Supplementary materials:

Full-length PARN and its mutants were assayed by the release of TCA-soluble products from homogeneously labelled poly(A) (Korner & Wahle, 1997). The reaction buffer was 20 mM HEPES pH 7.0, 10% (v/v) glycerol, 0.2 mg/ml mBSA, 0.02 % NP40, 120 mM KCl, 2 mM DTT, 1.5 mM magnesium acetate. A 20 µl reaction mix contained 0.05 mM (as mononucleotides) of unlabelled poly(A) and 10 000 cpm homogeneously labelled poly(A) as a substrate. This amount of poly(A) is about five fold above the K_M. The reaction was started by the addition of protein, incubated at 37°C for 10 minutes, and stopped by the addition of 60µl ice-cold 16% (w/v) TCA. The samples were centrifuged at 20,000g for 15 minutes at 4°C. AMP released into the supernatant was measured by scintillation counting. For measurements of K_M and k_{cat}, time courses of digestion were done with substrate concentrations varied over a suitable range. Kinetic constants were obtained by direct fitting of the data to the Michaelis-Menten equation with the help of Sigmaplot. Kinetic experiments were carried out at least twice for each enzyme variant, and numbers were reproducible within +/- 10%. Homogeneously labeled poly(A) used in these assays was prepared by the extension of 0.02 mM of an oligo$(A)_{20}$ primer in a 50 µl reaction containing 25 mM Tris/HCl pH 8.3, 40 mM KCl, 20% (v/v) glycerol, 0.05 mM EDTA, 0.2 mg/ml methylated BSA, 0.02 % NP40, 0.5 mM DTT, 1 mM ATP, 1 mM $MnCl_2$, 5µCi [α-^{32}P] ATP, 20 U RNasin (Promega) and 10 units of bovine poly(A) polymerase (a gift from U. Kühn) at 37°C for 10 minutes. Poly(A) was ethanol-precipitated and separated from residual ATP on a G50 Sephadex column (Amersham).