a few proteins have been shown to associate with c-Myc, and the exact mechanisms of c-Myc action are poorly understood. Inhibition of YY1 activity may be one mechanism by which c-Myc acts. YY1 regulates transcription of many genes, including the oncogenes c-*fos* and c-*myc*, and also acts as a transcription initiator (*8*). The amounts of c-Myc are highly regulated and differ markedly between dividing and nondividing cells. YY1 may function only when the amounts of c-Myc drop below some threshold level. Lack of YY1 activity would activate some genes and repress others. Concentrations of c-Myc compatible with YY1 activity may vary with cell type or with the YY1 target gene or with both. It is also interesting that c-Myc has recently been shown to inhibit the activity of another transcriptional initiator, TFII-I (*26*). In addition to affecting YY1 function, there is increasing evidence that c-Myc–Max heterodimers regulate gene transcription directly (*27*).

The YY1-Myc association gives a partial indication of the complex equilibria that appear to exist among transcriptional regulators. For example, c-Myc transcription is negatively autoregulated by c-Myc (*28*), c-*myc* transcription is activated by YY1 (*7*), and, as shown here, c-Myc blocks this activation, explaining in part the autoregulation mechanism. Thus, c-*myc* transcription is probably sensitive to changes in relative and absolute amounts of c-Myc and YY1.

Another regulatory loop may involve putative cellular E1A-like proteins. E1A and c-Myc compete for association with YY1. E1A relieves YY1 repression (*1*) and synergizes with YY1 activation (*7*), whereas c-Myc inhibits YY1 repression and activation. Cellular E1A-like proteins could have a strong effect on YY1 activity by simultaneously displacing the inhibitor (c-Myc) and directly activating YY1.

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Attachment of *Helicobacter pylori* to Human Gastric Epithelium Mediated by Blood Group Antigens

Thomas Borén,* Per Falk, Kevin A. Roth, Göran Larson, Staffan Normark

Helicobacter pylori **is associated with development of gastritis, gastric ulcers, and adenocarcinomas in humans. The Lewis^b (Le^b) blood group antigen mediates *H. pylori* attachment to human gastric mucosa. Soluble glycoproteins presenting the Le^b antigen or antibodies to the Le^b antigen inhibited bacterial binding. Gastric tissue lacking Le^b expression did not bind *H. pylori*. Bacteria did not bind to Le^b antigen substituted with a terminal GalNAcα1-3 residue (blood group A determinant), suggesting that the availability of *H. pylori* receptors might be reduced in individuals of blood group A and B phenotypes, as compared with blood group O individuals.**

Helicobacter pylori, a prevalent human-specific pathogen, is a causative agent in chronic active gastritis (*1*), gastric and duodenal ulcers (*2*), and gastric adenocarcinoma (*3*), one of the most common forms of cancer in humans. This genetically diverse bacterial species (*4*) has been estimated to infect the gastric mucosa of >60% of adults over the age of 60 in industrialized countries (*5*). In developing countries, most individuals are infected during childhood (*6*).

Attachment is a prerequisite for microbial colonization of epithelial surfaces and is mediated by molecules on the bacterial surface, adhesins (*7*), that recognize proteins or glycoconjugates on the surface of the eukaryotic cell (*8*). The specificity of this interaction and the limited distribution of receptors often results in a restricted range of hosts and tissues utilized for colonization. This phenomenon is known as tropism. Bacteria unable to adhere to epithelium tend to be rapidly removed by shedding of surface cells and mucus layer.

