Supplement:

Fig. 1 An inhibitor overlaps with the deadenylating activity in the DEAE column. 1 µg of 32P-labelled poly(A) was incubated in a 50 µl reaction with 1 µl of fraction 7 or 1 µl of the indicated fractions or the combination of both in standard deadenylation buffer for 5 min at 30°C. After TCA precipitation the amount of acid-soluble AMP was determined. The sum of the activities of the single fractions is compared with the measured activity of the combined fractions.

Fig. 2 Immunodepletion of poly(A) degrading activity with anti-CAF1 and anti-CCR4 antibodies. (A) Antibodies were directed against Drosophila CAF1 or against human PARN (control). 150 µl of a 50 % suspension of protein A-sepharose were incubated with 350 µl rabbit serum for 1 h at RT. The sepharose was pelleted by centrifugation, washed with 4 x 300 µl IP-buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.02 % NP-40) and resuspended in 100 µl IP buffer. 10 µl of the suspension was mixed with 15 µl of fraction 7 of the DEAE column and incubated for 1 h on ice with occasional mixing. The suspension was filtered through a spin column (BioRad), and the flowthrough was mixed with another 15 µl of immobilized immunoglobulins for 1 h on ice. The sepharose was removed again by filtration. Protein concentration of the filtrate was determined by the Bradford assay. 5 µl of the filtrate after the second round of immunodepletion was mixed in a 200 µl standard poly(A) degradation reaction with 32P-labelled poly(A) (20 ng/µl) and incubated at 30°C. At the time points indicated aliquots were withdrawn, and the amount of acid soluble radioactive material was determined. For comparison, the activity of fraction 7 was measured directly without immunodepletion. (B) Antibodies were directed against Drosophila CAF1 or CCR4. CCR4 pre-immune serum was used as a control. Depletion was carried out as in (A). Note that this experiment was carried out later than the one in (A), and the activity of DEAE fraction 7 had decreased upon storage.

Fig. 3 Analysis of the length of bulk poly(A) in S2 cells after RNAi against components of the CCR4/NOT-complex. S2 cells were incubated for four days with dsRNA directed against the individual mRNAs indicated or with a combination of all dsRNAs. ‘-RNAi’ indicates untreated control cells, additional control cells received dsRNA directed against luciferase mRNA. 1 µg of total RNA was radioactively labelled with 3´d ATP and digested with RNase A and RNase T1 for 30 min at 30 ºC. The products were separated on a 10 % urea-PAGE and visualized using a Phosphor Imager. As a control for the digestion, in vitro synthesized
L3preA(80) RNA was treated like the experimental RNA samples (nd = non-digested, d= digested).

Fig. 4 Analysis of the length of bulk poly(A) from *Rga*^K3j03834^ flies. Total RNA from two independent RNA preparations from wt or *Rga* mutant flies, respectively, was radioactively labelled with 3´dATP and digested with RNase A and RNase T1. The products were separated on a 10 % urea PAA gel and visualized using a PhosphorImager. As a control for the digestion, *in vitro* synthesized L3preA(80) RNA was treated like the experimental RNA samples (nd = non-digested, d= digested). The four panels on the right show pairwise combinations of linear PhosphorImager scans of the RNA samples.

The following primers were used for generating double-stranded RNA for RNAi experiments:

*caf1*: 1. 5' TAATACGACTCACTATAGGGAGATGAAATGGACAATGCGCCCTCG 3'
2. 5' TAATACGACTCACTATAGGGAGATTTGAAGGAGGACTGCGCCCTCG 3'

*ccr4*: 1. 5' TAATACGACTCACTATAGGGAGACAAAGACAAATACGACAGCGC 3'
2. 5' TAATACGACTCACTATAGGGAGACATAGGCTATCTCGCCCTCG 3'

*not1*: 1. 5' TAATACGACTCACTATAGGGAGAGCTCACTCAGCATCAGCCCTCG 3'
2. 5' TAATACGACTCACTATAGGGAGAGTAGGCGAAGGCCCATGCGCCCTCG 3'

*Rga/not2*: 1. 5' TAATACGACTCACTATAGGGAGAGCTCACTCAGCATCAGCCCTCG 3'
2. 5' TAATACGACTCACTATAGGGAGAGGAGGCTCACAGCCCTCG 3'

*not3/5*: 1. 5' TAATACGACTCACTATAGGGAGAGCTCACTCAGCATCAGCCCTCG 3'
2. 5' TAATACGACTCACTATAGGGAGATTTGGAAATGGACAATGCGCCCTCG 3'

*not4*: 1. 5' TAATACGACTCACTATAGGGAGAGCTCACTCAGCATCAGCCCTCG 3'
2. 5' TAATACGACTCACTATAGGGAGAGGAGGCTACAGCCCTCG 3'
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