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Modeling host–pathogen interactions: *H. sapiens* as a host and *C. difficile* as a pathogen

Namrata Tomar* and Rajat K. De

Many complex mechanisms in immunological studies cannot be measured by experiments, but can be analyzed by mathematical simulations. Using theoretical modeling techniques, general principles of host–pathogen system interactions can be explored and clinical treatment schedules can be optimized to lower the microbial toxin burden and side effects in the host system. In this study, we use a computational modeling technique that aims to explain the host–pathogen interactions and suggests how the host system tries to survive from the pathogen attack. The method generates data on reaction fluxes in a pathway at steady state. A set of constraints is incorporated and an objective function for the minimization of toxin expression, with respect to some parameters such as concentration of signaling molecules, is formulated. We have integrated the toxin expression regulatory pathway in *Clostridium difficile*, apoptosis and mitogen-activated protein kinase pathways in an infected host (*Homo sapiens*). We have found that due to the minimization of the toxin expression, the signal flow values for most of the survival genes are at the higher side, whereas it is the reverse for most of the proapoptotic genes. We have observed increased signal flow values of the molecules for extracellular regulated kinase as compared with the molecules present in c-Jun NH2-terminal kinase/p38 pathways. In light of these observations, we can hypothesize that lower toxin level in a pathogen implies higher chance of host survival. Copyright © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

Biological system has a high degree of complexity. The complexity arises from interaction among various components of a biological system (organism). Systems biology aims at understanding these interactions between various components of an organism as well as with its outside environment. The components of an organism may be at different levels. If we consider a living cell as a system, then various pathways form components, whereas cells form components of a tissue system. In this way, tissues and organs can be components of an organ and a whole organism respectively.

Likewise, an immune system involves interactions among various molecules and cells. The immune system has evolved to protect an individual from potential harmful outside invaders. Immune response to pathogen attack constitutes a complex interaction system. Signal activation, deactivation and coordination of immune responses constitute host–pathogen interactions. Immunological signaling is central in determining the success of host defense. Massive experimental efforts and increasingly sophisticated experimental methods have resulted in a large list of components involved in immunological signaling and their role in pathogenic infections. However, a good understanding of the principles of immune signaling and an integrated view of its role in larger-scale effect of infection require systems-oriented approaches.

There exist a few *in silico* studies performed on host–pathogen interactions. Impact of the host–pathogen interaction during a viral infection on the metabolism of host has been studied employing flux balance analysis (Jain and Srivastava, 2009). Through their model, the authors have predicted increase in metabolic activity of the pentose phosphate pathway, although there is a breakdown in the citric acid cycle. However, they have not predicted any change in the glycolysis pathway. Flux balance analysis and *in silico* gene essentiality analysis have been applied to investigate the growth of *Salmonella typhimurium LT2 (iRR1083)* under different environments (Raghunathan *et al*., 2009). The results suggest a robust minimal set of metabolic pathways that is required for replication of *Salmonella* inside the host cell. Similar kind of study has been carried out that reconstructs a genome-scale metabolic model for the biothreat agent *Francisella tularensis (iRS605)* using constraint-based modeling (Raghunathan *et al*., 2010). It also reveals a minimal set of metabolic genes that are functional during infection and shows the pathogen's metabolic capacity during infection. These kinds of studies identify the essential genes for survival and the minimal requirement for pathogen survival in the host cell. It subsequently identifies targets for new antibiotics. Two separate networks of interactions have been synthesized between host immune components and two closely related bacteria of the genus *Bordetellae*, *viz. B. bronchiseptica* and *B. pertussis* (Thakar *et al*., 2007). The model indicates that the infection time course of both *Bordetellae* can be separated into three distinct phases on the basis of the most active immune processes. They have also compared the effect of species-specific virulence factors on disrupting the immune response.

* *Correspondence to: N. Tomar, Machine Intelligence Unit, Indian Statistical Institute, 203 B.T. Road, Kolkata 700108, India. E-mail: namrata_t@isical.ac.in*

N. Tomar, R. K. De
Machine Intelligence Unit, Indian Statistical Institute, 203 B.T. Road, Kolkata 700108, India

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Balance should be maintained between cell survival and apoptosis to maintain cellular homeostasis in multicellular organisms. The responsiveness of individual cells to death signals can vary greatly and depends on the presence of survival cues from the extracellular environment. The perturbation of normal cell survival mechanisms, leading to an increase in cell death or cell survival, is responsible for the development of disease states. Apoptosis is a critical component of successful immune response as infected cells have to be efficiently removed without inciting an inflammatory reaction (Hildeman *et al*., 2007). Thus, we have integrated apoptosis pathway of *Homo sapiens* with toxin expression regulatory pathway of *Clostridium difficile*. The other signaling pathway that we have included for this study is the mitogen-activated protein kinase (MAPK) pathway of *H. sapiens*, which converges diverse extracellular stimuli to initiate inflammatory cellular responses.

Thus the aim of this work is to develop a computational model that captures several facets of apoptosis and MAPK signaling in host infected by the microbe *C. difficile* as a pathogen. The objective is to uncover the relative roles played by the host's system at the time of the pathogen attack, during the course of defending the pathogen and to have a better understanding of what drives the intensity of infection and infectivity of the pathogen in the host. Host–microbe interactions can range from the elimination of the microbe to the death of the host. It encompasses the states of latency, colonization and commensalism, which can cause disease (Casadevall and Pirofski, 2000). Host–pathogen interaction results in phagocytosis of the pathogen, release of cytokines, production of reactive oxygen species and secretion of toxins.

Pathway modeling has been performed for understanding the toxin expression in a pathogen and mechanism of infection spread in a host, which affects the signaling process of the host. This kind of modeling shows how a toxin expression regulatory pathway affects the host signaling system upon infection. We have applied a method based on the notion of flux balance analysis (FBA). This needs minimal amount of biological knowledge and data required to make quantitative inferences about network behavior. FBA involves conservation of mass in a network. It utilizes the stoichiometric matrix and a biologically relevant objective function to identify optimal reaction flux distributions. The objective function is to be maximized or minimized according to the requirement of the study.

Here we have considered a modified methodology of FBA that incorporates enzyme concentration into the formulation. The methodology has already been developed for a single metabolic and gene regulatory pathways (De *et al*., 2008; Das *et al*., 2010). For the current study, we set the toxin expression in *C. difficile* to be minimized as the objective function. The reason behind this is the following. We hypothesize that the lower toxin expression level in a pathogen implies higher survival chances of host upon infection. It has been shown that signal flow values for the survival genes are at the higher side with toxin minimization and are at the lower side for the proapoptotic genes that are present in an integrated pathway of toxin expression regulation and apoptosis. Here, it needs to be mentioned that the term signal flow have been used to describe the concentration of other molecules, such as enzymes in a metabolic pathway, which help in carrying out various biochemical conversions. Then we have integrated toxin expression regulatory pathway in pathogen with MAPK pathway of *H. sapiens*, and we have obtained signal flow values at the higher side for molecules that are the components of extracellular regulated kinase (ERK) pathway, whereas signal flow values are at the lower side for the components of c-Jun NH2-terminal kinase (JNK)/p38 MAPK pathway (where the range of signal flow value is 0–1). In the case of integrated toxin expression regulatory pathway in pathogen with apoptosis and MAPK pathways in host, we have compared the signal flow values of the common molecules present in apoptosis and MAPK pathways in integrated apoptosis and MAPK pathways, along with toxin expression regulatory pathway in *C. difficile*. We have found that the signal flow values for most of the common survival molecules are at the higher side and it was found to be at the lower side for proapoptotic molecules. We have also observed signal flow values for ERK and JNK/p38 pathways and found that the values for the molecules present in ERK pathway are at the higher side in integrated toxin expression regulatory, apoptosis and MAPK pathways.

