elk-1 to *H. pylori* induction of the promoter. The results showed that point mutations within the -85 SRE/EBS site diminished induction by *H. pylori* (Fig. 7B). Moreover, co-transfection with ddnElk-1 reduced both basal and inducible activation of the promoter by *H. pylori*, confirming that this factor is required for the induction.

It is known that Elk-1 binding is activated by translocation of Elk kinase to the nucleus and phosphorylation of this transcription factor (37). Therefore, to determine whether Elk-1 was phosphorylated during co-culture with *H. pylori*, an immunoblot was performed. The results showed that Elk-1 was phosphorylated at 3 and 24 h of treatment with *H. pylori* (Fig. 8). As a control for *H. pylori* induction, we examined the induction of Elk-1 by interleukin-1β (Fig. 8B). In both cases, induction of phosphorylated Elk accompanied Elk-1 protein induction, indicating that the increase was due not only to an increase in the phosphorylated form but also because of an increase in the amount of protein. Thus, we concluded that the mechanism of *H. pylori* activation of the villin promoter was by Mek-1 activation of Elk that, in turn, phosphorylates Elk-1.

Phosphorylation of Elk-1 facilitates its binding to DNA and cooperation with SRF (38). Therefore, electrophoretic mobility shift assays were performed to determine whether co-culturing with *H. pylori* increased Elk-1 binding to the inducible villin element. AGS nuclear extracts were prepared after incubating the cells with the bacteria for 0, 3, and 24 h. In untreated extracts, there was binding of YY1 and SRF and no binding of...
Elk-1 (Fig. 9). An increase in DNA binding to the inducible villin element was observed after 3 h of incubation with the bacteria. By 24 h, there was increased SRF binding in addition to the binding by Elk-1. Anti-GATA-4 antibody was used as a control and did not shift any of the complexes. The identity of the bound complexes was confirmed in the untreated and 24-h-treated samples by supershifting with specific antibody (Fig. 9). Moreover, the specificity of the complexes at each time point was confirmed by competition with the unlabeled element, which competed for all three of the major complexes. Collectively, the electrophoretic mobility shift assays revealed that YY1 and SRF bind to the element in untreated extracts (Fig. 10). After exposure to bacteria for 3 h, Elk-1 binds and YY1 binding is reduced. There is also a decrease in SRF, which correlates with greater occupancy of the element by the induced binding of Elk. By 24 h, both SRF and Elk-1 binding is prominent and YY1 binding remains low, suggesting sustained occupancy of this element by these inducible factors (Fig. 10).

DISCUSSION

In this study, we showed that induction of endogenous villin in the stomach occurs in atrophic human and mouse stomach in response to chronic H. pylori infection. These results are consistent with the concept that villin is an indicator of this pre-neoplastic lesion in the stomach and esophagus (10). Despite numerous reports that villin expression develops in tissues responding to chronic injury, little is known regarding the cis-acting elements that control its inducible expression. In the small intestine, villin is expressed during fetal development. Specific cis-acting transcriptional domains within the 6.7-kb promoter are responsible for regulating intestinal villin expression vertically (crypt versus villus) and horizontally (duodenum versus cecum) prior to birth (21). Based on the results reported here, this promoter also contains the elements capable of mediating regulation of villin expression in gastric cells.

