Supporting Information

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SI Text

Purification of *Drosophila* RdRP (D-elp1). S100 *Drosophila* embryo extract was prepared as previously described (1) with modifications. The S100 pellet was resuspended and extracted in buffer containing 1 M KoAc, 30 mM HEPES/KoAc pH 7.8, 5 mM EDTA, and 5 mM β-ME. Extract was spun at 50,000 rpm for 30 min and the supernatant solution was stored at −80 °C. The solution was thawed, spun for clarification at 10,000 rpm, then dialyzed overnight against 30 mM HEPES/KoAc pH 7.8 and 5 mM EDTA. This material was again spun at 50,000 rpm in TLA100 for 30 min and the supernatant, with a 260/280 absorbance ration ~1.0, was used to start the purification. All of the columns were prepacked (Amersham Pharmacia) and run on the AKTA chromatography system (Amersham Pharmacia) with the Unicorn 5.0 software. The S100 extract was fractionated sequentially on HiTrap DEAE FF and the peak of activity eluted between 280 and 380 mM NaCl. The pooled active fractions were precipitated at 60% of saturated ammonium sulfate and further fractionated on a Superose 6 10/300 column equilibrated in a buffer containing 150 mM KCl, 30 mM HEPES/KoAc pH 7.8, and 0.2 mM EDTA. The pooled active fractions, running as a high molecular weight complex, were then fractionated on a HiTrap Heparin HP column preequilibrated with the same buffer used in the Superose 6 column. Bound material was eluted with a step gradient from 450 mM to 600 mM NaCl. This material was then fractionated on 10–40% glycerol velocity gradient with the active fractions located in the middle of the gradient between the 25% and 33% glycerol. Two samples from the velocity gradient, one with no RdRP activity (fraction 4) and the most active fraction (fraction 6), were analyzed by mass spectroscopy (Dr. William Lane, Harvard Microchemistry Facility, Harvard University, Cambridge). Comparison of the proteins identified in each fraction indicated ~25 proteins (Table S1) were highly enriched or unique to the active fraction. CG10535 was unique to the active fraction and was chosen for further analysis. We named this protein, D-elp1 for *Drosophila* elp1 homolog, the largest protein in the RNA polymerase II core elongator complex.

Preparation of Recombinant D-elp1. The entire PCR amplified *Drosophila* D-elp1 reading frame with a C terminus Flag tag was cloned into a modified Bac-to-Bac vector, pFastBac-1, containing the HSP70 promoter instead of the polyhedrin promoter and was used to produce recombinant D-elp1 virus according the manufacturer's protocol (Invitrogen). Virus, at an m.o.i. of ~5, was used to infect Sf9 cultures at a cell density at 1×10^6 cells per mL (500 mL) for 48 h at 25 °C where upon cultures were heat shocked at 37 °C for 45 min. The cells were pelleted and snap frozen and stored at −80 °C. The cells were lysed in buffer containing 30 mM HEPES/KoAc pH 7.8, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA with the addition of protease inhibitors (Complete-C from Roche) and phosphatase inhibitors (1 mM NaF, 8 mM β-glycerophosphate, 0.1 mM NaVanadate, and 2 pills/50 mL of PhosSTOP from Roche). The lysate was spun at 12,000 rpm for 30 min at 4 °C in a Sorvall centrifuge, and the supernatant was bound to Flag-beads (Sigma, clone M2) preequilibrated in lysis buffer. Beads were washed four times with buffer containing 400 mM NaCl, 30 mM HEPES/KoAc pH 7.8, 1% Triton X-100, 10% glycerol, and 1 mM EDTA, and then eluted in buffer containing 500 mM NaCl, 30 mM HEPES/KoAc pH 7.8, 1% Triton X-100, 10% glycerol, and 1 mM EDTA with the addition of Flag peptide at the concentration of 2.5 mg/mL. Eluted protein was dialyzed overnight with three changes of buffer in 30 mM HEPES/KoAc pH 7.8, 10% glycerol and 0.2 mM EDTA to reduce the concentration of Flag peptide. Proteins were quick frozen and stored at −80 °C in aliquots. Protein concentrations were determined using the Nano-orange kit (Molecular Probes) with BSA as a standard. The same protein preparations were run with the BSA standard on polyacrylamide gels stained with Colloidal Blue (Invitrogen).

To prepare recombinant D-elp1 in *Escherichia coli*, Rosetta BL21-LysS cells, or BL21-CodonPlus (DE3)-RIPL were transfected with a D-elp1 Pet 28 plasmid containing the C-terminal Flag tagged D-elp1 cDNA and expression was carried out essentially as described (2) except that the induction temperature was 16 °C. D-elp1 and its deletions described in the text and Fig. 2*A* were detected by Western blot with T7 tag antibody, HRP conjugated, at a 1:10,000 dilution (Novagen). Proteins were transferred to nitrocellulose and blocked with PBST (PBS with 0.1% Tween and 3% BSA). Incubation with the antibody was performed in PBST for 1 h. ECL detection was performed after three washes with PBST.