A BRIEF DESCRIPTION OF RELEVANT PATHWAYS IN PATHOGEN (*C. DIFFICILE*) AND HOST (*H. SAPIENS*), AND THEIR INTERACTIONS

Since the present work involves toxin expression regulatory pathway in *C. difficile*, and apoptosis and MAPK pathways of the host (human), we describe them briefly. This is followed by a brief description of their interactions.

Toxin expression regulatory pathway in *C. difficile*

Bacterial induction of apoptosis has been studied extensively in recent decades. Many bacterial pathogens cause apoptosis in target immune cells such as macrophages (Zhang *et al*., 2005) and neutrophils (Blomgran *et al*., 2004). *C. difficile*, originally named as *Bacillus difficilis*, was first described in the mid-1930s (Hall and O'Toole, 1935). It is an anaerobic Gram-positive bacterium that has been identified in 1978 as the primary cause of pseudomembranous colitis (Bartlett *et al*., 1978; George *et al*., 1978). Original studies and observations have revealed *C. difficile* as an emerging pathogen that is capable of causing severe gastrointestinal disease in individuals undergoing antibiotic therapy. (Wren *et al*. 1987) have established, about 20 years back, a relationship between the symptoms of antibiotic-associated diarrhea and the toxigenic potential of *C. difficile* strain responsible for infection. Akerlund's group (Akerlund *et al*., 2006) has shown that the toxin levels are correlated with the severity of *C. difficile*-associated disease (CDAD).

Genetic manipulation is not possible in *C. difficile*. Hence, it is difficult to generate isogenic strains deficient in toxin production. Therefore, implication of *C. difficile* toxins in disease has taken more indirect and surrogate approaches. Detailed experimental investigations into the fundamental biology of these toxins have provided meaningful insight into *C. difficile*'s mechanism of pathogenesis. Molecular analysis of the pathogenicity locus PaLoc of the virulent strains is considered as one of the breakthroughs in elucidating the mechanism of toxin gene regulation. The toxin genes tcdA and tcdB are located in a pathogenicity locus, that is, in 19.6-kb, which also includes three accessory genes tcdR, tcdC and tcdE (Dove *et al*., 1990; Braun *et al*., 1996) as shown in Figure 1. TcdR has been shown to act as an alternative σ factor that mediates positive regulation of both the toxin genes and its own gene (Mani and Dupuy, 2001). Gene tcdC lies downstream of tcdA. Genetic and biochemical

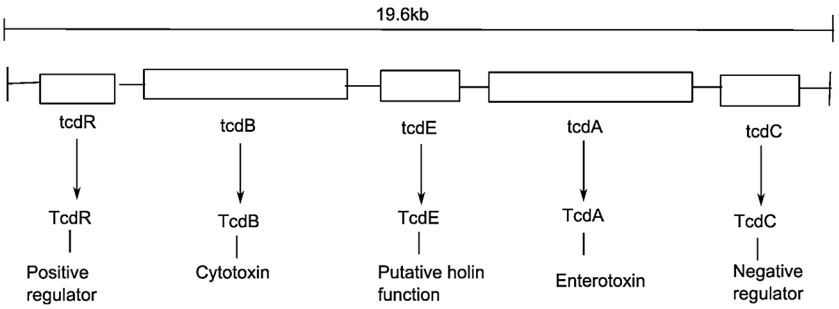

Figure 1. Genetic arrangement of *C. difficile* pathogenicity locus, adapted from Voth and Ballard (2005).

evidences have shown that TcdC interferes with TcdR-containing RNA polymerase and thus inhibits *C. difficile* toxin synthesis (Voth and Ballard, 2005; Matamouros *et al*., 2007). Further details on toxin effects on infected host can be found in Burdon *et al*., (1981), Mahe *et al*., (1987), McFarland *et al*., (1991), Ward and Young (1997) and Akerlund *et al*., (2006).

Apoptosis pathway in *H. sapiens*

Apoptosis forms an important defense mechanism against viral, bacterial and parasitic pathogens during innate and adaptive immunity. It occurs during development and immune cell proliferation, and maintains cellular integrity and tissue homeostasis (Vaux and Strasser, 1996; Reed, 2000). The complex interactions of pathogen/parasite proteins with cellular host proteins lead to the induction of apoptosis. It facilitates the survival of the host by diminishing the products of pathogen. Moreover, apoptosis in specific cells contributes to the regulation of pathogen-induced immune responses (Liles, 1997).

Apoptosis pathway is characterized initially by a series of morphological and biochemical alterations (Arends and Wyllie, 1991). Two major pathways of apoptosis are the extrinsic (Fas and other TNFR superfamily members, and ligands) and the intrinsic (mitochondria-associated) pathways present in the cytoplasm (Zimmermann and Green, 2001). The extrinsic pathway involves the interaction of death receptor, which initiates a signaling cascade mediated by Casp-8 activation. Caspase-8 activation leads to Casp-3 activation and stimulates the release of cytochrome *c* by the mitochondria. Caspase-3 activation leads to the degradation of cellular proteins necessary to maintain cell survival and integrity. Mitochondria participates in the intrinsic pathway. Proapoptotic and antiapoptotic members of the Bcl-2 family regulate the mitochondrial pathway. It occurs when various apoptotic stimuli trigger the release of cytochrome *c* from the mitochondria (independently of Casp-8 activation). Cytochrome *c* interacts with Apaf-1 and Casp-9 to promote the activation of Casp-3. Alterations in Ca^{2+} homeostasis and accumulation of misfolded proteins in the endoplasmic reticulum (ER) cause ER stress. It can result in the activation of BAD and/or Casp-12 and lead to the execution of apoptosis. We have downloaded apoptosis pathway of *H. sapiens* from KEGG pathway database (http://www.genome.jp/kegg/pathway/hsa/hsa04210.html).

MAPK pathway in *H. sapiens*

MAPKs are a family of conserved protein kinases that phosphorylate specific serine and threonine residues of target protein substrates. All eukaryotic cells possess multiple MAPK pathways in a large modular network form, which coordinately regulate diverse cellular activities, such as mitosis, differentiation, metabolism, motility, survival and apoptosis (Pearson *et al*., 2001a). It is a three-tiered cascade composed of an MAPK (MAPKK, MKK or MEK) and an MAPKK (MAPKKK or MEKK). Three MAPK pathways have been characterized in detail to date, *viz*. classical MAPK/ERK, JNK/stress-activated protein kinase and p38 MAPK (Pearson *et al*., 2001b; Roux and Blenis, 2004; Raman *et al*., 2007; Weston and Davis, 2007).

