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Signaling Perturbations Induced by Invading *H. pylori* Proteins in the Host Epithelial Cells:

A Mathematical Modeling Approach

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Abstract

Helicobacter pylori (*H. pylori*), a gram-negative bacterium, infects the stomach of approximately 50% of the world population. *H. pylori* infection is a risk factor for developing chronic gastric ulcers and gastric cancer. The bacteria produce two main cytotoxic proteins: Vacuolating cytotoxin A (*VacA*) and Cytotoxin-Associated gene A (*CagA*). When these proteins enter the host cell they interfere with the host MAP Kinase and Apoptosis signaling pathways leading to aberrant cell growth and premature apoptosis. The present study expanded existing quantitative models of the MAP Kinase and Apoptosis signaling pathways to take into account the protein interactions across species using the CellDesigner tool. The resulting network contained hundreds of differential equations in which the coefficients for the biochemical rate constants were estimated from previously published studies. The effect of *VacA* and *CagA* on the function of this network were simulated by increasing levels of bacterial load. Simulations showed that increasing bacterial load affected the MAP Kinase signaling in a dose dependant manner. The introduction of *CagA* decreased the activation time of *mapK* signaling and extended activation indefinitely despite normal cellular activity to deactivate the protein. Introduction of *VacA* produced a similar response in the apoptosis pathway. Bacterial load activated both pathways even in the absence of external stimulation. Time course of emergence of transcription factors associated with cell division and cell death predicted by our simulation showed close agreement with that determined from a publicly accesible microarray dataset of *H. pylori* infected stomach epithelium. The quantitative model presented in this study lays the foundation for investigating the affects of single nucleotide polymorphisms (SNPs) on the efficiency of drug treatment.

Keywords

H. pylori; Computational model; *mapK*; EGF signaling; FasL signaling

1 Introduction

Helicobacter pylori (*H. pylori*) is a gram-negative bacterium that inhabits the acidic conditions of the human stomach in 50% of the world's population [Hatakeyama and Brzozowski, 2006]. Chronic *H. pylori* infection is a major risk factor for gastric ulcers and gastric cancer [Hatakeyama and Brzozowski, 2006].

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H. pylori effects host stomach epithelial cells by producing two unique cytotoxic proteins: Vacuolating cytotoxin A (*VacA*) and Cytotoxin-Associated gene A (*CagA*) [Cover and Blanke, 2005, Hatakeyama and Higashi, 2005]. In previously published experimental studies, *VacA* has been implicated in hyper-vacuolization, small molecule leakage and apoptosis [Cover and Blanke, 2005]. *CagA* has been implicated in morphological changes (“hummingbird” phenotype) and induction of the MAP Kinase pathway [Hatakeyama and Higashi, 2005].

VacA acts as both a trans-membrane channel and a protein activator [Galmiche et al., 2000, Cover et al., 2003]. *VacA* associates with planar lipid membranes, such as the outer cell membrane, vesicles and mitochondrial membranes [Galmiche et al., 2000]. When *VacA* associates with vesicle membranes it creates a leak channel, which eventually leads to vesicle swelling [Cover and Blanke, 2005]. This hyper-vacuolization is a drastic phenotypic effect noticed *in vitro*, however, rarely under *in vivo* conditions [Cover and Blanke, 2005]. Blocking the hyper-vacuolization phenotype has no effect on the *VacA* induced apoptosis rates, [Yamasaki et al., 2006] since the effect is independent of vesicle activity. When *VacA* associates with the mitochondrial membrane it begins to activate the *BAX* protein [Yamasaki et al., 2006]. This protein catalyzes the release of *Cyt-C* from the mitochondria which then begins the apoptosis cascade through the type II pathway downstream of the traditional activators [Clements et al., 2001].

CagA is injected into the host cytosol through a type IV secretory system [Bourzac and Guillemin, 2005]. Within the cytosol it can interact with the *Grb-2* and *SOS* proteins to activate the MAP Kinase pathway in a *Ras*-dependent manner [Mimuro et al., 2002]. It is also phosphorylated by the host protein *SRC* Kinase [Fragale et al., 2004]. In the phosphorylated form, it can bind to the host protein *Shp-2* and relieve its structural inhibition. Activated *Shp-2* directly activates *mapK* in a *Ras*-independent manner [Tsutsumi et al., 2003]. *CagA* also activates the host protein *CSK* which then preferentially phosphorylates and deactivates *SRC* Kinase [Fragale et al., 2004], providing a feedback loop to modulate the activity of *CagA*.

MAP Kinase and Apoptosis pathways have been extensively studied and reviewed by others [Clarkson and Watson, 1999, Kohnno and Pouyssegur, 2006, Lamkanfi et al., 2006]. Quantitative models of these pathways have been developed using rate equations and experimentally determined binding constants [Bhalla and Iyengar, 1995, Hua et al., 2005]. We used this body of research as a foundation to computationally simulate the interaction of *H. pylori* proteins with the host epithelial cell protein networks, in order to identify the critical modes of this interaction that lead to increasing rates of growth and apoptosis. We have compared our findings in a time dependent manner with the transcription factor activation data obtained from publicly accessible microarray data [El-Etr et al., 2004] and PAINT analysis [Vadigepalli et al., 2003].

Our results indicate a bacterial load dependent activation of the MAP Kinase and Apoptosis pathways. This dependency varies based on whether the pathway was stimulated by physiological conditions. The time dependent transcription factor activation predicted by the model equations agrees with the outcome of the microarray data, indicating that present mathematical formulations of MAP Kinase and Apoptosis pathways are able to reproduce response to bacterial challenges to the host cell.

2 Method

2.1 Quantitative Model

Quantitative interactions of *H. pylori* bacterial proteins with the host epithelial cell proteins are described below in a pathway specific manner. The pathways affected by bacterial proteins were previously shown to be the Map Kinase and the Apoptosis pathways in a large number

of experimental studies as reviewed in [Censini et al., 2001, Cover et al., 2003]. Figure 1 shows a functional breakdown of the entire network model for visualization purposes.

