Supplemental figure S3, related to Figure 3.

(Legend on the next page)
Supplemental figure S3, related to Figure 3.

(A-E) Denaturing RNA gels of in vitro CPF polyadenylation assays using pcCYC1 RNA substrates (see Methods for detailed reaction conditions). 5’ fluorescently-labelled 120 nt pcCYC1 was used in (A), (C) and (D), unlabelled 180 nt pcCYC1 stained with SYBR green was used in (B), and 5’ fluorescently-labelled 42 nt pcCYC1 was used in (E). Adenylated and non-adenylated forms of the substrate are indicated as grey boxes on the righthand side of the gel panels. Native CPF is labeled as nCPF. All assays were repeated at least three times and representative gels are shown.

(A) Time course analysis of polyadenylation by CPF and the cleavage factors CF IA and CF IB, in the presence of different concentrations of Nab2p. The final Nab2p concentration in the reaction is indicated above each gel. (B) Time course analysis of polyadenylation by native CPF in the presence or absence of Nab2p and cleavage factors CF IA and CF IB, as indicated above the gel panels. Final concentration of Nab2p was 600 nM. (C) Time course analysis of polyadenylation by CPF with Nab2p. (D) Time course analysis of polyadenylation by CPF and the cleavage factors CF IA and CF IB, in the presence of different concentrations of Pab1p. The final Pab1p concentration in the reaction is indicated above each gel. Note that the gel panel for 1 μM Pab1p is the same as in Figure 3C and is reproduced here.

(E) Time course analysis of polyadenylation by native CPF and the cleavage factors CF IA and CF IB, in the presence of different concentrations of Pab1p.