

DWNN, a novel ubiquitin-like domain, is found at the N-terminus of the RBBP6 family of putative E3 ubiquitin ligases

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RBBP6 is a 250 kDa nuclear-associated protein that has been shown to interact with both p53 and pRb in human and mouse and to suppress the binding of p53 to DNA [1-4]. Homologues from all species contain a conserved N-terminal region of approximately 80 residues, which we have named the DWNN domain, followed by a CCHC zinc knuckle and a C3HC4-type RING finger domain. In higher organisms the protein contains additional p53 and pRb-interacting domains.

In order to shed light on the function of this protein, we have expressed a number of different fragments from the full-length human protein in bacteria to investigate their suitability for structure determination using heteronuclear NMR. Compact domains were identified on the basis of resistance to proteolysis and the degree of folding was assessed using ¹⁵N-HSQC spectra. We have determined the structure of the N-terminal DWNN domain and shown that it is a novel ubiquitin-like domain, despite the absence of any significant sequence homology. This appears to be highly significant, given the presence of the RING domain within the protein. To investigate the putative ubiquitin-ligase activity of the protein, we have recombinantly expressed an 11 kDa fragment containing the RING finger, and are currently in the process of determining its structure. Both the pRb-binding domain and the p53-binding domains of the protein have been solubly expressed and *in-vitro* interaction studies with pRb and p53 respectively are currently underway.

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