A Process Model of Rho GTP-binding Proteins in the Context of Phagocytosis

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Abstract

At the early stages of the phagocytic signalling, Rho GTP-binding proteins play a key role. With the stimulus from the cell membrane and with the help from the regulators (GEF, GAP, Effector, GDI), these proteins serve as switches that interact with their environment in a complex manner. We present a generic process model for the Rho GTP-binding proteins, and compare it with a previous model that uses ordinary differential equations. We then extend the basic model to include the behaviour of the GDIs. We discuss the challenges this extension brings and directions of further research.

Keywords: phagocytosis, GTP-binding proteins, stochastic π-calculus, process modeling

1 Introduction

Phagocytosis is a form of endocytosis by which a cell engulfs micro-organisms, large edible particles and cellular debris. Phagocytosis literally means 'cell eating'.

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Single-celled organisms such as amoeba obtain food in this way. Phagocytosis also occurs in multi-celled organisms when, for example, macrophages and other white blood cells defend the body against invasions of harmful viruses, bacteria, cancerous body cells, and other threats to health [1]. A key project in the newly-formed Centre for Integrative Systems Biology at Imperial is to analyse the fundamental mechanisms for phagocytosis, using the expertise of biologists, computer scientists and mathematicians. Our part of the project is to use stochastic process algebras, such as the stochastic π-calculus (see, e.g., [18]), to provide a compositional and scalable notation for simulating phagocytosis.

In this first paper, we give a process calculus model of Rho GTP-binding proteins which play an important role also in phagocytosis. These proteins act on the membranes of cells as molecular switches in several subcellular activities, regulating a variety of cell functions such as actin organisation, cell shape and cell adhesion. We study Goryachev and Pokhilko's paper [11] on an ordinary differential equation (ODE) analysis of the Rho GTP-binding protein cycle, first in isolation and then with their regulators GEF and GAP. Our process models provide a simple, compositional description of the Rho GTP-binding protein cycle, where the structure of models naturally follows the structure of the biological models. Using the Stochastic Pi Machine (SPiM) [17,16] and the rates of interaction described in [11], we provide simulations which precisely mimic the results given using ODEs. Following [11], we also extend our model to include the effectors which interact with these proteins at the membrane. Again, our results remain consistent with the results obtained from the ODE analysis. Our results provide an essential starting point for our investigation of the behaviour of Rho GTP-binding proteins using process models.

We further extend our model to include the interactions of the GTP-binding proteins with another class of regulators called GDIs, which were not incuded in the ODE analysis of [11]. Our initial aim was to analyse two biological models described in the survey paper [7]. Instead, based on discussions with Caron, a biologist at Imperial, we introduce a hybrid model which fits more closely with current knowledge. We extend our process model, again illustrating the compositional nature of our approach. The next step is to provide a systematic study of the rates, searching the biological literature and using SPiM to explore the parameter space leading to sensible behaviour. We begin this study, using SPiM to explore the effect of varying the quantity of the GDI protein on the behaviour of the protein cycle and the effectors. Ultimately, we require help from the biologists to fill the gaps in our current knowledge. Indeed, our ambitious goal is to create a research environment where the biological experiments corroborate and provide data for our process-model simulations, and our simulations guide the biological experiments.

2 Rho GTP-binding Proteins and their Role in Fc Receptor-mediated Phagocytosis

Phagocytosis is the process whereby cells engulf large particles, usually over $0.5\mu m$ in diameter, by a mechanism that is based on local rearrangement of the cytoskeleton. Phagocytosis plays an essential role in host defence against invading pathogens, and in clearance of cell corpses generated by programmed cell death or apoptosis.

Phagocytosis contributes to inflammation and the immune response [1].

Phagocytosis is a triggered process, often initiated by the interaction of particle-bound ligands (opsonins) with specific receptors on the cell membrane of 'professional' phagocytic white blood cells such as macrophages, neutrophils and dendritic cells [5]. Among the variety of surface proteins dedicated to phagocytosis, Fc receptors (FcRs) and receptors for complement fragments (Cr's) mediate the clearence of pathogens covered by the specific antibody or complement respectively [12].

2.1 Fc Receptor-mediated Phagocytosis

In the context of Fc receptor-mediated phagocytosis, the signalling cascade is triggered by antibodies, called immunoglobulin, e.g., IgG, which protect the organism by binding to the surface of infectious microorganisms to form a coat. In this situation, the tail region of each antibody molecule, called the *Fc region*, is exposed on the exterior. This antibody coat is recognised by specific *Fc receptors* on the surface of the cell. Their binding induces the phagocytic cell to extend pseudopods and extend its tips to form a phagosome while proceeding with binding its ligands in a zipper like fashion around the internalised particle [10].

As a result of FcR-Fc interaction on the exterior surface of the cell membrane, a protein tyrosine kinase of the Src family is activated. Following this, Src phosphorylates two tyrosine residues on the receptor's signalling subunits located on the internal tail of the Fc receptor. These tyrosine residues belong to immunoreceptor tyrosine-based activation motifs, or ITAMs. Another protein tyrosine kinase, Syk, is then recruited through its Src-homology 2 (SH2) domains by binding to the phosphorylated ITAMs. This results in autophosphorylation and activation of Syk. Among other tasks, activated Syk is responsible for the recruitment of the protein Vav [12], which then activates Rho GTP-binding protein Rac. In a parallel independent pathway, another Rho GTP-binding protein Cdc42 gets activated by an unknown protein [15]. Cdc42 and Rac then act at distinct stages to promote actin filament polymerisation and organisation at the site of particle ingestion: Cdc42 and Rac control actin filament polymerisation through proteins WASP (Wiskott-Aldrich Syndrome protein) and WAVE, respectively, that bind to and stimulate the activity of the Arp2/3 complex. Activation of Arp2/3 results in actin polymerisation and a network of actin filaments that extend the cytoskeleton around the internalised particle formed. While Rac is responsible for the branching structure of the actin polymerisation, Cdc42 causes the actin to polymerise in a linear structure [19].

