Supporting Information

Jia et al. 10.1073/pnas.1201085109

SI Materials and Methods

Sequences of RNA oligonucleotides (duplex regions are underlined). Note that some sequences contain adenosines (marked A) and others do not (marked R).

R16 (top strand for 16-bp duplex substrates): 5′AGCACCGUAAAGACGC3′
R16-bio (top strand for 16-bp duplex substrates, biotin with 9-carbon spacer at the 3′): 5′AGCACCGUAAAGACGC-C9-biotin3′
R16-dd (top strand for 16-bp duplex substrates, 2′,3′-dideoxycytosine at the 3′): 5′AGCACCGUAAAGACGddC3′
R41(A) (bottom strand for 16-bp duplex with 25-nt A-rich single-stranded region): 5′GCGUCUUUACGGUGCUUAAAACAAAACAAAACAAAACAAAA3′
R41(R) (bottom strand for 16-bp duplex with 25-nt single-stranded region containing no A): 5′GCGUCUUUACGGUGCUUGCCUGUUCGUGUCCUGUUGCUGCU3′
R22A(A) (bottom strand for 16-bp duplex with 6-nt A-rich single-stranded region): 5’GCGUCUUUACGGUGCUUAAAAA3′
R22(R) (bottom strand for 16-bp duplex with 6-nt single-stranded region containing no A): 5′GCGUCUUUACGGUGCUUGCCUG3′
R21(A) (bottom strand for 16-bp duplex with 5-nt A-rich single-stranded region): 5′GCGUCUUUACGGUGCUUAAAA3′
R21(R) (bottom strand for 16-bp duplex with 5-nt single-stranded region containing no A): 5′GCGUCUUUACGGUGCUUGCCU3′
R20(A) (bottom strand for 16-bp duplex with 4-nt A-rich single-stranded region): 5′GCGUCUUUACGGUGCUUAAA3′
R20(R) (bottom strand for 16-bp duplex with 4-nt single-stranded region containing no A): 5′GCGUCUUUACGGUGCUUGCC3′
R17 (bottom strand for 16-bp duplex with one unpaired nucleotide): 5′GCGUCUUUACGGUGCUU3′
R36 (top strand for 36-bp duplex substrate): 5′AGCACCGUAAAGACGCAAUCAUGCAGGGUCUGUCAG3′
R61 (bottom strand for 36-bp duplex with 25-nt single-stranded region): 5′CUGACAGACCCUGCAUGAUUGCGUCUUUACGGUGCUUAAAACAAAACAAAACAAAACAAAA3′

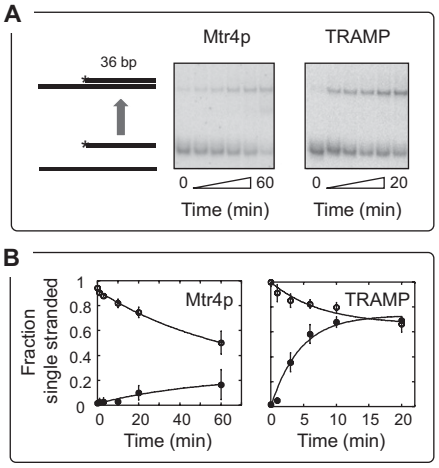

Fig. S1. Strand annealing activity by Mtr4p and Trf4/Air2/Mtr4 polyadenylation (TRAMP). (*A*) Representative PAGE of strand annealing reactions with the 36-bp duplex substrate. Annealing reactions were performed at temperature and buffer conditions identical to those for unwinding reactions. Duplex RNA substrates were denatured at 95 °C (3 min). Denatured single strands (0.5 nM final concentration) were incubated in reaction buffer for 5 min with 2 mM equimolar dATP and $MgCl_2$. Annealing reactions were started by addition of 400 nM Mtr4p or TRAMP. Aliquots were removed after 1, 3, 10, 20, and 60 min with Mtr4p (*Left*), and after 1, 3, 6, 10, and 20 min with TRAMP (*Right*). Reactions were quenched with the same buffer used to stop unwinding reactions. Duplex and single-stranded RNAs were separated as described for unwinding reactions. No notable strand annealing was observed in the absence of TRAMP or Mtr4p (not shown). (*B*) Time courses for strand annealing reactions (○) of the substrate used in *A* (○) compared with time courses for unwinding reactions at identical conditions for Mtr4p (*Left*) and TRAMP (*Right*). Data points are averages from three independent experiments; error bars indicate one SD. Curves represent best fits to the integrated first-order rate law. For Mtr4p, $A_{ann} = 0.70 \pm 0.15$, $k_{obs,\ ann} = 0.02 \pm 0.01$ min^{-1}; for TRAMP, $A_{ann} = 0.35 \pm 0.06$, $k_{obs,\ ann} = 0.13 \pm 0.05$ min^{-1}. Data for the unwinding reactions are from Fig. 2. For Mtr4p, $Amp_{unw} = 0.224 \pm 0.102$, $k_{obs,\ unw} = 0.02 \pm 0.02$ min^{-1}; for TRAMP, $Amp_{unw} = 0.733 \pm 0.057$, $k_{obs,\ unw} = 0.22 \pm 0.05$ min^{-1}. For both Mtr4p and TRAMP, unwinding and strand annealing reactions reached similar amplitudes (Amp_{unw} + Amp_{ann} ~1), indicating a steady state between unwinding and strand annealing that causes the reaction amplitude observed in Fig. 2.

