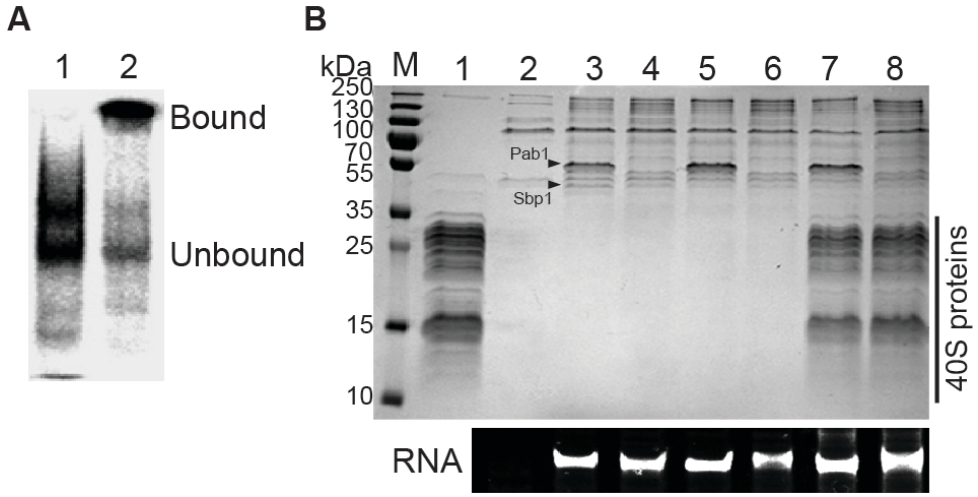
Supplemental Materials

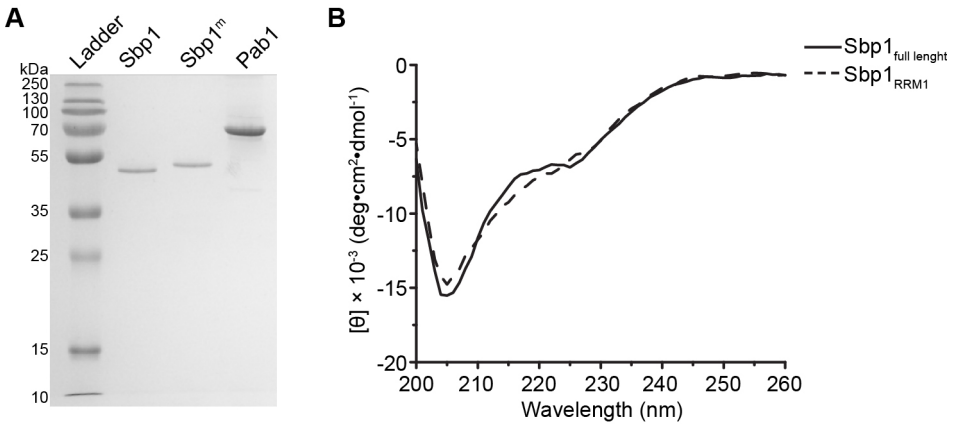


Supplemental Figure S1. The TRAP assay for pull-down of 5'UTR-assembly.

(A) An electrophoresis mobility shift assay (EMSA) on the binding of the ^{32}P-labeled 5'UTR$_{Pab1}$ (lane 1) to the 43S pre-initiation complex (43S PIC, which contains 40S•eIF2•GDPCP•Met-tRNA$_i^{Met}$•eIF1•eIF1A•^{32}P-5'UTR$_{Pab1}$, lane 2).

(B) Top: SYPRO Ruby stained gel showing protein profiles pulled down by TRAP in translation. Lane 1: 43S PIC; Lane 2: proteins washed off from empty beads incubated with the cell extract; Lane 3: proteins pulled down by 5'UTR$_{Pab1}$ alone; Lane 4: proteins pulled down by 5'UTR$_{Pab1\text{-}complement}$; Lane 5: proteins pulled down by 5'UTR of the Pab1-construct1 (5'UTR$_{Pab1\text{-}construct1}$) alone; Lane 6: proteins pulled down by the RNA with sequence complementary to the Pab1-construct1, 5'UTR$_{Pab1\text{-}construct1\text{-}complement}$; Lane 7: proteins pulled down by 43S PIC with 5'UTR$_{Pab1\text{-}construct1}$; Lane 8: proteins pulled down by 43S PIC with 5'UTR$_{Pab1\text{-}construct1\text{-}complement}$. The sequence after the start codon AUG in the mRNA was replaced by (AUG)AAAAAAAAA in the 5'UTR$_{Pab1\text{-}construct1}$ to facilitate ribosome loading.