2.2 Rho GTP-binding Proteins in Fc Receptor-mediated Phagocytosis

The proteins Cdc42 and Rac mentioned above belong to the Rho GTP-binding proteins. As in the context of phagocytosis, the family of Rho GTP-binding proteins serve as molecular switches in various subcellular activities, regulating a variety of cell functions, including actin organisation and cell shape, cell adhesion, cell motility, membrane trafficking and gene expression [5,3]. These proteins can be perceived as transmitting an incoming signal further to some effector in a molecular module by cycling between inactive and active states, depending on being GDP or GTP bound, respectively. As depicted in Figure 1, GDP/GTP cycling is regulated by

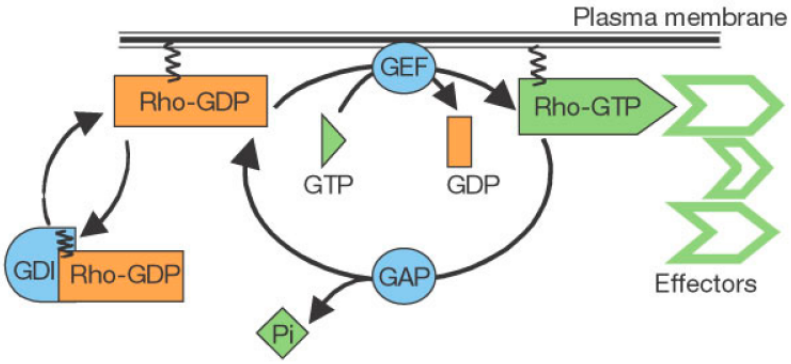

Fig. 1. Rho GTP-binding protein cycle. Adapted by permission from Macmillan Publishers Ltd: *Nature* [9], copyright 2002.

guanine nucleotide exchange factors (GEFs) that promote the GDP dissociation and GTP-binding, whereas GTPase-activating proteins (GAPs) have the opposite effect and stimulate the hydrolysis of Rho GTP into Rho GDP. In the active GTP-bound state, Rho proteins interact with and activate downstream effectors, e.g., to control actin polymerisation in the context of Fc receptor mediated phagocytosis [13]. Although the role of GDIs (Guanine Nucleotide dissociation Inhibitors) is not totally clear, there is evidence that these proteins are responsible for multiple tasks in the regulation of Rho GTP-binding proteins, including the inhibition of the GTP hydrolysis into GDP (see Section 4).

2.3 An ODE Model of Rho GTP-binding Proteins

In [11], Goryachev and Pokhilko give a computational model of the Rho GTP-binding proteins by means of ordinary differential equations (ODE). The structure and the differential equations of their model are given in Figure 2, respectively. In Figure 2, R denotes the Rho GTP-binding protein, whereas RD and RT denote its GDP and GTP bound forms respectively. A and E denote GAP and GEF, respectively. Thus, RDE, for example, denotes the protein complex formed by RD and E. After an inspection of the ODEs given in [11], we found the typos depicted with red ink in Figure 2. However, these typos seem to be only in the presentation of [11], not in the actual model, as suggested by the results in this paper.

In this model, the authors study GTP-binding proteins in isolation, disregarding the GDIs. The model uses mainly the quantitative biochemical data on Cdc42p. This results in an explanation of the experimentally observed rapid turn over in Rho GTP-binding proteins by means of an ODE model. In the following, based on this ODE model, we will give a process calculus model which compositionally builds and extends the ODE model. We will provide a comparison of two models.

3 A Process Calculus Model

We compositionally build a process model of Rho GTP-binding proteins by treating the components of the Rho GTP cycle as components of a stochastic π-calculus process (see, e.g., [18,2]). We first build a basic model, and then modularly extend

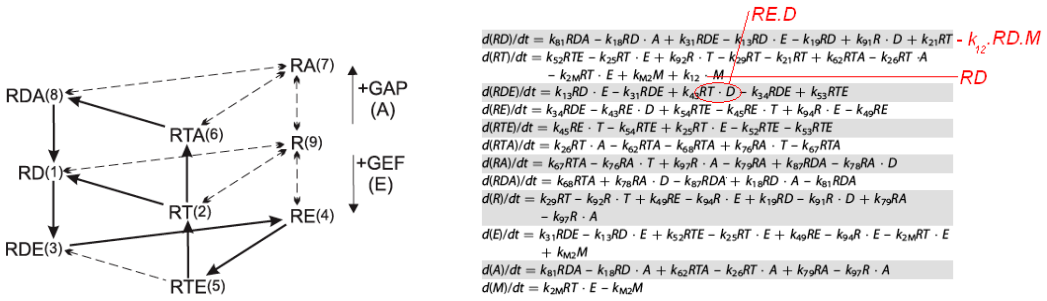

Fig. 2. The ODE model given in [11]. The diagram on the left depicts the chemical reactions underlying the ODEs on the right.

it with regulators. At each stage, we provide simulations of our models and compare our results with the corresponding ODE model [11].

3.1 Biological Processes as Computations

In the stochastic π-calculus, the basic building blocks are processes. Each process has a precise description of what actions it can take. Once a biological system has been modeled using these basic components, the model can be stochastically simulated in order to predict the evolution of the system over time. In this paper, the simulations are performed using the Stochastic Pi Machine (SPiM) [5] [17] which serves as a platform for implementing stochastic π-calculus processes and for running machine simulations. We use the SPiM notation throughout this paper.

Processes are viewed as the choice between zero or more processes. A process with n choices is written as `let P = do P1 or ... or Pn`. If there is only one choice, we write `let P = P1`. A process can perform an input `?x(m);Q` or output `!x(n);Q` on a channel `x` or perform a delay, written as `delay@r;Q`, where `r` is a real number value denoting the rate of an exponential distribution and `Q` is the continuation process. Complementary input and output actions interact by means of hand shake operations on channels declared with the syntax `new`. The operator `new x@r:t P` creates a fresh channel `x` of rate `r` to be used in the process `P` where `t` is the type of the channel `x` which can be, e.g., `chan(chan,chan)` denoting that the channel can pass the names of two channels. When a process is prefixed with a declaration of a fresh channel, that channel remains private to the process and does not conflict with any other channel. A process can be the empty process, written `()`. Two process components `P` and `Q` can be combined using parallel composition `P | Q`. This constitutes the basic form of compositionality which allows to compose processes in order to gradually build bigger models.