Elk-1 (Fig. 9). An increase in DNA binding to the inducible villin element was observed after 3 h of incubation with the bacteria. By 24 h, there was increased SRF binding in addition to the binding by Elk-1. Anti-GATA-4 antibody was used as a control and did not shift any of the complexes. The identity of the bound complexes was confirmed in the untreated and 24-h-treated samples by supershifting with specific antibody (Fig. 9). Moreover, the specificity of the complexes at each time point was confirmed by competition with the unlabeled element, which competed for all three of the major complexes. Collectively, the electrophoretic mobility shift assays revealed that YY1 and SRF bind to the element in untreated extracts (Fig. 10). After exposure to bacteria for 3 h, Elk-1 binds and YY1 binding is reduced. There is also a decrease in SRF, which correlates with greater occupancy of the element by the induced binding of Elk. By 24 h, both SRF and Elk-1 binding is prominent and YY1 binding remains low, suggesting sustained occupancy of this element by these inducible factors (Fig. 10).
atrophic epithelium. To address the effect of the organism on villin expression, we performed a series of co-culture experiments. These studies revealed that indeed \textit{H. pylori} was sufficient to stimulate villin expression and that the inducible expression required a SRE. These results are consistent with other reports indicating that SREs mediate regulation by the \textit{H. pylori} CagA protein (39). However, the results shown here are the first to document regulation of an SRE by \textit{H. pylori} within a native promoter and more specifically a reporter that correlates with a pre-neoplastic state. Although both Elk-1 and SRF inducibly bind to the SRE sites, multiple point mutations of the $\sim85$ element as well as a deletion did not completely abolish induction of the promoter. Therefore, we expressed kinase-deficient Elk-1 to abolish any contribution of Elk to the induction. This result was quite striking in that the dominant negative construct reduced the basal activity of the $\sim554$ reporter construct and completely blocked induction by \textit{H. pylori}. Despite inducible binding of SRF to this element, we concluded that inducible activation and binding of Elk-1 plays a critical role in the regulated expression of villin and, presumably, the intestinal metaplastic phenotype of the stomach.

Interestingly, the binding of both Elk-1 and SRF was sustained and did not return to the base line even after 24 h of contact with the bacteria, suggesting continued activation of the promoter or possibly setting the stage for further cooperativity with other factors involved in chronic expression of villin. These other factors may be involved histone modifications through the recruitment of histone deacetylases or histone acetyltransferases. In fact, it has been shown that the ternary complex interacts with repressor complexes that include histone deacetylases and activator complexes, which include histone acetyltransferases (34). The organization of the villin SRE is also flanked by at least three EBSs, which may add to the complexity of how these factors bind and regulate villin. Given that Elk-1 and SRF binding sites are known to overlap and exhibit cooperativity, the combination of the expression and DNA binding studies suggest that SRF may act as a scaffold protein. This notion is consistent with other promoters exhibiting cooperative binding between Elk-1 and SRF (34). YY1 acts as both a repressor and activator through the recruitment of transcriptional co-regulators and chromatin interactions (40). Given the decrease in YY1 binding with \textit{H. pylori}, it would appear that the predominant activity of YY1 on the villin promoter is as a repressor.

An important aspect of these results is the connection between Elk-1 activation and the pre-neoplastic condition of the stomach. It is clear that Elk-1 activation is regulated by the Mek-1-Erk pathway. Interestingly, Tarnawski and co-workers (41) showed that non-steroidal anti-inflammatory agents (NSAIDS), which inhibit Cox 2, also inhibit Erk activation in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig9.png}
\caption{\textit{H. pylori} stimulates nuclear protein binding. After 48 h of serum starvation, \textit{H. pylori} J99 bacteria were co-cultured with AGS cells. Electrophoretic mobility shift assays were performed with 6 $\mu$g of nuclear extracts after no treatment (Un) and 3 and 24 h of co-culture with the bacteria (lanes 1–3). Lane 4 contains the untreated extracts competed with the wild type element (WT). Lanes 5–8 contain extract from the 3-h-treated cells incubated with Elk-1, SRF, YY1, and GATA-4 antibody. The major complexes Elk-1, SRF, YY1 and supershifted SRF (SS) are indicated.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig10.png}
\caption{\textit{H. pylori} stimulates Elk-1 and SRE binding. After 48 h of serum starvation, \textit{H. pylori} J99 bacteria were co-cultured with AGS cells. Electrophoretic mobility shift assays were performed with 6 $\mu$g of nuclear extracts after no treatment (Un) and 24 h of co-culture with the bacteria. A, the extract from the untreated cells (lane 1) was incubated with unlabeled oligonucleotide (WT) (lane 2) and YY1, SRF, Elk, or GATA-4 antibodies (lanes 3–6). B, similarly, extract from cells co-cultured with bacteria for 24 h (lane 1) was competed using the wild type oligonucleotide (lane 2), YY1, SRF, Elk-1, and GATA-4 antibodies (lanes 3–6).}
\end{figure}
the stomach (41). This is consistent with the knowledge that NSAIDS exhibit potent effects in the stomach, i.e. ulcer formation and erosion. Moreover, prostaglandin E2, a product of elevated Cox 1,2 activity, phosphorylates the epidermal growth factor receptor and activates the Erk-2 signaling pathway in the colon (42). Thus, it is reasonable to consider that increased Erk-2 signaling in the stomach correlates with heightened cell proliferation. Because the results here demonstrate that induction of villin lies downstream of the Erk-Elk-1 pathway, its expression in the stomach is an increased cell signaling pathway. Although Helicobacter may be one of several triggers that increase epithelial proliferation through the Erk-Elk-1 pathway, clearly there is the potential for activating a variety of genes involved in increasing the proliferative rate in the stomach.

