Supporting material

Supporting methods.

Synthesis of spin labeled RNA for paramagnetic relaxation enhancement experiments. A 9mer RNA oligonucleotide with the sequence 5'-UUCAACGCC-3' containing a nitroxyl spin-label at the position 5 of the base of the 5'-uridine was synthesized on a 0,2 µmol scale on a rebuilt ABI 392 synthesizer (Applied Biosystems, Foster City, USA) with phosphoramidites purchased from Dharmacon (Lafayette, USA) (ACE chemistry). The RNA containing a 5-iodouridine at the 5'-position was synthesized completely without interrupting the synthesis after incorporation of the iodinated compound. For the Sonogashira cross-coupling the column was removed from the synthesizer and maintained under an argon atmosphere. 200 µl of a dried and deoxygenated solution of CH_2Cl_2/TEA (1.75/0.75 ml) and 9.5 mg copper(I)iodide were added under an argon atmosphere to a mixture of $Pd(II)(PPh_3)_2Cl_2$ (2.1 mg) and the spin label TPA (2.0 mg, 2,2,5,5-tetramethyl-pyrrolin-1-oxyl-3-acetylene). The yellow/orange solution was added to the column and moved back and forth using two syringes (34). After a reaction time of 2.5 h in the dark the column was washed with 10 ml dried CH_2Cl_2, dried for 10 min in vacuum and flushed with argon. The Sonogashira cross/coupling was repeated twice with the same amount of reagents. The methyl-protecting groups of the phosphate moieties were cleaved off using a 0.4 M solution of disodium-2-carbamoyl-2-cyanoethylene-1,1-dithiolate-trihydrate (S_2Na_2) in DMF/H_2O, 98/2 at room temperature for 30 min. Afterwards, the oligonucleotide was cleaved from the solid-support and the functional groups were deprotected simultaneously with methylamine (40% in water, 16 h at 4°C). Further purification was achieved by anion-exchange HPLC (DNAPack®PA-100, Semi-Prep, 9x250 mm from Dionex, Sunnyvale, USA) with 1 M LiCl. The RNA was desalted with a PD-10 Sephadex column (Amersham Biosciences, Piscataway, USA) and characterized by MALDI mass spectrometry. The final deprotection of the 2'-hydroxy groups was performed under sterile conditions with a TEMED/acetic acid buffer (pH 3.8) for 30 min at 60°C. After freeze-drying the probe was dissolved in NMR buffer.

Supporting Table 1.

Limited acidic cleavage fragments observed by MALDI mass spectrometry for the identification of the modified nucleotide position.

Observed mass	Mass difference	Sequence
3462,526	225,061	5’ GAUmΨCAACGCC 3‘
3237,465	305,041	5’ GAUmΨCAACGC
2932,424	345,047	5’ GAUmΨCAACG
2587,377	305,041	5’ GAUmΨCAAC
2282,336	329,053	5’ GAUmΨCAA
1953,283	329,053	5’ GAUmΨCA
1624,230	305,041	5’ GAUmΨC
1319,189	*320,041*	5’ GAUmΨ
999,148	0	5’ GAU
894,172	0	GCC 3‘
1199,213	305,041	CGCC 3‘
1528,266	329,053	ACGCC 3‘
1857,319	329,053	AACGCC 3‘
2162,360	305,041	CAACGCC 3‘
2482,401	*320,041*	mΨCAACGCC 3‘
2788,426	306,025	UmΨCAACGCC 3‘
3117,479	329,053	AUmΨCAACGCC 3‘
3462,526	362,054	5’ GAUmΨCAACGCC 3‘

Supporting figures – Figure captions

Figure S1. Conserved binding of *M. jannaschii* Nep1 to the consensus RNA motif 5'-UUCAAC-3' (RNA 1) identified for the yeast Nep1 homolog. (A) Fluorescence quenching of the *Mj*Nep1 Trp193 in the vicinity of the S-adenosylmethionine binding site upon titration with RNA 1. (B) Sections from $^{1}H,^{15}N$-HSQC spectra of *Mj*Nep1 in the absence (black) and in the presence of 0.5 (blue), 1 (red) and 2 (green) equivalents of RNA 1. Specific signals either display a gradual change in their chemical shift such as the one corresponding to the amide group of I90 or disappear entirely such as the one corresponding to L96. Other signals are unaffected by the presence of the RNA. This indicates binding of the RNA to a localized binding site in the fast to intermediate exchange regime on the NMR time scale.

Figure S2. Efficient methylation of a minimal substrate of *Mj*Nep1 (RNA 12) containing the Ψ directly at its 5'-end and only a 3'-end extension. Shown are MALDI mass spectra of a reaction mixture of S-adenosylmethionine, *Mj*Nep1 and RNA 12 directly starting with a pseudouridine at the 5'-position before the reaction (top) and after 10 min (bottom).

Figure S3. Involvement of the Nep1 extension elements of the SPOUT-class fold in RNA-binding. Top: Mapping of chemical shift perturbations induced by RNA 11 on the structure of *Mj*Nep1. Bottom: Ribbon representation of the *Mj*Nep1-dimer in the same orientation. One monomer is shown in blue, the other is shown in orange. The β–α–β-extension element of the blue monomer is coloured gray, the extension element of the orange monomer is coloured red orange.