We can model chemical reactions using processes [18]. Let us see this on the following example: consider the situation where the biological processes RD and E can interact to form RDE complex, which can return to the state where RD and E coexist. We depict this as the reaction $\mathsf{RD} + \mathsf{E} \underset{r'}{\overset{r}{\rightleftharpoons}} \mathsf{RDE}$. This reaction can be read in Figure 2 as the arrow from RD to RDE together with the arrow for GEF (E). Following the results in [11], we know that the left-to-right direction has the rate $r = 0.0054\,\mu\mathsf{M}^{-1}.\mathsf{min}^{-1}$, whereas the right-to-left direction has the rate

[5] `http://research.microsoft.com/~aphillip/spim/`

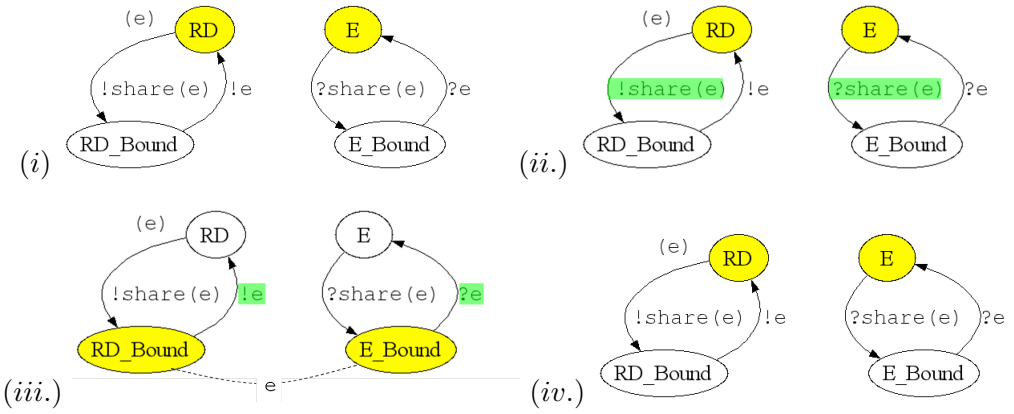

Fig. 3. Graphical representation of the evolution of the RD and E interaction model. Processes RD and E coexist (*i.*), and they can interact on channel share (*ii.*). When they interact, RD sends the private channel *e*, and E receives it. This way, they evolve to processes RD_Bound and E_Bound, respectively, which share the private channel *e*, resembling a covalent bond between two bio-chemical species (*iii.*). By interacting on channel *e*, they evolve back to the processes RD and E, respectively (*iv.*).

$r' = 0.136\,\mathsf{min}^{-1}$. This reaction is coded in SPiM as follows:

```
new share@0.0054:chan(chan)
let RD() = ( new e@0.136: chan() !share(e); RD_Bound(e) )
and RD_Bound(e:chan) = !e; RD()
let  E() = ?share(e); E_Bound(e) and E_Bound(e:chan) = ?e; E()
```

The first line of the code states that there is a channel `share` which takes another channel as argument. The second and third line state that the process `RD` can interact on channel `share` and broadcast the private channel `e`, and then evolve to process `RD_Bound`, which can send a message on channel `e` and evolve to `RD`. The fourth line states that process `E` can receive a message on channel `share`, and then evolve to `E_Bound` which can receive a message on channel `e` and evolve to `E`.

Figure 3 shows a run of a cycle of this reaction in the style of the graphical representation of the SPiM language, presented in [16]. There, the system is represented as two processes that interact over shared channels. Yellow marks indicate the state of the system, and green marks indicate the active channel.

When we run a simulation by using the code above with 1000 `RD` and 1000 `E` initially ($\mathsf{RD}_0 = 1000$ and $\mathsf{E}_0 = 1000$), we get the plot in Figure 4, where red and green are used for `RD` and `RD_Bound` molecules, respectively. We can read from this plot the recovery time, i.e., the time necessary for the system to reach stable state, as approximately 2.1 mins. At the stable state, the $\mathsf{RDE}/\mathsf{RD}_0$ ratio is 0.86.

3.2 Rho GTP-binding Proteins without GEF and GAP

At a first step towards compositionally building a model of Rho GTP-binding proteins, we first consider these proteins in isolation by disregarding the regulators GEF and GAP. This corresponds to the mid-layer of the graph on the left-hand side of Figure 2, which is also the left-most graph in Figure 5. Below, we encode this graph in the SPiM language by considering the possible reactions between the species as processes as shown on the left-hand-side of Figure 6.

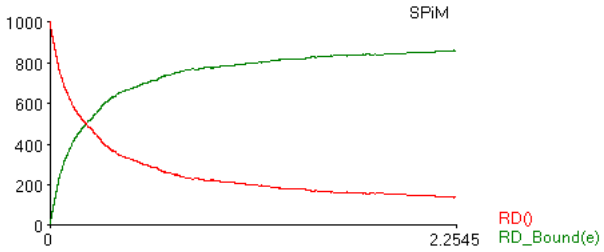

Fig. 4. SPiM plots of a simulation of the process with the graphical representation shown in Figure 3. The x-axis is the time in minutes and y-axis is the number of processes. The simulation is started with 1000 RD and 1000 E.

In the graph in Figure 5, the reactions between the species RD and R, and R and RT are bi-directional. However, there are only reactions from RT to RD with no reaction on the opposite direction. This is because GTP molecules can hydrolyse to GDP molecules by the disassociation of a phosphate group, but re-association of the phosphate group to GDP is not possible. Similar to the model in [11], we do not include the interactions with the GTP and GDP molecules explicitly in the structure of the model, this information is reflected in the quantitative data, as factors of the rates of the reaction which involve these molecules and set with respect to the constant concentrations of these molecules as reported in the literature ($500\mu M$ for GTP and $50\mu M$ for GDP [6]). The SPiM code of this model, including the quantitative data, is as follows:

```
val D = 50.0     val T = 500.0
let R()  = do  delay@0.033*D; RD()  or  delay@0.1*T; RT()
and RD() = delay@0.02; R()
and RT() = do  delay@0.02; R()  or  delay@0.02; RD()
```

The first line above states that the values of `D` and `T` are set to `50.0` and `500.0` respectively. The second line states that process `R` can evolve to `RD` or `RT` with the rates `0.033*D` and `0.1*T`, respectively. The third and forth lines state that `RD` can evolve to `R` with rate `0.02` and `RT` can evolve to `R` or `RD` with both rates `0.02`.