Recombinant *Drosophila* Dicer-2. *Drosophila* Dicer-2 cDNA was prepared by RT-PCR and cloned into the Bac-to-Bac baculovirus expression vector pFastBac Ht to prepare viral stocks in Sf9 cells for expression as described. Dicer-2 was prepared from infected Sf9 cells grown for 48–60 h using the same buffer used to prepare D-elp1. After centrifugation at 10,000 rpm for 30 min, the supernatant was bound and eluted from Flag-agarose beads, as described above. The eluted material was subsequently subjected to additional steps of purification. First, the proteins were loaded onto a MonoS column where the flow through was collected, then loaded onto a MonoQ column to concentrate the proteins for separation on a Superdex-200 column.

Primed Fill in Assay. The assay was performed using 716 bp GFP dsRNA with ~40 bp of 5′ overhang on each end, as previously described (3, 4). Short double stranded RNA was also used as a template in some reactions. In this instance, two RNA oligonucleotides of 30 bp and 48 bp were chemically synthesized (Invitrogen) with the following sequences: 30 bp: 5′-GCGUUCAA**C**UAGCAGACCAUUAUCAACAAA-3′; 48 bp: 5′-CAUCGCCAAUUGGAGUAUUUUGUUGAUAAUGGUCUGCUAGUUGAACGC-3′. Oligos were dissolved in TE at the concentration of 100 pmol/μL. Equimolar concentrations were annealed in TE pH 7.4 and 150 mM NaCl to generate dsRNA with an 18-bp 5′ extension. Two identical DNA oligonucleotides were chemically synthesized (Invitrogen), dissolved in TE, and annealed to form dsDNA with the same 5′ overhang. The annealed oligos were analyzed on a native 2% agarose gel. Both the dsRNA and dsDNA oligos were tested in the fill in assay.

Unprimed Synthesis Assay. Unprimed synthesis assays were performed using 50–100 ng of Flag-purified D-elp1 with 0.5 μg of an SP6 or T7-generated single-stranded RNA template in a reaction containing 30 mM HEPES-KOAc pH 7.8, 20 mM NH_4Ac, 6% (wt/vol) PEG4000, 5 mM MgOAc, 0.1 mM EDTA, 10 U RNase OUT, 1 mM ATP, 1 mM CTP, 1 mM GTP, 0.1 mM UTP, and 20 μCi of α-^{32}P UTP (3, 4). The 20-μL reaction was incubated at 25 °C for 1–2 h in the same buffer used in the fill-in assay. RNAs were purified as before and analyzed on 6% polyacrylamide-8M urea gels. The reactions were digested with

RNase I (NEB) or a mixture of RNase A/T1 (Ambion) according to the manufacturer's instructions.

Nearest Neighbor Analysis. Nearest neighbor analysis was essentially performed as previously described (4) with the exception that Ribonuclease T2 (NEB) digestion was replaced with RNase One. The digestion products were spoted on PEI TLC plates with fluor using 3′-ribonucleotide monophosphate standards and chromatographed as described (5).

Dicer-2 Processing Assay. The processing reaction was conducted in the presence of recombinant Drosophila Dicer-2 in a buffer containing 100 mM KoAc, 30 mM HEPES/KoAc pH 7.4, 5 mM DTT, 5 mM $MgCl_2$, and 40 U RNase OUT. The reaction was incubated for 2 h at 25 °C. After proteinase K treatment and phenol/chloroform/isoamylalcohol extraction, the aqueous phase was precipitated with three volumes of cold ethanol, resuspended in 4 μL of water, and heated at 65 °C for 5 min. Then 10 μL of formamide loading buffer was added and the sample heated to 95 °C. The RNA was loaded onto a 6% or 15% polyacrylamide gel with 8 M urea.

Primed Synthesis Assay. Primer extension was performed essentially as described (3). In brief, a synthetic GFP siRNA with 3′ overhangs complementary to GFP RNA, 5′-GCGTTCAATT-AGCAGACCA-3′, was 5′-labeled with T4-PNK and [γ-^{32}P] ATP on each strand and each strand was mixed separately with template GFP mRNA, heated to 85 °C for 5 min then annealed at 48 °C for 10 min. The extension assay was conducted with 40 μg/mL of D-elp1 and all four unlabeled ribo NTPs. The 3′ phosphorylated antisense siRNA strand was chemically synthesized by Dharmacon. The reaction products were separated on a 6% polyacrylamide gel with 8 M urea. An approximately 600-bp extension product is predicted with the antisense guide strand.

DsRNA Preparation. cDNA fragments from 150 to 600 bp for different candidate genes were cloned into the TOPO TA vector (Invitrogen). After linearization with the appropriate restriction enzymes, the linear plasmids were transcribed and the RNA products annealed, as previously described (1). The primers used to amplify the different cDNA fragments from genomic DNA are described in Table S2.

