Structural and Mechanistic Studies of Two Regulatory Factors in Actin Cytoskeletal Signaling: Vav and VopL

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Proper control of actin cytoskeletal dynamics is essential for cell survival. The goals of my thesis work have been to characterize the structural and biophysical properties of two regulatory proteins in actin cytoskeletal rearrangement pathway: Vav and Vibrio outer protein L (VopL).

Vav proteins are guanine nucleotide exchange factors for Rho family GTPases. They play key roles in actin regulatory pathways and control diverse cellular processes like T cell maturation and activation, cell migration and phagocytosis. They belong to a group of multi-domain signaling proteins which display complex behaviors because of the collective regulation from multiple domains. Previous work has shown that Vav is autoinhibited in the resting state through the cooperative suppression of N-terminal Calponin domain and Acidic region, with the physical mechanism yet to be determined. Here through structural, energetic and biochemical studies, I demonstrate that the Calponin homology domain of Vav binds to the Pleckstrin homology domain, restrains the inhibitory helix in the Acidic region, and shifts the Dbl homology domain - inhibitory helix equilibrium to a more closed state. This construction enables strong suppression and an efficient activation process. The energetic basis of Vav autoinhibition may turn out to be widespread in multi-domain systems.

VopL, a pathogenic effector from Vibrio parahaemalyticus, is an actin nucleation factor that induces stress fibers during bacterial infection. It contains three N-terminal Wiskott-Aldrich Homology 2 (WH2) motifs and a unique VopL C-terminal domain (VCD). It potently promotes actin filament nucleation in vitro. However, the physical basis of VopL mediated nucleation has not been understood. Here I performed structural and biochemical studies to investigate the mechanism of actin filament nucleation by VopL. I found that both the WH2 element and VCD are required for VopL activity. The crystal structure of VCD revealed a U-shaped dimer that is stabilized by a terminal coiled-coil. Dimerization of the WH2 motifs as well as contacts between VCD and actin contribute to the nucleation activity of VopL. My studies suggest the formation of a structurally organized actin cluster involving lateral contacts during nucleation. Stabilization of these lateral contacts may be a common feature of actin filament nucleation by WH2-based factors.