Purification of the START domain of the DLC1 protein

Lauren Frank, Shayaan Rasheed, Alex Bosher, Zach Shaffer, Blaise Koehler, Aman Husbands[1], Renee Bouley[1]

Affiliations: [1]Department of Molecular Genetics and [2]Department of Chemistry and Biochemistry, The Ohio State University

THE OHIO STATE UNIVERSITY

INTRODUCTION

The Rho family of GTPases, which is a subgroup of the Ras family of proteins, plays an important role in the regulation of cytoskeletal functions, cell cycle progression, cell polarity, and several other additional pathways in the body. Rho GTPases cycle between GTP-bound activate states and GDP-bound inactive states, which is regulated by other proteins (**Figure 1**). Rho-GAPs (GTPase-activating protein), are regulatory proteins and catalyze the hydrolysis of GTP bound to a Rho-GTPase protein, thus inactivating it. The down regulation of a set of Rho-GAPs known as Deleted in Liver Cancer (DLC) proteins, which are divided into 3 closely related genes (DLC1, DLC2, and DLC3) are correlated with tumor development of several cancers.[1] Specifically, the down regulation of DLC1 is highly correlated with poor cancer prognosis and tumor development in patients.[1]

Within DLC1, there are several domains that are critical for its activity and regulation, with our specific focus being on understanding the START (StAR-related lipid-transfer) domain. It is our hypothesis that the START domain plays a role in regulating the Rho-GAP activity of DLC1. In cancer, the DLC-START domain is often riddled with a number of cancer-related mutations, which affect residues evolutionary-conserved residues and affect the protein's tertiary structure.[2] We aim to determine the 3D structure of DLC1-START and determine its role in regulating the activity of DLC1. We hope to be able to better understand how mutations in the START1 domain contribute to the role DLC1 plays in several cancers.

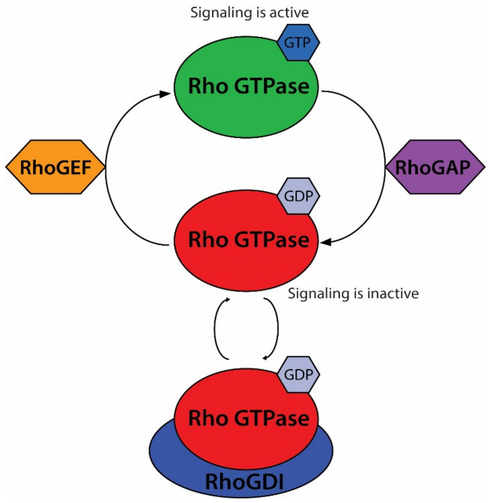

Figure 1. The Rho GTPase signaling module. Rho GTPases (red and green) are active while bound to GTP. RhoGAPs (purple) stimulate hydrolysis of bound GTP, stitching the RhoGAP to an inactive GDP-bound state. RhoGDIs (blue) bind to GDP-bound Rho GTPases and sequester them in the cytosol. Finally, RhoGEFs (orange) catalyze the exchange of bound GDP for GTP, activating Rho GTPase signaling.

REFERENCES & FUNDING

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1) Wang D, *et al*. (2016) *Oncotarget* **7**: 45144-45157
2) Holub AS, Bouley RA, Petreaca RC, Husbands AY. (2020) *Int J Mol Sci* **21**: 8175.

