Preface

THE RHO GTPASE FAMILY

ED MANSER
GSK-IMCB Group, Institute of Molecular and Cell Biology, 61 Biopolis Drive, SINGAPORE 138673.

1. THE CELL CYTOSKELETON

All organisms have cellular machinery dedicated to controlling cell shape, and the ability to dynamically change shape plays important roles in many biological processes. Muscle-based mechanisms are the best understood because the machinery is highly ordered and exerts force in a single dimension (ie. pulling between the two fixed ends of the cell). This utilizes interlaced actin and myosin II filaments that are distributed in fixed arrays. By contrast motile cells or cells with plastic processes (for example neurons) respond additionally by remodeling their internal actin cytoskeleton into many types of array. Actin, one of the most abundant eukaryotic proteins, is clearly a key protein in promoting structural change, being conserved from yeast to humans. Just recently proteins distantly related to actin have been discovered in prokaryotes such as *Bacillus subtilis* where they also regulate cell shape.

In the absence of internal structures the fluid lipid bilayer cell membrane would be spherical, but assymetry arises from both rigid elements within the cell (as exemplified by the well studied red blood cell) and external attachments. The internal cytoskeletal components work in combination with adhesive tension developed against other cells or the extracellular matrix. Actin-based structures are chiefly organized by Rho proteins, as appears to be the case for other important cytoskeletal components in mammalian cells, namely intermediate filaments, septin filaments and microtubules. All these are assembled from component protein subunits that under appropriate conditions can form various types of filaments. For many decades

microinjection of flourescently-labelled subunits, which incorporate into these filaments in vivo, has revealed a dynamic rather than static cytoskeleton. In the case of actin microfilaments and the microtubules, energy derived from hydrolysis of ATP or GTP respectively provides the filaments with polarity, and subunit addition occurs predominantly at one end. This produces a phenomena known as tread-milling whereby new monomers are added primarily at the 'plus' end to match loss from the 'minus' end. Recently exciting new discoveries are being made about the way in which Rho proteins control microtubules (Chapter 12), and we expect to similar levels of control to be found for intermediate filaments and the poorly characterized septin system.

2. SOME BACKGROUND

It is a decade since Alan Hall and his colleagues described how active versions of small GTP-binding proteins Rac1 and RhoA could assemble distinct actin arrays in mammalian cells. Significantly it was found external signals acting on transmembrane cell surface receptors use these GTPases to initiate similar changes in the actin cytoskeletal to promote changes in cell shape. Specifically Rac1 acting downstream of receptor tyrosine kinases generates peripheral extensions known as membrane ruffles or lamellipodia, while RhoA assembles more internally located actin stress fibres, and associated adhesion complexes. Shortly afterwards a related GTPase Cdc42, first described in budding yeast, was found to organize cell structures known as filopodia in mammalian cells - finger like projections containing an actin core.

Work from many laboratories including our own has established that Rho-GTPases alter the cytoskeleton by recruiting protein effectors. We discovered that kinases that belong to the ACK, PAK, MRCK and ROK families bind selectively to the GTP-form of certain Rho proteins. These kinases in turn act in phosphorylation cascades that are responsible for some of the changes to the actin, intermediate filament and microtubule networks initiated by the GTPases (see Chapters 6, 7 and 10). Non-kinase effectors such as WASP and formins play distinct but complementary roles. A working model is that many signals that impinge on cells to alter their shape route through a common sets of Rho switches. These proteins also have important functions beyond the cytoskeleton - in cell cycle control and transcriptional regulation, but such issues are less understood and not dealt with in this volume. Lessons learned about pathways regulated by Rho proteins are beginning to tell us how cells coordinate the complex internal reorganization required for processes such as cell movement.

3. THE BEHAVIOUR OF RHO PROTEINS

The reason small GTPases are referred to as molecular switches is that they exist in the cell in two forms, one bound to GDP and the other to GTP. In what is often referred to as the "active" state a GTPase may bind and allosterically activate an enzyme such as a PAK kinase (Chapter 10), alter the cellular location of effectors, or promote assembly of a multisubunit complex. The two inter-convertible states of these GTPases results from nucleotide-sensitive conformational changes in two regions of the protein referred to as switches I and II. The interaction of these regions with various types of protein are beautifully described and illustrated in Chapter 3. The presence of a permanent, covalently attached 16 or 20 carbon fatty acid at the C-terminal is essential to both membrane association and biological effects of Rho proteins (except perhaps RhoBTB).

The high degree of structural and functional conservation between mammalian Rho proteins and the orthologs in flies and even yeast is remarkable. More complex organisms in general have correspondingly increased numbers of Rho proteins although significant diversification can occur in apparently 'simple' organisms. These and other aspects of the evolution of the human Rho family are considered in Chapter 2.

The interconversion between the two states of a GTPase is promoted by two types of activities: guanine nucleotide exchange factors (GEFs) increase the level of activated (GTP-bound) GTPase and GTPase activating proteins (GAPs) speed the intrinsic hydrolysis of the bound GTP. Some of these Dbl-related RhoGEFs are discussed in Chapter 1 while a completely unrelated class of GEFs known as Dock180 proteins are covered in Chapter 4. The importance of diversity in the regulation of Rho proteins is evident for the GTPase activating proteins (RhoGAPs), whose bewildering diversity of domain structures is described in Chapter 5.

The Ras and Rho proteins behave in some ways like the alpha subunit of heterotrimeric G-proteins. Researchers initially applied techniques used in the heterotrimeric G-protein field to test whether small G-proteins behave as three-component systems (activator, G-protein, and effector) as for hormone- and light-activated heterotrimeric G systems.

The generally accepted view regarding guanine nucleotide dissociation inhibitors (GDIs) is that these proteins play a chaperone or negative regulatory role, though in one case the GDI can confer the Rac.GDP.GDI complex with Rac.GTP-like behaviour *in vitro*.

The overwhelming majority of studies to date have focused on roles and activities Rac1, Cdc42 and RhoA particularly since the latter two have orthologues well conserved across eukaryotes (see Chapter 2). These proteins play conserved roles in regulating actin assembly via effectors that

regulate myosin II, formin, and Arp2/3 pathways (as covered in detail in Chapters 6 and 7).

4. FINAL THOUGHTS

It is apparent that although the last 10 years have brought tremendous progress, really assessing how Rho and their partners regulate the cytoskeleton requires an understanding of how the numerous effectors actually integrate in the cellular context. Efforts recently underway to investigate global changes in phosphorylation promise to provide much more complete understanding of the role of kinase effectors of Rho. It is also important to appreciate that the cytoskeleton and membrane compartments are closely linked. Thus we are likely to see more input from colleagues working in the area of membrane trafficking (covered in Chapter 9).

We have plenty of work ahead to complete the molecular description of Rho actions in eukaryotes, and more particularly how these functions relate to vertebrate development (see Chapter 11). The use of knock-down as well as knockout techniques will no doubt produce a leap in the number of studies addressing Rho function in the whole animal.

ACKNOWLEDGEMENTS

Thanks must go to all the authors of this volume who have laboured to produce insightful chapters in their respective area of expertise. This effort will no doubt aid the expanding community of researchers who have an interest in Rho proteins. I would like to thank Jackson Zhao for excellent assistance in putting together and editing text and figures for each of the chapters in this book. I am grateful for support from the GSK-Singapore Research Fund.

Ed Manser
November 2004