ERK pathway is activated by a variety of mitogens and growth factors, whereas the JNK/SAPK and p38 pathways are stimulated mainly by environmental stress, pathogens and inflammatory cytokines. The function of ERK pathway is depicted as survival promoting, in essence by opposing the proapoptotic activity of JNK/p38 MAPK pathways. (Xia *et al*., 1995) have first introduced the idea that ERK and p38/JNK pathway activities oppose each other to regulate apoptosis. However, ERK pathway activity has been reported to be suppressed by JNK/p38 kinases during apoptosis induction (Friedman and Perrimon, 2006). ERK5 pathway also constitutes another module of MAPK pathway, and can be activated by serum, growth factors and extracellular stresses such as osmotic shock and oxidative stress. The regulation of survival by MAP kinase can be controlled by the activation of antiapoptotic pathways, such as the induction and/or activation of either Bcl-2 or inhibitor of apoptosis family members, or by the inhibition of proapoptotic pathways. We have downloaded MAPK pathway of *H. sapiens* from KEGG pathway database (http://www.genome.jp/kegg/pathway/hsa/hsa04010.html).

Molecular interactions between the toxin expression regulatory pathway of *C. difficile* and apoptosis and MAPK pathways of *H. sapiens*

There is a strong correlation between apoptosis and the host protein translation machinery. Some pathogens try to inhibit cell apoptosis and ensure their own survival, and later it can establish latent infection (in some cases). But it has also been shown that induction of apoptosis in the infected cells significantly protects the host from the pathogen (Hasnain *et al*., 2003). Receptor binding is the first essential step to enter the toxin molecule of *C. difficile* into the host cell. In order to elicit cytotoxic effects, TcdA and TcdB must be internalized into the host cell. This is performed via receptor-mediated endocytosis and requires an acidic endosome for translocation to the cytosol (Florin and Thelestam, 1983; Henriques *et al*., 1987). Both TcdA and TcdB target isoforms of Rho (RhoA, RhoB and RhoC), Rac and Cdc42 by glucosylation. It is performed via transfer of a sugar moiety to Thr-37 of the GTPase with UDP-glucose as a cosubstrate (Just *et al*., 1995). This results in actin condensation and consequent rounding of the cells, membrane blebbing and eventual

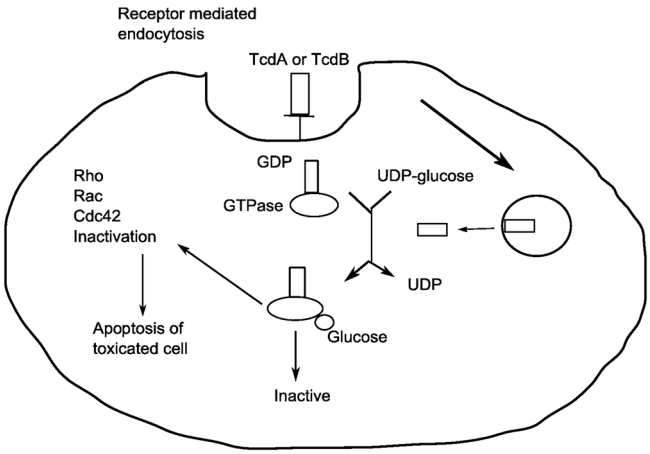

Figure 2. Overview of the intracellular modifications by TcdA and TcdB of *C. difficile*.

apoptosis of the target cell. This mechanism is shown in Figure 2. Here, we want to add that this interaction through which toxin molecules triggers apoptosis is one of the bases to model host–pathogen interactions that include the aforesaid pathways.

We model the aforementioned molecular mechanisms of host–pathogen interactions along with all the other interactions present in a designed integrated toxin expression regulatory, apoptosis and MAPK pathways in the form of stoichiometric matrix. We have shown the molecular interactions between the toxin and its effect on host's pathways in Figure 3. For modeling, we first consider the regulation of toxin proteins' (TcdA and TcdB) expression in a pathogen *C. difficile*. Then, we have shown in a stoichiometric matrix itself that how these toxin molecules enter the cell (through receptor-mediated endocytosis) and inactivate Rho GTPases through glucosylation. Functional

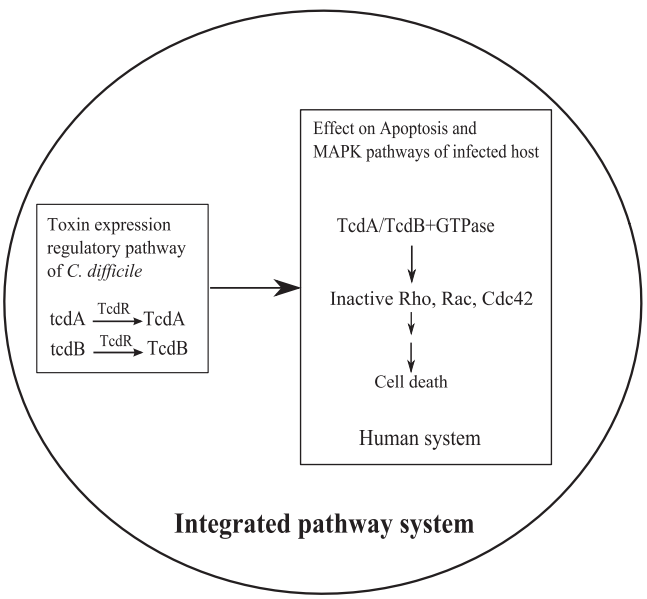

Figure 3. Molecular interactions in an integrated pathway system of toxin expression regulatory pathway of *C. difficile*, and apoptosis and MAPK pathways of the infected host (*H. sapiens*). It shows the regulation of toxin genes in *C. difficile* and molecular mechanism of toxicity in a host.

inactivation of Rho GTPases initiates the cascades of signaling events, for example, increased activity of Caspase-3 to execute apoptosis where Caspase-8 and Caspase-9 also participate. In the same manner of molecular mechanism, we add the MAPK pathway of human in the form of stoichiometric matrix as an input to the model, along with toxin expression regulatory and apoptosis pathways.

METHODOLOGY

Flux balance analysis is a constraint-based approach that attempts to derive a phenotype in the form of a steady-state flux distribution for the reactions in a given biological pathway (Lee *et al*., 2006). It assumes a system boundary drawn around all reactions that constitute internal fluxes operating inside the network. The system is closed to the passage of certain molecules whereas others are allowed to enter and/or exit the system. Here, a cellular objective function is considered that represents amount of a target molecule in a pathway (Segre *et al*., 2002). After fixing the objective function, the system of equations is solved to obtain a steady-state flux/signal flow distribution.

