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THE ASSEMBLY AND FUNCTION OF PWI DOMAIN CONTAINING COMPLEXES AND POXVIRUS PROTEINS

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This study describes the structural and functional studies of the proline-tryptophan-isoleucine (PWI) domain, which is found in several RNA processing factors and several proteins involved in tanapoxvirus replication. The PWI domain investigated is from RBM25 protein, which is incorrectly expressed in cancers and during heart failure. The PWI domain plays an important role in RBM25 function, but functional mechanism and contribution to protein functionality are unclear. Elucidating the mechanism of PWI domain function will contribute to our understanding of the molecular mechanisms of several diseases and may enable new therapeutic strategies. The tanapoxvirus is a poxvirus that is related to the medially important vaccinia, variola and Monkeypox viruses. The variola virus is the causative agent of smallpox that is a potential bioterrorism threat. Structural and functional insights into the proteins and biomolecular complexes that regulate the lifecycle of the virus will aid in the development of therapeutic agents against poxviruses and the development of poxviruses as therapeutic tools against cancer. The main goals of this study were to assess the structure and function of the RBM25 PWI domain and tanapoxvirus proteins using biochemical and biophysical methods. Nuclear magnetic resonance (NMR) spectroscopy was performed on several samples to assess their structural and conformational dynamic properties. Site directed mutagenesis was used to identify functional surfaces on the PWI domain, while circular dichroism spectroscopy, size exclusion chromatography and electrophoretic mobility shift assays were used to determine the roles of these surfaces in molecular function. The results reveal that the PWI domain of RBM25 has a complicated nucleic acid binding mechanism that involves both protein-protein and protein-nucleic acid interactions, and that tanapoxvirus proteins are generally insoluble when expressed alone. While biophysical studies of insoluble proteins are difficult, the samples generated are ideal for generating antibodies for in vivo functional studies.

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