Structure
Article

The Prp19 WD40 Domain Contains a Conserved Protein Interaction Region Essential for Its Function

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SUMMARY

Prp19 is a member of the WD40 repeat family of E3 ubiquitin ligases and a conserved eukaryotic RNA splicing factor essential for activation and stabilization of the spliceosome. To understand the role of the WD40 repeat domain of Prp19 we have determined its structure using X-ray crystallography. The domain has a distorted seven bladed WD40 architecture with significant asymmetry due to irregular packing of blades one and seven into the core of the WD40 domain. Structure-based mutagenesis identified a highly conserved surface centered around blade five that is required for the physical interaction between Prp19 and Cwc2, another essential splicing factor. This region is found to be required for Prp19 function and yeast viability. Experiments in vitro and in vivo demonstrate that two molecules of Cwc2 bind to the Prp19 tetramer. These coupled structural and functional studies provide a model for the functional architecture of Prp19.

INTRODUCTION

Prp19, an essential RNA splicing factor, is a core component of the spliceosomal NinTeen-associated complex (NTC), a subcomplex required for the activation of the spliceosome (Chan et al., 2003; Makarova et al., 2004). Prp19 plays a direct role in the activation and structural stabilization of the spliceosome (Tarn et al., 1994, 1993; Chan et al., 2003; Chan and Cheng, 2005). Temperature-sensitive mutants of Prp19 lead to destabilization of both the NTC and spliceosome as a whole (Ohi and Gould, 2002). Prp19 is a member of the U-box family of E3 ubiquitin ligases. Accumulating evidence supports the suggestion that Prp19 may regulate spliceosome activity by directly targeting proteins for ubiquitination and progress has been made in identifying putative Prp19 substrates and E3 ubiquitin ligase adaptors (Maeder and Guthrie, 2008; Ohi et al., 2005; Vander Kooi et al., 2006).

Prp19 contains three recognized domains: an N-terminal U-box domain, a central coiled-coil domain, and a C-terminal WD40 (40 residue Trp-Asp containing) β propeller repeat domain. Prp19 uses its N-terminal U-box domain to recruit Ubc3 and possibly other E2 conjugating enzymes (Aravind and Koonin, 2000; Hatakeyama and Nakayama, 2003; Ohi et al., 2003). The central coiled-coil domain of Prp19 mediates tetramerization of the protein, which is important for function in the context of the spliceosome (Ohi et al., 2005). The C-terminal WD40 repeat domain is the least understood both physically and functionally.

In fact, the role of the WD40 repeat domain and whether it is essential for Prp19 function is unknown. E3 ubiquitin ligases function in concert with E1-activating and E2-conjugating enzymes to covalently attach ubiquitin molecules to substrates. E3 ligases often possess a multidomain architecture and are distinguished by the different domain modules for recruiting E2 enzymes and substrates. E3 ligase proteins are distinguished on the basis of how they interact with E2 enzymes and have been grouped into three families based on the domain responsible for recruiting the E2 ligase: Really Interesting New Gene (RING)-finger (Freemont, 2000), U-box (Patterson, 2002), and Homologous to E6-AP Carboxyl Terminus (HECT) (Pickart, 2001). The substrate binding modules typically consist of well-known protein-protein interaction domains, such as WD40 repeat β propeller, leucine-rich repeat, or tetratric peptide repeat.

The diversity of substrate binding domains used by E3 ligases provides the critical element of substrate specificity. The structural differences in substrate binding domains are functionally significant because E3 ligases differ dramatically in the type and number of substrates they recognize, with certain ligases tuned to bind only one specific partner while others recognize many different substrates. WD40 domains are thought to not only directly interact with targets but may also position them in a catalytically competent orientation. For example, the structure

Table 1. Crystallographic Data and Refinement Statistics

	Native	SeMet		
		Edge	Peak	Remote
Beamline	PDX	PDX		
Space group	$P4_22_12$	$P4_22_12$		
Wavelength	1.381	0.9797	0.9793	0.9465
Unit-cell parameters				
a (Å)	83.110	83.426		
b (Å)	83.110	83.426		
c (Å)	126.641	126.307		
Unique reflections	14,158	14,092	14,059	14,063
Completeness (%)	99.2 (99.6)	99.9 (100.0)	99.8 (100.0)	99.8 (100.0)
Resolution (Å)	2.6 (2.60–2.69)	3.2 (3.20–3.31)	3.2 (3.20–3.31)	3.2 (3.20–3.31)
R_{merge} (%)	8.0 (44.5)	12.2 (43.6)	8.4 (27.1)	8.7 (29.0)
Redundancy	6.3 (5.5)	5.5 (5.0)	5.5 (5.3)	5.6 (5.3)
I/σ (I)	16 (3.5)	12.1 (3.6)	18.9 (6.8)	21.0 (7.3)
FOM (after density modification)		0.48 (0.62)		
Resolution limits (Å)	25.0–2.60			
Number of reflections used in refinement	13,408			
Number of reflections used to compute R_{free}	712			
R (R_{free})	21.9 (26.9)			
# solvent molecules	66			
# sulfate molecules	3			
Ramachandran				
Most favored	91.9			
Additionally allowed	8.1			
Generously	0.0			
Disallowed	0.0			
Rmsd				
Bond (Å)	0.011			
Angle (°)	1.397			

of the β-transducin repeat-containing protein (βTrcP) 1 SCF complex suggests that catalytic activity requires precise positioning of the E2 and substrate that is provided by orienting the E2 recruiting RING finger and the substrate recruiting WD40 repeat domains (Wu et al., 2003). We have previously proposed a mechanism for the activity of Prp19 that involves similar positioning of E2 and targeting by the U-box and WD40 domains from two antiparallel protomers in the tetramer (Vander Kooi et al., 2006).

Investigations into the function of Prp19 in the spliceosome have included the discovery of protein binding partners. Four such proteins have been shown to directly bind to the different domains of *S. cerevisiae* Prp19: the E2-conjugating enzyme Ubc3 to the U-box domain, the NTC components Cef1 and Snt309 to the coiled-coil domain, and Cwc2 to the WD40 repeat domain (Hatakeyama and Nakayama, 2003; Ohi and Gould, 2002; Ohi et al., 2005). A direct interaction with the WD40 domain has been shown for the essential splicing factor Cwc2 (Cwf2 in *S. pombe*) (Ohi and Gould, 2002), but the nature and significance of this interaction has not been investigated. Since Prp19 exists as a tetramer, it is particularly important to determine the relative stoichiometry of its binding partners because this has implications for its function and the overall organization and stability of the NTC.

The studies reported here use a coupled structural and functional approach to understand the WD40 domain of Prp19. An X-ray crystal structure shows that the domain adopts an asymmetric seven bladed fold. Conserved surface residues are identified and shown to be essential for protein interactions and function. These findings, along with determination of the stoichiometry of Prp19 and Cwc2, provide a model for the architecture of the Prp19-associated spliceosomal subcomplex.

RESULTS

Structure of the WD40 Repeat Domain of Prp19

The WD40 repeat domain of Prp19, encompassing residues 165–503, was subcloned, expressed, purified, and crystallized. The protein crystallized in a tetragonal unit cell and diffracted to 2.6 Å. Selenomethionine-labeled crystals were also produced, which diffracted to 3.2 Å. Phases were derived from selenium MAD experiments and the structure was fully refined to 2.6 Å (Table 1).

While previous sequence analysis identified varying numbers of WD40 repeats in the C terminus of Prp19, the X-ray crystal