Helicobacter pylori expresses sialic acid–specific hemagglutinins, the gene for one of which has been cloned (*9*). In addition to sialylated glycoconjugates, *H. pylori* binds to sulfatide (SO_3-Galβ1-1Cer) (*10*). However, *H. pylori* adhesion to HeLa cells appears to be independent of sialic acid (*11*). Cell-specific attachment of *H. pylori* to human gastric surface mucous cells is inhibited by human colostrum secretory immunoglobulin A (sc-IgA) (*12*, *13*), a glycoprotein carrying a highly variable set of N- and O-linked oligosaccharides (*14*). The less glycosylated serum IgA (S-IgA) (*15*) is devoid of such inhibitory properties. The adherence-inhibiting activity of sc-IgA is

T. Borén and S. Normark, Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.
P. Falk, Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.
K. A. Roth, Departments of Pathology, and Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.
G. Larson, Department of Clinical Chemistry, University of Göteborg, Sahlgren's Hospital, S-413 45 Göteborg, Sweden.

*To whom correspondence should be addressed.

reduced when sc-IgA is deglycosylated by digestion with α-L-fucosidase (*12*). However, selective removal of sialic acid or bacterial preincubation with sialylated glycoproteins does not reduce bacterial binding (*12*). These results imply that the *H. pylori* receptor on human gastric surface mucous cells contains fucose.

The fucosylated blood group antigens, typically found on erythrocytes, are also expressed on epithelial cell surfaces in humans (*16*). In the gastrointestinal tract, these antigenic structures are carried by two types of the lacto series core chains (*17*). The ABO and Lewis blood group antigens [see (*16–18*) for structural description] are formed by sequential addition of saccharide residues to both lipids (glycolipids) and proteins (glycoproteins) (*19*).

To delineate the fucosidase-sensitive receptor structure for *H. pylori*, we compared fucose-containing determinants of the sc-IgA and S-IgA molecules. Both sc-IgA and S-IgA were found to contain the H-2 antigen [terminal α1-2–linked fucose defining the blood group O phenotype of the ABO(H) system] (Fig. 1B) and Fucα1-6 substituted chitobiose (Fig. 1C), indicating that these structures do not constitute the *H. pylori* receptor. We found Le^b in the 90-kD secretory component of sc-IgA (Fig. 1D). The Le^a antigen together with Le^b was detected in a 120- to 150-kD glycoprotein (Fig. 1, D and E) that was present only in the sc-IgA preparation. The Le^b and Le^a antigens were hence fucosylated carbohydrate structures unique for the *H. pylori* adherence inhibitory sc-IgA preparation and could possibly be *H. pylori* receptors.

The glycosylation pattern of human colostrum (*20*) glycoproteins reflects the individual ABO and Lewis blood group phenotype. Colostrum samples from different individuals were screened for Lewis blood group antigens and for bacterial adherence inhibitory properties in situ (*12, 13*). We identified one individual (*1*) having high amounts of Le^a and no detectable Le^b and one individual (*2*) with low Le^a and high Le^b amounts. Colostrum sample 2 (10 μg/ml) abolished *H. pylori* P466 (*12, 21*) adherence, whereas sample 1 (100 μg/ml) only minimally reduced bacterial binding. This confirmed that the *H. pylori* receptor on human gastric surface mucous cells contains the Le^b antigen.

To assess the inhibitory properties of glycoproteins carrying defined carbohydrate structures, we analyzed a panel of fucosylated carbohydrate chains, chemically linked to albumin (Table 1 and Fig. 2). The Le^b-neoglycoprotein (Fig. 2H) and sc-IgA (Fig. 2C) were the only glycoconjugates that could eliminate *H. pylori* p466 binding [93% and 78% reduction, respectively (*22*)]. Adherence of the *H. pylori* VW229 (*12, 21*) was also eliminated by Le^b at a concentration of 20 μg/ml. A reduction in binding of 48% was observed with the H-1 neoglycoconjugate (Fig. 2D). The exclusive inhibitory activity of the Le^b and H-1 neoglycoconjugate demonstrates that the receptor epitope for *H. pylori* is confined to the terminally fucosylated lacto series type 1 chains. In addition, the weaker binding activity of the H-1 antigen shows that the

