Sqt1p is an eight-bladed WD40 protein

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Ribosome biogenesis in eukaryotes is a complex and highly orchestrated process involving more than 200 accessory factors in addition to ribosomal RNAs and ribosomal proteins. Among the many factors involved, Sqt1p has been reported to specifically bind to uL16 and to act as a chaperone. The crystal structure of full-length Sqt1p from the yeast *Saccharomyces cerevisiae* has been solved at 3.35 Å resolution. A SAD experiment at the Se K edge and an S-SAD experiment on the same selenomethionine-substituted protein crystal allowed unambiguous positioning of the selenomethionine and Cys residues. On the basis of the atomic structure of Sqt1p, the potential residues involved in uL16 interaction were identified and tested.

1. Introduction

Ribosomes are complex nanomachines that catalyse protein synthesis by decoding messenger RNAs (mRNAs). They are composed of two subunits, the large subunit (LSU) and the small subunit (SSU), each being an aggregation of ribosomal RNAs (rRNAs) and ribosomal proteins (r-proteins). In yeast, the SSU comprises the 18S rRNA and 33 r-proteins, while the LSU comprises the 25S rRNA, 5.8S rRNA, 5S rRNA and 46 r-proteins. The functions of the SSU are to decode mRNAs into proteins, bringing together mRNAs and the cognate aminoacyl-transfer RNAs. The LSU hosts the catalytic centre that allows peptide-bond formation and consequently protein synthesis itself. Ribosome formation lies upstream of protein synthesis. Ribosome biogenesis is a complex mechanism starting in the nucleolus with the transcription of a long pre-ribosomal RNA (pre-rRNA) by RNA polymerase I. This long precursor contains the non-mature forms of the 18S rRNA, 28S rRNA and 5.8S rRNA, which have to be further processed by numerous intertwined maturation events including methylations, nucleotide pseudouridylations, endonucleolytic and exonucleolytic cleavage and export through nuclear pore complexes (de la Cruz *et al.*, 2015; Henras *et al.*, 2015). The 5S rRNA is transcribed and processed independently. Among the r-proteins, more than 150 accessory factors are involved in ribosome biogenesis. Some of these 150 trans-acting factors possess defined enzymatic domains such as Ser/Thr kinases, GTPases and RNA helicases, and are probably involved in many of the checkpoint controls (Bassler *et al.*, 2001; Dragon *et al.*, 2002; Harnpicharnchai *et al.*, 2001; Milkereit *et al.*, 2003; Nissan *et al.*, 2002; Schäfer *et al.*, 2003). Many others have well predicted WD-repeat motifs with as yet poorly characterized functions. This holds true for Sqt1p, which has been reported to be a eight-bladed β -propeller structure (Pausch *et al.*, 2015). Sqt1p binds the N-terminal residues of uL16 and thus prevents

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Table 1
Crystallographic data and refinement statistics.

Values in parentheses are for the highest resolution shell.

	Se-SAD	S-SAD
Beamline	PROXIMA1	PROXIMA1
Space group	<i>P</i> ₃ ₂ ₁	<i>P</i> ₃ ₂ ₁
Unit-cell parameters (Å, °)	<i>a</i> = <i>b</i> = 166.04, <i>c</i> = 95.32, α = β = 90.0, γ = 120.0	<i>a</i> = <i>b</i> = 166.04, <i>c</i> = 95.32, α = β = 90.0, γ = 120.0
Wavelength (Å)	0.97883	1.907
Resolution range (Å)	47.90–3.35 (3.62–3.35)	33.80–3.65 (4.00–3.65)
Total reflections	207460 (43611)	200202 (48004)
Unique reflections	22101 (1268)	17121 (4037)
<i>R</i> _{p.i.m.} (%)	4.8 (29.1)	5.4 (46.7)
$\langle I/\sigma(I) \rangle$	9.1 (2.0)	11.2 (2.3)
Completeness (%)	99.9 (100.0)	100.0 (100.0)
Multiplicity	9.4 (9.7)	11.7 (11.5)
Wilson plot <i>B</i> factor (Å ²)	98.7	120
Mosaicity (°)	0.76	0.74
CC _{1/2}	0.924 (0.896)	0.895 (0.828)
Se sites	11	
FOM, acentric (before solvent flattening)	0.345	
Refinement statistics		
<i>R</i> _{free} (%)	22.21	
<i>R</i> _{work} (%)	17.13	
No. of non-H atoms	5879	
No. of protein residues	389	
No. of waters	0	
Average <i>B</i> factor (Å ²)	157	
Ramachandran statistics (%)		
Preferred regions	92.2	
Allowed regions	6.7	
Outliers	1.1	
R.m.s.d., bond lengths (Å)	0.015	
R.m.s.d., bond angles (°)	1.42	
PDB code	5ams	

