

Structural study of the RING domain of ARKADIA-2 and its Tryptophan mutants via NMR spectroscopy

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The ubiquitin-proteasome system is an essential tool for the regulation of protein levels in the cellular environment. Ubiquitination occurs through the concerted action of an ubiquitin-activating enzyme E1, an E2 ubiquitin conjugating enzyme and an E3 ubiquitin ligase. The E3 enzymes are the key components of the process as they are responsible for substrate recognition and poly-ubiquitination. ARKADIA and ARKADIA-2 proteins act as E3 Ubiquitin ligases via their C-terminal RING domains. ARKADIA is a positive regulator of the Transforming Growth Factor- β pathway whilst ARKADIA-2 is implicated in the Bone Morphogenetic Pathway [1,2].

The RING (Really Interesting New Gene) is a small domain which binds two zinc ions [Zn(II)] in a unique 'cross-brace' arrangement through its conserved cysteine and histidine residues: Cys-X2-Cys-X9-39-Cys-X1-3-His-X2-3-Cys/His-X2-Cys-X4-48-Cys-X2-Cys, where X can be any amino acid residue. Among all the types of E3 Ubiquitin ligases (HECT, U-box, RING) the RING family is the largest one [3].

Structural analysis of ARKADIA RING domain elucidated its enzymatic properties. Titration studies of ARKADIA protein with the UbcH5B E2 enzyme suggested that Tryptophan (Trp) is essential in the ubiquitin ligase enzymatic activity, consistent with the E2 recruitment [4]. Consecutively, protein mutations bearing Trp to Ala and Arg were studied, demonstrating significant differentiation in the protein activity. Subsequently, the wild type of ARKADIA-2 RING domain, as well as the same mutants of Trp, were studied in order to identify similar functionality.

References

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