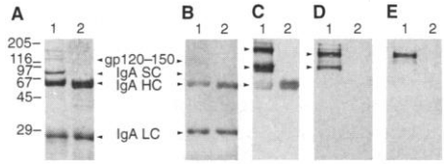
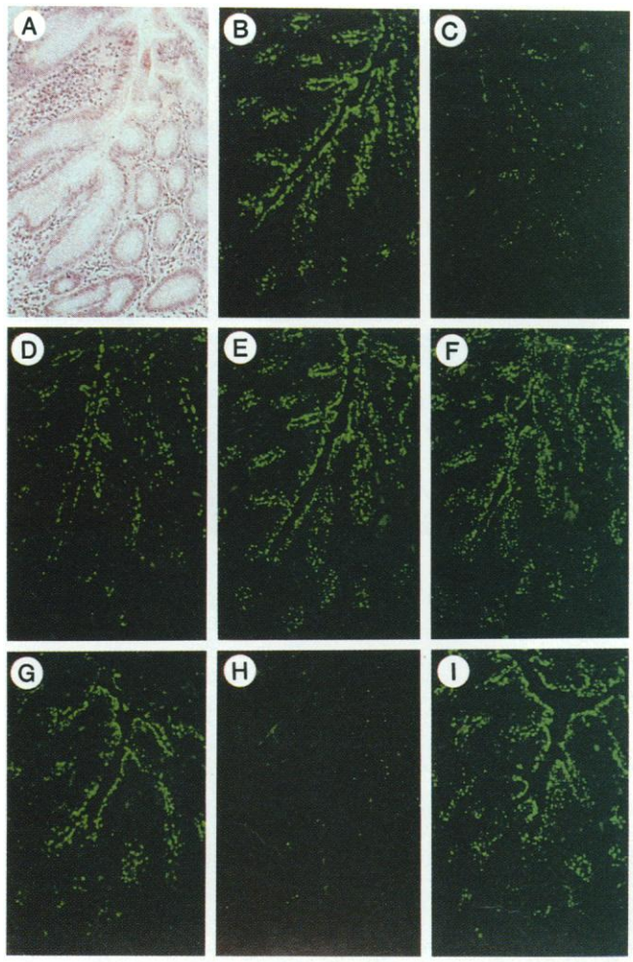


Fig. 1. Immunoblot analysis of fucosylated antigens on sc-IgA and S-IgA (Cappel Organon Teknika, West Chester, Pennsylvania). (**A**) SDS-PAGE–separated sc-IgA [(lanes 1); secretory component (SC), heavy chains (HC), and light chains (LC)] and S-IgA (lanes 2) (Coomassie blue) were transferred to nitrocellulose (**B** through **E**) and incubated for 1 hour in blocking buffer 2 (BB2) [tris-buffered saline (TBS), pH 7.5, 1% blocking reagent (Boehringer Mannheim), 1 mM $MnCl_2$, 1 mM $MgCl_2$, and 1 mM $CaCl_2$]. *Ulex europaeus* type 1 (UEA1) (B) and *Aleuria aurantia* (AAA) (C) lectins (Table 1) and mAbs to Le^b (D) and Le^a (E) (Table 1) were added in concentrations of 5 to 10 μg/ml for 6 hours and then washed seven times in TBS. We detected mAbs by alkaline phosphatase (AP)–conjugated goat antibody to mouse (Boehringer Mannheim). Lectins were detected with AP-conjugated sheep antibody to DIG or monoclonal Fab's to biotin (Boehringer Mannheim) and developed with BCIP/NBT. Molecular sizes are indicated at left in kilodaltons.