Protein Interaction Studies in S2 Cells. S2 cells were transfected as previously described (6) with with pITZ/V5-His vector constructs containing a V5 C-terminal tag for Dicer-1, Dicer-2, Argonaute 2, and a Flag-tag at the C terminus of D-elp1. Cells were transfected with FugeneHD (Roche) according the manufacturing protocol. After 72 h, cells were collected, washed with cold PBS once and lysed in a buffer containing 30 mM HEPES/KoAc pH 7.8, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, and inhibitors of phosphatases (Roche). Lysates were spun at 10,000 × *g* at 4 °C for 30 min, and the supernatant was bound to V5-agarose beads (Sigma-Aldrich) containing 10 μg/mL RNase A. After an overnight binding, the beads were washed four times with the lysis buffer and proteins, eluted by boiling the beads for 5 min in 2X Laemli buffer, were loaded on 4–12% polyacrilammide gels. Proteins were transferred to nitrocellulose using the iBlot Dry Blotting System (Invitrogen) and probed with Flag-HRP antibody, clone M2 (Sigma) at the dilution 1:10,000 using the One-Step IP Western Kit following the included protocol (GenScript Corp). For the reciprocal experiments, proteins were immunoprecipitated with Flag-agarose beads (Sigma) and blotted with V5 HRP antibody (Invitrogen) at the dilution 1:5,000 using the One-Step IP Western Kit.

Luciferase Assay. S2 cells were plated in 96-well culture plates and grown to 60% confluence. Typically 60,000 cells were resuspended in a 75 μL volume. Cells were incubated at 25 °C overnight and were then soaked with either D-elp1, D-elp2, D-elp3, Dicer2, or GFP dsRNA (40 μg/mL) for 4 days. Cells were then replated, and soaking was repeated with the same dsRNAs for an additional 4 days. On day 2 of the second knockdown cycle, cells were transfected with the Renilla and firefly luciferase reporters and dsRNA to firefly luciferase. After an additional 2 days, a portion of the cells were assayed for the knockdown of D-elp1, D-elp2, D-elp3, and Dicer2, while the remaining cells were subjected to the Luciferase RNAi assay using the Dual Gluo system (Promega) according to the manufacturer's protocol. Each assay was repeated at least three times, independently. The RNAi transfection mix contained 20 ng of *Pp*-Luc, 20 ng of Renilla luc, and Fugene 6 (Roche).

Immunolocalization. Indirect immunofluorescence was conducted on S2 cells expressing Flag-tagged D-elp1 from pIZT (Invitrogen). Cells were plated on slides coated with polylisine and processed for immunostaining, as previously described. Cells were fixed with 3.7% formaldehyde for 15 min then permeabilized with methanol for 10 min and stained with the anti-Flag monoclonal antibody, clone M2 (Sigma, Cat. # F3165) at a concentration of 25 ng/mL. S2 cells were also stained with antibody to the RNA polymerase II CTD repeat, YSPTSPS (Abcam, Cat. # 5408) at the concentration of 1 μg/mL (1:1,000). Pictures were taken on a Zeiss LSM 510 confocal (NCI core facility).

RT-PCR and Quantitative PCR. Methods have been described in ref. 7. Briefly, total RNA was isolated from D-elp1, D-elp2, D-elp3, and Dcr-2 depleted S2 cells using TRIzol (Invitrogen). For quantitative RT-PCR analysis, typically 2 μg of total RNA was digested with Turbo DNase I (Ambion) and reverse transcribed with SuperScript III (Invitrogen) according to the manufacturer's instructions. The cDNA was analyzed by RT-PCR in a DNA Engine OPTICON 2 (MJ Research, Bio-Rad) with a SYBER Green PCR kit (Bio-Rad). The primers used are listed in Table S3. The mRNA levels were calculated after normalization to Rrp49 levels and then to the value from GFP dsRNA-treated S2 cells using the $\Delta\Delta C_T$ method (8). Antisense transcript levels for RNAs derived from transposons 297 and mdg1 were determined by quantitative RT-PCR in S2 cells treated with dsRNA to GFP or D-elp1 or Dcr-2. In brief, antisense cDNAs were synthesized as before with primers specific for the antisense transcript for each transposon. The cDNA was analyzed as above. The antisense mRNA levels were calculated after normalization to Rrp49 RNA levels. The primers used to synthesize the antisense cDNAs were as follows: mdg1: F-5′-ACCAAGAAGATTGGT-TCACTT-3′, 297: F-5′-CATAGCTTCATTAATGAAGG-3′. The primers used for RT-PCR are listed in Table 3.

Total levels of 297, mdg1, and Het-A transposon RNAs were analyzed by quantitative RT-PCR from Canton S and D-elp1 null larvae, as described for S2 cells. Transposon antisense RNA levels for 297 and mdg1 in larvae were evaluated as described above.

Endo siRNA Analysis in S2 Cells. Analysis was essentially performed as described by Hamilton and Balcoumbe (9). Total RNA was extracted from S2 cells treated with dsRNA against GFP, Dicer2, and D-elp1 using TRIzol and was enriched for small RNAs using the mirVana kit (Ambion). The RNA was loaded on 15% PAGE/8 M Urea gels and transferred to Hybond N+ (Amersham) using the TransBlot SD (Bio-Rad). The membrane was UV crosslinked with a TransUV (Startagene) twice at 1,200 joules. The prehybridization was perfomed with the UltraHyb-

