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Structure of collagen-helix motif

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The triple helix is a specialized protein motif found in all collagens. Although X-ray diffraction studies of collagen began in the 1920s, the small amount of data available from fiber diffraction of collagen made the molecular structure determination difficult. In the early 1950s, two plausible fiber periods of about 20 and 30 Å were proposed, together with corresponding single-strand models having 7/2- and 10/3-helical symmetry, respectively. The first framework of the triple helix was proposed by Ramachandran and Kartha in 1955. Rich and Crick proposed another structure with the same framework to avoid some of the steric problems of the first model. Their framework, which involved a triple-helical structure with a period of 28.6 Å and 10/3-helical symmetry, was the same as one of two single-strand models proposed at that time, except for the number of strands. On the other hand, Okuyama et al. detected the triple-strand model with the other framework, with a fiber period of 20 Å and 7/2-helical symmetry, in the single crystal of $(Pro\text{-}Pro\text{-}Gly)_{10}$ in 1972. Although they proposed this structure as a new structural model for collagen, it has not been acknowledged as such, but instead has been regarded only as a model for a collagen-like peptide. Recently, we showed that both 7/2- and 10/3-helical models could explain X-ray diffraction data from native collagen quantitatively. Furthermore, the helical symmetries of collagen-model peptides analyzed at high resolution are very close to the ideal 7/2-helical symmetry, whereas no supporting data was found for the 10/3-helical model. This evidence strongly suggests that an average molecular structure of native collagen is the 7/2-helical model rather than the prevailing Rich and Crick (10/3-helical) model.

Keywords: collagen structure, fibre diffraction, single crystal structure analysis

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The structure of *Epiphyas postvittana* Takeout 1 suggests a ligand-carrying role for Takeout proteins

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Members of the Takeout / Juvenile Hormone Binding Protein (To/JHBP) superfamily are small proteins (~240 amino acids) found exclusively in insects, being present in all insect orders. JHBP bind juvenile hormones involved in the maintenance of the larval state and sexual development in adults. In contrast, very little information is available for the more diverse To proteins. Expression profiles and regulation patterns of some To proteins indicate that these proteins are ubiquitous, but their function(s) remain largely unknown. To and JHBP have similar predicted secondary structures, possess a conserved N-terminal disulfide bond and are likely to share a common fold. JHBP, however, have a second disulfide bond that is not present in To, while To contain two conserved C-terminal motifs that are not found in JHBP, suggesting distinctive roles for To and JHBP. Here we present the first crystal structure of a Takeout protein, To1 from the lightbrown apple moth *Epiphyas postvittana*, solved by in-house sulfur-SAD phasing and refined to 1.3 Å resolution. To1 adopts the unusual α/β fold recently described for JHBP, but major structural differences are observed i) for the takeout motifs and ii) for the internal cavity. To1 highlights a very large, purely hydrophobic, cavity buried inside the protein, in which a surrogate ubiquinone moiety from *Escherichia coli* is bound. This provides insights into the binding mode and chemical structure of To1 endogenous ligand(s) and suggests guidelines for the docking of potential ligands inside the cavity. Our structure represents the first experimental evidence that To proteins act as ligand carriers and confirms that the unusual α/β fold is shared by all members of the To/JHBP family.

Keywords: takeout, juvenile hormone binding protein, crystal Structure

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Structural basis for the RPEL motif interaction with G-actin

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Actin polymerisation plays a critical role in many important cellular processes including: cell motility, intracellular transport, control of cell shape and polarity. Monomeric forms of actin also have functional roles. For example, levels of G-actin regulate nucleocytoplasmic shuttling of the transcription factor MAL, a co-factor for SRF (serum-response factor). The actin-sensing mechanism resides within three consecutive RPEL motifs of MAL that together comprise a functional and regulated G-actin binding site. In this poster I report the molecular basis for RPEL motif interaction with G-actin by determining structures of two independent RPEL peptide:G-actin complexes at 1.45 Å and 2.3 Å resolution. The RPEL:G-actin structures explain concisely the sequence conservation defining the motif with the invariant RPEL arginine. Using a fluorescent polarisation assay we quantify the different affinities of individual MAL RPEL motifs and selected mutants that validate the structural data. Affinity differences may reflect different functional roles of the RPEL peptides in MAL regulation.

Keywords: G-actin, RPEL, MAL

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Structural basis for regulatory interplays between EB1, CLIP-170 and p150Glued

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Microtubule is essential for many cellular processes. Recent live-cell imaging studies have identified proteins that associate specifically with the growing-ends of microtubules, which are termed microtubule plus-end tracking proteins (+TIPs). +TIPs