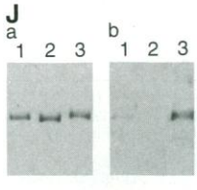


Fig. 2. In situ analysis of the receptor specificity of *H. pylori*. Oligosaccharides used for neoglycoconjugate preparation were purified by high-performance liquid chromatography, structurally identified and characterized with nuclear magnetic resonance spectroscopy, and more than 95% pure (IsoSep AB, Tullinge, Sweden). We verified the purity of all neoglycoproteins by immunoblots using the corresponding mAbs (Table 1). The ability of glycoconjugates to inhibit the bacterial adherence to human stomach in situ was analyzed as described (*12, 13*). Bacteria (*12, 21*) were preincubated with neoglycoconjugates (20 μg/ml) for 1 hour at room temperature, washed once in PBS (pH 7.6) to remove excess neoglycoconjugates, and then added to sections. (**A**) Section of human gastric mucosa stained with hematoxylin and eosin. (**B**) Noninhibited *H. pylori* P466 bound to gastric epithelium. (**C** through **I**) Inhibition experiments with sc-IgA (C), H-1 (D), H-2 (E), Le^a (F), Le^x (G), Le^b (H), and Le^y (I) neoglycoconjugates (Table 1). (**J**) Bacterial protein immunoblot overlay analysis. SDS-PAGE (a) was performed with 1 μg of H-1 (lane 1), Le^a (lane 2), and Le^b (lane 3). After transfer to nitrocellulose (b), the filters were incubated with BB2 overnight and washed two times in TBS. A DIG-labeled *H. pylori* P466 suspension of 0.1 OD_{600} (*13*) was added and incubated 8 hours at room temperature. Filters were washed six times for 5 min each in TBS. AP-conjugated antibody to DIG (Boehringer Mannheim) diluted 1:2000 was added and incubated for 1 hour, washed five times in TBS, and then developed (Fig. 1).

branched fucose residue on the Le^b chain is important for optimal receptor-bacterium interaction. The Le^a chain (Table 1), lacking the terminal fucose, is totally devoid of the receptor activity. The H-2, Le^a, Le^x, Le^y, and GlcNAcβ1-4(Fucα1-6)GlcNAcβ conjugates were inactive in concentrations up to 100 μg/ml.

Bacterial binding to glycoconjugates immobilized on protein immunoblots (Fig. 2J) confirmed the interaction with the Le^b antigen and showed a distinct but weaker binding to the H-1 conjugate, an observation that correlated with inhibition data. Binding of *H. pylori* to glycolipids separated on high-performance thin-layer chromatography (HPTLC) plates (*23*) was analyzed and confirmed the interaction with the Le^b and H-1 antigens.

In comparison, bacterial binding of the Le^b and H-1 neoglycoconjugates in solution was analyzed (*24*), and no differences in binding activity could be detected in concentrations of 1.0, 0.1, or 0.01 μg/ml. Inhibition experiments with free oligosaccharides also demonstrated a broader receptor specificity; both the monofucosylated H-1 and the difucosylated Le^b and Le^y oligosaccharides reduced bacterial binding at concentrations of 2.5 mM and almost eliminated binding at 25 mM, whereas Le^a and Le^x oligosaccharides did not reduce binding at concentrations of 25 mM (Table 1) (*25*). The slight shift in binding specificity to soluble, compared with immobilized, H-1 receptors could be influenced by the steric flexibility of the soluble receptors as described for several lectins (*26*).

Sections of human gastric mucosa were pretreated with monoclonal antibodies (mAbs) (Fig. 3) to the Le^b (anti-Le^b) and H-2 antigens (anti–H-2), respectively, and subsequently analyzed for bacterial binding. A 79% reduction (*22*) in bacterial binding was seen when the gastric tissue sections had been preincubated with anti-Le^b (Fig. 3C), whereas the reduction in binding to sections pretreated with anti–H-2 was 19% (Fig. 3D).