Flux balance analysis requires a stoichiometric representation of the biochemical network of interest. This representation can be in form of a matrix, **S**, where **S** is of the order of $m \times n$ (m being the number of components and n being the number of reactions) and is composed of stoichiometric coefficients that capture the underlying reactions of the biochemical network. The basic equation of FBA is $\mathbf{S.v} = 0$, **v** being a flux vector, which assumes that the concentration of the enzymes catalyzing various reactions in the network is present in the system at the required level. In other words, it assumes the genes that produce these enzymes express at the required level. However, in a real system, the concentration of molecules (e.g., transcription factors/enzymes) catalyzing various reactions in the network may not be expressed at the desired level in various cases. Thus, we consider a modified formulation for steady state and is given by $\mathbf{S}(\mathbf{C}.\mathbf{v}) = 0$ (De *et al*., 2008; Das *et al*., 2010). Here, **C** is an $n \times n$ diagonal matrix whose diagonal elements are the components of a vector **c**. That is, if $\mathbf{C} = [\gamma_{ij}]_{n \times n}$, then $[\gamma_{ij}] = \delta_{ij} c_j$, where δ_{ij} is the Kronecker delta. The term c_j indicates the level of concentration of the molecule catalyzing jth reaction. It may be mentioned here that the methodology has already been derived for single metabolic and gene regulatory pathways (De *et al*., 2008; Das *et al*., 2010).

We need to set a cellular objective function that gives the amount of a target molecule in a pathway. As in the case of FBA, we take the algebraic sum over the reactions $R_1, R_2, \ldots R_s$ that directly yield the target molecule and given by a linear equation, that is, $z = \sum_{j=1}^{s} c_j v_j$. It needs to be maximized or minimized according to the problem.

Following standard FBA, we consider $\mathbf{S.v} = 0$ for generating flux vectors. For this purpose, we first generate the basis vectors $\mathbf{v}_b$ and let the number of such basis vectors be l. Then we generate l number of positive random numbers α_p, $p = 1, 2, \ldots, l$. Finally, we generate flux vectors **v** as linear combination of the basis vectors using $\mathbf{v} = \sum_{p=1}^{l} \alpha_p v_{bp}$. All the internal fluxes are non-negative. The constraints on the exchange fluxes depend on their directions and can be expressed as $L_j \le v_j \le U_j$, L_j, and U_j being the lower and upper bounds of jth exchange flux (Schilling *et al*., 2000).

Here we consider an integrated system comprising toxin regulatory pathway of *C. difficile* (pathogen) and apoptosis and MAPK pathways of *H. sapiens* (host) and apply the methodology to this integrated system. The problem is to minimize the toxin expression/production in this integrated system. For this purpose, we consider the reactions through which toxin genes (tcdA and tcdB) are expressed into toxin proteins (TcdA and TcdB). These reactions are: (i) a reaction through which gene tcdA expresses toxin TcdA (a protein) and (ii) a reaction through which gene tcdB expresses toxin TcdB (a protein). The advantage of this method is that the minimization process of toxin molecule expression (or say toxin eradication) can be followed *in vitro* through manipulating the expression of regulators of toxin genes, tcdA and tcdB. Thus, the objective function is

$$y = z + \Lambda^T.(S.(C.v)) \tag{1}$$

Here y needs to be minimized with respect to the weighting factors c_j for all j. That is, c_j values corresponding to the minimization value of y represent the enzyme concentration pattern in the integrated system. The term $\Lambda = [\lambda_1, \lambda_2, \lambda_3, \ldots, \lambda_m]^T$ is the regularizing parameter. We consider $\lambda_1 = \ldots = \lambda_m = \lambda$ for simplicity. It is chosen empirically (De *et al*., 2008).

We have used gradient descent technique for the minimization of y (Haykin, 2001). Initially, a set of random values in [0, 1] corresponding to c_j's is generated. These c_j's are then modified iteratively using the gradient descent technique, where the amount of modification for c_j in each iteration is defined as

$$\Delta c_j = -\eta \frac{\delta \gamma}{\delta c_j} \tag{2}$$

The term η is a small positive quantity indicating the rate of modification. For computing the values of Δc_j's, we use the following expression.

$$\Delta c_j = -\eta \left[\frac{\delta z}{\delta c_j} + \frac{\delta}{\delta c_j} \left(\Lambda^T.(S.(C.v)) \right) \right] \tag{3}$$

The modified value of c_j is given by

$$c_j(t+1) = c_j(t) + \Delta c_j, \forall j \tag{4}$$

where $c_j(t+1)$ is the value of c_j at iteration $(t+1)$, which is computed on the basis of c_j value at iteration t. After convergence, that is, when c_j values in two successive iterations become nearly equal, we consider the c_j values as the solution and indicate the pattern of enzyme concentration in the integrated system upon minimization of toxin production. We have provided MATLAB codes for the implementation of the methodology as supporting information.

RESULTS

In this article, we demonstrate the effectiveness of the integrated model consisting of toxin expression regulatory pathway in *C. difficile* and apoptosis and MAPK pathways in *H. sapiens*. For this purpose, we have first constructed the integrated pathway comprising aforesaid toxin expression regulatory pathway in *C. difficile* and apoptosis and MAPK pathways of the infected host *H. sapiens* as given in Figure 3.

We have applied the aforementioned methodology in the following three integrated systems separately. They are: (i) an integrated pathway of toxin expression regulation in *C. difficile* and apoptosis pathway of *H. sapiens*, (ii) an integrated pathway of toxin expression regulation of *C. difficile* and MAPK pathway of *H. sapiens*, and (iii) an integrated system of toxin expression regulatory pathway of *C. difficile* and apoptosis and MAPK pathways of the infected host *H. sapiens*. As mentioned in the Section Introduction, there should be balance between cell survival and apoptosis to maintain cellular homeostasis. Thus, the aim of this study is to develop a computational model that captures several facets of apoptosis and MAPK signaling (studied individually as well as in an integrated fashion) upon infection. The reason behind this is the following. We want to study the behavior of apoptosis and MAPK pathways as separate systems along with toxin expression regulatory pathway upon infection. Then we want to investigate how and what extent the signal flow values change in comparison with earlier values (i.e., in case of (i) and (ii)) upon addition of apoptosis and MAPK pathways of human with toxin expression regulatory pathway of *C. difficile* as an integrated system. We hypothesize that lower toxin levels increase the survival chances in an infected host. In other words, the minimization of toxin levels in pathogen leads to increase the survival chances in the infected host.

Effect of toxin minimization on apoptosis pathway of *H. sapiens*

First, we study the integrated toxin expression regulatory pathway of the pathogen and apoptosis pathway of the host. The integrated pathway contains 81 molecules and 115 reactions. The objective is to minimize the expression of tcdA and tcdB (toxin-producing genes) of pathogen and to see the behavior of apoptotic pathway in the host. Thus, the objective function is $z = c_5 v_5 + c_8 v_8$, where c_5 = concentration of positive regulator, catalyzing one reaction for the expression of toxin gene tcdA, v_5 = rate of reaction at which gene tcdA is expressed to form protein TcdA (toxin molecule), c_8 = concentration of positive regulator, catalyzing the other reaction for the expression of toxin gene tcdB and v_8 = rate of reaction at which gene tcdB expressed to form protein TcdB (toxin molecule). That is, we find out whether minimization of the toxin production leads to the change in the concentration of the proteins coded by the survival genes/antiapoptotic genes in the host. We analyze the quantitative values of the signal flow for these proteins. The fluxes in metabolic pathway represent the rate of mass flow from one molecule to the other through a reaction. The flux vectors are replaced by signal flow vectors in context of a signaling pathway. We have observed that the signal flows have been at the higher side for most of the survival genes, whereas they have been on the lower side for most of the proapoptotic genes, where the range is in [0–1]. This change has been observed upon toxin minimization in *C. difficile*.

