Supporting Text

DNA template construction. By linearizing with different restriction enzymes, the DNA template of the U3 small nucleolar RNA (snoRNA) could be used to transcribe the full length U3 snoRNA and 5'-fragments thereof. The DNA template for *in vitro* transcription of the 3' domain of the U3 snoRNA containing nucleotides 77-333 (pUCU3-3') of the full length RNA was amplified from the template pGEMsnR17a provided by S. J. Baserga (Yale University, New Haven, CT) with *Sal*I (5') and *Bam*HI (3') restriction sites (New England Biolabs) using two primers (5'-GCGCGGTCGACTAATACGACTCACTATAGGCCAACTTGGTTGATGAGTCCCATAACC-3' and 5'-GCGCGGATCCGGTCTCTACTTGTCAGACTGCCATTTG-3'). The DNA template for the pre-rRNA fragment (nucleotides 469-722, pUCpre) was amplified from *Saccharomyces cerevisiae* genomic DNA (American Type Culture Collection) with *Eco*RI (5') and *Bam*HI (3') sites (New England Biolabs) using two primers (5'-GCGGAATTCTAATACGACTCACTATAGGCTCAAATCAGCGATATCAAACGTACC-3' and 5'-CGGGATCCCCCGCCCCCTACTGGCAGGATCAACCAGATAACTATC-3') and cloned into a pUC19 vector.

The Imp4-core representing amino acids 87-275 of Imp4p (pETImp4core) was amplified from ORF coding for full length Imp4p using two primers (5'-GCGGAATTCATGGATCCAAGAATCATCGTCACAAC-3' and 5'-GCGCAAGCTTTCACCACTCAACATCAGCGTCCTTATTTTC-3') and cloned into a pProExHTa vector.

RNA synthesis. The following designations were used for the U3 snoRNA: 5' domain plus the hinge region (nucleotides 1-76); the 3' domain (nucleotides 71-333) is the 3' remainder of the U3 snoRNA; and the pre-rRNA fragment (nucleotides 469-722) (Fig. 1). The full length U3 snoRNA (nucleotides 1-333), the 5' domain plus hinge region (nucleotides 1-76), the 5' domain (nucleotides 1-36), the 3' domain (nucleotides 71-333) and the pre-rRNA fragment (nucleotides 469-722) were produced by run-off transcription

using plasmid DNA templates linearized with the following restriction enzymes: *Bsa*I (U3 full length and 3' domain), *Bsm*FI (5' domain plus hinge region), *Fau*I (nucleotides 469-722 of the pre-rRNA) (New England Biolabs), and *Nmu*CI (5' domain) (Fermentas, Hanover, MD). The minimal RNA-binding site (nucleotides 4-50), herein designated U3 MINI, for Imp3p and Imp4p binding was transcribed from a synthetic oligonucleotide template (Integrated DNA Technologies, Coralville, IA). The RNA oligomers representing the ETS and 18S base pairing sites were synthesized by solid-phase oligonucleotide synthesis (Dharmacon, Lafayette, CO). Internally radiolabeled RNAs were made by *in vitro* transcription using published procedures (1). After shrimp alkaline phosphatase treatment, T4 kinase (Fermentas) was used in the presence of [γ-^{32}P]ATP (Perkin--Elmer, 150 μCi/μl) to label at the 5'-end of each RNA according to the manufacturer's instructions. Each RNA was purified by denaturing PAGE. The following RNA sequences were used in this paper: the ETS site (5'-AGUUUCUCAC-3'); the antisense ETS site (5'-UGUUUCUCAC-3'); the 18S site (5'-GGUUGAUCCUGCCAGUA-3'); the antisense 18S site (5'-CUUACUAGGACCCUCAU-3'); 5' hinge region (5'-GGCACUCUUUGACU-3'); U3 MINI (5'-*G*GACGUACUUCAUAGGAUCAUUUCUAUAGGAAUCGUCACUCUUUGACU-3'); U3 MINIAS1 (5'-*G*GACGUACUUCAUAGGAUCAUUUCUAUAGGAAUCGUCAGAGAAACUGA-3'); U3 MINIAS2 (5'-*G*GACGAUGAUCAGUCCUAGUUAGGACUAUCAAUCGUCACUCUUUGACU-3'); U3 MINIAS3 (5'-*G*GACGAUGAUCAGUCCUAGUUAGCUAUAGGAAUCGUCACUCUUUGACU-3'); the U3 hinge (5'-*G*GCACUCUUUGACUCUUCAAAAGAGCCACUGAAUCCAA-3'); the 5' domain (5'-*G*GUCGACGUACUUCAUAGGAUCAUUUCUAUAGGAAUCGUCACUC -3'), the 256 nucleotide fragment of the pre-rRNA that contains the ETS site, the 18S site and the intervening sequence (5'-*GG*CUCAAAUCAGCGAUAUCAAACGUACCAUUCCGUGAAACACCGGGGUAUCUGUUUGGUGGAACCUGAUUAGAGGAAACUCAAAGAGUGCUAUGGUAUGG