the otherwise unstable uL16 from unfolding. This qualifies Sqt1p as a ribosomal protein chaperone. Sqt1p is not a shuttling protein, which implies that the association of uL16 with the pre-ribosomal particle takes place in the cytoplasm (West *et al.*, 2005). This chaperone activity is common to other specific accessory factors such Yar1, which binds uS3 (Koch *et al.*, 2012), and Rrb1, which interacts with uL3 (Iouk *et al.*, 2001; Schaper *et al.*, 2001).

Here, we have investigated the structure of Sqt1p and its interaction with uL16. We have solved the crystal structure of Sqt1p to a resolution of 3.35 Å using a combination of SAD data collected from selenomethionine-substituted crystals at the Se *K* edge and at 1.9 Å wavelength for sulfur phasing (S-SAD). We have investigated the minimal domain of interaction with uL16 and have identified conserved residues on the surface of Sqt1p that may be involved in this interaction.

2. Experimental procedures

2.1. Constructs

The full-length open reading frame encoding Sqt1p was amplified from *Saccharomyces cerevisiae* DNA and subcloned into the NdeI and BamHI sites of a pET (Novagen) plasmid (Romier *et al.*, 2006) to produce an N-terminal His-tag fusion

protein containing a TEV cleavage site with antibiotic selection owing to an ampicillin-resistance marker.

2.2. Protein expression and purification

Full-length Sqt1p was overexpressed in *Escherichia coli* BL21(DE3) Gold cells following a screen of strains for optimal protein expression. The cultures were grown at 37°C in 2×YT medium supplemented with 100 mg l⁻¹ ampicillin and 34 mg l⁻¹ chloramphenicol. The cells were induced at 15°C with 0.5 mM IPTG for 14 h, collected by centrifugation at 4500g and resuspended in buffer consisting of 50 mM Tris pH 8.0, 150 mM NaCl, 1.0 mM DTT. Cell pellets were lysed with an EmulsiFlex-C3 (Avestin) and centrifuged at 50 000g. The clarified cell lysate was combined with His-Select Co²⁺ resin (Sigma) for 2 h at 4°C. The resin with bound proteins was washed with five column volumes of resuspension buffer and the proteins were eluted using a 10–250 mM imidazole gradient. After cleavage with TEV [1:100(*w:w*)] overnight at 4°C, the protein sample was further purified using a HiTrap Q Sepharose column (GE Healthcare) and a size-exclusion chromatography step on a Superdex 200 HR10/30 column (GE Healthcare) equilibrated in 25 mM Tris pH 7.5, 150 mM NaCl.

2.3. Co-precipitation experiments

E. coli BL21(DE3) cells were co-transformed with pET-15b-derived plasmids allowing the expression of His-tagged protein along with pET-28b-derived plasmids for the untagged

Figure 1
Overall structure of Sqt1p. The different blades of the eight-bladed β -propeller protein Sqt1p are coloured and labelled. Contiguous residues in the primary sequence contribute to the formation of each blade, with the exception of blades 1 and 7, which are formed by residues 53–68/395–431 and 24–34/306–346, respectively. This and all subsequent structural figures were generated with PyMOL (DeLano, 2002).

constructs. Cells grown at 37°C were induced with 1 mM ITPG. After overnight incubation at 15°C, the cells were harvested by centrifugation and resuspended in a buffer consisting of 1.5× PBS, 1 mM magnesium acetate, 0.1% NP-40, 20 mM imidazole, 10% glycerol. The cell pellet was sonicated for 5 s at 7 W. A fraction of the crude extract was saved at this stage and boiled in Laemmli buffer; the remainder was centrifuged and the supernatant was incubated with cobalt-affinity resin for 30 min at 4°C. The beads were washed three times with 1 ml lysis buffer. About 5% of the crude extract and 15% of the bound fraction were analyzed by 15% SDS-PAGE. Proteins were revealed by Coomassie Blue staining.

2.4. Crystallization and structure determination

Sqt1p was crystallized at 20°C by the sitting-drop method using a reservoir consisting of 100 mM Tris-HCl pH 8.5, 1.8 M ammonium sulfate and protein at 10 mg ml⁻¹ concentration.