The antigen Le^b is the predominant blood group–related antigen expressed on gastric surface mucous cells in stomach epithelium from individuals of positive secretor status (*27*). On the other hand, Le^a is the corresponding dominant blood group antigen in individuals of nonsecretor status. Individuals of positive secretor status may, therefore, be more susceptible to *H. pylori*

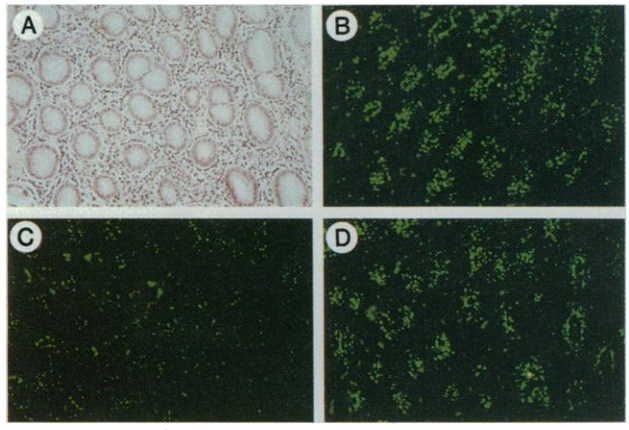


Fig. 3. Antibody inhibitions of bacterial binding in situ. The *H. pylori* P466 was overlayed on tissue sections pretreated with antibodies and detected as described (*13*). (**A**) Hematoxylin- and eosin-stained human gastric mucosa. (**B**) *Helicobacter pylori* binding to gastric epithelium. (**C** and **D**) *Helicobacter pylori* binding to tissue sections pretreated with anti-Le^b (C) and anti–H-2 (D).

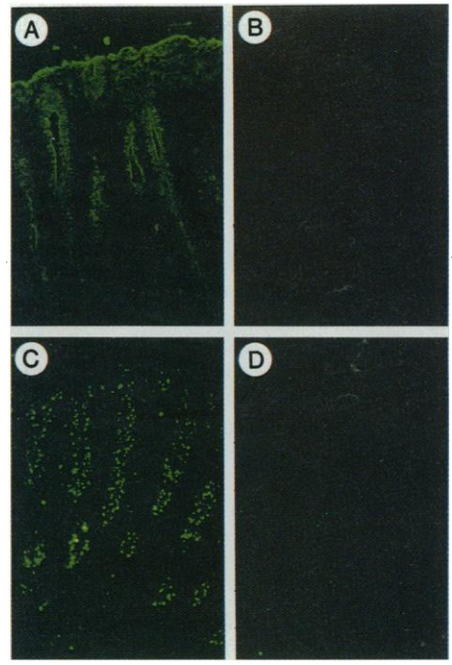


Fig. 4. Correlation between Le^b expression and bacterial binding to human gastric epithelium. Tissue sections of gastric mucosa of Le^b-positive (**A** and **C**) and Le^b-negative (**B** and **D**) phenotype, as determined by immunodetection (A and B) (*35*), were probed with *H. pylori* P466 (C and D).

Table 1. Neoglycoprotein conjugates, mAbs, lectins, glycosphingolipids, and oligosaccharides used in this study. N.D., not determined.

Sugar chains	Neoglycoprotein conjugates (*n*)*	mAb clones, lectins	Glycosphingolipids	Oligosaccharides
H-1	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4(Glc)-APD†–HSA‡ (35)	17-206§	$IV^2FucαLcOse_4Cer$	Fucα1-2Galβ1-3GlcNAcβ1-3 Galβ1-4Glc‡
H-2	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4(Glc)-BSA‡ (29)	92FR A2‖, UEA1#		
Le^a	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Glc)-APD†–HSA‡ (30)	CBM-LA1**	$III^4FucαLcOse_4Cer$	Galβ1-3(Fucα1-4)GlcNAcβ1-3 Galβ1-4Glc‡
Le^x	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Glc)-APD†–HSA‡ (30)	630/7H1‖	$III^3FucαnLcOse_4Cer$	Galβ1-4(Fucα1-3)GlcNAcβ1-3 Galβ1-4Glc‡
Le^b	Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3 Galβ1-4(Glc)-APD†–HSA‡ (32)	T218§, CBM-LB1**	$III^4,IV^2(Fucα)_2LcOse_4Cer$	Fucα1-2Galβ1-3(Fucα1-4) GlcNAcβ1-3Galβ1-4Glc‡
Le^y	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-*O*-APE†–HSA‡ (26)	672/7E3‖		Fucα1-2Galβ1-4(Fucα1-3) Glc‡
A-Le^b	GalNAcα1-3(Fucα1-2)Galβ1-3(Fucα1-4) GlcNAcβ1-3Galβ1-4(Glc)-APD†–HSA‡ (22)	40/5G7‖, 81 FR 2.2††		
Fucα1-6	GlcNAcβ1-4(Fucα1-6)GlcNAcβ-BSA‡ (N.D.)	AAA‡‡		