When we run a simulation using this code with 1000 `R` ($R_0 = 1000$), we get the left-most plot in Figure 7, where red, green and blue colours are used for `RD`, `R` and `RT` molecules, respectively. We can then read from this plot that the recovery time, i.e., the time necessary for the system to reach stable state, is approximately

[6] M (Mol) is the chemical unit for the number of molecules per unit solution, known as the Avogadro's number. $1M$ denotes approximately $6.02 * 10^{23}$ molecules, determined by the number of atoms in $12g$ of carbon-12. μM denotes micromoles, i.e., $1M * 10^{-6}$.

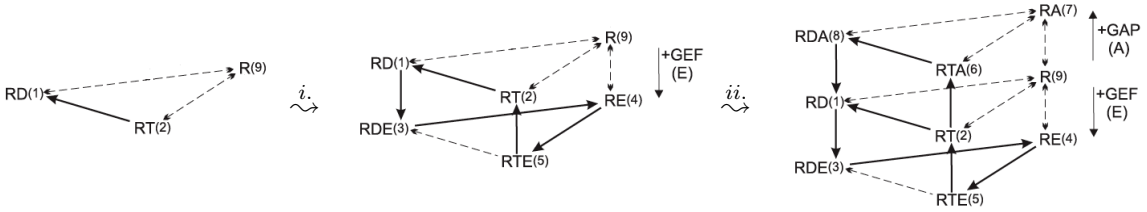

Fig. 5. A graphical representation of modular construction of the interactions of Rho GTP-binding proteins with respect to the ODE model in [11]. A basic model excluding the regulators GEF (E) and GAP (A) is extended first with GEF ($i.$) and then with GAP ($ii.$).

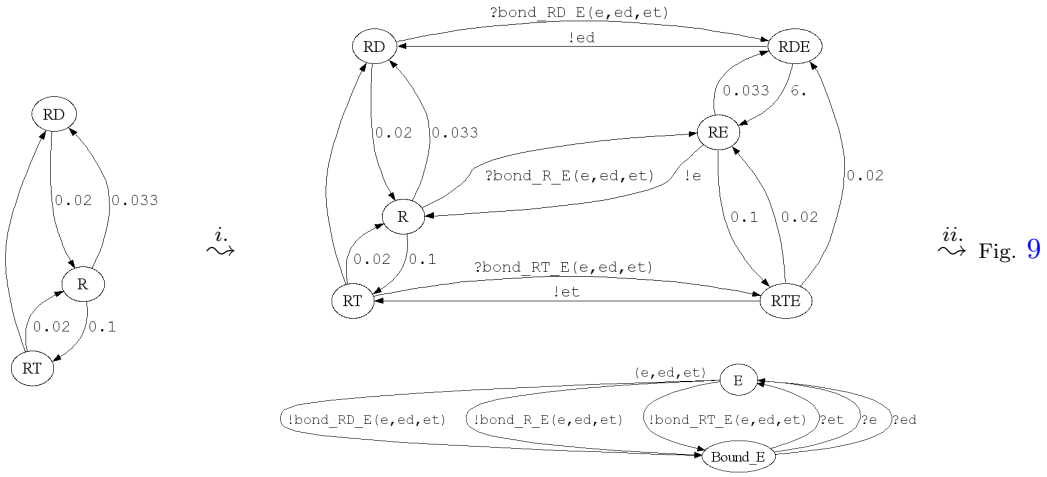

Fig. 9

Fig. 6. Compositional construction of the process model for the Rho GTP-binding proteins with GEF and without GAP with respect to $\overset{i}{\rightsquigarrow}$ in Figure 5.

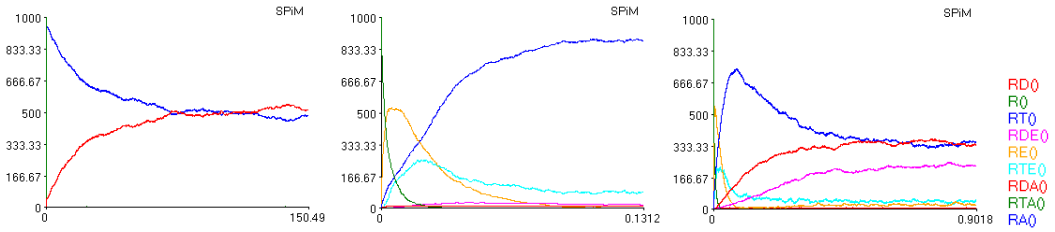

Fig. 7. SPiM plots of some simulations. The x-axis is the time in minutes and y-axis is the number of processes. The legend on the right is for all the three plots.

90 mins. At the stable state, the RT/R_0 ratio is 0.5.

3.3 Rho GTP-binding Proteins with GEF and without GAP

We extend the Rho GTP-binding protein process model, given in Subsection 3.2, to a process that also models GEF regulation. This corresponds to the middle diagram in Figure 5 and process model given in Figure 6. Here, we have two interacting processes, one for the Rho GTP-binding protein, which extends the process model given in the previous subsection, and one for GEF (the E process). The SPiM code of the model in Subsection 3.4 extends the code of this model, however that code can also be used for this model by taking the initial number of `A` (GAP in Figure 2) as 0. When we run a simulation using this code with 1000 `R` and 1000 `E` processes ($\mathsf{R}_0 = 1000$ and $\mathsf{E}_0 = 1000$), we get the middle plot in Figure 7, where red, green, blue, pink, yellow, and light blue colours are used for `RD`, `R`, `RT`, `RDE`, `RE`, and `RTE` molecules, respectively. We can then read from this plot that the recovery time, i.e., the time necessary for the system to reach stable state, is approximately 0.12 mins. At the stable state, the RT/R_0 ratio is 0.87.