Villin, as a proliferative marker in the stomach, will be useful in identifying relevant signaling pathways and transcription factor networks during the early stages before gastric transformation. Despite microarray studies of the human stomach, specific transcription factors have yet to be discovered (43). Thus, the signaling networks revealed here will, hopefully, become important tools to employ in dissecting the molecular steps leading to gastric transformation and, eventually, uncovering more effective and specific suppressive therapy.

REFERENCES

1. Blaser, M. J., and Parsonnet, J. (1994) J. Clin. Investig. 94, 4–8
2. Houben, G. M., and Stockbrugger, R. W. (1995) Scand. J. Gastroenterol. Suppl. 212, 13–18
3. Correa, P. (1992) Cancer Res. 52, 6735–6740
4. Hampson, S. J., Falzon, M., and Cowie, A. G. (1992) Br. J. Urol. 69, 323–324
5. Albores-Saavedra, J., Nadji, M., Henson, D. E., and Angeles-Angeles, A. (1988) Pathol. Res. Pract. 183, 279–281
6. Malhotra, S. L. (1976) Med. Hypotheses 2, 279–281
7. Moll, R., Robine, S., Dudouet, B., and Louvard, D. (1987) Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 54, 155–169
8. Robine, S., Huet, C., Moll, R., Sabuquillo-Merino, C., Coudrier, E., Zweibaum, A., and Louvard, D. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 8488–8492
9. Mizoshita, T., Tsukamoto, T., Nakaniishi, H., Inada, K., Ogasawara, N., Yoshida, T., Itsh, M., Yamamura, Y., and Tatematsu, M. (2003) J. Cancer Res. Clin. Oncol. 129, 727–734
10. Tsukamoto, T., Inada, K., Tanaka, H., Mizoshita, T., Mihara, M., Ushijima, T., Yamamura, Y., Nakamura, S., and Tatematsu, M. (2004) J. Cancer Res. Clin. Oncol. 130, 135–145
11. Ezzell, R. M., Chafel, M. M., and Matsudaira, P. T. (1989) Development (Camb.) 106, 407–419
12. Landry, C., Huet, C., Mangeat, P., Sabuquet, A., Louvard, D., and Crine, P. (1994) Differentiation 56, 55–65
13. Maunoury, R., Robine, S., Pringault, E., Lebrun, N., Gaillard, J. A., and Louvard, D. (1992) Development (Camb.) 115, 717–728
14. Braunstein, E. M., Qiao, X. T., Madson, B., Pinson, R., Dunbar, L., and Gumucio, D. L. (2002) Dev. Dyn. 224, 90–102
15. Pinto, D., Robine, S., Jaisser, F., El Marjou, F. E., and Louvard, D. (1999) J. Biol. Chem. 274, 6476–6482
16. Friederich, E., Pringault, E., Arpin, M., and Louvard, D. (1996) BioEssays 12, 403–408
17. Pinson, K. L., Dunbar, L., Samuelson, L., and Gumucio, D. L. (1998) Dev. Dyn. 211, 109–121
18. Ferrary, E., Cohen-Tannoudji, M., Pehau-Arnaudet, G., Lapillonne, A., Athman, R., Ruiz, T., Bouldou, L., El Marjou, F., Doyle, A., Fontaine, J. J., Antony, C., Babinet, C., Louvard, D., Jaisser, F., and Robine, S. (1999) J. Cell Biol. 146, 819–830
19. MacLennan, A. J., Orringer, M. B., and Beer, D. G. (1999) Mol. Carcinog. 24, 137–143
20. Regalado, S. P., Nambo, Y., Iannettoni, M. D., Orringer, M. B., and Beer, D. G. (1999) Mol. Carcinog. 22, 182–189
21. Madisson, B. B., Dunbar, L., Qiao, X. T., Braunstein, K., Braunstein, E., and Gumucio, D. L. (2000) J. Biol. Chem. 275, 32973–32983