2.2 Map Kinase Signaling Pathway

The Map Kinase signaling pathway equations used in our study were previously presented by [Bhalla and Iyengar, 1995]. Their mathematical model contained 120 nodes (proteins in various phosphorylation states) and 200 reactions between the nodes. The rate equations used in the model consisted of simple association reactions and Michaelis-Menten enzyme kinetics. We used Locus Link ID comparison to confirm that the nodes in [Bhalla and Iyengar, 1995] were actually present in the KEGG Map Kinase pathway [Kanehisa and Goto, 2000]. Moreover we were able to confirm the interaction connections between these nodes of the Map Kinase pathway using the Ingenuity Pathway Analysis (IPA [www.ingenuity.com]). The rate equations governing the network nodes and their interactions with *H. pylori* proteins are presented in the supplementary information. The Map Kinase parameters and equations appearing in the supplementary information were previously published by [Bhalla and Iyengar, 1995] and more recently used by [Pant and Ghosh, 2005a,b].

The equations governing the interaction of the host signaling networks with the invading *H. pylori* proteins used in this study are described in Equations 1-7 in Table 1. In these equations the bacterial protein *CagA* interacts with *GBR-2* and *Shp-2* within the Map Kinase pathway [Censini et al., 2001] and also *CagA* is phosphorylated at EPIYA motifs by the host protein *SRC* Kinase [Naito et al., 2006]. The network diagram shown in Figure 1 indicates the need of prescribing 15 rate constants describing the interactions of *CagA* with the host. The two Michaelis-Menten rate constants, (k_1 , k_2) from Equation 1 in Table 1, for the enzymatic phosphorylation of *CagA* by *SRC* Kinase were previously estimated by [Jeansonne et al., 2005] using affinity purification to isolate and characterize pure *SRC* Kinase.

The forward and reverse binding constants, from Equation 2 in Table 1, involved in the interaction between phosphorylated *CagA* and *c-SRC* Kinase (*CSK*) (k_3 , k_4) were estimated from data on immunoprecipitation and an *in vitro* kinase assay [Tsutsumi et al., 2003]. Previous studies show that active *CSK* phosphorylates and deactivates *SRC* Kinase, suggesting the presence of a feedback loop modulating the amount of active *CagA* [Hatakeyama and Higashi, 2005] as described in Equation 3 in Table 1. The rate constants for this interaction (k_5 , k_6) were estimated based the activity of *SRC* Kinase as previously mentioned above [Jeansonne et al., 2005].

The next set of rate constants (k_7 , k_8) involves the binding interaction between phosphorylated *CagA* and the host protein *Shp-2* [Hatakeyama, 2002, Higashi et al., 2002, Zhou, 2002]. The constant k_7 refers to the binding affinity between *CagA* and *Shp-2* and k_8 refers to the dissociation of the complex in Equation 4 in Table 1. Both k_7 and k_8 were estimated from on *in-vitro* kinetics on purified proteins [Hatakeyama, 2002]. Furthermore, *Shp-2* activation produces a *Ras*-independent activation of *mapK* [Neel et al., 2003]. This leads to the next set of rate constants (k_9 , k_{10}) for Equation 5 in Table 1 which describes the rate of activation of *mapK* by active *Shp-2*. In the absence of experimental data in the literature these constants were assumed to be the same as those regulating the activation by activated *mapKK* [Bhalla and Iyengar, 1995].

Non-phosphorylated *CagA* can also interact with the host *GBR-2* protein [Mimuro et al., 2002] as per Equation 6 in Table 1. This interaction allows *GBR-2* to recruit *SOS* and subsequently convert the inactive *GDP-Ras* to active *GTP-Ras*. *GTP-Ras* will then continue down the Map Kinase pathway in a *Ras*-dependent nature. The parameters (k_{11} , k_{12}) were estimated from experiments using *in-vitro* purified proteins by [Mimuro et al., 2002]. It is

assumed that the *CagA-SOS-GBR-2* complex activates *Ras* at the same rate as the *SHC-SOS-GBR-2* complex [Bhalla and Iyengar, 1995].

2.3 Apoptosis Pathway

The Apoptosis Pathway equations used in this study were previously presented [Hua et al., 2005] and again we confirmed the constituency of its nodes with the corresponding KEGG pathway. Moreover an IPA search also identified the node connections specified. Overall this pathway has 56 nodes and 114 reaction equations. The Apoptosis pathway of the host epithelial cell is affected by the bacterial protein *VacA* as reviewed in [Cover and Blanke, 2005]. In this study it is assumed that *VacA* can enter the host cell cytoplasm through such mechanisms and focuses on its interaction with the Apoptosis pathway proteins. Estimates for the rate constants of *BAX* activation by *VacA* (k_{13} , k_{14}) for Equation 7 in Table 1 were derived from experiments on isolated mitochondria and purified cellular proteins [Galmiche et al., 2000].

Production of bacterial proteins *CagA* and *VacA* as represented by $K^{prod}X_{BL}$ in the model equations were assumed to have occurred at a constant rate proportional to X_{BL} . This variable is a measure of bacterial load (*CagA* and *VacA*) and corresponds to the Multiplicity of Infection (MOI) [Tsutsumi et al., 2003, Yamasaki et al., 2006].

2.4 Numerical Simulation

The mathematical model incorporating the interaction of invading bacterial proteins with the host Map Kinase and Apoptosis pathways was constructed with the CellDesigner tool [Funahashi et al., 2003]. This program creates an SBML [Hucka et al., 2003, Kitano et al., 2005] compliant model which can be simulated by a number of tools. The ordinary differential equations (ODEs) resulting from the model were solved using the MatLab 7.0.4 (www.mathworks.com) ode23s function with default values given for ode23s. The protein levels described by the initial conditions were kept constant in the simulations modeling the signal processing through the Map Kinase and Apoptosis pathways. The proteins in these pathways were allowed to change state as the during the transmission of the signal as defined by network equations.