In order to compare our process model with the ODE model given in [11], we ran the SPiM simulations on a range of initial number of molecules, where R_0 and E_0 range between $10^{-2}\mu M$ and $10^{6}\mu M$. In these simulations, the rate values are given

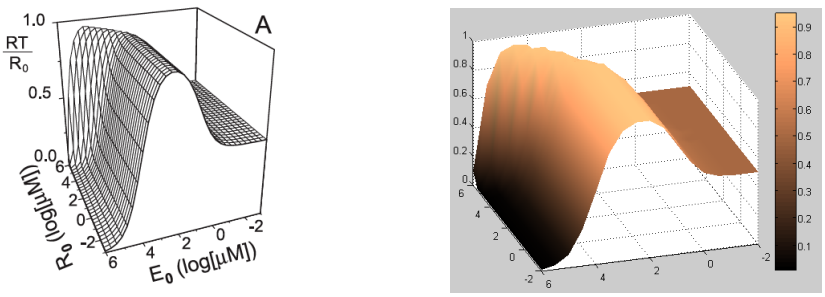

Fig. 8. Graphs displaying the RT/R_0 ratio as the output of the ODE [11] and process simulations, respectively, for the models for Rho GTP-binding proteins with GEF and without GAP.

with the unit μM^{-1}. Because of this, we encode $1\mu M$ of a species as 1 instance of the process in the model at the start of the simulation. For instance, when we start the simulation with $\mathsf{E}_0 = 1000$, this corresponds to $1000\mu M$ in the ODE model. In order to be able to run simulations for the situations where the initial concentration of species are too low for meaningful stochastic simulations or too high from the point of view of computational resources, we do a scaling by means of a factoring constant. This scaling can be seen to be performed on the underlying chemical reactions, that is, we divide the rates of the underlying binary chemical reactions and multiply the initial concentrations of the species with a factoring constant [20]. For instance, in order to run a simulation for the case where there are $10^{-2}\mu M$ of R and $10^{-2}\mu M$ of E, we scale the rate values by a factor of 10^4, which allows to give the initial values as $10^{-2} * 10^4 = 10^2$. For this purpose, we divide the rates of the interaction channels in the process model with our scaling factor, e.g., 10^4.

The outcome of our SPiM simulations, reflecting the RT/R_0 ratio at the stable state, are depicted as the graph on the right-hand-side of Figure 8. In Figure 8, the graph on the left-hand-side is the outcome of the ODE simulations taken from [11]. In both graphs, the values are given in logarithms of the concentrations in the ODE model and the amount of present processes in the process model, at the start of the respective simulations. For instance, the point in the plot where $\mathsf{E}_0 = 4$ and $\mathsf{R}_0 = 2$ is the case where the simulation is started with $10^4 = 10000$ E processes and $10^2 = 100$ R processes. We observe that the outcome of our simulations is consistent with the outcome of the ODE simulations. In order to obtain this match between the different models, the quantitative data consisting of the initial concentrations and rate values of the reactions had to be carefully analysed and fine-tuned. This turned out to be a challenging task which required a non-trivial interpretation of the data given in [11] in terms of processes.

3.4 Rho GTP-binding Proteins with GEF and GAP

We extend the model in Subsection 3.3 as in Figure 5 (*ii.*), and obtain process model for Rho GTP-binding proteins with GEF and GAP with the SPiM code given below. The graphical representation of this model is depicted in Figure 9 with three interacting processes: one for the Rho GTP-binding protein, which extends the model given in the previous subsection, one for GEF (E) and one for GAP (A).

```
directive sample 0.1 1000   directive graph
directive plot RD(); R(); RT(); RDE(e,ed,et); RE(e,ed,et); RTE(e,ed,et);
                                RDA(a,ad,at); RA(a,ad,at); RTA(a,ad,at)
```

```
val D = 50.0  val T = 500.0

new bond_RD_A@1.0:chan(chan,chan,chan)   new bond_RD_E@0.0054:chan(chan,chan,chan)
new bond_RT_A@1.0:chan(chan,chan,chan)   new bond_RT_E@0.0075:chan(chan,chan,chan)
new bond_R_A@1.0:chan(chan,chan,chan)    new bond_R_E@0.43:chan(chan,chan,chan)

let R() = ( do delay@0.033*D; RD()  or  delay@0.1*T; RT()
            or ?bond_R_A(a,ad,at); RA(a,ad,at)
            or ?bond_R_E(e,ed,et); RE(e,ed,et) )

and RA(a:chan,ad:chan,at:chan) =
      do delay@0.1*D; RDA(a,ad,at)  or  delay@0.0085*T; RTA(a,ad,at)  or  !a; R()

and RE(e:chan,ed:chan,et:chan) =
      do delay@0.033*D; RDE(e,ed,et)  or  delay@0.1*T; RTE(e,ed,et)  or  !e; R()

and RT() = (  do delay@0.02; R()  or  delay@0.02; RD()
              or  ?bond_RT_A(a,ad,at); RTA(a,ad,at)
              or  ?bond_RT_E(e,ed,et); RTE(e,ed,et)  )

and RTA(a:chan,ad:chan,at:chan) =
      do  delay@0.0002; RA(a,ad,at)  or  delay@2104.0; RDA(a,ad,at)  or  !at; RT()

and RTE(e:chan,ed:chan,et:chan) =
      do delay@0.02; RDE(e,ed,et) or delay@0.02; RE(e,ed,et) or !et; RT()

and RD() = (  do delay@0.02; R()  or  ?bond_RD_A(a,ad,at); RDA(a,ad,at)
              or  ?bond_RD_E(e,ed,et); RDE(e,ed,et)  )

and RDA(a:chan,ad:chan,at:chan) =  do delay@0.02; RA(a,ad,at)  or  !ad; RD()

and RDE(e:chan,ed:chan,et:chan) =  do delay@6.0; RE(e,ed,et)  or  !ed; RD()

let A() = (  new a@500.0:chan  new ad@500.0:chan  new at@3.0:chan
    do !bond_R_A(a,ad,at); Bound_A(a,ad,at)
    or !bond_RT_A(a,ad,at); Bound_A(a,ad,at)
    or !bond_RD_A(a,ad,at); Bound_A(a,ad,at)  )

and Bound_A(a:chan,ad:chan,at:chan) =  do ?a; A() or ?ad; A() or ?at; A()

let E() = (  new e@1.074:chan  new ed@0.136:chan  new et@76.8:chan
    do !bond_R_E(e,ed,et); Bound_E(e,ed,et)
    or !bond_RT_E(e,ed,et); Bound_E(e,ed,et)
    or !bond_RD_E(e,ed,et); Bound_E(e,ed,et)  )

and Bound_E(e:chan,ed:chan,et:chan) =  do ?e; E() or ?ed; E()  or  ?et; E()

run 1000 of R()  run 10 of A()  run 1000 of E()
```

