

Structural and Biochemical Characterization of TRAF2, a TNF-Receptor-Associated Factor Protein

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Zinc is found in a number of structural roles, such as in the folding of zinc fingers, and in catalytic roles, such as in metalloproteinases. In the former, a set of cysteine and histidine residues directly ligate the Zn^{2+} and as a result cause drastic conformational changes in the protein. In the Tumor Necrosis Factor α (TNF α) signaling pathway, adaptor proteins recruited to the TNF receptor upon stimulation partly rely on a series of properly folded TRAF-like zinc fingers to interact with and activate downstream components. These TRAF-like zinc fingers consist of the sequence Cys-X₂₋₆-Cys-X₁₁₋₁₂-His-X₃₋₄-Cys/His and are present in the TNF-Receptor-Associated Factor (TRAF) family of proteins. One specific member under study, TRAF2, contains five TRAF-like zinc fingers spaced by six amino acids between domains and also has an additional motif known as a Really-Interesting-New-Gene (RING) finger which binds two atoms of zinc in a crisscrossing manner. This RING finger portion at the N-terminus of TRAF2 is believed to have ubiquitin-ligase function while the highly conserved C-terminal domain is involved in self-trimerization and in interaction with the receptor. From homology modeling based on the known structures of other zinc-domain-containing proteins RAG1 and Siah2 as well as the structures of some TRAF protein fragments, we propose a model for the TRAF2 protein which incorporates two zinc atoms from the RING finger domain, plus five more zincs from each of the TRAF-like zinc fingers. The sequentially linked TRAF-like zinc finger domains are proposed to be disposed in a relatively rigid head-to-tail arrangement stabilized by peptide NH to cysteinate hydrogen bonds involving the terminal cysteine of each domain as well as by finger domain-finger domain interactions. The modeling suggests an elongated structure consistent with the structures of other ubiquitin ligases. In addition, biochemical characterization of this protein is under way to understand the properties of zinc binding and its implications in binding other partners in the TNF α pathway.