Clostridial toxins: Molecular probes of Rho-dependent signaling and apoptosis

David A. Bobak
Departments of Medicine and Microbiology, University of Virginia School of Medicine, Charlottesville, VA, USA

Abstract

The Rho family small GTPases are members of the Ras superfamily of small GTPases. Rho proteins were first determined to act as key regulators of many types of actin cytoskeletal-dependent cellular functions. Recent work by several investigators indicates that Rho GTPases are also critical modulators of several important intracellular and nuclear signal transduction pathways. Certain clostridial toxins and exoenzymes covalently modify, and thereby inactivate, specific types of Rho family GTPases. As such, these microbial enzymes have proven invaluable in helping to identify structural and functional attributes of Rho GTPases. (Mol Cell Biochem 193: 37–42, 1999)

Key words: Rho, GTPase, toxins, Clostridium, signal transduction, apoptosis

Introduction

In the past 30 years or so, study into the molecular mechanisms of action of microbial toxins has not only shed light on the etiology of many important diseases, but has directly led to the elucidation of many important and basic biologic functions as well. There are several notable examples from this field of investigation: cholera and pertussis toxins and the discovery of heterotrimeric G-proteins, diphtheria toxin and insight into the role of elongation factor II in protein synthesis, and molecular details of the neuroexocytotic process brought to light by the study of botulinal and clostridial neurotoxins. Recently, a variety of toxins and exoenzymes secreted by certain species of Clostridium bacteria have been discovered to covalently modify and inactivate several members of the Rho family of signal-transducing small GTPases [1–3]. Using these toxins and exoenzymes as molecular probes, several investigators have been able to determine that Rho GTPases are critical regulators of a wide variety of important cellular and nuclear signal transduction pathways.

Rho small GTPases

Rho family proteins are members of the Ras superfamily of signal-transducing small GTPases [4, 5]. In 1985, a gene was cloned from Aplysia and, on the basis of its sequence similarity to the ras oncogene, was named Rho (i.e. ‘Ras homologue’). Subsequently, several forms of rho genes were isolated from yeast and human cDNA libraries. In addition, several rho-related genes have also been discovered. A listing of the currently known Rho and Rho-like proteins is outlined in Table 1. Of these several forms of Rho, the three forms known as Rho, Rac, and Cdc42 have been the most well-studied among the group [6–8].

The Rho family of proteins belongs to a larger superfamily of small GTPases related to the product of the ras oncogene. These GTPases are all of generally similar size and overall structure to Ras and are particularly homologous in the domains of proteins known to be involved in the binding and hydrolysis of GTP. Using structural and functional similarities, the Ras superfamily GTPases can be grouped into several different subfamilies: Ras/Ras-related proteins, Rho/
Rho-related proteins, ARFs, Rabs, and a group of Misc. small GTPases [4, 5].

**Regulation of Rho activity**

As is generally true of most small GTPases, the activity state of Rho proteins is governed by a cycle of guanine nucleotide binding and modulated by complex interactions with a number of regulatory factors [4, 6–11]. Some of the key features of this activation/deactivation cycle for Rho GTPases are shown in Fig. 1. Basal, or ‘inactive,’ Rho is found in the cytosolic phase, bound to GDP and complexed with a factor known as Rho guanine nucleotide dissociation inhibitor (GDI). RhoGDI is believed to sustain the ‘off’ phase of Rho by stabilizing the GDP-bound state of the Rho protein and masking Rho’s COOH-terminal membrane targeting domain. In response to upstream activating signals, the exchange of GDP for GTP occurs on Rho, shifting the tertiary structure of the protein into an activated, or ‘on,’ state. In some instances, this exchange can be facilitated by another type of regulatory factor, known as a guanine nucleotide exchange factor (GEF). At, or about, the same time, RhoGDI dissociates from Rho and the fully activated GTPase then translocates to specific subcellular, membrane-associated compartments within the cell.

Activated Rho-GTP recruits one of several known Rho effector molecules to Rho’s subcellular location. Currently characterized Rho effectors include protein kinases, lipid kinases, phospholipases, putative adaptor proteins, and phosphatases [6, 7, 12–14]. The specificity of Rho signaling is believed to result from Rho’s putative ability to recruit a specific effector, or complex of effectors, to a particular subcellular compartment in a time- and space-dependent manner. Termination of the Rho-dependent signal likely results in a return of the Rho protein to its basal, GDP-bound state. Some hydrolysis of GTP to GDP occurs intrinsically, but specialized factors that accelerate this reaction, known as GTPase accelerating proteins (GAPs), have been identified for Rho family GTPases [9]. Rho-GDI can assist in the release of Rho-GDP from the target membrane and likely helps complete the activation cycle. A cycle for Rho is illustrated here, but the same general cycle and compliment of regulatory factors have been described for the Rho-related proteins Rac and Cdc42 as well. Although the simple version of this cycle presented here appears intricate enough, the true complexity is much greater because many of the GEFs and GAPs for the Rho family GTPases probably have effector activities themselves. For example, several Rho GEFs are known to be oncogenes (e.g. Vav, Db1, Ost, Lbc) [10, 13]. The intricacies of the relationships between Rho GTPases and their associated factors and effectors helps to explain how Rho proteins can be implicated in the regulation of such a wide variety of cellular functions.

Ras was the first of the small GTPases to be crystalized and analyzed at the structural level [4, 5, 15]. Since that time, the three-dimensional structures of a number of wild type and mutant small GTPases, including members of the Rho family, have been solved and published. When compared with Ras at the amino acid sequence level, Rho small GTPases exhibit identities in the range of ~30 to ~60%, with most of the identity occurring in the domains involved in binding and hydrolysis of GTP. The overall topological structure of the small GTPases, though, appears to be highly conserved, and, from the results of numerous structural- and mutagenesis-based studies, several important functional domains have been identified. A cartoon outlining these particular domains for the Rho GTPases is shown in Fig. 2.

The Rho protein motifs responsible for the binding and hydrolysis of GTP are critical for activation and function of the molecule [2, 4, 6–8, 15, 16]. These regions include the amino acid sequences GXXXXGKS/T, DXXGQ, NKXD, and SXK (as are outlined in Fig. 2). Several well-characterized mutations have been described within these GTP-binding domains. In some cases, the mutated proteins bind GTP with high avidity and are resistant to GTP hydrolysis, resulting in a GTPase that is locked in the ‘on’ conformation. These activated forms of small GTPases are commonly described as constitutively active or dominant active forms. Conversely,