Retraction and Corrections

RETRACTION

BIOCHEMISTRY

The authors wish to note the following: “In our article, we reported that recombinant subunit 1 of the Drosophila elongator complex, D-elp1, had RNA-dependent RNA polymerase (RdRP) activity and was involved in RNAi, transposon suppression, and endo-siRNA production, but not miRNA targeting. RdRP activity was identified using three assays to interrogate the reaction products: Dcr2 digestion, RNase sensitivity, and nearest neighbor analysis. Although we do see differential Dcr2 cleavage and RNase resistance, the gold standard for second strand RNA synthesis is nearest neighbor analysis, as described previously for the Neurospora crassa RdRP, QDE-1 (1). Subsequent studies revealed that the nearest neighbor analysis was misinterpreted because of two factors unknown to us at the time: First, T7 run-off transcripts used as single-stranded RNA templates have heterogeneous 3′ termini (2, 3); Second, the Flag-tag affinity purified recombinant D-elp1 protein preparations used in our study contain a ribonucleotide terminal transferase activity able to catalyze the addition of single a-labeled ribonucleotide triphosphates to the 3′ hydroxyl terminus of the template RNA. Therefore, nearest neighbor analysis measured the heterogeneous 3′ termini of the template RNA and not second strand RNA synthesis, as initially reported. Given this result, we wish to rescind the interpretation that D-elp1 can be considered an RNA-dependent RNA polymerase. The basis for the ribonucleotide terminal transferase activity is under investigation. The interpretation of the results does not detract from the fact that D-elp1 is involved in RNAi, endo-siRNA production, and transposon suppression, but not miRNA targeting as shown in Figs. 3 and 4. By clarifying this issue, we hope to promote further productive investigation into the role of elongator subunit 1 in RNAi and transposon suppression. We apologize for any difficulties our original interpretation may have caused other investigators and hereby retract the paper.”

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CORRECTIONS

BIOCHEMISTRY

The authors note that the following statement should have been added to the Acknowledgments: “Financial support was provided by the Deutsche Forschungsgemeinschaft (DFG, Ni 111812108).”

Additionally, the keyword “RNA interface” should instead appear as “RNA interactions.” The online version has been corrected.

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Identification of an RNA-dependent RNA polymerase in *Drosophila* involved in RNAi and transposon suppression

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Here, we show that recombinant *Drosophila* elp1 (D-elp1) produced in Sf9 cells or *Escherichia coli*, corresponding to the largest of the three subunits in the RNA polymerase II core elongator complex, has RNA-dependent RNA polymerase (RdRP) activity. D-elp1 is a noncanonical RdRP that can synthesize dsRNA from different ssRNA templates using either a primer-dependent or primer-independent initiation mechanism. Of the three core subunits, only D-elp1 depletion inhibits RNAi in S2 cells but does not affect micro RNA function. Furthermore, D-elp1 depletion results in increased steady state levels of representative transposon RNAs and a decrease in the corresponding transposon antisense transcripts and endo siRNAs. In contrast, although Dcr-2 depletion results in increased transposon RNA levels and a reduction in the corresponding endo siRNAs, there is no change in the transposon antisense RNA levels. In D-elp1 null third instar larvae transposon RNA levels are also increased and the corresponding transposon antisense RNAs are reduced. D-elp1 associates tightly with Dcr-2, similar to the Dicer-RdRP interaction observed in lower eukaryotes. These results identify an aspect of the RNAi pathway in *Drosophila* that suggest transposon derived endo siRNAs, critical for transposon suppression, are produced, in part, in a D-elp1 dependent step that converts transposon RNA into dsRNA that is subsequently processed by Dcr-2. The generality of this mechanism in genome defense and RNA silencing in higher eukaryotes is suggested.