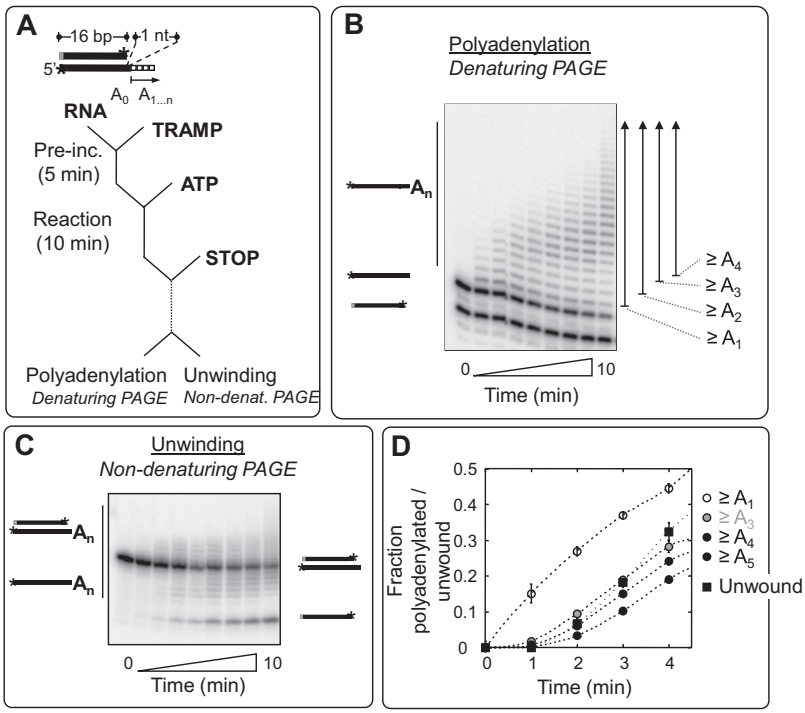

Fig. S2. Simultaneous measurement of polyadenylation and unwinding reaction in solution. (*A*) Reaction scheme. The cartoon shows the substrate, a 16-bp duplex containing a single unpaired nucleotide at the 3′ terminus that was radiolabeled on both strands (marked by the asterisks) .The top strand also contained a 2′,3′-dideoxy modification (light gray bar) at the 3′ end of the top strand to prevent polyadenylation of this strand. Reactions were performed with 0.5 nM RNA substrate, 150 nM TRAMP, and 2 mM equimolar ATP-Mg^{2+}. Two aliquots were simultaneously removed 1, 2, 3, 4, 5, 6, 7, and 10 min after the reaction start, and analyzed for polyadenylation and unwinding. (*B*) Denaturing PAGE to monitor polyadenylation. Schematics for the species observed are marked; the lines on the right highlight the species plotted in *D* (fraction of all species equal or larger than the value indicated). (*C*) Nondenaturing PAGE to monitor unwinding. Lines on the right show the respective RNA species. (*D*) Overlay of plots for polyadenylation and unwinding time courses. The fraction of species with at least 1, 3, 4, and 5 adenines were quantified from denaturing gels as illustrated in *B* at the times indicated (open and filled circles). The fraction of single strand at the same time was quantified from the nondenaturing PAGE shown in *C* (squares). Data points are averages from three independent measurements; error bars indicate one SD. The dashed curves mark trends. Unwinding time courses corresponded to polyadenylation time courses seen for species with at least three or four adenylates, i.e., species with four or five nucleotides.

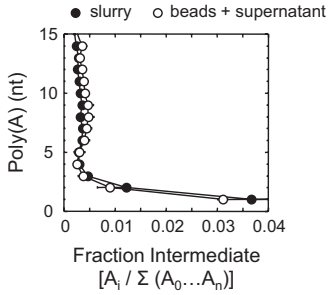

Fig. S3. No significant loss of material following sample separation during simultaneous measurements of polyadenylation and unwinding on beads (Fig. 4). Quantification of sample slurry before centrifugation (●, lane 2 in Fig. 4*B*), and the sum of beads and supernatant samples after centrifugation (○, lanes 3 and 4 in Fig. 4*B*) were plotted for each adenylated species ($A_1 \ldots A_n$). The signal from each species was normalized to the total signal from all species [A_i/Σ ($A_0 \ldots A_n$)]. Data are averages from three independent repeats; error bars indicate one SD. The substrate (A_0) contained a fraction of $f = 0.896 \pm 0.007$ of the total signal in the slurry before centrifugation and a fraction of $f = 0.892 \pm 0.020$ of the total signal after centrifugation.