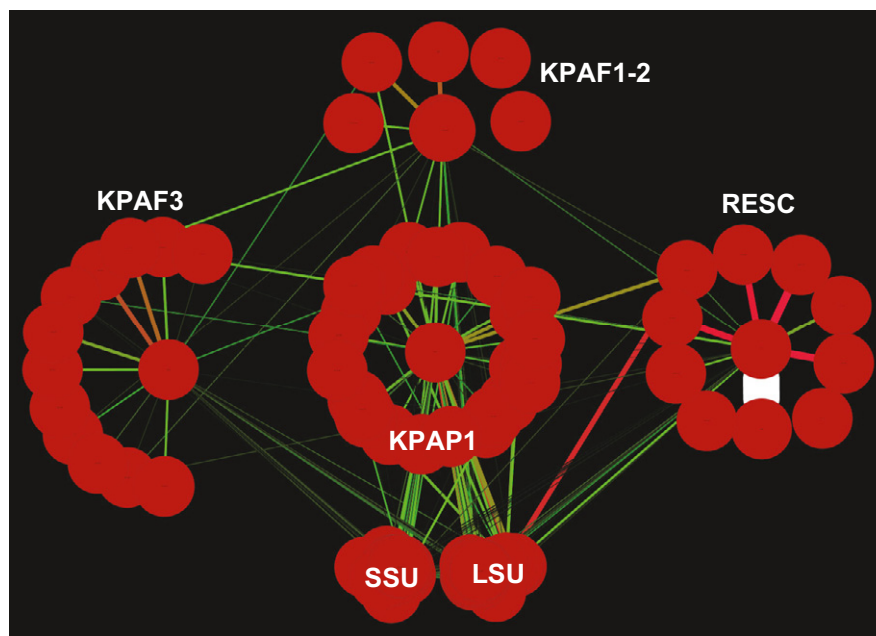


Expanded View Figures

A



B

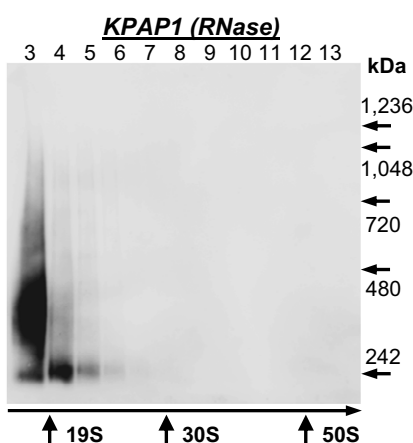


Figure EV1. Model of RNA-independent interactions between KPAP1 poly(A) polymerase, KPAF 1-2, KPAF3, RNA editing substrate binding complex (RESC), and the ribosome.

A KPAP1, KPAF2, KPAF3, GRBC1, L3, and S17 proteins were affinity purified from mitochondrial lysates in the presence of RNase A (0.1 mg/ml) and RNase T1 (500 U/ml). The network was generated in Cytoscape software from bait-prey pairs in which the prey protein was identified by five or more unique peptides (Table EV1). The edge thickness correlates with NSAF values.

B Mitochondrial fraction was extracted with detergent in the presence of RNase A (0.1 mg/ml) and RNase T1 (500 U/ml), and soluble contents were separated for 5 h at 178,000 *g* in 10–30% glycerol gradient. Each fraction was further resolved on 3–12% Bis-Tris native gel. Positions of native protein standards are denoted by arrows. KPAP1 was visualized by immunoblotting. Thyroglobulin (19S) and bacterial ribosomal subunits were used as apparent S-value standards.

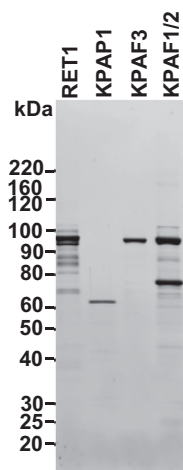


Figure EV2. Recombinant proteins used in reconstitution reactions.

Recombinant proteins were purified from *Escherichia coli* by sequential metal affinity, ion exchange, and size exclusion chromatographic steps. Final fractions were separated on 8–16% SDS–PAGE and stained with Sypro Ruby. Positions of molecular mass markers are indicated.



Figure EV3. Nucleotide frequency per each position in KPAF3-CLAP sequences.

Nucleotide frequency was calculated for each position of KPAF3-protected RNA sequence.