We have observed this fact for Bcl-2 family that includes proapoptotic members, *viz*., Bax, Bak, Bad, Bcl-Xs and Bid, and antiapoptotic members, *viz*., Bcl-XL. It has been found that the signal flow value for Bcl-XL and PKA, PKB and NF-κB (survival genes) has been larger, whereas it has been just the opposite for Casps and Bax (apoptosis-promoting genes; Tables 1 and 2).

Effect of toxin minimization on MAPK pathway of *H. sapiens*

There are 164 molecules and 204 reactions present in the integrated pathway comprising toxin regulatory pathway of

Table 1. Survival genes and their respective signal flow values

Survival genes	Signal flow values
Bcl-2	0.71899
Bcl-XL	0.64765
P13K	0.92612
IKK	0.66527
PKA	1.0
PKB	0.67466
NF-κB	0.59606
cAMP	1.0

Table 2. Apoptosis-promoting genes and their respective signal flow values

Apoptosis-promoting genes	Signal flow values
Casp8	0.44121
Casp3	0.56519
Casp6	0.081091
Casp10	0.68612
Bid	0.67289
CytC	0.40447
Bax	0.548882

C. difficile and MAPK pathway of *H. sapiens*. The objective function is to minimize the expression of tcdA and tcdB (toxin-producing genes) in a pathogen. Thus, the objective function is $z = c_5v_5 + c_9v_9$, where c_5 = concentration of positive regulator, catalyzing one reaction for the expression of toxin gene tcdA, v_5 = rate of reaction at which gene tcdA is expressed to form protein TcdA (toxin molecule), c_9 = concentration of positive regulator, catalyzing the other reaction for the expression of toxin gene tcdB and v_9 = rate of reaction at which gene tcdB expressed to form protein TcdB (toxin molecule).

As mentioned in the Section 2.3 on MAPK pathway in *H. sapiens*, the family of MAPKs comprised three major groups, *viz*., ERK, JNK and p38 MAPK pathways. It has been well established that the dynamic balance between growth factor-activated ERK and stress-activated JNK and p38 pathways may be important in determining whether a cell survives or undergoes apoptosis. Thus we have decided to compare the signal flow values for the components present in ERK and JNK/p38 pathways. The corresponding signal flow values for the components present in ERK (responsible for cell survival) and JNK/p38 pathways (responsible for cell apoptosis) are given in Table 4. Most of the genes related to cell survival (in ERK pathway) have larger signal flow values than those present in JNK/p38 pathway.

Effect of toxin minimization on integrated apoptosis and MAPK pathways of *H. sapiens*

There are 223 molecules and 312 reactions present in the integrated pathway (toxin regulatory pathway of *C. difficile* and an integrated apoptosis and MAPK pathway of *H. sapiens*). The objective is to minimize the expression of tcdA and tcdB (toxin-producing genes) in pathogen. Thus the objective function is $z = c_5v_5 + c_9v_9$, where c_5 = concentration of positive regulator, catalyzing one reaction for the expression of toxin gene tcdA, v_5 = rate of reaction at which gene tcdA is expressed to form protein TcdA (toxin molecule), c_9 = concentration of positive regulator, catalyzing the other reaction for the expression of toxin gene tcdB and v_9 = rate of reaction at which gene tcdB expressed to form protein TcdB (toxin molecule). Table 3 compares the signal flow values for survival genes present in apoptosis and an integrated apoptotic and MAPK pathways in host along with toxin expression regulatory pathway in the pathogen. It also provides the signal flow values for the apoptosis-promoting genes present in the aforesaid pathways. We have found 17 common molecules in an integrated toxin expression regulatory and apoptosis pathway, an integrated toxin expression regulatory and MAPK pathways and an integrated toxin expression regulatory pathway in a pathogen, and apoptosis and MAPK pathways of an infected host. The comparison among signal flow values of these common molecules present in aforesaid pathways is given in Table 4. We want to investigate the changes in the behavior (quantitatively, *i.e*., in terms of signal flow values) of these molecules, thus we have considered them to study separately as well as in integration with another pathway upon infection. Comparison of signal flow values for molecules present in ERK and JNK/p38 pathways of both an integrated toxin expression regulatory and MAPK pathways, and in integrated toxin expression regulatory, apoptosis and MAPK

Table 3. Signal flow values for survival genes and apoptosis-promoting genes present in apoptosis, and in integrated apoptosis and MAPK pathways in an infected host (human). Both pathways have been integrated with toxin expression regulatory pathway in C. difficile.

Survival genes (series 1)	Signal flow values		Apoptosis-promoting genes (series 2)	Signal flow values	
	Apoptosis pathway	Integrated MAPK and apoptosis pathway		Apoptosis pathway	Integrated MAPK and apoptosis pathway
Bcl-2	0.71899	0.92814	Casp8	0.44121	0.4254
Bcl-XL	0.64765	0.74548	Casp3	0.56519	0.46851
P13K	0.92612	0.93215	Casp6	0.081091	1.0
IKK	0.66527	0.35922	Casp10	0.68612	0
PKA	1.0	0	Bid	0.67289	0
PKB	0.67466	0.6436	CytC	0.40447	0.3064
NF-κB	0.59606	0.53051	Bax	0.548882	0.48641

Table 4. Comparison of signal flow values for common molecules present in apoptosis, MAPK and integrated apoptosis and MAPK pathways in infected host, all the three pathways are integrated with toxin expression regulatory pathway in *C. difficile*

Name of molecules	Apoptosis pathway	MAPK pathway	An integrated apoptosis and MAPK pathway
TNF	0.76463	0.9926	0.41114
TNFR	1.0	0.8713	0.35922
TNF : TNFR	0.700	0.39955	1.0
IL1	0.7004	0.65667	0.82822
IL1R	0.064201	0.14039	0.2779
IL1 : IL1R	0	0.423	0.55444
NGF	0.32422	0	1.0
TrkA	0.76883	0	1.0
NGF : TrkA	0.29907	0	1.0
Akt	0.67466	0.31962	0.74185
NIK	0.99057	0.69835	0.10963
IKK	0.66527	0.41858	0.75922
NF-κB	0.59606	1.0	0.53051
p53	0.60295	0.75622	0.5983
TRAF	0	0.72977	0.18692
PKA	1.0	0.75862	0.65759
cAMP	1.0	0	1.0

pathways is given in Table 5. We have obtained signal flow values on the lower side for most of the genes present in JNK/p38 pathway in integrated toxin expression regulatory, apoptosis and MAPK pathways in effect of toxin minimization in the *C. difficile* that affecting the host.