METHODS & RESEARCH DESIGN

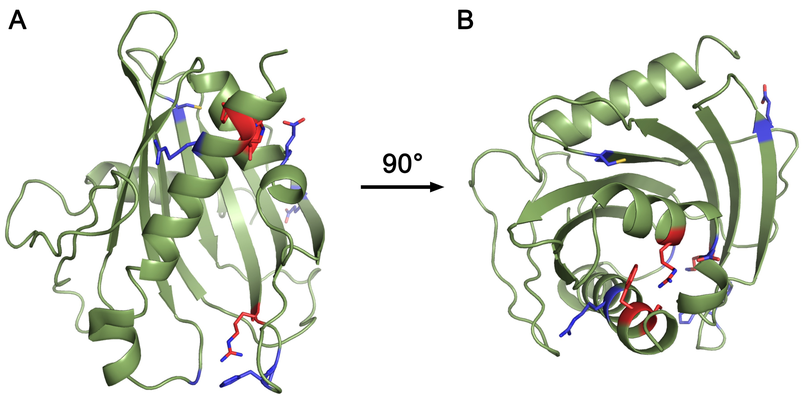


Figure 2. Homology model of DLC1-START domain. (A) Side and (B) top-down views of DLC1 START domain. Blue indicates conserved residues with missense mutations in cancers. Red indicates conserved arginine, serine, and phenylalanine residues mutated in DLC1, as well as highly conserved Arg988 mutated in cancer.[2]

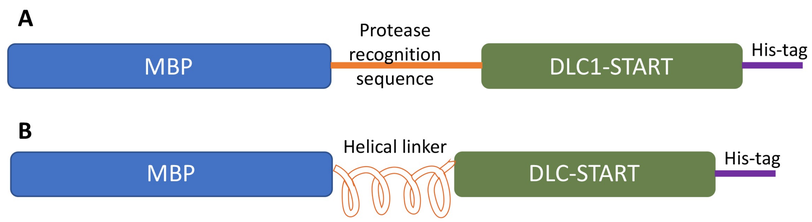
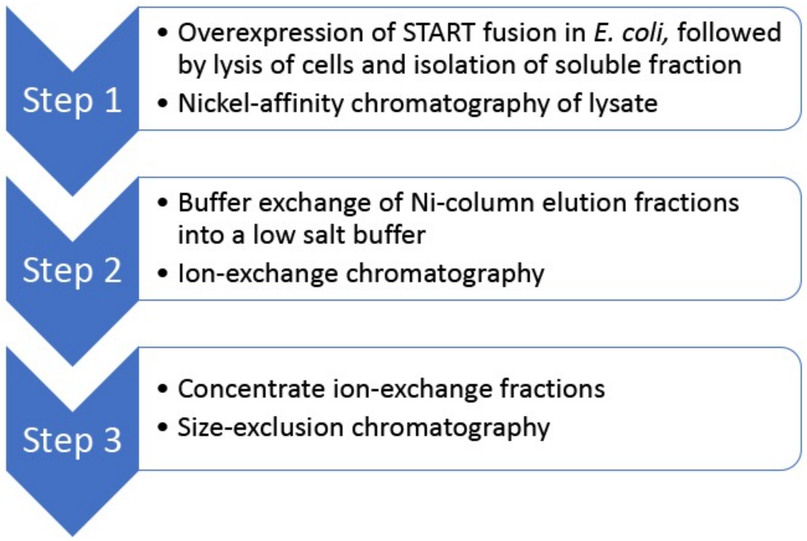


Figure 3. Design of MBP-START fusion protein constructs. Maltose-binding protein (MBP) was fused to DLC1-START to facilitate expression and solubility. Two constructs were designed; (A) a linker consisting of 5 glycines, a TEV protease recognition sequence, and another 5 glycines, and (B) a 7-amino acid rigid helical linker. The helical linker construct was expressed and purified for crystallography.

Purification Protocol.