Results and Discussion

Identification of D-elp1 as an RdRP. To characterize the proteins involved in siRNA-primed dsRNA synthesis identified previously (8), *Drosophila* embryo extract was fractionated by ion exchange chromatography (DEAE and Heparin Sepharose), gel filtration (Superose 6), and a final velocity sedimentation step (15–45% glycerol gradient) (Fig. S1A). Fractions were dialyzed against reaction buffer and tested for primed fill in synthesis using full length GFP dsRNA with approximately 40 bp of single-stranded 5′ overhang on each end as the template, and 0.1 to 2.0 μg of protein under reaction conditions outlined in *SI Text* and described previously, with minor buffer modifications (8) (Fig. S1B). Reaction products were analyzed on 1.5% agarose gels due to the size of the labeled RNA. Fill-in specific activity of siRNA-primed dsRNA synthesis came from (i) observation of siRNA-primed dsRNA synthesis in *Drosophila* (9, 10) and (ii) copy number-dependent post transcriptional silencing of an *Adh* transgene that is correlated with the appearance of *Adh* siRNAs, derived from *Adh* dsRNA generated in response to increased levels of *Adh* mRNA (10). Deep sequence analysis of endogenous *Drosophila* siRNAs (endo siRNAs) from embryos, larval discs, and adult structures, as well as from S2 cells and Kc cells, indicates almost twenty percent of the endo siRNAs are derived from transposable elements in a Dcr-2-dependent process and represent both the sense (41%) and antisense strand (59%) of the transposon RNAs along the entire body of the transposable element, consistent with the formation of transposon dsRNA (11, 12). In line with these observations, significant amounts of cytoplasmic, polyadenylated dsRNA, representing the entirety of the transposons mdg1 and mdg3, was identified in the *Drosophila* cell line 67J25D by the Georgiev laboratory nearly 30 years ago (13). At that time, the authors proposed either symmetric transcription or RNA-dependent RNA synthesis gave rise to the mdg1/mdg3 dsRNAs. In this study we show that elongator subunit 1 of the *Drosophila* pol II core elongator complex, D-elp1, has RdRP activity and plays a role in both RNAi and the Dcr-2 dependent formation of transposon specific endo-siRNAs correlated with transposon silencing.

### Results and Discussion

**Identification of D-elp1 as an RdRP.** To characterize the proteins involved in siRNA-primed dsRNA synthesis identified previously (8), *Drosophila* embryo extract was fractionated by ion exchange chromatography (DEAE and Heparin Sepharose), gel filtration (Superose 6), and a final velocity sedimentation step (15–45% glycerol gradient) (Fig. S1A). Fractions were dialyzed against reaction buffer and tested for primed fill in synthesis using full length GFP dsRNA with approximately 40 bp of single-stranded 5′ overhang on each end as the template, and 0.1 to 2.0 μg of protein under reaction conditions outlined in *SI Text* and described previously, with minor buffer modifications (8) (Fig. S1B). Reaction products were analyzed on 1.5% agarose gels due to the size of the labeled RNA. Fill-in specific activity of siRNA-primed dsRNA synthesis came from (i) observation of siRNA-primed dsRNA synthesis in *Drosophila* (9, 10) and (ii) copy number-dependent post transcriptional silencing of an *Adh* transgene that is correlated with the appearance of *Adh* siRNAs, derived from *Adh* dsRNA generated in response to increased levels of *Adh* mRNA (10). Deep sequence analysis of endogenous *Drosophila* siRNAs (endo siRNAs) from embryos, larval discs, and adult structures, as well as from S2 cells and Kc cells, indicates almost twenty percent of the endo siRNAs are derived from transposable elements in a Dcr-2-dependent process and represent both the sense (41%) and antisense strand (59%) of the transposon RNAs along the entire body of the transposable element, consistent with the formation of transposon dsRNA (11, 12). In line with these observations, significant amounts of cytoplasmic, polyadenylated dsRNA, representing the entirety of the transposons mdg1 and mdg3, was identified in the *Drosophila* cell line 67J25D by the Georgiev laboratory nearly 30 years ago (13). At that time, the authors proposed either symmetric transcription or RNA-dependent RNA synthesis gave rise to the mdg1/mdg3 dsRNAs. In this study we show that elongator subunit 1 of the *Drosophila* pol II core elongator complex, D-elp1, has RdRP activity and plays a role in both RNAi and the Dcr-2 dependent formation of transposon specific endo-siRNAs correlated with transposon silencing.