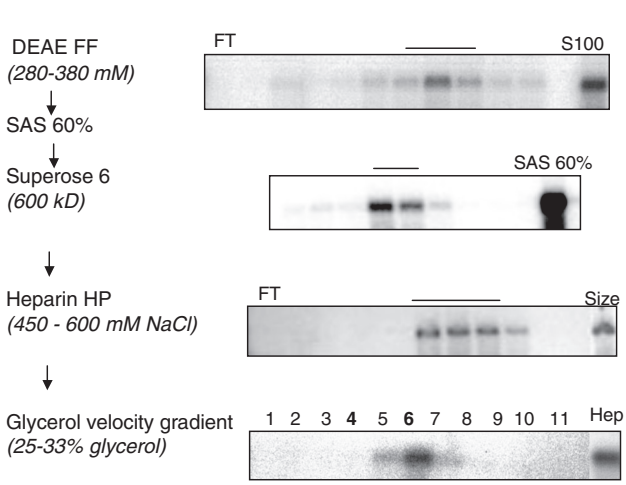
oligo buffer (Ambion) for 1–2 h. The sense and antisense probes against transposons 297 and mdg1 were transcribed with the Ambion T3 and T7 MegaScript kits using high specific activity α^{32}P-UTP and TOPA-TA (Invitrogen) plasmids containing a 149-bp insert for mdg1 and a 193-bp insert for 297. The RNA probe was hydrolized in alkali (Na_2Co_3 and $NaHCO_3$) and heated 1 min at 95 °C, just before it was added to the hydrization solution. Hybridization was carried out for 16 h. Washes at low strincency were carried out in 2× SSC and 0.2% SDS at 42 °C, twice briefly to remove the probe, and twice for 30 min. The membrane was reprobed for each endo siRNA strand after stripping with 0.2% SDS, 10 mM Tris/HCl pH 7.5 1 min at 90 °C and checking for complete removal of previous probe. Rrp49 was used as an RNA loading control.

1. Lipardi C, Wei Q, Paterson BM (2001) RNAi as random degradative PCR: siRNA primers convert mRNA into dsRNAs that are degraded to generate new siRNAs. *Cell* 107:297–307.
2. Tolia NH, Joshua-Tor L (2006) Strategies for protein coexpression in *Escherichia coli*. *Nat Methods* 3:55–64.
3. Makeyev EV, Bamford DH (2002) Cellular RNA-dependent RNA polymerase involved in posttranscriptional gene silencing has two distinct activity modes. *Mol Cell* 10:1417–1427.
4. Lipardi C, Baek HJ, Wei Q, Paterson BM (2005) Analysis of short interfering RNA function in RNA interference by using *Drosophila* embryo extracts and schneider cells. *Methods Enzymol* 392:351–371.
5. Volckaert G, Fiers W (1977) A micromethod for base analysis of 32P-labeled oligoribonulcleotides. *Anal Biochem* 83:222–227.
6. Wei Q, Marchler G, Edington K, Karsch-Mizrachi I, Paterson BM (2000) RNA interference demonstrates a role for nautilus in the myogenic conversion of Schneider cells by daughterless. *Dev Biol* 228:239–255.
7. Ghildiyal M, et al. (2008) Endogenous siRNAs derived from transposons and mRNAs in *Drosophila* somatic cells. *Science* 320:1077–1081.
8. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.
9. Hamilton AJ, Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286:950–952.

A

	Total Volume (ml)	Total Protein (mg)	Total Activity (PSL/mm2)	Specific Activity	Purification	Recovery %
S100	60	600	120,000	200	1	"100"
S100 after dialysis	40	200	224,000	1,120	5.6	100
DEAE FF	110	22	180,000	8,200	41	80
SAS 60%	3.2	18	130,000	7,222	36	58
Size	22	11	110,000	8,500	42	49
Heparin	10	2	90,000	45,000	225	40
Velocity Gradient	10	0.5	87,000	174,000	870	39