RESULTS

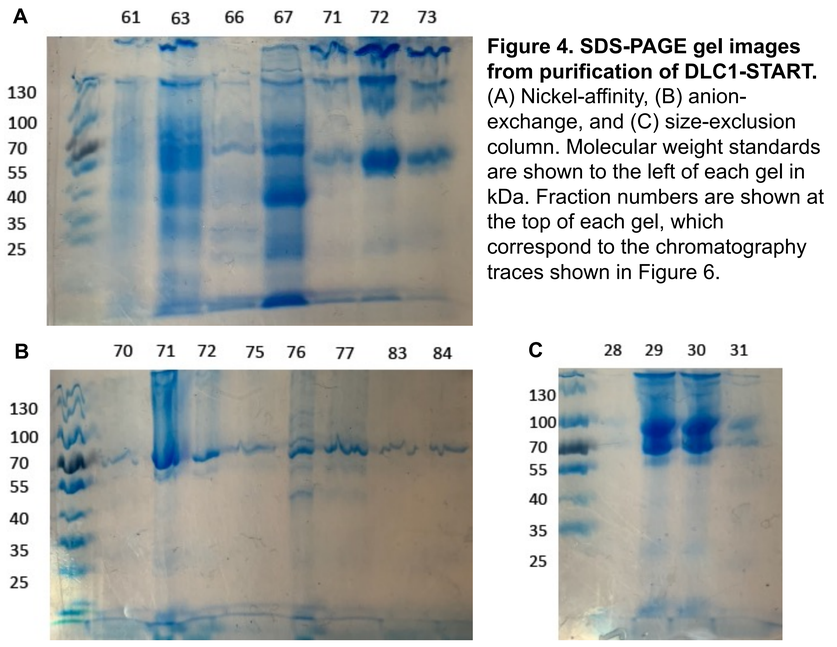


Figure 4. SDS-PAGE gel images from purification of DLC1-START. (A) Nickel-affinity, (B) anion-exchange, and (C) size-exclusion column. Molecular weight standards are shown to the left of each gel in kDa. Fraction numbers are shown at the top of each gel, which correspond to the chromatography traces shown in Figure 6.

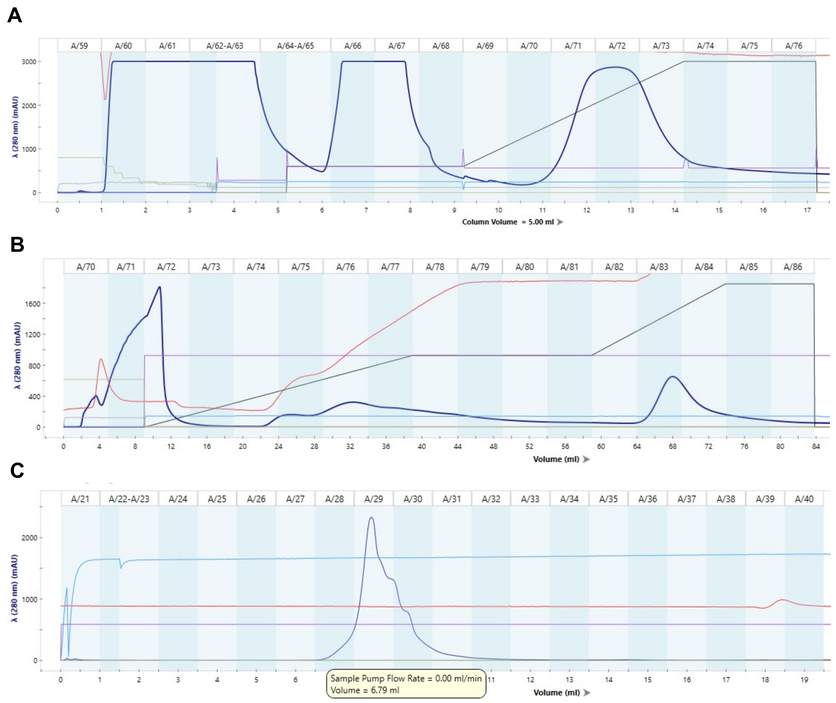


Figure 5. Chromatography traces for the purification of DLC1-START. (A) Nickel-affinity, (B) anion-exchange, and (C) size-exclusion column. The dark blue line shows the absorbance at a wavelength of 280 nm. The red trace shows the conductivity of the sample. Samples from fractions that showed absorbance were ran on SDS-PAGE, shown in Figure 4.