Crystals were transferred to a cryoprotectant consisting of 100 mM Tris-HCl pH 8.5, 1.8 M ammonium sulfate supplemented with 25% glycerol, flash-cooled in liquid nitrogen and maintained at 100 K in a nitrogen cryostream during data collection.

The crystals belonged to space group $P3_221$, with unit-cell parameters $a = b = 166.04$, $c = 95.32$ Å, and contained two molecules per asymmetric unit. Data were collected on the PROXIMA1 beamline at the SOLEIL synchrotron. Data were processed with *iMosflm* (Battye *et al.*, 2011) and reduced using *AIMLESS* (Evans & Murshudov, 2013). 11 selenium sites were located with *SHELXC/D* (Sheldrick, 2008) and their respective positions were refined with the *autoSHARP* procedure (Vonrhein *et al.*, 2007). The final model was refined with *BUSTER* (Smart *et al.*, 2012); it contains residues 23–34 and 53–431 for molecule *A* and residues 53–431 for molecule *B*, and has a final *R* factor of 17.13% and a free *R* factor of 22.21% (Table 1).

Figure 2

Surface properties of Sqt1p. (a, d) Ribbon diagrams of Sqt1p. The colour-coding is as in Fig. 1. (b, e) Residue conservation on the surface of Sqt1p as calculated using the *CONSURF* server (Ashkenazy *et al.*, 2010). The most conserved residues are labelled. The conservation is shown as a gradient from white for nonconserved residues to red for fully conserved residues. (c, f) Electrostatic surface properties of Sqt1p from positively charged (blue) to negatively charged (red) residues. The calculation was performed using *APBS* as implemented in *PyMOL* (Baker *et al.*, 2001). The views of Sqt1p in (d), (e) and (f) are related to those in (a), (b) and (c) by a 180° rotation around the plan axis.

3. Results

3.1. Overall structure determination and description

Full-length Sqt1p was prepared and crystallized as described in §2. Phases were obtained from a SAD data-collection experiment on selenomethionine (SeMet)-substituted Sqt1p (Table 1). In order to help in assigning the amino-acid sequence, a second data set set was collected at a longer wavelength, allowing S-SAD phasing and allowing the SeMet residues to be distinguished from Cys residues. The model was refined to R and R_{free} values of 17.1 and 22.2%, respectively, at a final resolution of 3.35 Å. The model comprises residues 23–34 and 53–431 for molecule *A* and residues 53–431 for molecule *B* of Sqt1p. Residues 53–431 of

Sqt1p contribute to the folded eight-bladed β -propeller WD40 domain. As originally observed for β -transducin (Fong *et al.*, 1986), each blade comprises four antiparallel β -strands that have been named *A*, *B*, *C* and *D* in the outward direction (Fig. 1). Each blade interacts with the following and the preceding blade by using the flat surface of the β -sheet. Overall, the eight β -sheets are curved to form a closed circle or disc.

3.2. Sqt1p harbours highly negatively charged surfaces

The WD-repeat proteins can use a number of interaction modes for substrate recognition (Stirnemann *et al.*, 2010). Having solved the crystal structure of Sqt1p, we therefore analyzed both its surface conservation and its electrostatic

Figure 3

Sequence alignment of Sqt1 orthologues. The primary sequence of Sqt1 orthologues from *S. cerevisiae*, *Homo sapiens*, *Tetraodon nigroviridis*, *Xenopus laevis*, *Dario rerio*, *Drosophila melanogaster*, *Gallus gallus* and *Anopheles gambiae* were aligned using *ClustalX*. The point mutants used in the study are indicated above the sequence. The secondary-structure elements are shown beneath the sequence alignment and are coloured according to Fig. 1.

properties (Fig. 2). Sqt1p displays several clusters of conserved residues on each side of the β -barrel, for example regions that include residues Glu110 and Glu156 on one side of the barrel or Glu120 and Lys214 on the opposite side (Figs. 2*b* and 2*e*). Interestingly, one of the two sides displays an overall negatively charged surface, as shown in Figs. 2(*c*) and 2(*f*).