We have experimented extensively with different values of λ and η, and checked the behavior of the integrated system. When we have varied λ, η has been kept fixed and vice-versa. It has been found that with the variation of λ and η, although *c* values for certain molecules change a little, overall pattern of *c* values remains unaltered. Thus we can say that the model is robust.

BIOLOGICAL VALIDATION OF THE RESULTS

In this section, we provide the biological validation of the results on the effect of toxin minimization on apoptosis, MAPK and integrated apoptosis and MAPK pathways of the infected host.

Effect of toxin minimization on apoptosis pathway of host

Many investigations suggest that apoptosis is the mechanism of death in cells that are exposed to TcdA and TcdB. Apoptosis-inducing signals are carefully processed and evaluated against antiapoptotic factors in the target cells. If the proapoptotic elements defeat their counterparts, a death program is then activated and the cell will undergo apoptosis. This is the reason, we have compared the signal flow values for cell survival and proapoptotic signaling molecules present in the host's apoptosis signaling pathway.

We have already mentioned that the signal flow values for the survival genes should be higher with toxin minimization in a pathogen. We have found higher signal flow value for Bcl-2 (0.71899) and for cAMP (1.0). One may refer to Table 1 in this regard. A study by (Hippenstiel *et al*., 2002) has shown that the inhibition of Rho reduces the expression of antiapoptotic Bcl-2 and increases proapoptotic Bid. They have also found that Casp-3 activity and apoptosis, induced by TcdB-10463, have

Table 5. Comparison of signal flow values for molecules present in ERK and JNK/p38 pathways of both MAPK and integrated apoptosis and MAPK pathways in an infected host along with toxin expression regulatory pathway in *C. difficile*

Molecules present in ERK pathway	Signal flow values		Molecules present in JNK/p38 pathway	Signal flow values	
	MAPK pathway	Integrated MAPK and apoptosis pathway		MAPK pathway	Integrated MAPK and apoptosis pathway
ERK	0.52052	0.61599	TNF-α	0.9926	0.41114
NGF	0	1.0	IL1	0.65667	0.082822
BDNF	0.46306	0.83292	FASL	1.0	0
PKC	0.2386	0.67001	Casp	0.77159	0.93451
PKA	1.0	0.65759	GADD45	0	1.0
MEK1/2	0.67282	0.53801	TRAF2	0.72977	0.18692
Akt	0.31962	0.74185	DAXX	0.36161	0.81331
Ras	0.83687	0.40121	ASK1	0.83063	0.53555
Raf	0.95072	0.63217	MEKK1	0.53928	0.46795
CREB	1.0	1.0	MKK4	0.7249	0.27071
NIK	0.69835	0.10963	JNK	0.64532	0.32294
IKK	0.41858	0.60811	p38	0.65098	0.51879
NF-κB	1.0	0.53051	p53	0.75622	0.5983
cAMP	0	1.0	c-Jun	0	1.0
EGFR	0.70921	1.0	NFAT2/4	0.56131	0.87203
c-Fos	0.56121	0.89317	ATF2	0.26783	0.041425
RSK2	0.50585	0.59616	TGF-β	0	1.0
c-Myc	0.33567	0.20563	JunD	0	0
Ca^{2+}	1.0	1.0	TAK1	0.26886	0.79276

been abolished by cAMP elevation. The value of the signal flow for Bcl-2 is evidently more than the value of signal flow for CytC (cytochrome *c*). Antiapoptotic Bcl-2 members act as repressors of apoptosis by blocking the release of CytC, whereas proapoptotic members act as promoters. These effects are more dependent on the balance between Bcl-2 and Bax than on the quantity of Bcl-2 alone (Reed, 1997).

Our results show that toxin minimization leads to increase in the lifespan of the infected cell, as we have obtained lower values for the signal flow for Casps and Bid (apoptosis-promoting genes). We have obtained signal flow values of 0.081091 for Casp-6, 0.56519 for Casp-3, 0.44121 for Casp-8 and 0.68612 for Casp-10 (Table 3). The signal flow values are 0.56519 for Casp-3 for an integrated toxin expression regulatory and apoptosis pathway and 0.46851 for integrated toxin expression regulatory, apoptosis and MAPK pathways (Table 3). The signal flow value for Casp-3 has been found to be decrease when we have integrated MAPK along with integrated toxin expression regulatory and apoptosis pathways. As mentioned earlier, a possible candidate mechanism for TcdA-induced activation of Casp-3 is via the proapoptotic molecule Bid. We have mentioned comparative signal flow values for Bid in Tables 1 and 3. (Solomon *et al*., 2005) have found an increase in Casp-3 activity in TcdA-stimulated monocytes. Their study has demonstrated that *C. difficile* toxin TcdA has a cell-specific effect, in which monocytes exhibit greater susceptibility than lymphocytes. Isolated normal human intestinal lamina propria T-cells exposed to *C. difficile* TcdA underwent cell death by apoptosis, which was prominent after 72 h (Mahida *et al*., 1998). Moreover, a study by (Carneiro *et al*., 2006) also confirms the involvement of both intrinsic and extrinsic apoptotic pathways in TcdA-induced apoptosis and their convergence at Casp-3.

Induction of apoptosis via death receptors results in the activation of Casps cascade, which is responsible for the cleavage of the key cellular proteins, such as cytoskeletal proteins, that leads to the typical morphological changes (Reed, 2000; Friedlander, 2003; Ghobrial *et al*., 2005). The death process begins its terminal phase when executioner Casps activate the machinery that degrades DNA (Liu *et al*., 1997). (Brito *et al*., 2002) have observed that TcdA induces the apoptotic pathway in T84 intestinal cells via Casps-3, 6, 8 and 9 and Bid activation, which mediates mitochondrial damage followed by cytochrome *c* release in the T84 epithelial cell line. TcdA induces activation of Casps-3, 8 and 9 in HT-29 cells and thereby triggers apoptotic cell death (Gerhard *et al*., 2008). In this study, authors have found that the activation of the executioner Casp-3 is strongly correlated with the glucosylation of Rac1.

We have minimized the toxin expression in the microbe and thereby, we have found that it has led to lower signal flow values of the proapoptotic signaling molecules. For example, we have obtained the signal flow value of 0.40447 for CytC. CytC is released by the mitochondria in response to proapoptotic stimuli. A study by (Matte *et al*. 2009) has utilized two cell culture models involving human colonic carcinoma and ovarian carcinoma cell lines to examine the mechanism of TcdA-induced cell death, and has demonstrated that TcdA-induced cell death is independent of death receptor pathway but strongly depends on the activation of the mitochondrial pathway. They have also found that the overexpression of the antiapoptotic proteins Bcl-2 and Bcl-XL significantly inhibits TcdA-induced cell death. We have also found increased signal flow values for antiapoptotic proteins with toxin minimization in microbe. TcdA strongly induces colonocyte BAK expression (proapoptotic member of the Bcl-2 gene family) and regulates mitochondrial CytC release and subsequent activation of Casp-3, which leads to apoptosis (Kim *et al*., 2005).

