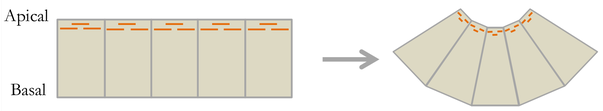
Structural Insights into the Shrm-Rock Interaction

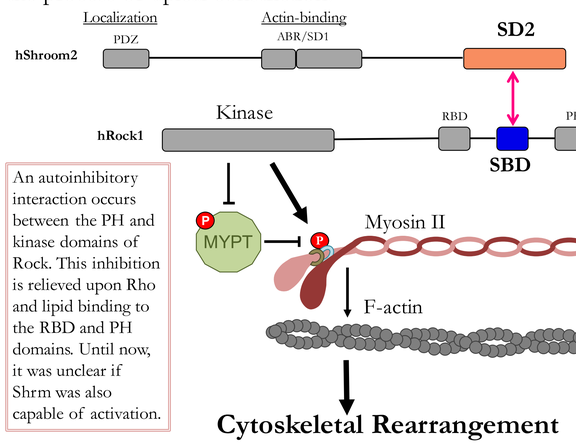
Jenna K. Zalewski[1], Joshua Mo[1], Simone Heber[1], Annie Héroux[2], Jeffrey D. Hildebrand[1], Andrew P. VanDemark[1]
[1]Department of Biological Sciences, The University of Pittsburgh [2]Department of Biology, Brookhaven National Laboratory

Introduction

Alterations in cell shape drive the drastic changes seen throughout morphogenesis. During neural tube development, polarized epithelial cells must constrict the apical surface to form the tube that becomes an organism's brain and spinal cord.

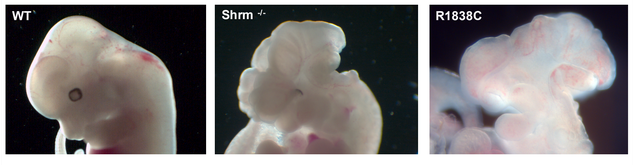


Actin and myosin form a cytoskeletal network that controls cell shape. Rho Kinase (Rock) is a key regulator of this network, and its interaction with the SD2 domain of the Shroom (Shrm) protein is required for proper development of the neural tube[1], eye[2], and gut[3] through the process of apical constriction.



An autoinhibitory interaction occurs between the PH and kinase domains of Rock. This inhibition is relieved upon Rho and lipid binding to the RBD and PH domains. Until now, it was unclear if Shrm was also capable of activation.

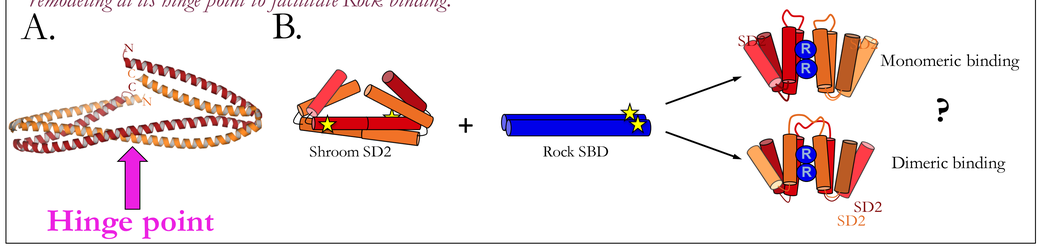
A point mutation within Shrm (R1838C) that disrupts Rock binding leads to developmental defects that phenocopy a Shrm-null mutant[4]. The interaction between these two domains is critical to proper development.



We want to determine the molecular basis for SD2-SBD complex formation to understand the role these domains play in development.

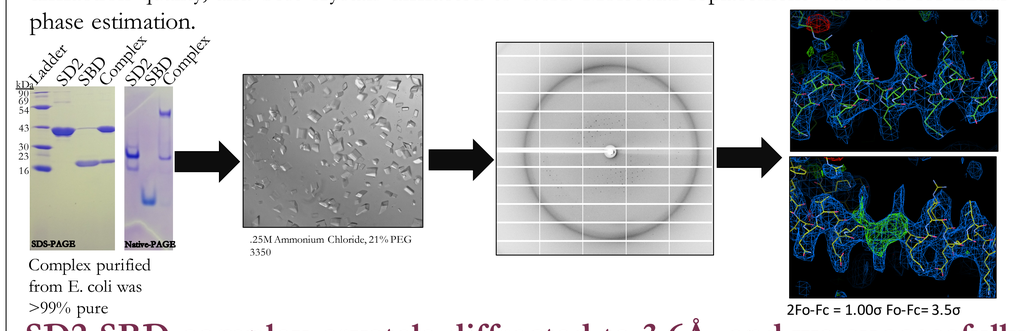
What is the Rock-binding conformation of Shrm?

Shrm's binding sites on Rock SBD exist in adjacent positions within a *parallel* coiled-coil[5]. Recent structural data from our lab has revealed that the Shrm SD2 domain adopts an *anti-parallel* coiled-coil composed of two identical half-dimers each containing a Rock-binding site[6]. It is unclear how this arrangement could allow both sites to contact Rock. *We hypothesize that SD2 must undergo a dramatic conformational change, bending or remodeling at its hinge point to facilitate Rock binding.*



Determining the structure of the SD2-SBD complex

SBD and SD2 were individually purified from *E. coli*, then mixed in a 2:1 SBD:SD2 molar ratio. The complex was then concentrated and used for crystallization reactions. Crystals were screened for diffraction quality, and best crystals diffracted to 3.6Å. Molecular replacement was used for initial phase estimation.