*(*n*) represents the number of oligosaccharides per molecule of human serum albumin (HSA) or bovine serum albumin (BSA), as determined by the manufacturer (IsoSep AB). †APE (aminophenylethyl) and APD (*N*-acetyl-*p*-phenylenediamine) indicate the nature of the spacer used to attach the oligosaccharides to the albumin molecule. The APE spacer is attached to the carbohydrate glycosidically, whereas the APD spacer is attached to the carbohydrate by reductive amination, and thus the terminal reducing monosaccharide unit of the oligosaccharide is reduced and is present as an aminoalditol. ‡IsoSep AB, Tullinge, Sweden. §Signet Laboratories, Dedham, Massachusetts. ‖Accurate Chemical & Scientific Corporation, Westbury, New York. #Sigma, St. Louis, Missouri. **Immucor, Incorporated, Norcross, Georgia. ††Dakopatts A/S, Glostrup, Denmark. ‡‡Boehringer Mannheim, Indianapolis, Indiana.

infection. Epidemiological studies of individuals with gastric ulcers demonstrate a higher prevalence of *H. pylori* infections in Le^b-positive individuals (*28*). In our assay, bacteria only bound to gastric epithelium if Le^b was expressed (Fig. 4).

The Le^b carbohydrates may serve as a therapy for *H. pylori* infections and gastric ulcer disease. Soluble receptor analogs (*29*) could competitively inhibit pathogenic bacterial attachment without interfering with the indigenous flora, circumventing the negative effects of broad-spectrum antibiotics (*30*).

The Le^b antigen substituted with a terminal GalNAcα1-3 residue, A-Le^b [blood group A determinant (Table 1)] (*16*), did not bind to bacteria in solution (*24*) nor did it inhibit bacterial adherence in situ. The terminal sugar residues GalNAcα1-3 (blood group A) or Galα1-3 (blood group B) will, in addition to the H antigen, also substitute the Le^b antigen (A-Le^b and B-Le^b, respectively) (*16*). Thus, there might be fewer available *H. pylori* receptors in individuals of blood group A and B phenotypes, as compared with blood group O individuals. This may explain epidemiological observations (*31*) that individuals of blood group O phenotype run a greater risk for developing gastric ulcers. Distinctive differences in carbohydrate compositions of natural glycoconjugates are genetically regulated in individuals and populations (*18*). It is an interesting notion that the glycosylation patterns of soluble glycoconjugates, that is, natural receptor analogs in secretions such as breast milk and saliva (*32*), may act as clearance factors and consequently govern the susceptibility for bacterial adherence and colonization.