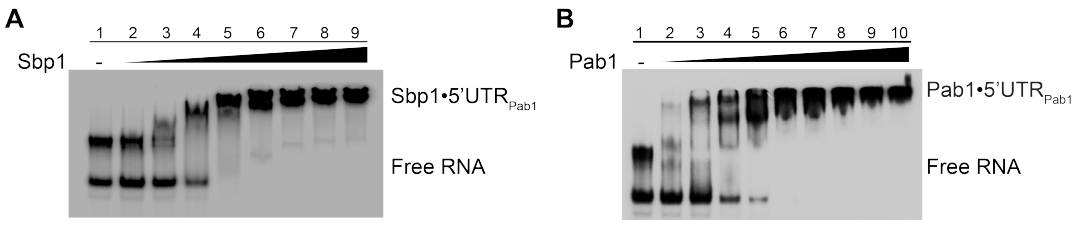
Bottom: ethidium bromide-stained gel showing the integrity of 5'UTR$_{Pab1}$ after the TRAP assay.



Supplemental Figure S2. Quality of the recombinant proteins and protein mutants.

(A) Coomassie Brilliant Blue staining SDS-PAGE gel showing purified Sbp1, $Sbp1^{m}$ and Pab1 proteins.

(B) Circular dichroism (CD) spectra of the wild type Sbp1 and its RRM1 domain mutant ($Sbp1_{RRM1}$) show the RRM domain mutant folds correctly.

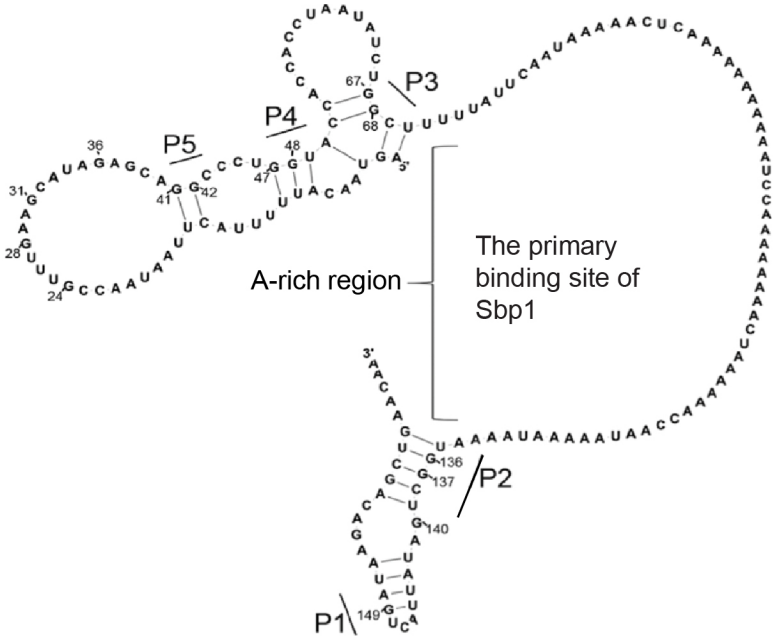


Supplemental Figure S3. The binding constant of Sbp1 and Pab1 to the 5'UTR$_{Pab1}$. An excess amount of the protein at increasing concentrations was added into a fixed amount of ^{32}P-labeled 5'UTR$_{Pab1}$. The mixture was subjected to EMSA gel.