Author contributions: C.L. and B.M.P. designed research, performed research, analyzed data, and wrote the paper. The authors declare no conflict of interest. This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

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A homolog of the Lipardi and Paterson D-elp1 protein expressed in Sf9 cells has RdRP activity. Baculovirus rD-elp1 was tested with different single-stranded RNA templates, including B, Center showed that the knobdown of D-elp1 strongly inhibited RNAi reaction had no effect on the labeled RNA (8) (Fig. S2A). Nearest neighbor analysis measures the distribution of the nucleosides that are 5′-adjacent to the α-labeled position in the RNA product after RNase A/T1 digestion (Right). (C) Nearest neighbor analysis (n.n.a.) confirms template directed synthesis. Black arrowheads mark the ribonucleotide-3′-monophosphates. (D) Dcr-2 digests the 350-bp dsRNA to yield 21–25 bp siRNAs. (E) D-elp1 directs primed dsRNA synthesis to give the expected ~400 bp extension product. MW, 25-bp DNA ladder end-labeled with 32P dCTP.

**Fig. S2B)** resulted in the identification of approximately 25 proteins highly enriched or unique to the active fraction (Table S1). These included members of the COP9 signalosome complex, initiation factors, DNA polymerase sigma and epsilon, heat shock proteins, an ATP-dependent helicase, mcm4 and mcm6, and eight peptides for CG10535, corresponding to the Drosophila homolog of the RNA polymerase II core elongator complex subunit, elp1 (D-elp1), also known as IKAP in mammals (Fig. S1C). The other two subunits of the core elongator complex usually associated in a stochiometric ratio with D-elp1, the Drosophila elp2-2 (CG11887) and elp-3 (CG15433) homologs, were not detected in either fraction at the single peptide level.

Since Drosophila does not contain a canonical RdRP gene, we screened selected proteins in the active fraction for their ability to inhibit siRNA-mediated silencing of a GFP reporter in S2 cells, by soaking the cells with double stranded RNA for the various candidate proteins. Using a mixture of dsRNAs previously screened for the absence of off target effects against D-elp1 (D-elp1.A and D-elp1.B) (14, 15), the knockdown of D-elp1 strongly inhibited RNAi (see below). After two rounds of dsRNA treatment the cells were healthy and did not take up trypan blue. Taken together with the evidence indicating elp-1 has multiple roles in transcription, cytoplasmic kinase signaling, exocytosis, tRNA modification and disease (16) we felt further analysis of the Drosophila elp1 (D-elp1) was warranted.

**Baculovirus Recombinant D-elp1 Has Primed Fill-In Activity.** Flag-tagged D-elp1 protein was expressed in S9 cells using a modified baculovirus vector containing the heat shock inducible HSP70 promoter (17). High level recombinant D-elp1 (rD-elp1) expression from the polh promoter in the standard baculovirus vector was toxic to S9 cells. Acrylamide/SDS gel analysis of Flag-tag purified rD-elp1 showed a single major band of the expected molecular weight (~142 kD) that reacted with anti-Flag antibody (Fig. L4). Western blot analysis demonstrated a small amount of Hsp83 protein remained tightly bound to the affinity isolated rD-elp1. S9 cells infected with a GFP control baculovirus yielded no high molecular weight proteins when processed similarly (Fig. S2B, Center).