We have found that with toxin minimization, the quantity of signal flow for Akt/PKB (survival gene) is 0.67466, which is at the higher side. The highly conserved serine/threonine kinase, Akt/PKB is an important pro-survival factor that is activated by growth factors. It contributes to cell survival by phosphorylation, inactivation of Bad and Casp-9 and activation of the NF-κB pathway via phosphorylation and inactivation of IκB (Wada and Penninger, 2004).

Effect of toxin minimization on apoptosis and MAPK pathways of host

Several members of the MAPK family respond to environmental signals and thereby control cell proliferation and death. This complex pathway is prime targets of numerous pathogens, which try to modulate the host cell fate (Wada and Penninger, 2004).

C. difficile TcdA activates MAPK signaling in monocytes (Warny *et al*., 2000). All the three MAPK pathways were activated within 1–2 min of stimulation, but the duration of activation was quite different. In monocytic cells exposed to TcdA, ERK activity peaked after 2–4 min and returned to control levels after 5 min. JNK1 activity returned to control level after 30–60 min, whereas p38 remained highly activated after 1 h of stimulation. These results have shown that *C. difficile* TcdA activates all the three MAPK pathways and p38 has the strongest sustained activation. This time course supports the hypothesis that interaction of toxin with a cell-surface receptor may trigger MAPK activation. Cell apoptosis induced by the Rho-directed *C. difficile* toxin has been shown to be prevented by activation of Raf-1 and ERK1/ERK2 MAPK in a highly growth factor-dependent line of lung fibroblasts (Gall *et al*., 2000). This finding highlights the ability of ERK1/ERK2 MAPK to generate survival signals that opposes the cell death induced by the loss of matrix contact and cytoskeletal integrity. This is the reason we have chosen MAPK pathway to find out its interaction with apoptosis pathway (in the host) along with toxin expression regulatory pathway in a pathogen.

We have found that EGFR has higher signal flow value of 0.70921 in integrated toxin expression regulatory and MAPK pathways, whereas it is 1.0 in integrated toxin expression regulatory, apoptosis and MAPK pathways. Taking the case of ERK, we have obtained signal flow value of 0.52052 in integrated toxin expression regulatory and MAPK pathways and 0.61599 for integrated toxin expression regulatory, apoptosis and MAPK pathways (Table 5).

C. difficile toxins, *viz*., TcdA and TcdB mediate acute inflammatory diarrhea characterized by neutrophil infiltration and intestinal mucosal injury. In a xenograft animal model, TcdB was shown to induce interleukin-8 (IL-8) gene expression in human colonic epithelium (Savidge *et al*., 2003). TcdB activates ERK MAPK in human colonic epithelial cells via a pathway, dependent on EGFR tyrosine phosphorylation (Na *et al*., 2005). This subsequently leads to the release of IL-8 from human colonocytes. Increased IL-8 gene expression, a major proinflammatory cytokine, indicates the pathophysiology of *C. difficile*-associated colitis. (Na *et al*., 2005) have demonstrated that phosphorylation of EGFR and ERK1/2 has increased dose dependently after TcdB stimulation with a maximum of 20 nmol/l TcdB exposure. They have also shown that TcdA and TcdB activate ERK in colonic epithelial cells using different receptors and signaling pathways. Thus we can say that TcdB signals acute proinflammatory responses in

colonocytes by activation of EGFR and ERK/MAPK pathways. It is to be mentioned here that EGFR transactivation and MAPKs promote cell proliferation and tissue repair.

We have obtained signal flow value of 0.65098 for p38 in integrated toxin expression regulatory and MAPK pathways and 0.51879 in integrated toxin expression regulatory pathway with MAPK and apoptosis pathways (Table 5). The TcdB-induced activation of p38 MAPK and ERK1/2 was detected by western blotting against the doubly phosphorylated forms of these kinases. Meyer *et al.* (2007) have shown that TcdB (6 nM) has led to an activation of p38 MAPK and ERK1/2 after 1 h. A study showed that the TcdA has strongly induced the phosphorylation of ERK1/2, p38 and JNK in HT-29 cells (Lee *et al.*, 2007). The authors have found that all the three MAPK pathways were activated within 5–10 min of stimulation.

We have obtained higher signal flow value of 0.83687 for Ras in integrated toxin expression regulatory and MAPK pathways and 0.40121 in integrated toxin expression regulatory pathway with MAPK and apoptosis pathways (Table 5). With toxin minimization, Ras need not be expressed at higher level, thus we have found lower signal value in integrated toxin expression regulatory, apoptosis and MAPK pathways. (Palsson *et al.*, 2000) have studied the role of small G proteins (Ras, Rac, Rap, Cdc42 and Rho) in the activation of p38 MAPK by IL-1, and their results strongly indicate that Ras protein (a proto oncogene) has a role in it, whereas Rap has an antagonistic effect.

The signal flow value of 0.9926 has been found for TNF-α in integrated toxin expression regulatory and MAPK pathways and 0.41114 for integrated toxin expression regulatory, apoptosis and MAPK pathways along with apoptosis pathway (Table 5). We have considered the integrated pathway with toxin expression minimization in microbe as an objective function. Thus, the signal flow value in integrated toxin expression regulatory pathway along with apoptosis and MAPK pathways (0.41114) is justified. (Sun *et al.*, 2009) have demonstrated a crucial role of glucosyltransferase activity of *C. difficile* toxins in the induction of TNF-α in macrophages. They have found a dose-dependent TNF-α secretion in murine macrophage cell line RAW 264.7 after exposure to TcdA or TcdB. More reports are also available (Filho *et al.*, 1997; Rocha *et al.*, 1997), which show that both TcdA and TcdB induce an intense neutrophil migration that is mediated by macrophage-derived TNF-α. Another study by (Calderon *et al.*, 1998) has also shown that mast cells release TNF-α as initial inflammatory response to *C. difficile* TcdA.

We have obtained the signal flow value of 0.64532 (higher) for JNK present in integrated toxin expression regulatory pathway with MAPK and 0.32294 (lower) in integrated toxin expression regulatory pathway with MAPK and apoptosis pathways (Table 5). A study has shown that the inhibition of JNK activity has reduced epithelial cell apoptosis in colitis model (Assi *et al.*, 2006). The inhibition of Rho family GTPases by TcdB also blocks JNK activation in gentamycin-treated auditory hair cells (Bodmer *et al.*, 2002) and IL-1-induced activation of JNK, p38 MAPK and NF-κB in murine EL-4 thymoma cells (Dreikhausen *et al.*, 2001). JNK pathway has been implicated in both apoptosis and survival signaling. Ultraviolet-induced apoptosis in fibroblasts requires JNK for CytC release from the mitochondria (Tournier *et al.*, 2000).

