Recombinant expression, full backbone assignment and structure determination of the human DWNN domain using heteronuclear NMR

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The cellular levels of a number of proteins have been found to be regulated by the ubiquitin-proteasome pathway. In this pathway, proteins are covalently tagged by ubiquitin, which acts as a signal for degradation by the proteasome. A number of key cellular processes, including cell-cycle progression, transcription and DNA repair, are regulated in this way. In recent years a number of cellular proteins resembling ubiquitin in structure or function, the so-called ubiquitin-like proteins, have been identified. We have identified a novel ubiquitin-like domain, the DWNN domain, which occurs at the N-terminus of RBBP6, a protein that has been shown to interact with p53 and Rb. RBBP6/DWNN was shown to be involved in mRNA processing and apoptosis.

We have over-expressed the human DWNN domain in E.coli using the GST fusion protein system. The domain was labelled with 15N and 13C to perform triple-resonance heteronuclear NMR experiments, from which full backbone assignments were obtained. DWNN domain backbone resonances were assigned using 15N-HSQC, 3D-CBCA(CO)NH and CBCANH spectra. The backbone assignments, together with NOESY data were used for the subsequent structure determination, which confirmed that the DWNN domain is a novel ubiquitin-like domain. The RBBP6 protein may therefore represent a novel E3 ubiquitin ligase that plays a role in regulating the cellular levels of p53 and Rb, and this suggests mechanisms for the role of DWNN in apoptosis.