B



C

MRNLKLRYCKELNAVAHPQHLLLQPELNGGASDIYFVVADNK**IYAVQESGDVR**LKVIADLPDIVGVEFLQ
LDNAICVASGAGEVILVDPQTGATSEGTFCDVGIESMAWSPNQEVVAFVTRTHNVVLMTSTFDVIAEQPL
DAELDPDQQFVNVGWGKKETQFHGSEGKQAAKQKESDSTFIRDEQELNQDVSISWRGDGEFFVVSYVAAQ
LGRTFKVYDSEGKLNHTAEKSANLKDSVVWRPTGNWIAVPQQFPNKSTIALFEKNGLRHR**ELVLPFDLQE**
EPVVQLRWSEDSDILAIRTCAKEEQRVYLYTIGNYHWYLKQVLIFEQADPLALLHWDTRCGAEHTLHVLK
ESGKHLVYRWAFAVDRNNSIVGVIDGKRLLLTDFDEAIVPPPMSKIVLKFETYINAFISHGTSLWVYTCD
RKIYLNEHIHTLGKELQKPIMLMPDAELSGLHLANLTHFSPHYLLATHSSAGSTRLLLLSYKDNDNKPGE
WFYRVHSSVRINGLVNAVAVAPYAMNEFYVQTVNNGHTYEVSLKADKTLKVERSYVQLHEPADQIDWVIV
KGCIWDGYTGALVTLRNQHLLHIDGYRIGEDVTSFCVVTNYLVYTQLNAMHFVQLDDRRQVASRNIERGA
KIVTAVARKARVVLQLPRGNLEAICPRVLVLELVGDLLERGKYQKAIEMSRKQRINLNIIFDHDVKRFVS
SVGAFLNDINEPQWLCLFLSELQNEDFTKGMYSSNYDASKQTYPSDYRVDQKVEYVCRLLEQQMNRFVSR
FRLPLITAYVKLGCLEMALQVIWKEQQEDASLADQLLQHLLYLVDVNDLYNVALGTYDFGLVLFVAQKSQ
KDPK**EFLPYLNDLK**ALPIDYRKFRIDDHLKRYTSALSHLAACGEQHYEEALEYIRKHGLYTDGLAFYREH
IEFQKNIYVAYADHLRAIAKLDNASLMYERGGQLQQALLSAKHTLDWQRVLVLAK**KLSEPLDQVAQSLVG**
PLQQQGRHMEAYELVKEHCQDRKRQFDVLLEGHLYSR**AIYEAGLEDDDVSEK**IAPALLAYGVQLESSLQA
DLQLFLDYKQRLLDIRRNQAKSGEGYIDTDVNLKEVDLLSDTTSLHSSQYSGTSRRTGKTFRSSKNRRKH
ERKLFSLKPGNPFEDIALIDALHNHVTKIAQQQQPVRDTCK**ALLQLANAADADPLAAALQR**EFKTLLQAV
DAALDEIWTPELRGNGLMADHLTGPNVDYLALQKEQRYALLSPLKRFKPQLIMMDWQHEILQ

Fig. S1. Purification of *Drosophila* D-elp1 RdRP. (*A*) Cell free extract was prepared from *Drosophila* embryos as described in *SI Text*. A table of purification is shown starting from the high salt extract of the ribosomal pellet (S100) described in ref. 1. (*B*) The dialyzed extract from the ribosomal pellet was further fractionated sequentially on DEAE FF, Superose 6, and Heparin HP columns. The last step of purification was a glycerol velocity gradient (10–40%). The proteins in each fraction from the columns and gradient were tested for RdRP primed fill in activity, as described in *SI Text*, and initilly analyzed on denaturing formaldehyde 1.5% agarose gels. The apparent molecular weight, concentrations of salt or glycerol in the pooled active fractions are in parentheses and correspond to the horizontal bars over each panel. Active (fraction 6) and inactive (fraction 4) glycerol fractions are marked with bold numbers. (*C*) The amino acid sequence of *Drosophila* D-elp1 is shown and the peptides identified by mass spectroscopy analysis are indicated in bold (W. Lane, Harvard Microchemistry Laboratory). The two peptides found twice are underlined.