When we run a simulation using this code with 1000 `R`, 10 `A` and 1000 `E` processes ($\mathsf{R}_0 = 1000$, $\mathsf{A}_0 = 10$ and $\mathsf{E}_0 = 1000$), we get the right-most plot in Figure 7. We can then read from this plot that the recovery time is approximately 0.5 mins. At

Fig. 6 $\overset{ii.}{\rightsquigarrow}$

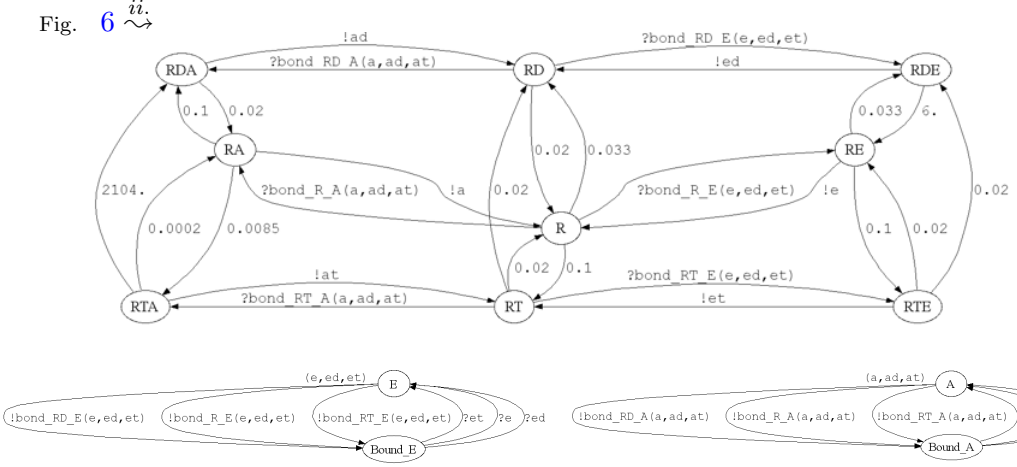

Fig. 9. Compositional construction of the process model for the Rho GTP-binding proteins with GEF and GAP with respect to $\overset{ii}{\rightsquigarrow}$ in Figure 5.

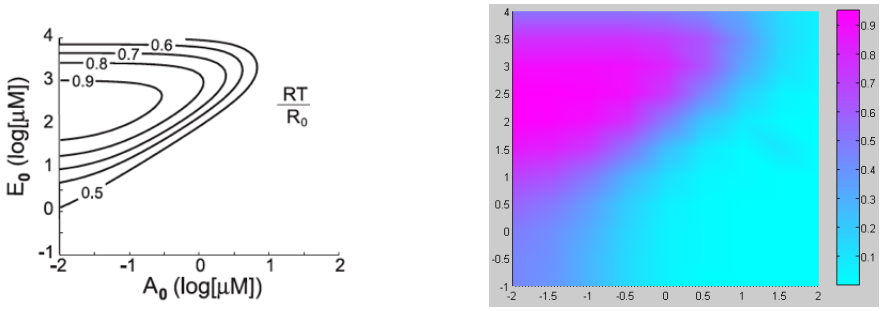

Fig. 10. Graphs displaying the RT/R_0 ratio as the output of the ODE [11] and process simulations, respectively, for the models for Rho GTP-binding proteins with GEF and GAP.

the stable state, the RT/R_0 ratio is 0.35.

In order to compare this model with the model in [11], we ran simulations on a range of initial number of molecules, where R_0 is 1000 and E_0 ranges between $10^{-1}\mu M$ and $10^{4}\mu M$, and A_0 ranges between $10^{-2}\mu M$ and $10^{2}\mu M$. For some simulations, we performed a scaling as described for the simulations in Subsection 3.3.

The outcome of our simulations are depicted as the graph on the right-hand-side of Figure 10, where the graph on the left-hand-side is the outcome of the ODE simulations taken from [11]. In both graphs, the values are given in logarithms of the concentrations in the ODE model and the amount of present species in the process model, at the beginning of the respective simulations. Again, the outcome of these simulations is consistent with the outcome of the ODE simulations.

4 Extending the Model with Effectors and GDI

Besides the regulators GEF and GAP, the Rho GTP cycle depicted in Figure 1 is also affected by the interactions with the effectors and another regulator called GDI. The effectors of the Rho GTP-binding proteins, e.g., WASP (also in the context of phagocytosis), change their structural conformation and gain the ability to bind to other proteins while they are associated with the active GTP-bound Rho protein attached to the membrane. The GDIs (Guanine-nucleotide Dissociation Inhibitors) form a class of regulatory proteins for the Rho GTP cycle [6,7,8]. GDIs play two roles: they down-regulate the Rho activity by preventing membrane association of these proteins, and they serve as shuttling mechanisms for the active GTP-bound and passive GDP-bound Rho proteins to and from membranes.

