Supplementary information

Materials and Methods

Protein expression and purification

All R17 fusion proteins were cloned in the pGEX 2T *E. coli* expression vector and contained the following: (1) the GST tag followed by the R17 coding region at the N terminus; (2) different regions of the U2AF 65 coding sequence (1-475; 1-148; 1-85; 1-85Δ17-47; 17-47; 30-47; 17-30) at the C terminus; (3) a glycine hinge (SGGGGG) placed between the R17 and the sequence coding for U2AF 65 to improve protein flexibility. All the numbers listed above correspond to the amino acid coordinates found in the natural protein. All R17 proteins were expressed in *E. coli* at 25°C overnight and purified to homogeneity by glutathione agarose chromatography. All fractions were then screened for their ability to specifically bind to substrate RNA. The bovine PAP (residues 1-694) and PAPΔC (residues 1-562) were histidine-tagged at the carboxyl terminus, expressed in *E. coli* and purified to homogeneity by Ni^{2+}-NTA chromatography as described (Gunderson *et al.*, 1994).

The BAC-TO-BAC Baculovirus expression system from Life Technologies was used to produce recombinant CF I_m subunits. The ORF of the 25 kDa subunit of CF I_m was ligated into the BamHI and XhoI sites of pFASTBAC HTb. A modified pFASTBAC DUAL vector in which a sequence for the 6×histidine tag was inserted into the MCS I was used to insert the ORFs corresponding to the 25 kDa and 59 kDa subunits of CF I_m in order to co-express both proteins. The resultant plasmids were used to generate recombinant baculoviruses as described by the manufacturer. Sf9 cells were infected with the recombinant baculoviruses

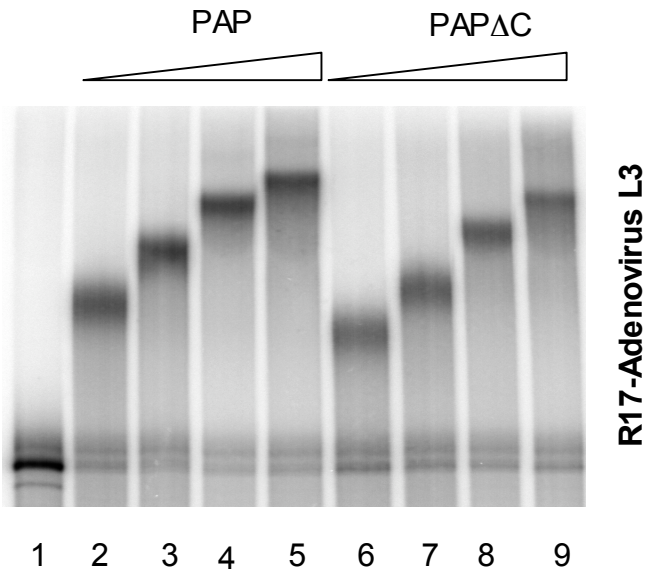
and harvested 3–5 days postinfection. The proteins were purified under native conditions on Ni-NTA agarose (Qiagen) and dialyzed against buffer D.

Nitrocellulose filter binding assays

An increasing amount of purified proteins was added to an *in vitro* transcribed ^{32}P-labelled RNA in a total volume of 10 µl GS binding buffer containing 400 ng of yeast tRNA. The mixture was allowed to incubate 10 min at room temperature. 8 µl of each binding reaction were applied on a pre-soaked nitrocellulose membrane on a slot dot apparatus (hybrislot manifold, BRL) under moderate succion. Each slot dot was washed with 200 µl of room temperature GS buffer and the membranes were dried for 1 hour at room temperature. The filters were exposed in a phosphoimager cassette (Molecular Dynamics) for 3 hours and revealed. The quantifications were performed with the Image Quant v1.1 software and the data were corrected for the background (RNA retention without any added protein) which was < 2%. The fraction of RNA bound was plotted against the protein concentration.

Supplementary Figure S1

Polyadenylation reactions comparing the activity of PAP and PAPΔC. The reactions were performed using increasing amounts of recombinant PAP (lanes 2-5; Two fold serial dilution starting at 1.2 pmoles on lane 5) or PAPΔC (lanes 6-9; Two fold serial dilution starting at 1.2 pmoles on lane 9)



Supplementary Figure S2

Polyadenylation reactions were performed using recombinant PAP (lanes 2-4; 0.1 pmol) in the presence of the baculovirus expressed CF I_m 25 (lanes 2-4; 2 pmol) and increasing amounts (0.5 and 1 pmol) of the R17-U2AF 65 fusion protein (lanes 3-4).