Version 2.0

Abstract

Back to Hit List

Grant Number:	1R01GM056322-01
PI Name:	ROSEN, MICHAEL K.
PI Email:	rosen@mrnmr1.mskcc.org
PI Title:	ASSISTANT MEMBER
Project Title:	STRUCTURAL STUDY OF RHO-GTPASE REGULATORS AND EFFECTORS

Abstract: The Rho-family GTPases Cdc42, Rac and Rho regulate cytoskeletal structure, adhesion and gene expression in cells. Aberrant signaling through these proteins can lead to cellular transformation and tumor invasiveness. Research in this proposal focuses on comparative structural characterization by NMR spectroscopy of two ligands for the Cdc42Hs GTPase, the negative regulator, RhoGDI and CRIB domain of the putative effector, Wiskott-Aldrich Syndrome protein (WASP). Preliminary studies of RhoGDI indicate the protein is composed of two distinct regions with different, but possibly overlapping functions. The N-terminal domain binds the protein portion of Cdc42Hs, while the C-terminal domain binds to isoprenyl groups. The backbone and sidechain 1H, 15N and 13C chemical shift assignments of a 16 kDa C- terminal fragment of RhoGDI (GDIC, residues 60-204) are nearly complete. The structure of this domain will be determined to high resolution using NOE and 3J coupling information derived from 3- and 4-dimensional NMR experiments. The structure of a complex of GDIC with an isoprenylated peptide representing the C-terminus of Cdc42Hs will also be determined. These structures will explain the mechanism by which RhoGDI and its homologs extract GTPases from membranes, thus negatively regulation Rho-family signaling. The N-terminal domain of RhoGDI (GDIN) will be studied in complex with Cdc43Hs through a series of experiments designed to provide structural information at increasing levels of resolution. The GDIN-binding surface of the GTPase will be mapped, followed by structure determination of the bound regulator, and ultimately of the full complex. The relatively large size of this system (26kDa) will require extension of recently developed deuterium-aided NMR methods to analysis of multi-protein complexes. Structural and biochemical analyses of RhoGDI domains will explain the protein's ability to bind all Rho-GTPases, inhibit dissociation of nucleotides, and block the actions of other regulatory molecules. The complex of the WASP CRIB domain and Cdc42Hs will also be studied in a similar manner, utilizing novel deuterium-aided NMR methods. Comparison of structural data on RhoGDI and WASP will explain the different binding properties of these two ligands, including their requirements for GTPase isoprenylation, sensitivity to nucleotide state, and specificity for different Rho-family members. These studies will provide fundamental insights into the

mechanisms of signal transduction through Rho- GTPases, and will open new avenues for development of diagnostic and therapeutic agents that act on these pathways.

Thesaurus Terms:
Wiskott Aldrich syndrome, enzyme activity, enzyme induction /repression, guanosinetriphosphatase
protein biosynthesis
nuclear magnetic resonance spectroscopy

Institution:	SLOAN-KETTERING INSTITUTE FOR CANCER RES FOR CANCER RESEARCH NEW YORK, NY 10021
Fiscal Year:	1997
Department:	
Project Start:	01-AUG-1997
Project End:	31-JUL-2002
ICD:	NATIONAL INSTITUTE OF GENERAL MEDICAL SCIENCES
IRG:	BBCA