4.1 Extending the Model with Effectors

The biological function of the GTP-binding proteins is performed only by the active GTP-bound form that binds and activates a broad range of effector proteins. By disregarding the role played by the GDIs, [11] gives a model that extends the model in Subsection 3.4 with effectors. The model is obtained by extending the model of GTP-binding protein cycle with reactions that capture the behaviour of these proteins together with the effectors; a scaffold-effector complex forms a stable complex with GEF (E) at all times. The binding of E to the RT results in the formation of an activated tripartite complex, consisting of RT, E and the effector protein. In this model, M denotes this complex. Due to the lack of detailed data in

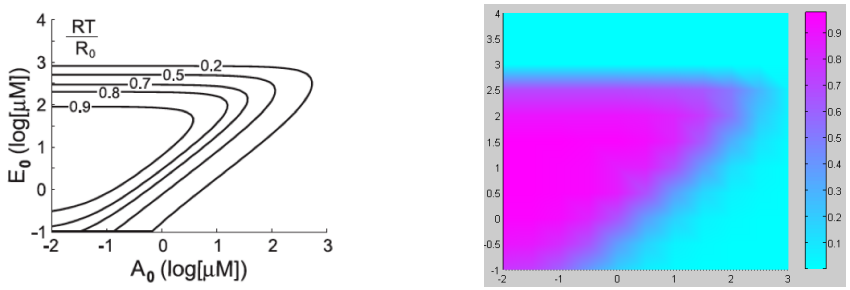

Fig. 11. Graphs displaying the RT/R_0 ratio as the output of the ODE [11] and process simulations, respectively, for the models for Rho GTP-binding proteins with GEF, GAP and effectors.

the literature, the authors suggest that such a representation provides a sufficiently abstract model of the actual biological system. This results in the introduction of 3 reactions which sufficiently abstract away from the actual biological kinetics that would involve 54 more reaction rate constants. Thus, this simplified model extends the model depicted in Figure 2 with the reactions

$$\mathsf{r}_1 : \mathsf{RT} + \mathsf{E} \to \mathsf{M} \qquad \mathsf{r}_2 : \mathsf{M} \to \mathsf{RT} + \mathsf{E} \qquad \mathsf{r}_3 : \mathsf{RD} + \mathsf{M} \to \mathsf{RT} + \mathsf{M}$$

where the rates of r_1, r_2, and r_3 are estimated and set as $600\,\mu\mathsf{M}^{-1}.\mathsf{min}^{-1}$, $18\,\mathsf{min}^{-1}$ and $0.6\ \mu\mathsf{M}^{-1}.\mathsf{min}^{-1}$, respectively.

Using the reactions and rates given above, we extended the process model of Subsection 3.4, analogously. For a comparison with the model of [11], we ran simulations on a range of initial number of molecules, where R_0 is 1000 and E_0 ranges between $10^{-1}\mu M$ and $10^{4}\mu M$ and A_0 ranges between $10^{-2}\mu M$ and $10^{3}\mu M$.

The outcome of our simulations are depicted as the graph on the right-hand-side of Figure 11, where the graph on the left-hand-side is the outcome of the ODE simulations taken from [11]. In both graphs, the values are given in logarithms of the concentrations in the ODE model and the amount of present species in the process model, at the beginning of the respective simulations. Again, the outcome of these simulations is consistent with the outcome of the ODE simulations.

4.2 Extending the Model with GDIs

GDIs were initially identified as down-regulators of GTP-binding proteins due to their ability to prevent the dissociation of GDP or GTP from the GTP-binding proteins [6]. However, this ability turns out to be a crucial mechanism with which GDIs serve as transport proteins, shuttling GTP-binding proteins between membranes. It is now believed that the complementary structures of GTP-binding proteins and GDIs is crucial in this transport mechanism [8]. When associated to the membrane, GTP-binding proteins are anchored to the membrane by lipid modification on their C-terminus. However, when GTP-binding proteins interact with GDIs, they establish a bond such that C-terminal domain of GDI binds with the C-terminus and recognises switch 2 region of the GTP-binding protein, while N-terminal domain of the GDI binds to the switch 2 region of the GTP-binding protein and recognises its switch 1 region as well. This interaction results in a blocking mechanism that prevents the anchoring of the GTP-binding protein to the membrane. By interacting with and covering the switch 1 and switch 2 region of the GTP-binding protein, GDIs also inhibit the dissociation of the GDP or GTP [6]. We can thus summarise

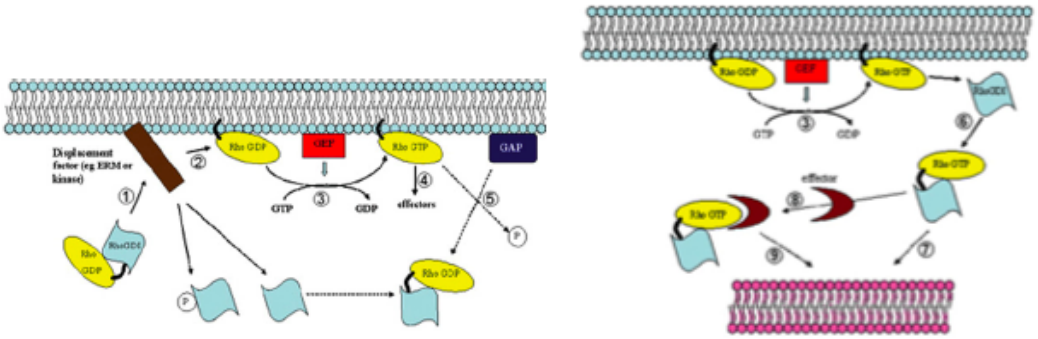

Fig. 12. Models for the regulation of Rho family GTP-binding proteins by RhoGDI molecules [7].

the function of the GDIs as inhibiting the disassociation of GDP, inhibiting GTP hydrolysis, and stimulating the shuttling of Rho GTP proteins such as Cdc42 and Rac to and from cellular membranes.

Along these lines, there are various models of the exact role of the GDI. We adopt a model which is hybrid between the two models given in Figure 12 [7], based on a discussion with Caron, a biologist at Imperial College. We describe this model by means of reactions $\mathsf{r}_4, \ldots, \mathsf{r}_7$ given in Figure 13. During the interaction of the GTP-bound Rho protein with an effector, GTP hydrolysis facilitated by a GAP protein terminates the signal and allows the membrane extraction of the resulting GDP-bound Rho protein by binding to a GDI (reaction r_4, 5 in Figure 12). A complex formed by GDP-bound Rho and GDI is then in the cytosol; a displacement factor or signal at the membrane localizes the complex proximal to a membrane compartment (reaction r_5, 1 in Figure 12). At some membrane, GDI might extract the Rho protein from the membrane in its GTP-bound form to either terminate signal prematurely (reaction r_6, 6 in Figure 12) or to redirect the Rho protein to a distinct membrane within the cell where GTP-bound Rho anchors to the cell membrane and GDI is released (reaction r_7, 9 in Figure 12).

