Characterization of the interaction between protein Snu13p/15.5K and the Rsa1p/NUFIP factor and demonstration of its functional importance for snoRNP assembly

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SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

GST pull-down assays

Recombinant proteins GST-Snu13p, wild-type or variant $E_{72}D_{73}K_{74}$/AAA, (0.35 nmol) were loaded onto 15 µl of beads of Glutathione-Sepharose 4B (GE Healthcare©) in 100 µl of binding buffer (10 mM phosphate pH 7.0, 150 mM NaCl). Recombinant $_{His6}Rsa1p_{230-266}$ (0.7 nmol) was treated in the same way. The protein partners were incubated for 30 min at room temperature. Beads loaded with proteins were washed three times with the binding buffer. Proteins were eluted by boiling in Laemmli loading buffer. Protein fractions were resolved by SDS-PAGE on 15% polyacrylamide gels and visualized by Coomassie staining.

Isothermal Titration Calorimetry

Binding of purified Snu13p and synthetic $Rsa1p_{238-259}$ was measured at 20°C in 10 mM NaPi, pH 5.6, 150 mM NaCl on a VP-ITC microcalorimeter (Microcal) with a cell volume of 1.4228 mL. 28 injections of 10 µL peptide solution (1.4 mM in the needle) were performed into 130 µM of protein in the chamber. The syringe speed was set at 300 rpm and a 200 s

delay time was maintained between each injection. After subtraction of dilution heats, calorimetric data were analyzed using a non-linear least square curve fitting algorithm provided by the manufacturing (Origin version 7) with four parameters: stoichiometry (n), association constant (K_a), variation in enthalpy (ΔH), and in entropy (ΔS). Gibbs free energy (ΔG) was calculated by using Gibbs free energy equation (ΔH-TΔS).

SUPPLEMENTARY FIGURES (S1-S4)

Figure S1. GST-pull down experiments. A His6-tagged version of the conserved peptide Rsa1p$_{230-366}$ within the yeast Ras1p protein was over-expressed and purified in *E. coli*. It was assayed for binding to the indicated recombinant GST fusions. The gel was stained with Coomassie blue.