The simulations take into account the possibility that host stimulation of the effected pathways modulate bacterial pathogenesis [Hatakeyama and Brzozowski, 2006]. To model host stimulation of the respective pathways, the host cells were assumed to be exposed to a concentration of $EGF = 0.5$ uM and $FasL = 0.1$ uM, resulting in subsequent stimulation of the Map Kinase [Bhalla and Iyengar, 1995] and Apoptosis pathways [Hua et al., 2005].

2.5 Parameter Scan

The parameters appearing in our simulated Map Kinase and pathways were investigated previously in a parameter search space by [Pant and Ghosh, 2005a,b]. This study focuses on the impact of parameters (k_1 - k_{14}) governing the direct interaction of host and invading proteins. The standard values of these 14 parameters were multiplied by a modifying constant (K_{mod}) that ranged in value between 0.1 and 4.0. Simulations were then executed by altering these parameters one at a time and quantifying the time course of *mapK* and *Casp-3* activity. Note that *Casp-3* stands as a phenotypic marker for apoptosis effects and *mapK* for growth.

2.6 Microarray analysis

Publicly accessible microarray datasets involving global gene expression profiles of human epithelial cells in the presence and absence of *H. pylori* infection were acquired from Stanford Source Microarray Database [Gollub et al., 2006]. Specifically, these data sets corresponded to microarray data on T84 polarized monolayers taken at discrete time intervals following

infection with wild-type *H. pylori* and their controls. The Stanford gene chip used in the microarray study consisted of cDNA spots for approximately 43,000 genes [El-Etr et al., 2004]. The data sets were normalized based on the standard Stanford procedure [Gollub et al., 2006] into log2 normalized data. Table 2 summarizes the microarray data used in this study.

Microarray data samples from infected cells were grouped and compared to the entire time-course of the corresponding data for mock infected cells (control data). This analysis was performed using the t-test module of the TIGR MEV tool [Welch, 1947, Dudoit et al., 2002, Pan, 2002]. Genes which had a p-value less than 0.01 regardless of the direction of expression change were saved for further analysis. The pairings used in the analysis are listed in Table 2.

Since both the Map Kinase and Apoptosis pathways effect transcription of functional genes the gene sets should have an over-representation of promoters for either apoptosis or growth. The PAINT promoter tool [Vadigepalli et al., 2003] is a high-throughput bioinformatics algorithm for promoter analysis. The PAINT analysis retrieved the 2000 bases upstream of each gene within the test set and searched the public TRANSFAC database [Matys et al., 2003] for known promoter recognition sites and their corresponding transcription factors.

3 Results and Discussion

This section presents the simulation results concerning perturbations of the Apoptosis and MAP Kinase pathways, which are due to *H. pylori* infection. The parameters that govern the interaction of bacterial proteins with the host protein network are altered one at a time to gauge the possible effects of bacterial and host genotypes on the predicted results. The last subsection compares model predictions with findings based on microarray data of epithelial cells infected with *H. pylori* and their controls.

3.1 MAP Kinase pathway

The bacterial protein *CagA* interacts with the proteins of the host cell, and these proteins lay in the MAP Kinase Pathway as described above. *CagA* can directly activate *mapK* through activation of *Shp-2* or activate *RAS* through interaction with *GBR-2* and *SOS*. Consistent with the extent of interaction of *CagA* with the host protein network, our results indicate a complex set of perturbations to the MAP Kinase pathway, as quantified in simulations by the time variant concentration of active *mapK* (Figure 2). The simulations, shown in Figure 2, were generated by solving the MAP Kinase pathway rate equations for a time-span of 32400 second (9 hours) under *EGF* stimulated (A) and non-stimulated (B) conditions. Note that *EGF* (Epidermal Growth Factor) regulates the growth activity of many types of epithelial cells, including those affected by *H. pylori* infection. The figure indicates that the level of activation of *mapK* changes with bacterial load in a dose dependent manner. Furthermore, bacterial load shortens the time to *mapK* activation in a dose dependent manner. The time of activation of MAP Kinase pathway due to bacterial infection is shorter in the presence of *EGF* stimulation at moderate to low bacterial loads, but time to activation decreases rapidly with increasing bacterial load as shown in Figure 2 C. In contrast, the time for deactivation is independent of the level of bacterial load.

The time course of MAP Kinase activation due *H. pylori* infection can be better understood when the simulation results are plotted as a function of time for the critical nodes appearing within the pathway (Figure 3). The MAP Kinase nodes of the figure include *GBR-2-SOS-SHC* complex, GTP-*RAS*, active *Raf*, *RAS-Raf* complex, active *mapKK*, active *mapK*, *CagA-Shp-2* complex, inactivated *GBR-2-SOS* and aberrantly activated *GBR-2-SOS-CagA*. The time variant concentration curves of the nodes within the MAP Kinase pathway under *EGF* stimulation are shown as a black line with stars at specific data points; corresponding curves due to bacterial loading only are shown as a red line with circles at specific data points; the same curves with both bacterial loading and *EGF* stimulation conditions are shown as a blue

line with triangles at specific data points. The results shown in the diagram point to a feedback loop of active *mapK* deactivating the *GBR-2-SOS* complex and modulating the activation length of the MAP Kinase pathway. *MapK* activated by bacterial proteins causes an aberrant feedback loop, which deactivates the *GBR-2-SOS* complex and prevents the other regulatory functions of the MAP Kinase pathway from deactivating *mapK*.

