Accession numbers. Homo sapiens rDNA promoter (X01547), Mus musculus rDNA repeat unit (BK000964), Rattus norvegicus rDNA promoter (X00677), Sus scrofa rDNA promoter (L31782).

**RNase footprinting**. pRNA was transcribed with T7 RNA polymerase, labeled at the 5'-end with T4 polynucleotide kinase, and purified by electrophoresis on 8% polyacrylamide/7M urea gels. Prior to binding to TIP5, 1 fmol pRNA was renatured in binding buffer at 55°C for 20 min, slowly cooled to 20°C and incubated with 20-500 nM TIP5<sub>510-611</sub> for 10 min on ice. After addition of 0.1  $\mu$ g *E. coli* tRNA and either RNase V1 (0.0003 units) or RNase T1 (0.05 units), incubation was continued for 10 min at 20°C. RNA was recovered by ethanol precipitation and analyzed by electrophoresis on 10% polyacrylamide/7 M urea gels along with partial alkaline hydrolysis or RNase T1 cleavage products as size markers.

## LEGENDS TO SUPPLEMENTARY FIGURES

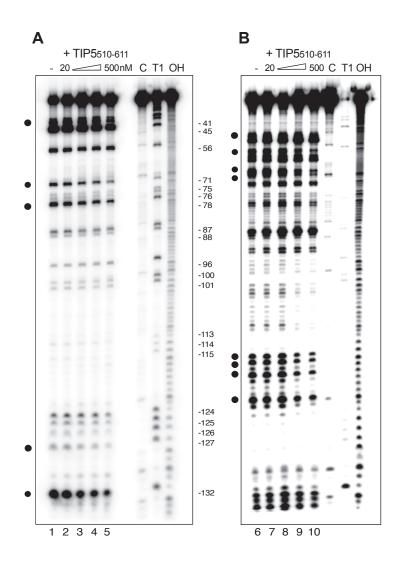
Figure S1. RNase footprinting of TIP5/pRNA complexes.

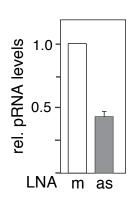
(A) Electrophoretic analysis of partial RNase T1 cleavage of pRNA. 1 fmol of radiolabeled pRNA (-143/-39) was incubated with increasing amounts (20-500 nM) of purified hTIP5<sub>510-611</sub> and subjected to partial digestion with 0.05 u RNase T1 (lanes 1-5). RNA incubated with buffer only was used as control (C). A partial alkaline hydrolysis ladder (OH) and RNase T1 ladder (T1) were run alongside as size markers. The positions of guanosines are indicated.

(B) Same as in (A), except that the incubation mixture was digested with 0.0003 u RNase V1.

Figure S2. Depletion of pRNA from NIH3T3 cells.

Knockdown of pRNA by antisense LNA-DNA gapmers. NIH3T3 cells were transfected with control RNA (light bar, m) 50 nM LNA-DNA gapmers in antisense orientation (dark bar, as). The amount of pRNA levels was determined by RT-qPCR and normalized against GAPDH mRNA. Data are from two independent experiments. Error bar indicates +/-SD.





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