22. Frost, J. A., Geppert, T. D., Cobb, M. H., and Feramisco, J. R. (1994) Proc. Natl. Acad. Sci., U. S. A. 91, 3844–3848
23. Gille, H., Kortenjann, M., Thomas, O., Moonaw, C., Slaughter, C., Cobb, M. H., and Shaw, P. E. (1995) EMBO J. 14, 951–962
24. Gille, H., Kortenjann, M., Strahl, T., and Shaw, P. E. (1996) Mol. Cell. Biol., 1094–1102
25. Fox, J. G., Beck, P., Dangler, C. A., Whary, M. T., Wang, T. C., Shi, H. N., and Nagler-Anderson, C. (2000) Nat. Med. 6, 536–542
26. Basque, J. R., Chenard, M., Chailler, P., and Menard, D. (2001) J. Cell. Biochem. 81, 241–251
27. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
28. Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6419
29. Hagen, S. J., Yanaka, A. and Janssens, R. (1994) Cell Tissue Res. 275, 255–267
30. Zavros, Y., Rieder, G., Ferguson, A., and Merchant, J. L. (2002) Infect. Immun. 70, 2630–2639
31. Keates, S., Keates, A. C., Warzy, M., Peak, R. M., Jr., Murray, P. G., and Kelly, C. P. (1999) J. Immunol. 163, 5552–5559
32. Meyer-ter-Vehn, T., Covacci, A., Kist, M., and Pahl, H. L. (2000) J. Biol. Chem. 275, 16064–16072
33. Abdel-Latif, M. M., Windle, H. J., Fitzgerald, K. A., Ang, Y. S., Eidhin, D. N., Li-Weber, M., Sahra, K., and Kelleher, D. (2004) Infect. Immun. 72, 3549–3560
34. Buchwalter, G., Gross, C., and Wasylyk, B. (2004) Gene (Amst.) 324, 1–14
35. Hipskind, R. A., Rao, V. N., Mueller, C. G., Reddy, E. S., and Nordheim, A. (1991) Nature 354, 531–534
36. Treisman, R. (1994) Curr. Opin. Genet. Dev. 4, 96–101
37. Keenan, S. M., Beilone, C., and Baldassare, J. J. (2001) J. Biol. Chem. 276, 22404–22409
38. Puel, T., Wu, J. J., Zimmerman, T. L., Zhang, L., Ehrlich, B. E., Berchtold, M. W., Hoeck, J. B., Karpen, S. J., Nathanson, M. H., and Bennett, A. M. (2002) J. Biol. Chem. 277, 27517–27527
39. Hirata, Y., Maeda, S., Mitsuoka, Y., Tateishi, K., Yanai, A., Akunoma, M., Yushida, H., Kawabe, T., Shiratori, Y., and Omata, M. (2002) Gastroenterology 123, 1862–1971
40. Thomas, M. J., and Seto, E. (1999) Gene (Amst.) 236, 197–208
41. Jones, M. K., Wang, H., Peskar, B. M., Levin, I., Itani, R. M., Sarfie, I. J., and Tarnawski, A. S. (1999) Nat. Med. 5, 1418–1423
42. Pai, R., Sureghan, B., Staubo, I. L., Pavilka, M., Baatar, D., and Tarnawski, A. S. (2002) Nat. Med. 8, 289–293
43. Kim, B., Bang, S., Lee, S., Kim, S., Jung, Y., Lee, C., Choi, K., Lee, S. G., Lee, K., Lee, Y., Kim, S. S., Yeon, Y. I., Kim, Y. S., Yoo, H. S., Song, K., and Lee, I. (2003) Cancer Res. 63, 8245–8255
Helicobacter-induced Intestinal Metaplasia in the Stomach Correlates with Elk-1 and Serum Response Factor Induction of Villin
Gabriele Rieder, Arthur J. Tessier, Xiaotan T. Qiao, Blair Madison, Deborah L. Gumucio and Juanita L. Merchant

*J. Biol. Chem.* 2005, 280:4906-4912.
doi: 10.1074/jbc.M413399200 originally published online December 2, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M413399200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 42 references, 15 of which can be accessed free at
http://www.jbc.org/content/280/6/4906.full.html#ref-list-1