3.2 Apoptosis pathway

The interaction of bacterial protein *VacA* with the *BAX* protein of the Apoptosis pathway leads to a perturbation of the Apoptosis network, as shown in Figure 1. The end result of this perturbation is exhibited in the concentration of the active form of *Casp-3*, which is considered to be a marker for upcoming apoptosis [Xia et al., 2001]. Our results, obtained by solving the 114 rate equations of the apoptosis model for a time span of 86400 seconds (24 hours), show that *Casp-3* activation is dependent on the presence of *FasL*, an Apoptosis stimulating hormone, stimulation of the host cell and the bacterial load, as measured by the concentration of *VacA* in the host cell (Figure 4A). *FasL* concentration in this computational simulation was held constant at 0.1uM (I) or zero (II), which is consistent with standard experimental techniques [Kelly et al., 2002]. Bacterial load was then varied for both stimulated and non-stimulated conditions from 0.1 to 1.0. Our results indicate that *FasL* activation is much more efficient in a time-wise manner for inducing apoptosis compared to *H. pylori* infection in short time periods (7 hours vs. > 2 hours), but bacterial infection is sufficient to cause apoptosis by itself within the first twenty-four hours of infection.

Infection induced apoptosis is dependent on the presence *FasL* stimulation of the host epithelial cells, which might have been caused by a number of factors including inflammation. Figure 4B shows that *FasL* induced, *Casp-3* activation proceeds independent of the level of bacterial infection, whereas, the bacterial infection induced apoptosis is highly dependent on the cellular concentration of the invading *VacA* protein. The 80% activation time, shown in the figure, identifies the time in which 80% of *Casp-3* had been converted to its active form. These results point to the importance of bacterial load in the progression *H. pylori* induced gastritis.

The model simulations presented here bring a deeper understanding of the detailed interrelations between various nodes within the Apoptosis pathway. The diagram in Figure 5 shows the predicted time variant concentrations of critical nodes within the Apoptosis pathway under *FasL* stimulated and bacterial loading conditions. Each curve in a node box in Figure 5 represents the concentration of the node under bacterial load only (red with circles), stimulated only (black with stars) and stimulated and bacterial loading (blue with triangles) conditions. The numerical simulation indicates two distinct modes of flow of the apoptosis inducing signal through the pathway. The preferred mode of flow for *FasL* stimulation is through activation of *Casp-3* directly by *Casp-8*, whereas, the bacterial load induced signal perpetuates through an alternative cycle that begins with *BAX* activation by *VacA* and goes through *Cyt-C* release and subsequent activation of *Casp-3*. These results are in agreement with medical practice of eradicating *H. pylori* infections to prevent progression from gastric ulcers to cancerous regions [Xia et al., 2001].

Activation of the Apoptosis pathway by *H. pylori* infection bypasses the primary mode of transmission that utilizes the interaction between *Casp-8* and *Casp-3*. Under bacterial loading only conditions *Casp-8* never activates and interactions 2, 7, and 8 never occur, since *VacA* perturbations activate the pathway downstream of *Casp-8*, as shown in Figure 5. The *VacA* induced activation of *BAX* increases *Cyt-C* release through interaction 4. This causes a slower rate *Casp-9* activation, as compared to *FasL* stimulation, due to the interaction of *XAIP*, which inhibits *Casp-9* activation (not shown). Active *Casp-9* then activates *Casp-3* through interactions 6 and 9. The results of this simulation demonstrate that bacterial induced apoptosis bypasses the primary cellular modes of apoptosis, avoiding various checkpoints for cell death.

When the simulated host cell is exposed to both *FasL* stimulation and bacterial loading conditions, the *FasL* mode of transmission dominates. Figure 5, *FasL* stimulation leads to activation of *Casp-8* and subsequent activation of *Casp-3* after approximately three hours. Even under high bacterial loads the *Casp-3* activation by *Casp-8* preempts the hyper-activation of *Casp-9* by bacterial induced *Cyt-C* release.

3.3 Bacterial Parameters and Their Effect of Host Cell Response

Parameters that govern the interaction of bacterial proteins with the host cell proteins were described above. We have changed these parameters one at a time by multiplying their standard values with a following coefficients (0.1, 0.2, 0.5 1, 2, 4). Results of this large scale simulation show that 12 out of the 14 parameters described above could be varied by large amounts without significant alteration to the time course of *mapK* activation. On the other hand, as shown in Figure 6, the parameter k_9 , representing the rate at which the *CagA-Shp-2* complex converts inactive *mapK* into activated *mapK* (Figure 3, Interaction 6), strongly influences time to activation of *mapK*. Similarly, the decreasing values of the rate constant k_8 , governing the dissociation rate of the *CagA-Shp-2* complex, shortened the activation time of *mapK*. These results are consistent with medical data, indicating genotype dependence of *H. pylori* as a risk factor for infection induced gastritis [Bhat et al., 2005]. Moreover, our simulation results point to the importance of single nucleotide polymorphisms that affect the formation and enzymatic efficiency of *Shp-2*.

3.4 Comparison of Simulation Results to Microarray Data

The end result of signal transduction through the MAP Kinase pathway is the emergence of a series of transcription factors that modulate the expression of growth related genes. The surrogate markers for the transcription factors involved in our simulation are *mapK* and *PKA*. Increasing concentrations of these nodes point to increasing expression of genes related to the transcription factors such as *AP-1* and *elk-1*. Similarly, the end result of apoptosis pathway is *Casp-3* activation, which leads to a sequence of events such as activation of the transcription factors *Oct-1* and *NFkB*. Using PAINT and IPA tools and publicly accessible microarray data on *H. pylori* infected epithelial cells and their controls [El-Etr et al., 2004], we determined the time course of the emergence of transcription factors that are linked to the concentration of *MapK*, *PKA*, and *Casp-3*, as shown in Table 3. The impetus for this study was to see if the time course of emergence of MAP Kinase and Apoptosis related transcription factors matched with the predicted time course of signal transduction presented in this study.