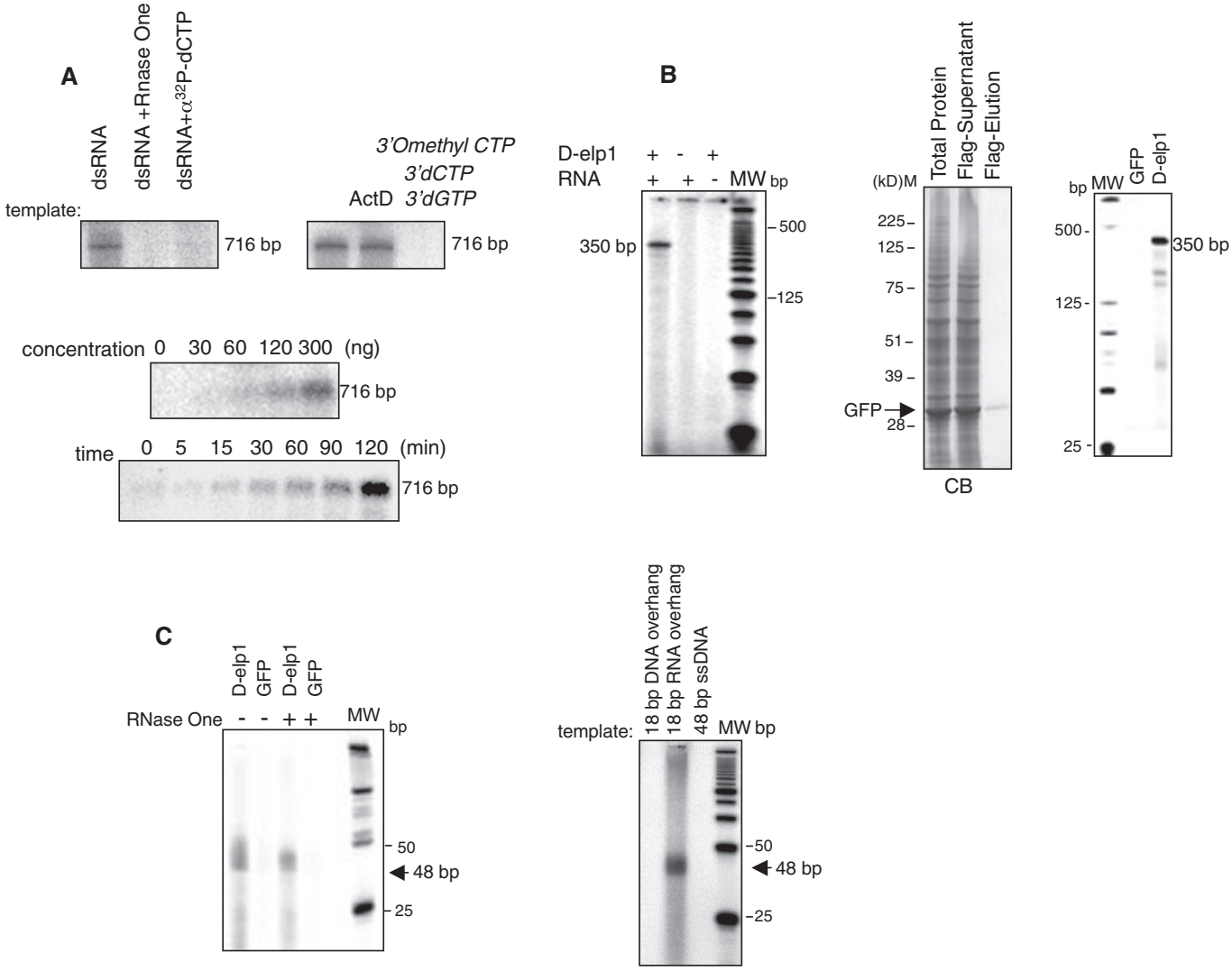


Fig. S2. Recombinant D-elp1 from Sf9 cells has RdRP activity. (*A*) The GFP substrate used in the primed fill-in assay was also used by baculovirus recombinant D-elp1. Substitution of α^{32}P-UTP with α^{32}P-dCTP resulted in no labeling and predigestion of the template with RNase I to remove the 5′ overhangs blocked incorporation of α^{32} P -UTP, as described in ref. 5 and in *SI Text* (*Upper Left*). Actinomicin D at concentrations that inhibited RNA synthesis in S2 cells >95% did not inhibit D-elp1 primed fill in activity but substitution of CTP and GTP with either dCTP, dGTP, or 3′-O-Methyl CTP blocked activity entirely (*Upper Right*). Labeling of the primed fill-in substrate was concentration and time dependent (*Center* and *Lower*), as given in the text. (*B*) D-elp1 protein and the ssRNA template are both required for unprimed RNA synthesis (*Left*). Sf9 cells were infected with a GFP virus as a control and processed similarly to cells infected with D-elp1 virus. Proteins were stained with Colloidal Blue, CB (*Center*). D-elp1 and the GFP negative control, prepared identically from Sf9 cells infected with a GFP baculovirus, were tested for unprimed synthesis and only D-elp1 produced labeled RNA (*Right*). (*C*) In a variation of the primed fill-in assay using a 30 bp dsRNA with an 18-bp 5′ extension (described in *SI Text*) only D-elp1, but not proteins prepared from Sf9 cells infected with the GFP negative control virus, was able to produce the expected RNase One resistant 48-bp product. A dsDNA version of the same substrate with a 5′ extension identical to the dsRNA substrate used in the *Left*, as well as a 48-bp single-stranded DNA oligo were not labeled by D-elp1 (*Right*); only the dsRNA primed fill in substrate was labeled.