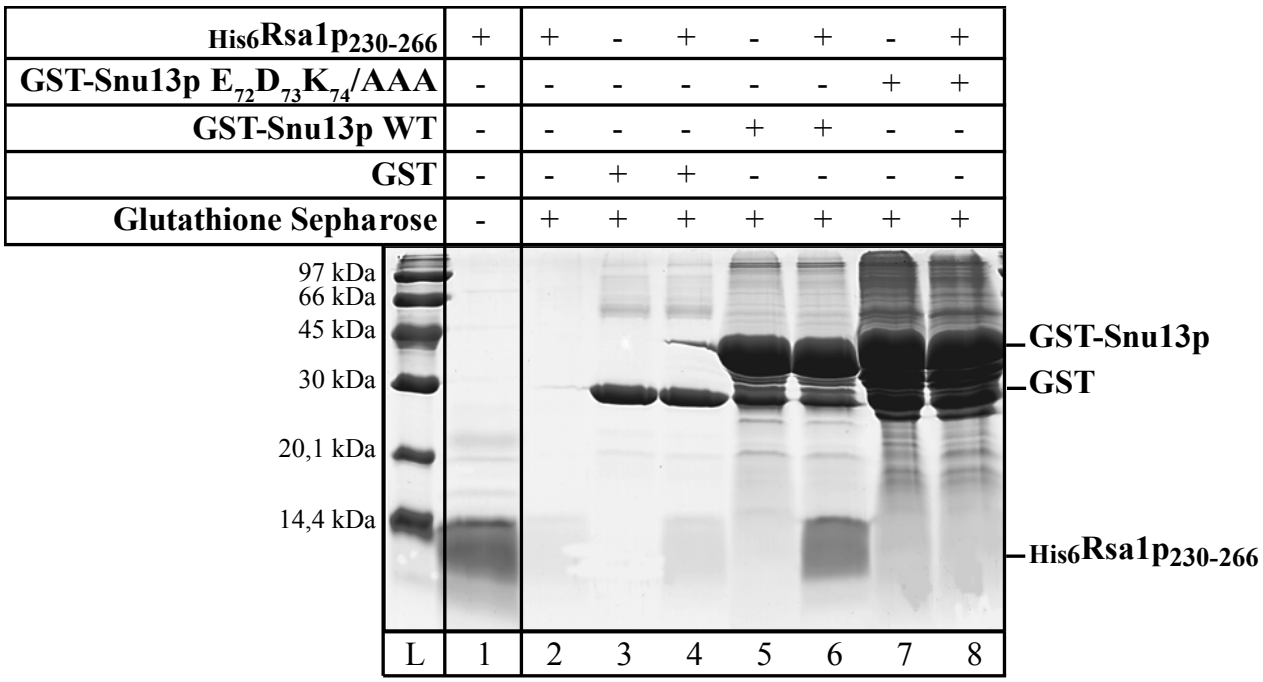


Figure S2. The PEP sequences. Multiple amino acid sequence alignments were constructed using CLUSTALW. Numbering corresponds to residues of the *S. cerevisiae* Rsa1p protein. Identical and similar residues are highlighted in dark and light grey respectively.

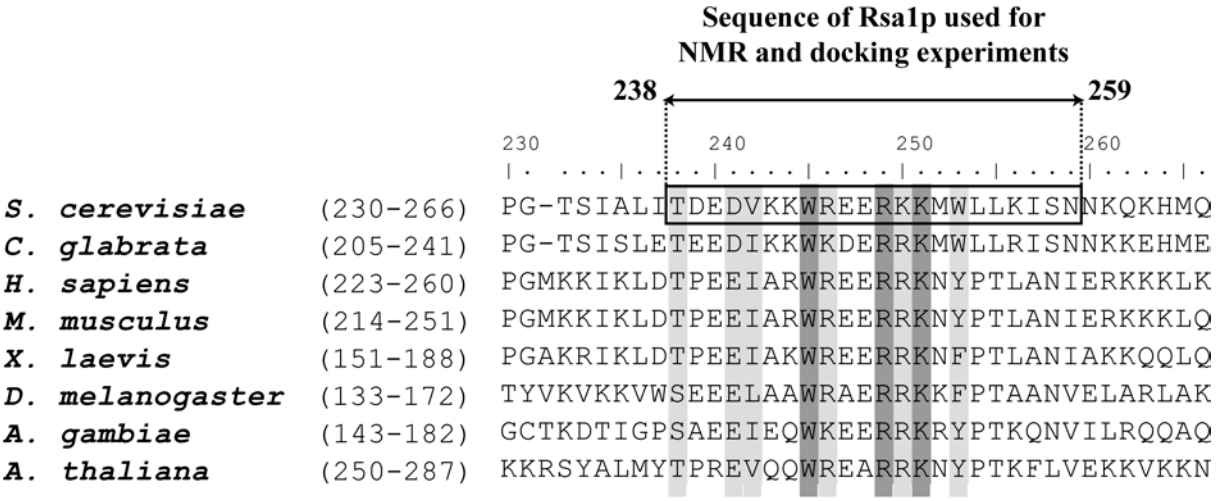


Figure S3. Results of the isothermal titration calorimetry experiment performed between Snu13p and the synthetic Rsa1p$_{238-259}$ peptide at 20°C. N, ΔH, ΔS and Kd values depict respectively the site number, the enthalpy, the entropy and the dissociation constant of the system. Top panel: Heat values measured from 28 injections of 10 µL of a 1.4 mM peptide solution into the 1.4288 mL chamber containing 130 µM of Snu13p. Bottom panel: Non-linear least-square fit (using one set of sites model) of the integrated heat data from the top panel as a function of molar ratio.

