

A Process Model of Rho GTP-binding Proteins

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Rho GTP-binding Proteins

The family of Rho GTP-binding proteins serve as molecular switches in various subcellular activities, regulating a variety of cell functions, including cell adhesion, cell motility, actin organisation and cell shape (see, e.g., [1]). These proteins transmit 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. GDP/GTP cycling is regulated by 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 activate downstream effectors.

GDI (Guanine Nucleotide dissociation Inhibitors) 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 (see, e.g., [2]). This ability turns out to be a crucial mechanism resulting in two roles of GDIs: 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.

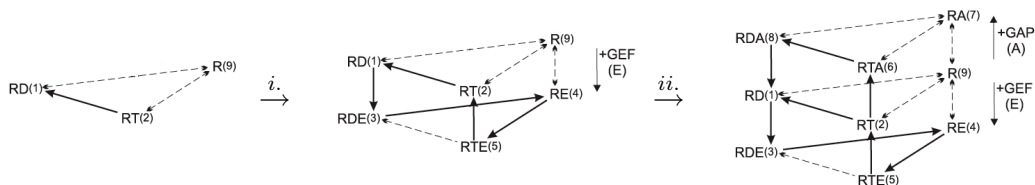


Figure 1: A graphical representation of modular construction of the interactions of Rho GTP-binding proteins with respect to the ODE model in [3]. A basic model excluding the regulators GEF (E) and GAP (A) is extended first with GEF (*i.*) and then with GAP (*ii.*).

A Process Model of Rho GTP-binding Protein Cycle

We give a process calculus model of Rho GTP-binding proteins. We study and build on Goryachev and Pokhilko's ordinary differential equation (ODE) analysis [3] of the Rho GTP-binding protein cycle, first in isolation and then with their regulators GEF and GAP. The structure of the model in [3], reflecting the underlying chemical reactions, is given as the right-most diagram in Figure 1. There, 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. 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.

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., [4]). We first consider these proteins in isolation by disregarding the regulators GEF and GAP. This corresponds to the left-most diagram in Figure 1. We extend this model to a process that also models GEF regulation. This corresponds to the middle diagram in Figure 1. We then extend this model to obtain a process model including GEF and GAP, corresponding to the right-most diagram in Figure 1.

We then extend our process model with respect to a biological model, hybrid between the two given in [2]: 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_1). A complex formed by GDP-bound Rho and GDI is then in the cytosol; a signal localizes the complex proximal to a membrane compartment (reaction r_2). At some membrane, GDI might extract the Rho protein from the membrane in its GTP-bound form to terminate the signal prematurely (reaction r_3) or to redirect the Rho protein to a distinct membrane within the cell where GTP-bound Rho anchors to the membrane and GDI is released (reaction r_4).



These reactions provide 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

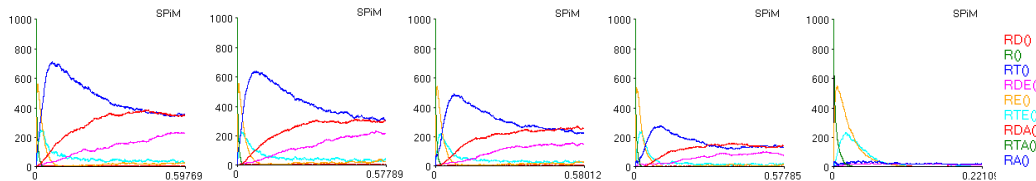


Figure 2: SPiM plots of simulations with the model extended with GDIs. The x -axis is the time in minutes and y -axis is the number of processes. In all the simulations, R_0 and E_0 are 1000; A_0 is 10. From left to right, the G_0 is 0, 100, 300, 600 and 1000.

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.

Simulations

We ran simulations with our model by using the Stochastic Pi Machine (SPiM)¹ (see, e.g., [4]) with the rates of interaction given in [3]. We compared our results with the ODE model of [3]. We observed that our simulations remain consistent with the simulations of [3]. This provided an essential calibration between our process-algebra techniques and the ODE analysis of the Rho GTP-binding protein cycle. We then compositionally extended this model to include the interactions with GDIs and ran simulations on this extended model. Although the ODE approach can also be extended, we believe the extension is less natural and will not scale to larger systems.

The molar amount of GDI is in excess of any single Rho protein, but roughly equal to the total levels of the Rho proteins in these cells. Our model captures this behaviour, because, for lower concentrations of GDIs, our simulations conservatively capture the inhibitory role of the GDIs (see Figure 2).

References

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¹<http://research.microsoft.com/~aphillip/spim/>