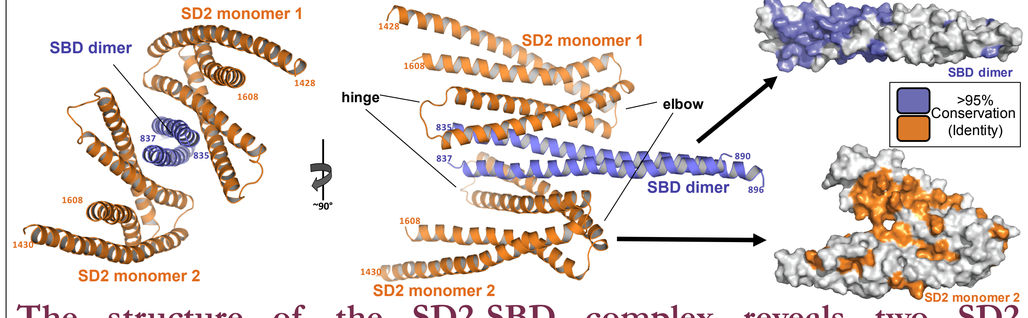


Complex purified from E. coli was >99% pure

SD2-SBD complex crystals diffracted to 3.6Å, and we successfully used molecular replacement to estimate phases.

Conserved interactions drive SD2-SBD binding

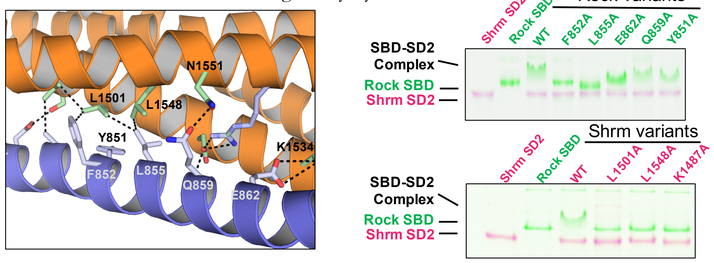
The structure of the SBD-SD2 complex reveals an arrangement in which two monomers of Shrm SD2 bind to opposing faces of the Rock SBD. conserved binding interface. The amino acids within the binding interface are highly conserved across species.



The structure of the SD2-SBD complex reveals two SD2 monomers bound to one SBD dimer via a conserved interface.

Biochemical analysis of the hSD2-hSBD interface revealed residues important for recognition

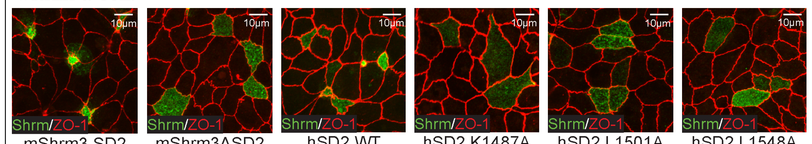
Our structure revealed an interface with many interacting amino acids. We wanted to pin-point the residues that were most important for SD2-SBD binding, so we designed point mutations in each domain and tested their binding ability by Native PAGE.



hRock1 SBD L855, E862, Y851, and F852 and hShrm2 SD2 L1501, L1548, and K1487 are all important for SD2-SBD binding *in vitro*.

SBD-SD2 interface is important for cell shape change

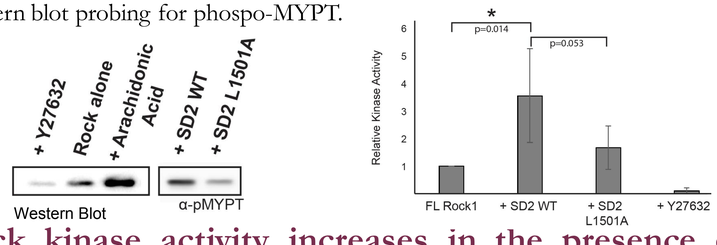
After biochemically identifying amino acids seen in our structure that contribute to binding, we wanted to determine the effect they have on cytoskeletal regulation. We utilized an apical constriction assay[7] to evaluate Shrm function within a cell.



The interface observed in our structure is important for cytoskeletal regulation and cell shape change.

Shrm SD2 can activate Rock kinase activity

Prior to this study, it was not known if Shrm proteins could directly activate the catalytic activity of Rock. I tested this by performing kinase assays with purified components. I used MYPT as a substrate, and evaluated kinase activity via western blot probing for phospo-MYPT.



Rock kinase activity increases in the presence of wild-type Shrm SD2 domain

References

[1]Hildebrand and Soraino. *Cell.* 99(1999):485-97.
[2]Lee et al. *Dev Dyn.* 238(2009):1480-1491.
[3]Chung, M. I. et al. *Dev.* 137(2010):1339-1349.
[4]Das, D. et al. *Biol Open.* 3.9(2014):850-860.
[5]Mohan, S., et al. *PlOS One.* 8.12(2013):e81075.
[6]Mohan, S., et al. *MBoC.* 23.11(2012):2131-42.
[7]Hildebrand. J Cell Sci. 118(2005):5191-203.
Conservation images made using ESPript 3.0 Structure figures made using PyMol
Molecular Replacement/Refinement performed in Phenix, models built in Coot

Acknowledgements

We would like to thank the Hildebrand lab for their collaboration and guidance, as well as the Berman lab for helpful discussions and encouragement. Thank-you to Annie Héroux for data collection. We would also like to express our gratitude to Tom Harper for printing this poster and taking pictures of our crystals in the cold room. This work was funded by NIH grant GM097204 and an Andrew Mellon Predoctoral Fellowship from the University of Pittsburgh.