Upon submitting to PAINT the significant genes found in the analysis of the 2 and 4 hour time points, PAINT returned a list of transcription factors which showed a statistical over-representation; these are listed in Table 3. The transcription factors that appear at this early stage of infection include the *mapK* activated *elk-1* ($p=0.00000$) and *AP-1* ($p=0.00000$), a transcription known to be activated by *mapKK* [Takeuchi et al., 2006]. These results closely match with the time course of signaling of the MAP Kinase pathway predicted in this study (Figure 7).

The transcription factor *NFkB* appeared as significantly over-represented ($p=0.0083$) in microarray data at the 8 and 12 hour comparison. This transcription factor controls survival and inflammatory genes and is activated in response to apoptosis [Clarkson and Watson, 1999]. Recent studies have suggested that *NFkB* can be activated by *Casp-8* [Lamkanfi et al., 2006]. Figure 7 shows the *Casp-8* response to infection and stimulation. *Oct-1* was also significantly over expressed at 12 to 24 hour time points; this transcription factor is known to be down-regulated by *PKA* activity and activated in response to DNA damage [Heckman et al., 2006]. During the early time-points, *Oct-1* activity is repressed by high levels of *PKA* activity (Figure 7). However, active *Casp-3* will begin apoptotic events and increase DNA

damage [Lamkanfi et al., 2006]. Again, these results match with the predicted time course of Apoptosis signaling events using the CellDesigner simulation, as shown in Figure 7.

4 Conclusion

This study integrates detailed analysis of signaling pathways related to *H. pylori* infection of epithelial cells with global gene expression patterns that are associated with infection and the time course of the emergence of transcription factors which modulate the growth and death of infected epithelial cells. The signaling pathway models used in this study are composed of sets of ODEs with time as an independent variable and concentrations of proteins appearing in these signaling pathways as dependent variables. These equations and the estimates of rate parameters associated with them were previously published. We have incorporated a set of new rate equations and associated rate constants to these equations in order to quantitatively describe the binding interactions of *H. pylori* proteins with the protein network of human epithelial cells. One of the most important findings of our study is the fact that existing mathematical models of MAP Kinase and Apoptosis pathways stood up to the challenge of comparison with microarray data on *H. pylori*. Note that the signaling pathways investigated in this study are implicated in the transformation of human cells to a cancer phenotype.

Our study showed the importance of the feedback loop in MAP Kinase mediated signaling as a modulator of bacterial activation of the pathway. Our simulations show that bacterial load activates the MAP Kinase pathway and subsequently a feedback loop of *mapK*; specifically, the feedback loop operates by deactivating the *RAS-RAF* complex and results in aberrant *mapK* signaling. The two constants that showed significant effect on the time course of simulated infection are linked to the binding of *Shp-2* to *CagA* and the *Shp-2* mis-activation of *mapK*. These proteins might be the loci of SNPs which modulate host response and the time course of infection. On the bacterial side *CagA* SNPs have already been shown to have drastic effects on the host response [Israel et al., 2001, Höcker and Hohenberger, 2003, Naito et al., 2006].

The transcription factors that emerge due to the MAP Kinase signaling perturbations by bacterial infection lead to an increased transcription of Apoptosis inhibitors *XAIP* and *FLIP* [Kasid and Dritschilo, 2003, Shelton et al., 2003]. In addition, the bacterial protein *VacA* specifically binds and activates *BAX*, a protein that appears in the *FasL* stimulated Apoptosis pathway. Our simulations concerning the time course of Apoptosis signaling are consistent with aforementioned mentioned microarray data on *H. pylori* infected epithelial cells and their controls. Our simulations indicated that SNPs in the *BAX* protein are likely to affect the interactions of the *VacA* protein and therefore, the time course of infection. Genotyping of patients with *H. pylori* infection are only beginning. The use of recently developed high-throughput genotyping tools will lead to testing and validating the findings of this study concerning the SNPs in the human *BAX*, *Shp-2* and *mapK* proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Diagram of the host signaling network (Map Kinase and Apoptosis pathways) perturbed by the invading bacterial proteins which are marked in red. The pathways are divided into functional groups for representative purposes. A diagram the entire network and its connections is available in the supplementary information.

Fig. 2.

The time course of active *mapK* due to bacterial loading in the presence and absence of *EGF*. The symbol BL refers to the simulated level of bacterial load. **A:** The system was stimulated by a constant *EGF* concentration ($EGF = 0.5$ uM) and varying bacterial loads for the entire duration. **B:** The pathway was stimulated by varying levels of bacterial loads. **C:** The time at which 80% of *mapK* is in the active form as a function of bacterial loading and *EGF* stimulation.

Fig. 3.

A diagram indicating the simulation of the Map Kinase pathway under stimulation by EGF only (black starred line), Bacterial load only (red circled line) and both (blue triangled line). Bacterial load was held at 0.1 for the simulation. Each node represents the time-dependant concentration over 8 simulated hours and heights are scaled for visualization purposes. Interaction 1: *GBR2-SOS-SHC* causes GDP-RAS phosphorylation to GTP-RAS. Interaction 2: *CagA* forms a complex with *SOS* and *GBR-2* to mis-activate *RAS*. Interaction 3a and 3b: Activated *Raf* and GTP-RAS bond to form an active *RAS-RAF* complex. Interaction 4: Active *RAS-RAF* complex activates *mapKK*. Interaction 5: Active *mapKK* activates *mapK* which leads to transcription of growth factors. Interaction 6: *CagA-Shp-2* complex mis-activates *mapK*.

Interaction 7: Active *mapK* converts *SOS* to an inactivated form which attenuates the signal. The green arrows denote activation of molecules while red denotes deactivation.

Fig. 4.