A hypothesis and its possible biological validation

Here, we hypothesize that lower toxin level implies higher chance of survival. In other words, lower toxin levels may have signal flow values for survival genes at the higher side, in comparison with apoptosis-promoting genes. It may be a reason for increased survival chances. This hypothesis may be validated through laboratory experiments by experimental biologists. Here, we study the behavior of several molecules present in apoptosis and MAPK signaling pathways upon infection.

The signal flow values for most of the apoptosis-promoting genes have been found at the lower side and the reverse for the survival genes (Table 3). We have also found some common molecules that are present in integrated toxin expression regulatory and apoptosis pathways, integrated toxin expression regulatory and MAPK pathways and in integrated toxin expression regulatory in a pathogen, apoptosis and MAPK pathways of an infected host. The respective signal flow values have been given in Table 4. From this table, we have found that the signal flow values for most of the survival genes are on a higher side for integrated toxin expression regulatory, apoptosis and MAPK pathways. Apoptosis and MAPK pathways function in accordance, which lead to cell survival/apoptosis as per the requirement of the cell. As we have minimized toxin expression in the pathogen, we can hypothesize in favor of the increased signal flow values of the survival genes in integrated toxin expression regulatory pathway of pathogen and apoptosis and MAPK pathways in an infected host.

MAPK pathway has been studied as a three-tiered pathway composed of classical, JNK/p38 and ERK5 pathways. ERK pathway is depicted as a survival-promoting pathway whereas JNK/p38 pathway corresponds to apoptosis activity. It is mentioned earlier in this article that the activities of ERK and JNK/p38 oppose each other to regulate apoptosis (Xia *et al.*, 1995). Based on this, we have compared the signal flow values of molecules present in ERK and JNK/p38 pathways for integrated toxin expression regulatory pathway of pathogen and MAPK pathway in an infected host and for integrated toxin expression regulatory pathway of pathogen, apoptosis and MAPK pathways in an infected host. The signal flow values for most of the molecules present in ERK pathway have increased compared with that in JNK/p38 pathway, as toxin expression has been minimized in the pathogen (Table 5). The signal flow values of the survival genes have also increased when we have integrated MAPK and toxin expression regulatory pathways with apoptosis pathway of the host. The fact of increased signal flow values of survival genes supports that MAPK pathway of the host helps in an infected cell's survival. Thus we can say that if one minimizes the toxin expression in a pathogen, chances of survival of infected cell will be increased.

DISCUSSION

It has been suggested that TcdA can induce apoptosis outside of inactivating Rho proteins. Cells intoxicated with TcdA result in the accumulation of toxin in the mitochondria within 5 min of exposure. It is found that this localization event occurs before detectable glucosylation of Rho proteins (He *et al.*, 2000). Thus TcdA may induce apoptosis by disrupting the mitochondria, which promotes proapoptotic events.

Now, we take the case of TcdB, which is also capable of triggering apoptosis. It has been shown that HeLa cells being intoxicated by TcdB undergo Casp-3-dependent apoptosis with a concurrent loss in host cell vimentin (Qa'Dan *et al.*, 2002). TcdB is able to induce Casp-dependent and Casp-independent

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apoptosis specifically because of substrate inactivation. The proteome of TcdB-treated cells contains fragments of intermediate filaments that result from the cleavage by Casp-3. These results collectively demonstrate some of the potential effects of GTPase inactivation on the host cell due to intoxication by the large clostridial toxins (TcdA and TcdB). This implicates that the apoptosis is a major process by which the toxins induce cell death in the host.

Most of the pathogens possess multilevel mechanisms to interfere with these signaling pathways to subvert host cell death. Paradoxically, some bacterial pathogens also inhibit apoptosis for their survival in the host cells. *Shigella* and *Salmonella* possess ability to protect epithelial cells from cell death but both of them have different means to kill macrophages in order to escape the immune response of the infected host (Fink and Cookson, 2005). Another example is *Mycobacterium tuberculosis* that induces apoptosis in macrophages via a TNF-α and Casp-1-dependent pathway (Rojas *et al*., 1999). But it also protects cells against apoptosis via two key pathways: (i) through induction of TLR-2-dependent activation of the NF-αB cell survival pathway (Aliprantis *et al*., 1999) and (ii) by enhancing the production of soluble TNF receptor (TNFR) that neutralizes the proapoptotic activity of TNF-α (Balcewicz-Sablinska *et al*., 1998).

CONCLUSION

In this article, we have modeled an integrated system comprising pathways from two different organisms. One is toxin expression regulatory pathway from a pathogen (*C. difficile*) and the other one is apoptotic and MAPK pathways from an infected host (*H. sapiens*). This is a way to model host–pathogen interaction. The methodology involves generating data on reaction flows/fluxes in the integrated pathway based on steady state condition. A set of constraints is incorporated with weighting coefficients, and an objective function is set according to the requirement of the study.

It may be mentioned here that the methodology has already been developed for single metabolic and gene regulatory pathways (De *et al*., 2008; Das *et al*., 2010). We have chosen *C. difficile* as a model organism and studied its mode of toxin infection in the host. *C. difficile* elicits its pathogenesis via infecting the host through its toxins. Thus we have taken minimization of the toxin expression/production in the pathogen as the objective function.

We have hypothesized that lower toxin level implies higher chance of survival. It has been validated by observing the effect of toxin on target molecules of apoptotic and MAPK pathways (of host) upon infection, if the toxin expression is minimized in the pathogen. MAPKs regulate cell responses to growth factors and stress stimuli and transmit signals from the cell surface to the nucleus. It is performed via three distinct but related pathways. These culminate in the selective activation of ERK, p38 and JNK pathways. All the three signaling cascades have been implicated in the control of apoptosis and cytokine transcription. Thus we have integrated MAPK pathway with apoptosis and toxin expression regulatory pathways to investigate their contrasting role upon pathogen's infection on host's cell.

We have found that the quantity of signal flow values for survival genes has been increased as toxin production is minimized in the pathogen in the case of an integrated toxin expression regulatory pathway in pathogen and apoptosis pathway in host. In the case of integrated toxin expression regulatory and MAPK pathways, we have obtained higher signal flow values for the molecules, which are the components of ERK pathway, whereas it is the reverse for the components of JNK/p38 MAPK pathway.

One major characteristic of immune signaling is a balance between proinflammatory responses to pathogens and anti-inflammatory regulation that stabilizes and modulates immunity. Here, we have observed that upon toxin expression minimization, the quantity of signal flow values for survival genes has been increased, whereas the values have decreased for proapoptotic genes. Thus it is clear that if immune signals do not permit sufficient protection against bacterial clearance, the infection can indefinitely persist in a latent state. This represents a potential survival strategy for a pathogen.

This study provides an understanding of the mechanisms by which intracellular bacterial pathogens induce and/or block the host cell apoptotic pathways. Thus the role of inducing and/or blocking apoptosis in modulating the outcome of the struggle between the host and the pathogen is better understood. Simulating host–pathogen interactions and modulating signaling pathways of host may help in finding the key defense cells that are necessary to eradicate the pathogen, inhibit pathogen replication and toxin expression or promote inflammation, which aids clearance or prevents spread of the pathogen within other tissues of the host further.

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