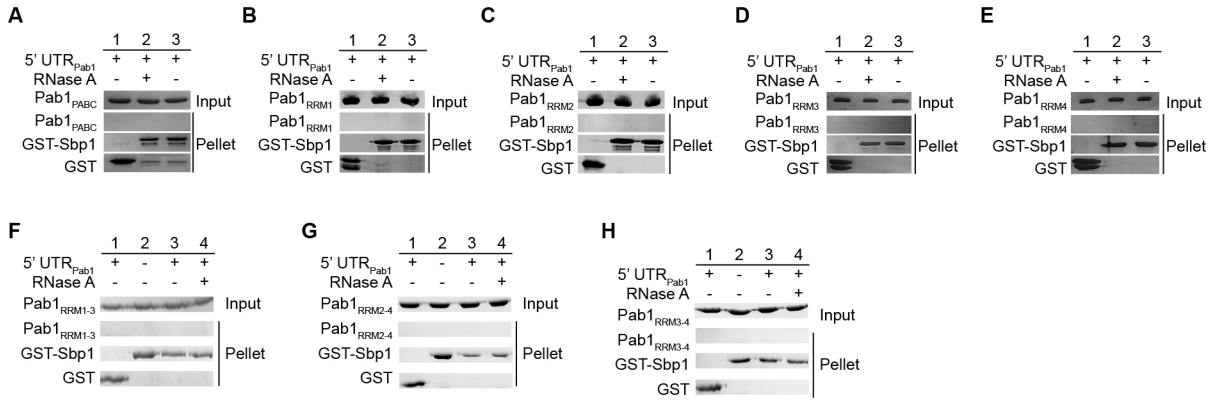
(A) The molar concentrations of Sbp1 are 10, 20, 50, 100, 150, 200, 250 and 300 nM for lanes 2 to 9, respectively.

(B) The molar concentrations of Pab1 are 10, 20, 50, 100, 150, 200, 250, 300 and 400 nM for lanes 2 to 10, respectively.

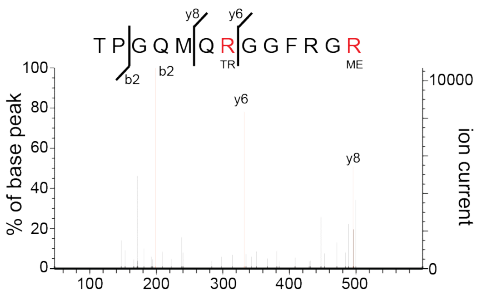
The apparent dissociation constant (K_d) of 5'UTR$_{Pab1}$ and Sbp1 is 26.5 ± 1.1 nM. The K_d of 5'UTR$_{Pab1}$ and Pab1 is 31.2 ± 2.5 nM. The results are the average of three independent experiments.



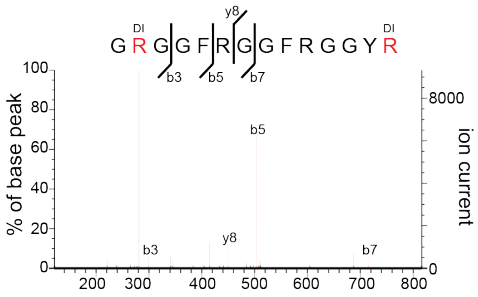
Supplemental Figure S4. The secondary structure of 5'UTR$_{Pab1}$. The secondary structure of 5'UTR$_{Pab1}$ was obtained by an analysis of the cleavage pattern from in-line probing and confirmed by using experimentally determined double-stranded and single-stranded regions as restraints in the mFold program (Zuker, 2003). Sbp1 targets the A-rich region in the 5'UTR$_{Pab1}$.



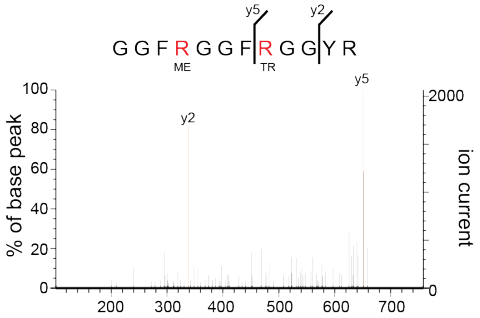
Supplemental Figure S5. GST-pulldown assay showing no interaction of individual domains of Pab1 with Sbp1 in the presence of 5'UTR$_{Pab1}$. No interactions were detected for Sbp1 with PABC (A), RRM1 (B), RRM2 (C), RRM3 (D), RRM4 (E) and tandem domains RRM1-3 (F), RRM2-4 (G) and RRM3-4 (H). Lane 1 is the negative control containing GST only.