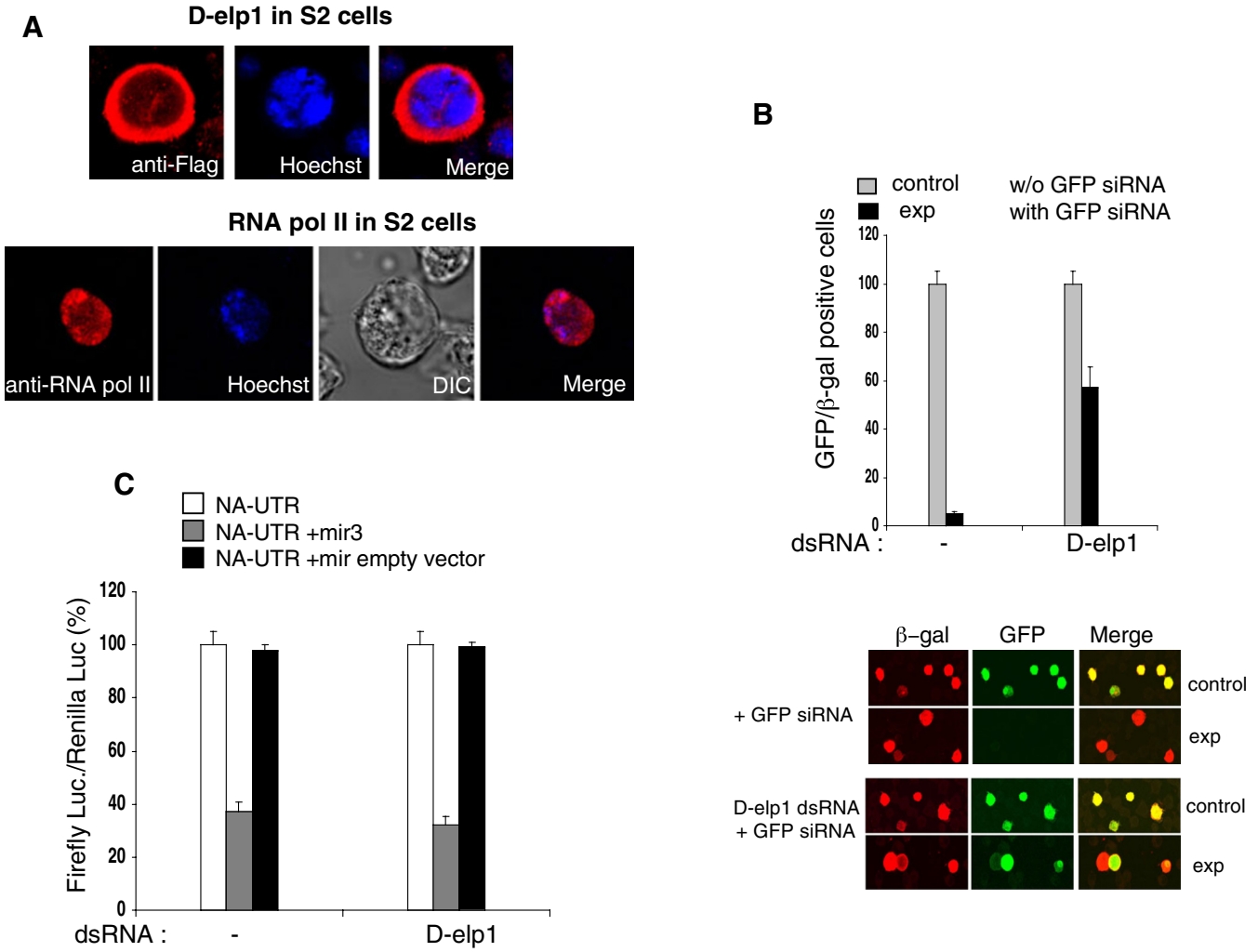


Fig. S3. D-elp1 is localized in the cytoplasmic compartment and inhibits RNAi but not miRNA function. (*A*) S2 cells expressing Flag-tagged D-elp1 were incubated with M2 Flag-antibody. D-elp1 is predominately in the cytoplasmic compartment (α-Flag: red) rather than in nuclei (Hoechst: blue) but a minor amount of nuclear staining is observed. As a control for RNA pol II and the location of core elongator, S2 cells were stained with antibody against the CTD of DNA-dependent RNA polymerase II. Note the cytoplasmic localization of D-elp1 and the nuclear localization of pol II CTD. (*B*) D-elp1 depleted S2 cells were cotransfected with GFP and β-galactosidase reporters with and without a siRNA to GFP, as described in ref. 4. D-elp1 depletion inhibits siRNA-mediated RNAi. (*C*) Depletion of D-elp1 does not affect miR-3 targeting of the *nautilus* gene transcript. The ratios of firefly to Renilla luciferase activity, in the presence or absence of mir3, were determined in three separate experiments.