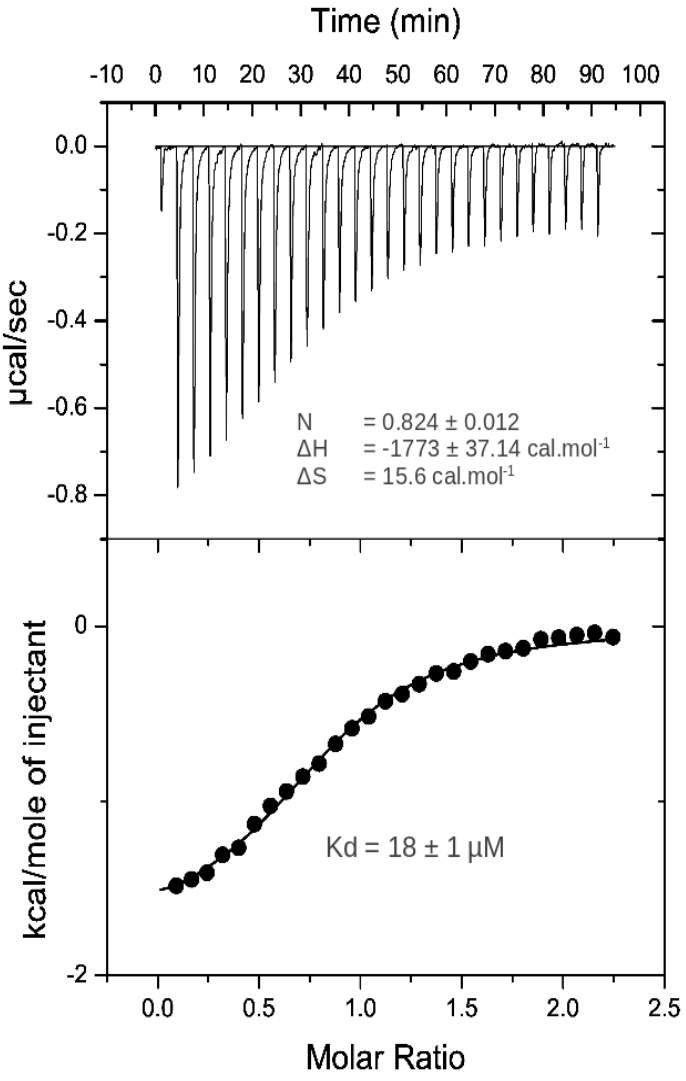
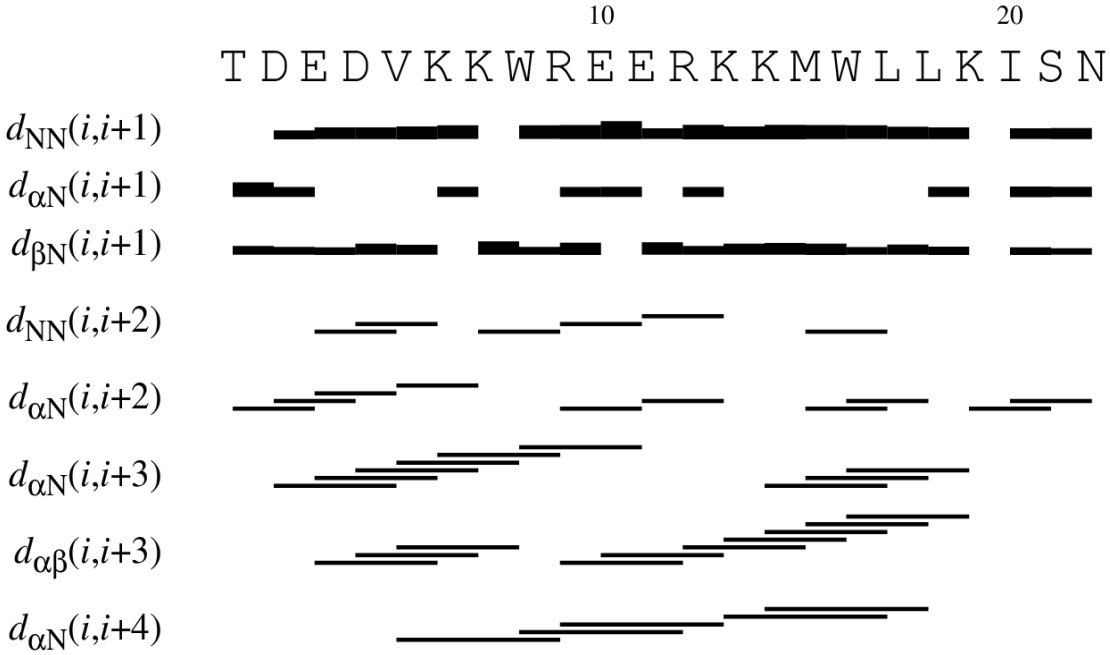


Figure S4. NOE connectivities for amide proton amide (N), alpha (α) and beta (β) protons of Rsa1p$_{238\text{-}259}$. The NOE intensities are represented by lines with different thicknesses; the intensities of the lines reflect the intensities of the connectivities.