The time course of active *Casp-3* under bacterial loading in the presence and absence of *FasL* stimulation. The symbol BL refers to the simulated level of bacterial load. **A I:** The system was stimulated by a constant *FasL* concentration $FasL = 0.1\mu\text{M}$ and varying bacterial loads for the entire duration. **A II:** The pathway was stimulated only by varying levels of bacterial loads. **B:** Time to 80% activation.

Fig. 5.

Simulation of the Apoptosis pathway under FasL stimulation (black starred line), bacterial only (red circled line) and both (blue triangled line). Bacterial load was held at 0.1 for the simulation. Each node represents the time-dependant concentration over 24 simulated hours and heights are scaled for visualization purposes. Interaction 1: *FasL* stimulation leads to *Casp-8* activation through the *FAS-FADD* complex. Interaction 2: Active *Casp-8* will lead to host activated *BAX* through a truncation of *BID* (not shown). Interaction 3: Activated *BAX* (*BAX* bound to *tBID*) will cause *Cyt-C* release from the membrane. Interaction 4: *VacA* activated *BAX* will cause *Cyt-C* release into the membrane. Interaction 5: *Cyt-C* leads to an activation of *Casp-9* through a complex with *APAF:ATP* (not shown). Interaction 6a, 6b and 9: Activated *Casp-9*

will form a complex with inactive *Casp-3* and subsequently cause *Casp-3* activation. Interaction 7a, 7b and 8: Activated *Casp-8* will form a complex with inactive *Casp-3* and cause a subsequent cleavage and activation of *Casp-3*. Interaction 10: Active *Casp-3* will lead to the physical acts of Apoptosis.

Fig. 6.

The top panel shows time course curves of mapK activation due to variation in k_9 and the bottom panel indicates the time course of mapK due to variation in k_8 . Simulation was performed under non-stimulated conditions with a $BL=1$.

Fig. 7. Normalized activities of selected proteins which have been associated with the *AP-1*, *Elk-1*, *NfKb*, *Oct-1* transcription faction. The simulation was integrated for 86400 seconds (24 hours) with a BL = 1.0 and (*FasL* = 0.1 uM, *EGF* = 0.5 uM).

The equations that follow describe the direct interactions between host and bacterial proteins in the simulation. The terms in bold are taken directly from either Bhalla and Iyengar [1995] or Hua et al. [2005] The entire set of 200 differential equations are available in the supplementary material.

Table 1

$$\begin{aligned}
 \frac{dX_{CagA}}{dt} &= -\frac{k_1 X_{CagA} X_{SRC}}{X_{CagA} + k_2} + \frac{prod X_{BL} - K_{CagA}^{deg} X_{CagA}}{K_{CagA}} \\
 \frac{dX_{CagA}_P}{dt} &= \frac{k_1 X_{CagA} X_{SRC}}{X_{CagA} + k_2} \\
 \frac{dX_{CagA}_{pp}}{dt} &= \frac{k_1 X_{CagA} X_{SRC}}{X_{CagA}_P + k_2} \\
 \frac{dX_{CagA}_{pp}}{dt} &= \frac{X_{CagA}_P + k_2}{X_{CagA}_P} \\
 \frac{dX_{CagA}_{pp}}{dt} &= -k_3 X_{CagA} X_{CSK} + k_4 X_{CagA}_{pp} X_{CSK} + \frac{k_1 X_{CagA} X_{SRC}}{X_{CagA}_P + k_2} \\
 k_1 &= 233(\text{uM/s}), k_2 = 3.1(\text{uM}) \text{ [Jeansonne et al., 2005]} \\
 \frac{dX_{CagA}_{pp}}{dt} &= \frac{dX_{CagA}_{pp}^{CSK}}{dt} = k_3 X_{CagA} X_{CSK} - k_4 X_{CagA}_{pp} X_{CSK} \\
 \frac{dX_{CSK}}{dt} &= -k_3 X_{CagA} X_{CSK} + k_4 X_{CagA}_{pp} X_{CSK} \\
 k_3 &= 5(\text{uM/s}), k_4 = 0.1(\text{uM/s}) \text{ [Tsutsumi et al., 2003]} \\
 \frac{dX_{SRC}_{in}}{dt} &= \frac{k_5 X_{SRC}_{act} X_{CagA} X_{CSK}}{X_{SRC}_{act} + k_6} \\
 \frac{dX_{SRC}_{act}}{dt} &= \frac{k_5 X_{SRC} X_{CagA} X_{CSK}}{X_{SRC}_{act} + k_6} \\
 k_5 &= 233(\text{uM/s}), k_6 = 3.1(\text{uM}) \text{ [Hatakeyama and Higashi, 2005]} \\
 \frac{dX_{CagA}_{pp}}{dt} &= -k_7 X_{CagA} X_{CagA}_{pp}^{shp-2} + k_8 X_{CagA}^2 - k_3 X_{CagA} X_{CSK} + k_4 X_{CagA}_{pp} X_{CSK} + \frac{k_1 X_{CagA} X_{SRC}}{X_{CagA}_P + k_2} \\
 \frac{dX_{CagA}_{pp}^{shp-2}}{dt} &= -k_7 X_{CagA} X_{CagA}_{pp}^{shp-2} + k_8 X_{CagA}^2 \\
 \frac{dX_{CagA}_{pp}^{shp-2}}{dt} &= \frac{dX_{CagA}_{pp}^{shp-2}}{dt} = k_7 X_{CagA} X_{CagA}_{pp}^{shp-2} - k_8 X_{CagA}^2 \\
 k_7 &= 5.0(\text{uM/s}), k_8 = 0.1(\text{uM/s}) \text{ [Hatakeyama and Higashi, 2005, Higashi et al., 2002, Zhou, 2002]}
 \end{aligned}$$