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13. Bacterial in situ adherence assay was as described (*12*). Human stomach samples were obtained from the Department of Pathology at Washington University. Bacteria were labeled as described (*12*) or with digoxigenin-3-*O*-succinyl-ε-aminocaproic acid *N*-hydroxysuccinimide ester (DIG-NHS; Boehringer Mannheim). Protease inhibitors were included in the labeling reaction; 1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM EDTA-Na_2, and 10 mM benzamidine-HCl. Bacterial suspensions were diluted to 0.05 OD_{600} (optical density at 600 nm) in blocking buffer (*12*), and 200 μl was applied to the sections, incubated for 1 hour at room temperature, and washed six times for 5 min each in phosphate-buffered saline (PBS) (pH 7.6). DIG-labeled bacteria were then incubated for 1 hour with fluorescein isothiocyanate (FITC)–conjugated sheep antibody to DIG (Boehringer Mannheim) and washed three times for 5 min each in PBS.
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20. Human colostrum samples were obtained from Children's Hospital, St. Louis, MO. Colostrum samples were delipidated, and cellular debris was removed by centrifugation two times for 30 min each at 20,000 rpm in a Sorvall SS-34 rotor. SDS–polyacrylamide gel electrophoresis (SDS-PAGE) of human colostrum samples was done with 4 to 20% gels (Bio-Rad), and immunoblots were probed with mAbs to Le^b and Le^a antigens as in Fig. 1.
21. The *H. pylori* P466 and WV229 are clinical isolates from patients with acute gastritis and gastric ulcer, respectively.
22. We estimated reduction in bacterial binding by counting the number of adherent bacteria in two different fields under magnification ×20 in two independent inhibition experiments. The controls where bacteria were not preincubated with glycoconjugates were defined as 100% binding.
23. Binding of *H. pylori* P466 to glycosphingolipids was analyzed on HPTLC plates (Merck Kieselgel 60, EM Separations, Gibbstown, NJ) (*33*). Isolation, identification, and structural characterization of glycolipids (Table 1) was as described (*34*).
24. Bacterial suspensions (*13*) were incubated with biotin-labeled (Biotin-X-NHS, Calbiochem, San Diego, CA) Le^b, H-1, H-2, and A-Le^b neoglycoconjugates for 1 hour at room temperature, washed three times in TBS (pH 7.5), and applied in twofold serial dilutions to nitrocellulose with a slot-blotter. Neoglycoconjugates bound to bacteria were detected with peroxidase-labeled Fab's to biotin with ECL detection (Amersham, Arlington Heights, IL).
25. The ability of free oligosaccharides to inhibit bacterial adherence in situ was analyzed by preincubation of DIG-labeled *H. pylori* P466 for 3.5 hours at room temperature with oligosaccharides (Table 1). Bacterial suspensions (*13*) containing the H-1, Le^a, Le^b, Le^x, and Le^y oligosaccharides were added to tissue sections, excluding the prewashing step.
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35. Blood group antigens were detected in situ with mAbs to the Lewis antigens (Table 1) (Immucor) and FITC-conjugated rabbit antibody to mouse immunoglobulins (Dakopatts).

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Separate GTP Binding and GTPase Activating Domains of a Gα Subunit

David W. Markby, René Onrust, Henry R. Bourne*

Most members of the guanosine triphosphatase (GTPase) superfamily hydrolyze guanosine triphosphate (GTP) quite slowly unless stimulated by a GTPase activating protein or GAP. The α subunits (Gα) of the heterotrimeric G proteins hydrolyze GTP much more rapidly and contain an ~120-residue insert not found in other GTPases. Interactions between a Gα insert domain and a Gα GTP-binding core domain, both expressed as recombinant proteins, show that the insert acts biochemically as a GAP. The results suggest a general mechanism for GAP-dependent hydrolysis of GTP by other GTPases.

Heterotrimeric G proteins couple cell surface receptors to intracellular signaling pathways through a GTP-dependent cycle in which α and βγ subunits regulate effectors (*1–3*). Gα subunits belong to a large, diverse family of GTPases whose members include Ras, the product of the *ras* proto-oncogenes, and bacterial elongation factor Tu (EF-Tu). GTPase family members share conserved structures and mechanisms: a core GTP-binding domain probably similar in topology to the α-β folds of Ras and EF-Tu and a GTPase cycle that controls protein-protein interactions (*3, 4*). In the GTPase cycles of all these proteins, exchange of GTP for bound guanosine diphosphate (GDP) initiates activation and GTP hydrolysis terminates activation. For individual GTPases, either of these two

Departments of Pharmacology and Medicine, Cell Biology Program, and Cardiovascular Research Institute, University of California, San Francisco, CA 94143.

*To whom correspondence should be addressed.