UGACGGAGUGCGCUGGUCAAGAGUGUAAAAGCUUUUUGAACAGAGAGCAU
UUCCGGCAGCAGAGAGACCUGAAAAAGCAAUUUUUCUGGAAUUUCAGCUG
UUUCCAAACUCAAUAAGUAUCUUCUAGCAAGAGGGAAUAGGUGGGAAAAA
AAAAAAGAGAUUUCGGUUUCUUUCUUUUUUACUGCUUGUUGCUUCUUCUU
UUAAGAUAGUUAUCUGGUUGAUCCUGCCAGUA-3'); the U3 snoRNA 5' domain plus hinge region (5'-
*G*GUCGACGUACUUCAUAGGAUCAUUUCUAUAGGAAUCGUCACUCUUUGAC
UCUUCAAAAGAGCCACUGAAUCCAACU-3'; the U3 snoRNA (5'-
*G*GUCGACGUACUUCAUAGGAUCAUUUCUAUAGGAAUCGUCACUCUUUGAC
UCUUCAAAAGAGCCACUGAAUCCAACUUGGUUGAUGAGUCCCAUAACCUU
UGUACCCCAGAGUGAGAAACCGAAAUUGAAUCUAAAUUAGCUUGGUCCGC
AAUCCUUAGCGGUUCGGCCAUCUAUAAUUUUGAAUAAAAAUUUUGCUUUG
CCGUUGCAUUUGUAGUUUUUUCCUUUGGAAGUAAUUACAAUAUUUUAUGG
CGCGAUGAUCUUGACCCAUCCUAUGUACUUCUUUUUUGAAGGGAUAGGGC
UCUAUGGGUG GGUACAAAUG GCAGUCUGACAAGUA-3'); the 3' domain of the U3 snoRNA (5'-
*GG*CCAACUUGGUUGAUGAGUCCCAUAACCUUUGUACCCCAGAGUGAGAAA
CCGAAAUUGAAUCUAAAUUAGCUUGGUCCGCAAUCCUUAGCGGUUCGGCC
AUCUAUAAUUUUGAAUAAAAAUUUUGCUUUGCCGUUGCAUUUGUAGUUUU
UUCCUUUGGAAGUAAUUACAAUAUUUUAUGGCGCGAUGAUCUUGACCCAU
CCUAUGUACUUCUUUUUUGAAGGGAUAGGGCUCUAUGGGUGGGUACAAAU
GGCAGUCUGACAAGUA-3'). The underlined nucleotides were added or mutated to G to aid high-level *in vitro* transcription.

Nitrocellulose filter binding and electromobility shift assays. RNA fragments were annealed in water before use: U3 MINI (nucleotides 4-50), the ETS site, the 18S site, and and the 5' domain (nucleotides 1-36) were incubated at 100°C (2 min), then on ice (10 min); other RNA constructs were incubated at 100°C (3 min), cooled at room temperature (3 min), then incubated with 10 mM $MgCl_2$ at 55°C (3 min). Before the binding assays, ribonucleoprotein (RNP) complexes were assembled under the following

conditions: increasing amounts of protein (100 nM to 5 μM) were incubated with up to 10 nM radiolabeled RNA for 5 min in binding buffer [20 mM Tris (pH 8.0)/30 mM NH_4Cl/100 mM KCl/0.5 mM $MgCl_2$/1 mM DTT/4% (vol/vol) glycerol/0.1% (wt/vol) Nonidet P-40/0.2 units/μl RNasin (Promega)] in 12-μl final volume. Nitrocellulose filter-binding assays were done by using published procedures (1). For electromobility shift assays, the RNP complexes (8 μl) were resolved on a 6% nondenaturing PAGE [40 mM Tris•acetate (pH 8.0)/1 mM EDTA/50 mM KCl/2.5% (vol/vol) glycerol)] run for 50 min at 125 V at 4°C. The results of the assays were visualized by autoradiography by using a Fuji imaging plate (Bas-III) and a Molecular Dynamics Storm 860 PhosphorImager (Amersham Pharmacia). Radioactivity was quantified with the IMAGEQUANT 5.0 (Amersham Biosciences) software package. Dissociation constants were fit by ORIGIN 6.0 (Microcal, Amherst, MA) to Eq. **1**:

$$f = \frac{[protein]}{[protein] + K_d}, \qquad [\mathbf{1}]$$

where f is the fraction bound; typically, saturating values of f range from 0.7 to 1.0.

RNase protection assays. RNP complexes containing protein and 1,000 cpm 5'-radiolabeled RNA were assembled in binding buffer. RNA fragments were refolded before the assays (see above). Nuclease protection patterns were generated by mixing 5 μl of RNP complexes (assembled as above) with 1 μl of either RNase T1 or snake venom ribonuclease (RNase V_1) (Ambion, Austin, TX). After 5-min incubation at room temperature, the reactions were stopped with 24 μl of urea dye [9 M urea/50 mM EDTA/0.02% xylene cyanole/0.02% bromophenol blue/0.65× 90 mM Tris-borate (pH 8.0)/2.0 mM EDTA]. Sequencing markers were generated by partial RNase T1 digestion [0.005 units of RNase T1 in 50 mM Tris (pH 7.5)/ 0.05% (vol/vol) Triton X-100] or partial alkaline hydrolysis [1 mM glycine (pH 9.5)/0.5 mM $MgCl_2$] of the RNA template. Samples were resolved on 10% denaturing PAGE run for 1.5 h at 20 W. Control reactions in the annealing assays used 1 μM of either the RNA-binding domain of the human U1A protein (2) or *Bacillus subtilis* RNase P protein (3). The U1A and RNase P proteins were gifts from B. L. Golden (Purdue University, West Lafayette, IN).

1. Dresios, J., Chan, Y. L. & Wool, I. G. (2002) *J. Mol. Biol.* **316,** 475-88.

2. Oubridge, C., Ito, N., Evans, P. R., Teo, C. H. & Nagai, K. (1994) *Nature* **372,** 432-438.

3. Niranjanakumari, S., Kurz, J. C. & Fierke, C. A. (1998) *Nucleic Acids Res.* **26,** 3090-3096.