$$\frac{dX_{mapK_{pa}}}{dt} = \frac{k_9 X_{mapK_{in}} X_{CagA}^2}{X_{mapK_{in}} + k_{10}} + \frac{V_{177}^{max} X_{MKP1} X_{mapK_{pa}}}{K_{177}^m + X_{mapK_{pa}}} + \frac{V_{83}^{max} X_{mapKK_{pp}} X_{mapK_{pa}}}{K_{83}^m + X_{mapK_{pa}}} - \frac{V_{85}^{max} X_{mapKK_{pp}} X_{mapK_{pa}}}{K_{85}^m + X_{mapK_{pa}}} \quad (5)$$

$$\frac{dX_{mapK_{pa}}}{dt} = - \frac{k_9 X_{mapK_{pa}} X_{CagA}^2}{X_{mapK_{pa}} + k_{10}} - \frac{V_{83}^{max} X_{mapKK_{pp}} X_{mapK_{pa}}}{K_{83}^m + X_{mapK_{pa}}} + \frac{V_{86}^{max} X_{MKP1} X_{mapK_{pa}}}{K_{86}^m + X_{mapK_{pa}}}$$

$$\frac{dX_{mapK_{pp}}}{dt} = - \frac{V_{85}^{max} X_{mapKK_{pp}} X_{mapK_{pa}}}{K_{85}^m + X_{mapK_{pa}}} + \frac{V_{86}^{max} X_{MKP1} X_{mapK_{pp}}}{K_{86}^m + X_{mapK_{pp}}}$$

$k_9 = 0.15(\text{uM/s})$, $k_{10} = 0.0463(\text{uM})$, $V_{86}^{max} = 1(\text{uM} / \text{s})$, $K_{86}^m = 0.066667(\text{uM})$, $V_{85}^{max} = 0.15(\text{uM} / \text{s})$, $K_{85}^m = 0.046297(\text{uM})$, $V_{83}^{max} = 0.15(\text{uM} / \text{s})$, $K_{83}^m = 0.046297(\text{uM})$, $V_{177}^{max} = 1(\text{uM} / \text{s})$, $K_{177}^m = 0.066667(\text{uM})$. [Bhalla and Iyengar, 1995]

$$\frac{dX_{CagA-GBR-2}}{dt} = k_{11} X_{GBR-2} X_{CagA} - k_{12} X_{CagA-GBR-2} - \frac{k_1 X_{CagA} X_{SRC}}{X_{CagA} + k_2} + K_{CagA}^{prod} X_{BL} - K_{CagA}^{deg} X_{CagA} \quad (6)$$

$$\frac{dX_{GBR-2}}{dt} = -k_{11} X_{GBR-2} X_{CagA} + k_{12} X_{CagA-GBR-2} - K_{11}^b X_{GBR-2} X_{SOS} + K_{11}^f X_{GBR-2} - SOS_P X_{GBR-2} + K_{13}^b X_{SOS_P} X_{GBR-2} + K_{13}^f X_{GBR2} - SOS_P$$

$k_{11} = 0.75(\text{uM/s})$, $k_{12} = 0.075(\text{uM/s})$ [Mimuro et al., 2002], $K_{13}^b = 0.0168(\text{uM} / \text{s})$, $K_{13}^f = 4.1667 \cdot 10^{-8}(\text{uM} / \text{s})$, $K_{11}^b = 1(\text{uM} / \text{s})$, $K_{11}^f = 4.1667 \cdot 10^{-8}(\text{uM} / \text{s})$ [Bhalla and Iyengar, 1995]

$$\frac{dX_{VacA}}{dt} = K_{VacA}^{prod} X_{BL} - K_{VacA}^{deg} X_{VacA}$$

$$\frac{dX_{BAX_{act}}}{dt} = \frac{k_{13} X_{BAX_{in}} X_{VacA}}{X_{BAX_{in}} + k_{14}}$$

(7)

$$\frac{dX_{BAX_{in}}}{dt} = - \frac{k_{13} X_{BAX_{in}} X_{VacA}}{X_{BAX_{in}} + k_{14}} - \frac{K_{232}^f X_{tBID} X_{BAX_{in}} + K_{232}^b X_{BAX_{in}}}{X_{BAX_{in}}} - \frac{K_{233}^f X_{BCL-2} X_{BAX_{in}} + K_{233}^b X_{BCL-2} X_{BAX_{in}}}{X_{BAX_{in}}} - \frac{K_{234}^f X_{BAX_{in}} + K_{234}^b X_{BAX_{in}}}{X_{BAX_{in}}} - tBID$$

$k_{13} = 0.0004(\text{uM/s})$, $k_{14} = 6.0(\text{uM})$, $K_{232}^f = 0.2(\text{uM} / \text{s})$, $K_{232}^b = 0.02(\text{uM} / \text{s})$, $K_{233}^f = 2(\text{uM} / \text{s})$, $K_{233}^b = 0.02(\text{uM} / \text{s})$, $K_{234}^f = 0.2(\text{uM} / \text{s})$, $K_{234}^b = 0.02(\text{uM} / \text{s})$ [Galmiche et al., 2000]

Table 2

A summary of microarray data from El-Etr et al. used in the analysis.

Number of cDNA probes per chip: 43,196			
Number of chips per time point: 2			
Number of control replicates: 12			
Time points sampled: {2, 4, 8, 12, 24}			
t-test pairings			
<i>T2 U T4</i> vs. Ctl	<i>T4 U T8</i> vs. Ctl	<i>T8 U T12</i> vs. Ctl	<i>T12 U T24</i> vs. Ctl

Microarray data indicating the time course of emergence of transcription factors (TF) due to *H. pylori* infection. The table shows the results of the promoter analysis of the paired time points vs. the entire mock time-course using the PAINT software (p<0.1). The p-value is a significance measure comparing the measured frequency of the binding sites to the frequency of the entire database.