SUPPLEMENTARY TABLES (S1-S2)

Table S1. NMR-Derived Geometrical Restraints and Structural Statistics of $Rsa1p_{238-259}$.

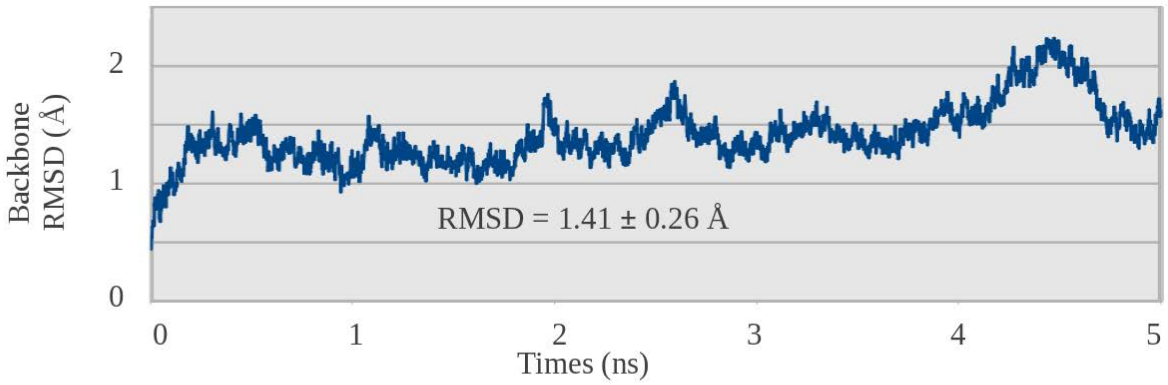
Restraints	number
Distance restraints	
total of distance restraints	439
intra	133
short	149
medium	157
Dihedral angles	
Ψ	20
Φ	20
X_1	4

CNS Violation	Occurrence
distance violation> 0.5 Å	0
dihedral violation> 5 degrees	0
Ramachandran statistics (PROCHECK-NMR)	
residues in the most favorable region (%)	99.5
residues in additionally allowed regions (%)	0.5
residues in generously allowed regions (%)	0
residues in disallowed regions (%)	0
Atoms (residues 3 to 20)	rmsd (Å)
backbone	0.47 ± 0.14
heavy	1.17 ± 0.22

Table S2. Analysis of the NAMD trajectory calculated on the Snu13p/Rsa1p$_{238-259}$ 3D model.

(A) Backbone RMSD calculated along the trajectory. The reference structure was the previously structure coming from haddock. (B) Mean distance between Rsa1p$_{238-259}$ and Snu13p atoms along the trajectory. (C) Salt bridges analysis between Rsa1p$_{238-359}$ R_{249} and K_{250} residues and Snu13p E_{72} and D_{73} residues. A cut-off of 3.8 Å between acceptor and donor atoms has been defined to evaluate the time occupancy of the salt bridges.

A



B

Rsa1p$_{238-259}$	Snu13p	Mean distance (Å)	Standard-deviation (Å)
W253 Cζ3	K7 Cβ	7.61	0.69
W253 Cζ3	F9 Cβ	5.46	0.32
W253 Cζ3	L65 Cβ	7.26	0.63
W253 Cζ3	L69 Cβ	8.08	0.54
W253 Cζ3	E72 Cγ	7.19	0.51
K256 Cδ	F9 Cγ	5.99	0.73
I257 Cδ1	L65 Cγ	4.98	0.50

C

Rsa1p$_{238-259}$	Snu13p	Time occupancy
R249	E72	91.4 %
K250	D73	98.6 %