3.3. Sqt1p interacts with the N-terminal portion of uL16

uL16 was shown to require Sqt1p in order to associate with the 60S subunits (Eisinger *et al.*, 1997), with this interaction being further restricted to the N-terminal residues of uL16 (West *et al.*, 2005; Pausch *et al.*, 2015). On the basis of the sequence alignments (Fig. 3) and the structure of the yeast ribosome (Ben-Shem *et al.*, 2011), we have generated truncations of uL16 and tested whether the reported interaction could be reproduced *in vitro*. In Fig. 4(*a*), we show that a His-tagged version of full-length uL16 co-precipitates with Sqt1p

when co-expressed. We then decided to identify the minimal region of uL16 required for Sqt1p interaction. From the various truncations of uL16 that were tested, the shortest fragment recapitulating the interaction with Sqt1p corresponded to residues 1–27 of uL16 (Fig. 4*a*).

For the Sqt1p protein, the N-terminal residues of Sqt1p (1–43) could be modelled for only one of the two molecules in the asymmetric unit. In order to test the importance of this region for uL16 interaction, we produced a truncated construct that removes the first 42 N-terminal residues of Sqt1p. As shown in Fig. 4(*b*), this shorter version of Sqt1p retains the ability to form a complex with uL16, suggesting that residues 1–42 are not critical for uL16 recruitment.

On the basis of the conserved residues located on the surface of Sqt1p (Figs. 2 and 3), point mutants aimed at reversing the charge or the basic contribution were introduced into the open reading frame of Sqt1p. The effect of the mutations on the interaction with uL16 were tested by

Figure 4

Sqt1–uL16 interaction mapping and the effect of Sqt1p point mutants. (*a*) Different truncations of His-tagged uL16 were generated and co-expressed together with full-length Sqt1p. After extensive washes, the eluted proteins were loaded onto an SDS–PAGE gel. The minimal fragment retaining Sqt1p interaction corresponds to the N-terminal 27 residues of uL16. (*b*) A shorter version of Sqt1p retains the ability to form a complex with uL16, as shown by pull-down experiments. The 42 residues at the N-terminus are observed in only one of the two molecules in the asymmetric unit. (*c*) Point mutants of conserved and surface-exposed residues of Sqt1p were generated and tested for their effect on the interaction with uL16. The E156K mutation shows an abolished interaction with uL16 compared with the other constructs.

pull-down experiments following co-expression (Fig. 4c). Of the four single-point mutations tested, only the E156K mutation strongly affected the interaction with uL16.

4. Discussion

WD40 repeats are protein motifs that are widely represented in all kingdoms of life. Although also found in bacteria, they are much more abundant in eukaryotes, where they participate in a large range of functions from signal transduction to cytoskeleton assembly, RNA processing and DNA repair (Stirnemann *et al.*, 2010). Currently, the WD40 domain remains a prototypical interaction module since it has not yet been reported to harbour an enzymatic activity. The ribosome-biogenesis pathway is rich in proteins containing WD40 repeats, with an over-representation in the UTPA and UTP B complexes. Here, we have solved the crystal structure of full-length Sqt1p to a resolution of 3.35 Å. The structure is similar to the recently reported structures of ctSqt1 and Sqt1p (Pausch *et al.*, 2015), with r.m.s.d. of 1.62 and 0.55 Å, respectively. In addition to the Sqt1 model reported by Pausch and coworkers, we have been able to model and to assign a portion of the N-terminal residues of Sqt1p that is missing in their models. Those residues fold on the external side of blade 7 (Fig. 1). This conformation might not reflect a mandatory organization of the protein since it is observed in only one of the two molecules in the asymmetric unit. Furthermore, the first 42 residues are not necessary to maintain normal yeast growth (Pausch *et al.*, 2015). We also note that the binding of Sqt1p to uL16 is not compatible with the positioning of uL16 in the mature 60S ribosomal subunit. Indeed, if Sqt1 were to interact with uL16 in the 60S mature subunit, it would clash with helix H89 of the 28S rRNA (Supplementary Fig. S1). This observation argues in favour of dissociation of Sqt1 from uL16 and suggests that H89, at least, has not yet acquired its final position when uL16 is delivered to the pre-60S ribosomal subunit.

Overall, we report that Sqt1p is an eight-bladed β -propeller protein that binds the N-terminal residues of uL16. We showed that the negatively charged and conserved residue Glu156 of Sqt1p is involved in interaction with uL16. This observation is consistent with the observations in another study (Pausch *et al.*, 2015). Since this electrostatic property is reminiscent of the negatively charged RNA phosphodiester backbone, we can speculate that the Sqt1p–uL16 interaction may be released upon the interaction of uL16 with the rRNA without the requirement for any additional factors.

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