2-4 hour	4-8 hour	8-12 hour	12-24 hour	p-value
TRE\ExternalGroup	TRE\ExternalGroup	TRE\ExternalGroup	TRE\ExternalGroup	
AP-1/V/\$AP1_C	AP-1/V/\$AP1_C	AP-1/V/\$AP1_C	AP-1/V/\$AP1_C	0
AP-1/V/\$AP1_Q4	AP-1/V/\$AP1_Q2	AP-1/V/\$AP1_Q2	AP-1/V/\$AP1_Q2	0
AP-4/V/\$AP4_01	AP-1/V/\$AP1_Q4	AP-1/V/\$AP1_Q4	AP-1/V/\$AP1_Q4	0
AP-4/V/\$AP4_01	AP-4/V/\$AP4_01	AP-4/V/\$AP4_01	AREB6/V/\$AREB6_01	0
Barbie Box/V/\$BARBIE_01	AREB6/V/\$AREB6_01	Barbie Box/V/\$BARBIE_01	Ami/V/\$ARNT_01	0
CCAAT box/V/\$CAAT_01	Ahr/Ami/V/\$AHRARNT_01	CCAAT box/V/\$CAAT_01	CCAAT box/V/\$CAAT_01	0
CDP CR3/V/\$CDPCR3_01	C/EBP/V/\$CEBP_C	CDP CR1/V/\$CDPCR1_01	CDP CR1/V/\$CDPCR1_01	0
CDP/V/\$CDP_01	CCAAT box/V/\$CAAT_01	CHOP-C/EBPalpha/V	CDP CR3+HD/V	0
CHOP-C/EBPalpha/V	CDP CR1/V/\$CDPCR1_01	\$CHOP_01	\$CDPCR3HD_01	0
\$CHOP_01	CDP CR3+HD/V	COMP1/V/\$COMP1_01	CHOP-C/EBPalpha/V	0
COMP1/V/\$COMP1_01	CDP CR3+HD/V	COMP1/V/\$COMP1_01	\$CHOP_01	0
CRE-BP1/V/\$CREBP1_Q2	CDPCR3HD_01	CP2/V/\$CP2_01	COMP1/V/\$COMP1_01	0
CRE-BP1/c-Jun/V	COMP1/V/\$COMP1_01	CRE-BP1/V/\$CREBP1_Q2	CP2/V/\$CP2_01	0
\$CREBP1CJUN_01	CP2/V/\$CP2_01	CRE-BP1/c-Jun/V	CRE-BP1/V/\$CREBP1_Q1	0
E2/V\$E2_Q6	CRE-BP1/V/\$CREBP1_Q1	CREB/V/\$CREB_01	CRE-BP1/V/\$CREBP1_Q2	0
Elk-1/V/\$ELK1_01	CRE-BP1/V/\$CREBP1_Q2	E2/V\$E2_Q6	CRE-BP1/c-Jun/V	0
Elk-1/V/\$ELK1_02	CRE-BP1/c-Jun/V	ER/V\$ER_Q6	\$CREBP1CJUN_01	0
E47/V/\$E47_Q2	\$CREBP1CJUN_01	ER/V\$ER_Q6	CREB/V/\$CREB_01	0
GATA-1/V/\$GATA1_Q6	CREB/V/\$CREB_01	Elk-1/V/\$ELK1_02	E2F/V\$E2F_Q2	0
AP-1/V/\$AP1_Q2	E2/V\$E2_Q6	CRE-BP1/V/\$CREBP1_Q1	ER/V\$ER_Q6	0
AREB6/V/\$AREB6_01	ER/V\$ER_Q6	E2F/V\$E2F_Q2	Elk-1/V/\$ELK1_01	0
CDP CR1/V/\$CDPCR1_01	Elk-1/V/\$ELK1_01	NF-kappaB/V	Elk-1/V/\$ELK1_Q2	0
Bmi-2/V/\$BRN2_01	Elk-1/V/\$ELK1_Q2	\$NFKAPPA_B_01	Evi-1/V/\$EVI1_Q4	0
E2F/V/\$E2F_Q2	Elk-1/V/\$ELK1_Q2	c-Myc/Max/V/\$MYC/MAX_Q2	FOXD3/V/\$FOXD3_Q1	0
CREB/V/\$CREB_01	CHOP-C/EBPalpha/V	Elk-1/V/\$ELK1_01	Gfi-1/V/\$GFI1_01	0.00002
E2F/V/\$E2F_Q2	\$CHOP_01	NF-kappaB (p50)/V	Ahr/Ami/V/\$AHRARNT_01	0.00003
CREB/V/\$CREB_01	E2F/V\$E2F_Q2	\$NFKAPPA_B50_01	Oct-1/V/\$OCT1_Q7	0.00111
C/EBP/V/\$CEBP_C	FOXJ2/V/\$FOXJ2_01	HNF-1/V/\$HNF1_C	ARP-1/V/\$ARPI_01	0.00258
E47/V/\$E47_Q1	Lmo2 complex/V	PPARalpha/RXR-alpha/V	C/EBP/V/\$CEBP_C	0.00314
Ami/V/\$ARNT_01	\$LMO2COM_02	SPPARA_01	Cart-1/V/\$CART1_01	0.00335
HFH-1/V/\$HFH1_01	SREBP-1/V/\$SREBP1_Q1	MEF-2/V/\$MEF2_Q2	Bmi-2/V/\$BRN2_01	0.01369
Cart-1/V/\$CART1_01	NF-Y/V/\$NFY_Q6		PPARalpha/RXR-alpha/V	0.01871
Evi-1/V/\$EVI1_Q4			\$PPARA_01	0.01871
USF/V/\$USF_Q6			GATA-1/V/\$GATA1_Q5	0.0209
GATA-X/V/\$GATA_C			Evi-1/V/\$EVI1_Q5	0.03262
PPARalpha/RXR-alpha/V			USF/V/\$USF_Q6	0.0515
SPPARA_01			Barbie Box/V/\$BARBIE_01	0.05182