Supplementary Material

For

Structural insights into the interaction of the nuclear exosome helicase Mtr4 with the pre-ribosomal protein Nop53

Sebastian Falk¹, Jan-Niklas Tants²³, Jerôme Basquin¹, Matthias Thoms⁴, Ed Hurt⁴, Michael Sattler²³ and Elena Conti¹

¹ Max-Planck-Institute of Biochemistry, Department of Structural Cell Biology, Am Klopferspitz 18, D-82152 Martinsried, Germany
² Center for Integrated Protein Science Munich at Chair of Biomolecular NMR Spectroscopy, Department Chemie, Technische Universität München, 85748 Garching, Germany
³ Institute of Structural Biology, Helmholtz Zentrum München, 85764 Neuherberg, Germany
⁴ Heidelberg University Biochemistry Center (BZH), INF 328, D-69120 Heidelberg, Germany
**Supplemental Figures**

**Figure S1.** Limited proteolysis of the Mtr4 arch domain Nop53 complex.

The complex of the Mtr4 arch domain (Mtr4$^{\text{arch}}$, residues 613-874) and Nop53$^{\text{AIM}}$ (residues 48-99) was subjected to limited proteolysis with subtilisin. 10 µg of the Mtr4$^{\text{arch}}$-Nop53$^{\text{AIM}}$ complex was incubated with 30 ng of subtilisin at 0°C. Samples were taken at different time points and the reaction was stopped by the addition of SDS sample buffer and incubation at 95°C for 5 min. The samples were analysed on a 16% SDS-PAGE and proteins were visualized by Coomassie staining.

To determine the sequence of the proteolysed Nop53 fragment, the reaction was upscaled to 500 µg Mtr4$^{\text{arch}}$-Nop53$^{\text{AIM}}$ and 1.5 µg subtilisin. The reaction was allowed to proceed for 30 min at 0°C, was subsequently quenched by the addition of 1 mM AEBSF (4-(2-Aminoethyl)benzensulfonylfluorid) and was then submitted to gelfiltration on a S75 (10/300) column in 20 mM Tris/HCl, 150 mM NaCl, 2 mM DTT, pH 7.5. Peak fractions were analyzed by ESI-MS to determine the mass and sequence of the Nop53 fragment from the proteolysis experiment.
Figure S2. Comparison of the Nop53 AIM motif and the Air 2 AIM-mimicking motif.

(A) Snapshot of the refined electron density from the Mtr4-ΔN – Nop53 AIM interface. The refined 2mFo-DFc map around the Nop53 AIM peptide (sharpened with -90 Å² B-factor) is contoured at 1.0 σ and superposed with the final model. Mtr4 is colored green and the Nop53 AIM peptide in pink. (B) Snapshot of the simulated annealing composite omit map (calculated with Phenix) from the Mtr4-ΔN – Nop53 AIM interface. The 2mFo-DFc map is contoured at 1.0 σ and superposed with the final model. Mtr4 is colored green and the Nop53 AIM peptide in pink. (C)
Superposition of the KOW domains in the Nop53-bound structure and in the Trf4-Air2-bound structure. The AIM-mimicking motif from Air2 that forms a crystal lattice contact in the Trf4-Air2-bound structure (in blue) superposes well with the Nop53 AIM motif (in pink). The Mtr4 KOW domain from the Trf4-Air2-bound structure is shown in yellow the Mtr4 KOW domain of the Nop53 bound structure in green. (D) Zoom in of the superposition showing the details of the interacting residues. The sulfate residue is part of the Trf4-Air2-bound structure and coordinated by Arg774 of Mtr4 and Thr15 of Air2. (E) Sequence alignment of the Nop53 and Utp18 AIM motifs with the Air2 AIM-mimicking motif from S. cerevisiae.

**Figure S3.** NMR structural analysis.

(A) $^{13}$C secondary chemical shifts recorded for the KOW domain plotted against residue numbers of the full-length Mtr4 protein. Positive values indicate α-helical, negative β-strand regions. The secondary structure elements based on the crystal structure are plotted above as cartoon. (B) $^{1}H$-$^{15}N$-heteronuclear NOE data for the free KOW domain. Lower values for residues 701-711 indicate a higher flexibility observed for the loop.
Figure S4. Mtr4 KOW domain titration with various ligands.

$^{1}$H,$^{15}$N-HSQC of free KOW (black) and in presence of a ligand (red): (A) dsRNA, (B) Phe-tRNA, (C) Nop53 peptide, (D) Nop53 peptide and dsRNA.