The reactions shown in Figure 13 are an abstraction of the interactions of GDIs with the GTP-binding protein cycle in the actual biological system. It is possible to work with more complicated models: for example, those involving reactions for the association of different combinations of R, RD and RT with A and E together with G. We do not have precise information about the rates of these reactions except that they have been observed to have very low affinity. We therefore work with a model which abstracts away from these reactions.

We implemented the reactions above in the SPIM language in the same way as in Section 3. By using the quantitative data at hand on the concentration of the GDIs, we ran simulations. The outcome of 8 simulations, where G_0 is set to 0, 10, 30, 60, 100, 300, 600 and 1000, are shown in Figure 14. In these simulations, the initial number of molecules for R_0 and E_0 are 1000 and the number of molecules for and A_0 is 10. The rate of the reactions above are set to 1.0.

In [8,6], it is reported that GDIs are out numbered by GEF and GAP regulators.

$$\mathsf{r}_4 : \mathsf{RD} + \mathsf{G} \to \mathsf{RDG} \qquad \mathsf{r}_5 : \mathsf{RDG} \to \mathsf{RD} + \mathsf{G}$$

$$\mathsf{r}_6 : \mathsf{RT} + \mathsf{G} \to \mathsf{RTG} \qquad \mathsf{r}_7 : \mathsf{RTG} \to \mathsf{RT} + \mathsf{G}$$

Fig. 13. Reactions of a model that extends the GTP-binding protein cycle with GDI (G).

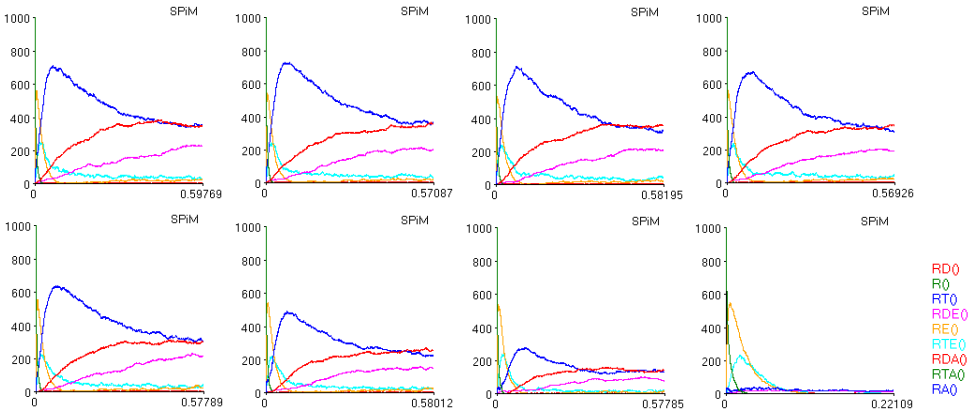

Fig. 14. SPiM plots of simulations with the model which extends GTP-binding protein cycle with GDI and effectors. The x-axis is the time in minutes and y-axis is the number of processes. The legend on the right is for all the the plots. In all the simulations, R_0 and E_0 are 1000; A_0 is 10. From left to right, the G_0 value is 0, 10, 30, 60, 100, 300, 600 and 1000.

The molar amount of GDI is in excess of any single Rho protein, but roughly equal to the total levels of the RhoA, Rac1 and Cdc42 GTP-binding proteins in these cells. In human neutrophils, RhoA, Rac1/Rac2 and Cdc42 are also equimolar with overall GDI levels, and exist largely as cytosolic GDI complexes. Our simulations capture this behaviour, because, for lower concentrations of GDIs, our model captures the inhibitory role of the GDIs while preserving the behaviour pattern of the model of Subsection 3.4. However, a more comprehensive analysis of the model above requires more accurate quantitative data in terms of rates of reactions, which is the topic of on-going work.

5 Discussion

We have given a process model of the Rho GTP-binding protein cycle, and run simulations of our model using SPiM [17]. Our model closely follows Goryachev and Pokhilko's paper [11], which provides an ODE analysis of the Rho GTP-binding protein cycle, both in isolation and with effectors. The use of our techniques to model and simulate biological systems, and the comparison with the ODE analysis is not new, see for example [14,4]. Our results do however provide an essential calibration between our process-algebra techniques and the ODE analysis for the basic model of the Rho GTP-binding protein cycle. With this initial calibration, we now have the freedom to exploit the compositionality of the process-algebra approach to study additional features—in this paper adding effectors in the cytosol—on top of our basic model. Although the ODE approach can also be extended, we believe the extension is less natural and ultimately will not scale to large biological systems.

We extend our basic model to capture also the effect of the GDIs in the Rho GTP-binding protein cycle. We expected to use the biological models described in a survey paper [7]. Instead, based on discussions with Caron, a biologist at Imperial, we introduce an abstract, hybrid model which better reflects the current knowledge. At the moment, we lack the quantitative data for the rates of the reactions for this model. We give an initial analysis of the behaviour of the protein cycle by setting the unknown rates as 1.0 and varying the quantity of GDIs. A thorough assessment of the usefulness of SPiM as a tool for systematically searching the parameter space

for plausible rates is left for future research. We hope SPiM will eventually assist in the literature search and wet-lab experiments necessary to complete the missing quantitative data.

Rho GTP-binding proteins serve as molecular switches in various subcellular activities, including phagocytosis. Our long-term goal is to use the model of this paper as a generic model for these proteins which can be compositionally plugged into larger models. By gradually extending the model and moving between levels of abstractions, we hope to eventually deliver useful process models of phagocytosis. However, biological knowledge is in a state of constant flux, and is often incomplete. Our aim is to work closely with biologists at Imperial to overcome these challenges.

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