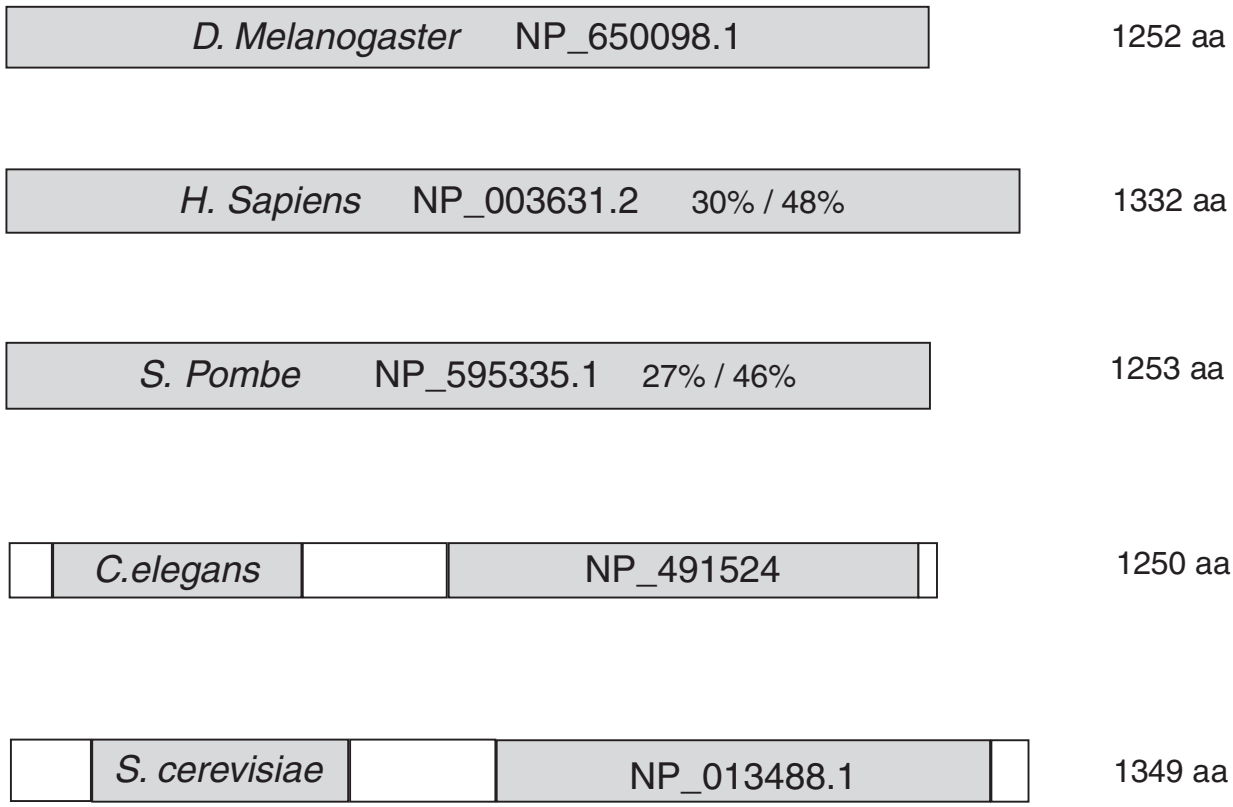


Fig. S4. Sequence conservation among the elongator protein-1 (elp-1) related proteins. The accession number, the protein length, and the percentage of residues with positive identity/similarity are indicated for each protein. The *S. cerevisiae* and *C. elegans* elp-1 homologs have two extensive gaps compared to the other elp-1 related proteins so the percentage of positive identity/similarity has not been calculated. Results to be presented elsewhere indicate all of the elp-1 related proteins shown, with the exception of *S. cerevisiae* elp-1, have RdRP activity.

Table S1.

CG number	Functions	No. of peptides
CG10535	Immune defence	8
eIF3		
CG9805	Stress granule marker	46
CG4878	Stress granule marker	26
CG9124	Stress granule marker	17
CG9769	Stress granule marker	15
COP9 complex		
CG3889	COP9S1b	9
CG9556	COP9S2	21
CG18332-PA	COP9S3	20
CG8725-PA	COP9S4	26
CG14884	COP9S5	22
CG6932-PA	COP9S6	12
CG8309	probable COP9S7	23
CG2038	COP9S7	12
CG13383-PA	COP9S8	4
DNA polymerase		
CG1091-PB	DNA polymerase sigma	16
CG6768	DNA polymerase epsilon	2
Heat Shock Protein		
CG4264-PF	Heat shock protein cognate 4	26
CG5525	TCP-1 chaperonin family	2
CG1242	Hsp83	11
Mcm proteins		
CG4039	mcm6	2
CG1616	mcm4	1
Helicase		
CG8103	ATP dependent helicase Mi-2	6

Table S2. List of primers for cloning dsRNA

	Forward	Reverse
D-elp1.A (CG10535)	AATCAGCATCTACTACATATCG	GTTTGGACGCATCATAATTACT
D-elp1.B (CG10535)	GGAGAGCAGCATTACGAGGAA	TTCGATCCTGACAGTGCTCCTAGGAGCACTGTCAGGA
Ago-2 (CG7439)	AGCACAACCTGGATCCGACCA	AGGATAGGCGTTACGGTACTC
Dicer-2 (CG6493)	AAGGATCCGTCGCATTTGCTTAGCTGCT	AGATCTACTTCAAGATGCTAGAG TACTTC
D-elp2 (CG11887)	TGA CAA GAC CGT CAT AAT TTG	AGT TGT TTG GTC TGC AGA CGC
D-elp3 (CG15433)	ACT CGA CAC AGT CGT ACA CAG	CCA TAG CTA AGC ATA TCC GAT
297	AAAGGGCGTTCATACAAATG	TGTGCACATAAAATGGTTCG
mdg1	CACATGTTCTCATTCCCAACC	TTCGCTTTTTATATTTGCGCTAC

Table S3. List of primers for RT-PCR and quantitative PCR

	Forward	Reverse
D-elp1 (CG10535)	ATAGCGCCCGCTTTGTTAGCA	GGAGTGGAGACTGGTGGTGTCCACAGTAGGTCCAC
Ago-2 (CG7439)	GTCGATCGCACCATTGTGCAT	GTCAACTGCTGCAACAAGTCG
Dicer-2 (CG6493)	GAGCTGCTCCATCAGTTTCA	TCCCAGTCAAAGCATTTCTGT
D-elp2 (CG11887)	ATC TCC GTG AA C TG G CGC TGG	CCT ACG AGA ATG TCC TGT TCA
D-elp3 (CG15433)	CAA AGG AGC TTC CGT GCC TTC	GGC CAT AAA GCT TCT TCA GCT
Rrp49 (CG7939)	TACAGGCCCAAGATCGTGAA	ACCGTTGGGGTTGGTGAG
297	AAAGGGCGTTCATACAAATG	TGTGCACATAAAATGGTTCG
mdg1	CACATGTTCTCATTCCCAACC	TTCGCTTTTTATATTTGCGCTAC
Het-A	CGCGCGGAACCCATCTTCAGA	CGCCGCAGTCGTTTGGTGAGT
F-element	GCTGGTAGATACCGCTGAGG	GTAGTCGTCCTCCGTTTTCG