
Article:
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Expanded View Methods

Protein purification

S. cerevisiae $Rrp6_{122\text{-}518,}$ $Rrp6_{1\text{-}518} – Rrp47_{\Delta C}$ and $Rrp6_{1\text{-}518} – Rrp47$ were expressed recombinantly using *E. coli* BL21-Gold (DE3) pLysS cells (Stratagene) grown in TB medium and induced overnight at 18 °C. The Rrp6 constructs were expressed with an N-terminal His tag and the complexes were co-expressed such that the Rrp47 constructs are untagged. The proteins were purified using Cobalt-based affinity chromatography, and in the case of $Rrp6_{122\text{-}518,}$ and $Rrp6_{1\text{-}518} – Rrp47_{\Delta C}$ followed by cleavage of the His-tag with Human Rhinovirus 3C protease. After affinity purification, all samples were subjected to anion exchange chromatography (HiTrap Q HP, GE Healthcare). Size-exclusion chromatography on a Superdex 200 column (GE Healthcare) was performed as a final step of purification in buffer comprising of 20 mM Tris pH 7.5, 100 mM NaCl and 1 mM DTT. An additional 10% glycerol was added to the size-exclusion buffer when purifying $Rrp6_{1\text{-}518} – Rrp47$. Rrp6 mutants were verified by DNA sequencing, and purified using the protocol for the wild-type protein.

Nuclease Assay

The exonuclease activity assays in figure E3B were carried out similarly as described in the main text in a buffer containing 25 mM Tris pH 8.0, 20 mM KCl, 0.4 mM MgCl2, 10% (v/v) glycerol, 0.02% (v/v) NP40, 1 mM DTT and 0.05 mg/ml BSA. The reactions contained protein at a final concentration of 4 nM while the concentration of RNA substrates was 200 nM.

Expanded View Figure legends

Table E1

Direct interactions of the nuclear cofactors of the yeast exosome

Detailed analysis of the chromatography profiles shown in Fig 1B-1G of the main text.

Figure E1

Biochemical characterization of nuclear exosome cofactors from yeast

(A) Formation of a *N. crassa* Mtr4 (FRH) – $Rrp6_N$ (residues 1-111) – $Rrp47_{\Delta C}$ (residues 1-133) complex by size-exclusion chromatography. The experiment was carried out as described in Figure 1B in the main text.

(B) A purified binary complex of $Rrp6_N$ and $Rrp47_{\Delta C}$ was incubated with a peptide corresponding to Mtr4 1-20 ($Mtr4_N$) in a 1:1.5 molar ratio and eluted as a complex in size-exclusion chromatography. The size-exclusion chromatography profile (Superdex 200) is shown on the left with a Coomassie stained 14% Tris-Tricine SDS-PAGE gel of the peak fraction (right panel). The peptide is too small to be visualized in the gel, but was identified by total mass analysis of the peak fraction by ESI-TOF-MS (lower panel). For $Mtr4_N$ the peak of the mono-isotopic mass (Mr) is labeled, followed by different isotopic peaks.

(C) Limited proteolysis experiments of a purified $Rrp6_{1-518}$ – $Rrp47_{1-133}$ complex, using the protease trypsin, showed the presence of stable fragments. The fragments were identified by N-terminal sequencing and mass spectrometry as corresponding to residues 1-106 of Rrp6 and residues 1-103 of Rrp47.

Figure E2

Crystallographic analysis of the $Rrp6_N$ – $Rrp47_{\Delta C}$ and of the $Rrp6_N$ – $Rrp47_N$ – $Mtr4_N$ complexes

(A) Three independent copies of $Rrp6_N$ – $Rrp47_{\Delta C}$ from the tetragonal crystal form of this binary complex (with Rrp6 in red and Rrp47 in orange). Two copies (A and B) are related by a non-crystallographic two-fold axis. The third copy (C) is not related by rotational or translational symmetry axes and has generally poorer electron density as compared to the A and B complexes. In the B complex, $Rrp47_{\Delta C}$ shows well-ordered electron density up to residue 102. In the A complex, the C-terminal helix of Rrp47 is well ordered up to residue 120. In this copy, the C-terminal end of Rrp47 is engaged in a crystal packing contact with a symmetry-related molecule. This lattice contact occurs at the concave surface of the heterodimer and mimics the binding of Mtr4.

(B) Three independent copies of $Rrp6_N$ – $Rrp47_N$ – $Mtr4_N$ comprise the asymmetric unit of the merohedrally twinned trigonal crystal form of this ternary complex (with Rrp6 in red, Rrp47 in orange and Mtr4 in blue). The twinning is generated by a two-fold axis that is perpendicular to a crystallographic 3-fold axis. The symmetry-related copies are shown in grey and the unit cell boundaries are indicated by the black box. Packing within the crystal lattice generates a ring organization of the complex, where 6 copies of the complex make up an outer ring, which obeys 6-fold symmetry and 3 copies of the complex make up an inner ring of 3-fold symmetry.

(C) Stereo view of the Mtr4-binding site in $Rrp6_N$ – $Rrp47_N$, with the electron density of $Mtr4_N$ (Fo-Fc, contoured at 2.0 σ in Pymol).

Figure E3

Biochemical properties of the $Rrp6_N$ – $Rrp47_N$ – $Mtr4_N$ complex

(A) Isothermal titration calorimetry experiments (ITC) of complexes of mutant $Rrp6_N$ protein and $Rrp47_{\Delta C}$ with the $Mtr4_N$ peptide. The experiments were carried out as described in Figure 4B in the main text.

(B) Nuclease activity of $Rrp6_{\Delta NLS}$ – $Rrp47_{\Delta C}$ in complex with Mtr4, Exo-9, Mpp6 and $Rrp44_{D171}$ (endoribonuclease activity mutant) as indicated towards duplex RNAs with 3' overhangs. Substrates were designed to have a 17 base pair GC-rich duplex (ds17), corresponding to the 3' end of $tRNA^{Tyr}$ (Lorentzen *et al*, 2008; Vincent & Deutscher, 2006), and either a 3' overhang of 10 adenine nucleotides (A10) in the upper panel or a 3' overhang of 35 adenine nucleotides (A35) in the lower panel. The experiment was carried out as described in Figure 6C in the main text. A Coomassie stained gel with the proteins used is shown on the right.

Figure E4

Mutation of conserved surface residues within Rrp6 and Rrp47 cause a defect in 5.8S rRNA processing

Northern blot hybridization analyses of RNA from *rrp6* and *rrp47* mutants. Total cellular RNA was isolated from strains grown in selective medium at 30 °C and resolved through acrylamide gels. Consecutive hybridizations of a single northern blot are shown in each panel. The major species detected with each probe are indicated on the right. The 5S* species is a truncated degradation intermediate.

(A) Analyses of *rrp6* mutants.

(B) Analyses of *rrp47* mutants.