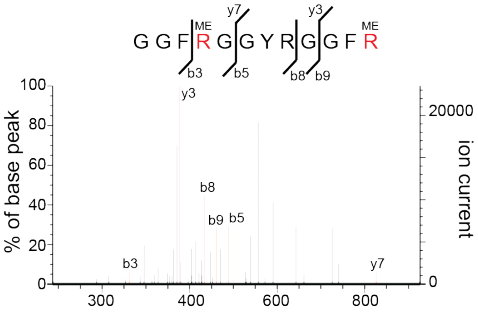
y8 m/z:989.5752
y8 (unmethylated) m/z:933.0432



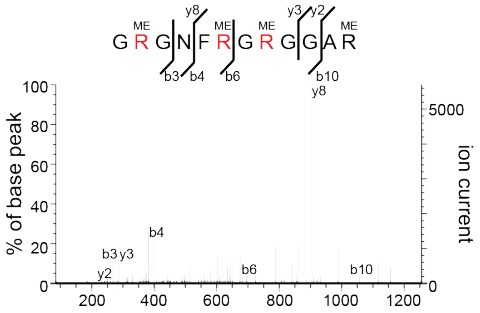
y8 m/z:897.4690
y8 (unmethylated) m/z:868.9876
b3 m/z:316.1826
b3 (unmethylated) m/z:288.3124



y5 m/z:650.3733
y5 (unmethylated) m/z:607.6754



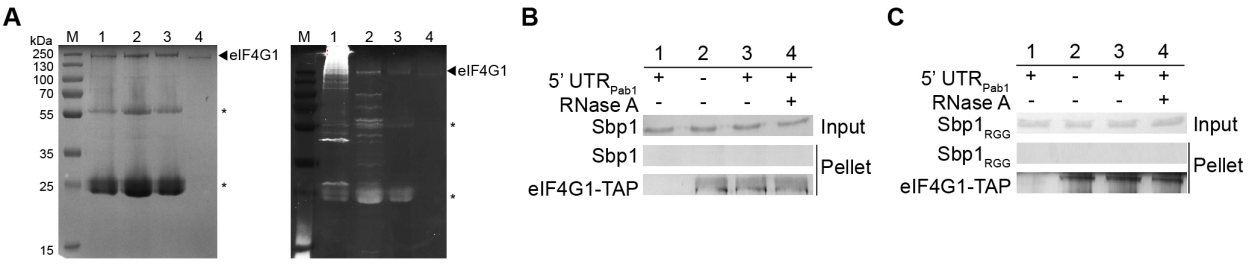
y3 m/z:393.2245
y3 (unmethylated) m/z:378.43
b5 m/z:506.5441
b5 (unmethylated) m/z:492.54



y8 m/z:904.5224
y8 (unmethylated) m/z:875.9908
b6 m/z:732.3951
b6 (unmethylated) m/z:705.7763

Supplemental Figure S6. The mass/charge ratio of peptides containing RGG repeats in Sbp1 co-expressed with Hmt1 showing methylation of arginines.

Samples of the mass/charge ratio of fragmented peptides with methylated and unmethylated arginines are shown. The b fragment peaks extended from the amino terminus and the y fragment peaks from the C-terminus of the peptide are indicated.



Supplemental Figure S7. No interactions were detected between Sbp1 and eIF4G1.

(A) Purification of RNA-free eIF4G1.

Left gel: Coomassie Brilliant Blue staining showing protein bands; right gel: ethidium bromide staining showing RNA bands. The asterisk (*) denotes IgG protein. TAP-tagged eIF4G1 was expressed in yeast. eIF4G1 (left gel) and bound RNAs to eIF4G1 (right gel) were shown after affinity purification (lane 1), followed by RNase A treatment (lane 2), stringent wash steps (lane 3) and passing through heparin column (lane 4).

(B and C) Neither Sbp1 (B) nor RGG domain of Sbp1 (C) binds to eIF4G1. Purified RNA-free proteins were incubated in the absence of 5'UTR$_{Pab1}$ (lane 2), in the presence of 10× 5'UTR$_{Pab1}$ (lane 3) and RNase A (lane 4). Lane 1: proteins were incubated with IgG sepharose alone as a negative control.

REFERENCE

Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res* **31**: 3406-3415.