rD-elp1 protein directed the labeling of the primed fill-in substrate with α32P-UTP in a concentration and time dependent manner (PSL/mm2, FujiFilm FLA-5100) using 30–250 ng protein per reaction incubated for 2 h, or 100 ng of protein in reactions incubated for various times up to 2 h, respectively (Fig. S2A). Labeling depended upon the 5′ overhangs since pretreatment of the substrate with RNase One to generate blunt-ended dsRNA eliminated incorporation entirely but nuclease treatment after the reaction had no effect on the labeled RNA (8) (Fig. S2A). Nearest neighbor analysis had previously confirmed internal labeling of the filled in 5′ overhang (8). Nearest neighbor analysis measures the distribution of the nucleosides that are 5′-adjacent to the α-labeled position in the RNA product after RNase One digestion and TLC of the resultant nucleoside-3′-monophosphates. Actinomycin-D and α-amanitin at concentrations that inhibited uridine incorporation >95% in S2 cells and inhibit the activity of all three DNA-dependent RNA polymerases, respectively, did not affect rD-elp1 activity (Fig. S2A) (8). Replacement of CTP and GTP with deoxynucleotides or 3′-O-methyl ribonucleotides prevented α32P-UTP incorporation (Fig. S2A) (18). When synthetic 30-bp dsRNA or dsDNA templates with a single 18-bp 5′ overhang were tested with rD-elp1 or the GFP control proteins eluted from the flag beads, only the dsRNA template incubated with rD-elp1 protein produced the expected 48-bp fill in product resistant to RNase One digestion (Fig. S2C, Left). Furthermore, neither single- or double-stranded DNA templates served as substrate for rD-elp1 directed labeling (Fig. S2C, Right).

**Baculovirus rD-elp1 Has RdRP Activity.** Baculovirus rD-elp1 was tested with different single-stranded RNA templates, including unmodified, capped, or capped and polyadenylated RNAs of 250–380 bp. Template length labeled RNA was produced that was resistant to RNaseA/T1 concentrations that completely degraded the input RNA, as shown by analysis on 6% polyacrylamide-8M
urea gels (Fig. 1B). To independently confirm the RNaseA/T1 digestion results and to rule out possible nuclease titration artifacts, rD-elp1 reaction products were also digested with Dcr-2 (recombinant Dcr-2 prepared in Sf9 cells, SI Text), the ribonuclease III-related enzyme that digests dsRNA to produce siRNAs (19). Dcr-2 cleaved the labeled RNAs into siRNA length fragments of ~22–25 bp, as shown here for the 380-bp labeled RNA derived from a polyadenylated template, confirming independently that the rD-elp1 reaction products are dsRNAs (Fig. 1D). Material prepared similarly from Sf9 cells infected with a GFP control virus did not produce labeled RNA in response to any of the template RNAs, and rD-elp1-dependent dsRNA synthesis required the presence of both the ssRNA template and rD-elp1 protein in the reaction (Fig. S2B, Right).

Nearest neighbor analysis of the 250-bp and 380-bp labeled RNAs confirmed that α-UTP was incorporated internally into the newly synthesized RNA strand and gave the expected nucleoside-3’-monophosphate ratios predicted by the template sequence for each template normalized to 3’-CMP (FujiFilm FLA 5100, PSL/mm²); 250-bp RNA with Cp, Ap, Up and Gp ratios distributed as 1.0 to 1.3 to 1.5 to 1.0, and 380-bp RNA (380-bp polyadenylated template) ratios distributed as 1.0 to 1.1 to 2.2 to 1.0 (Fig. 1C). Thus, baculovirus rD-elp1 is similar to N. crassa Qde-1 and can initiate primer-independent, template-directed dsRNA synthesis on a variety of ssRNA templates (2). But unlike Qde-1, we did not observe significant back-primed or self-primed synthesis that generated product larger than the input template using template RNAs from 50–700 bp.

To ascertain if baculovirus rD-elp1 was also able to initiate primer-dependent RNA synthesis, as describe for N. crassa Qde-1 and S. pombe Rdp-1 (20, 21), GFP ssRNA (716 bp) was annealed either with the 5’-32P-labeled sense or anti-sense strand of a GFP siRNA and tested as substrate in reactions with rD-elp1 protein and unlabeled ribonucleotide triphosphates. First, only the antisense primer directed dsRNA synthesis to give the expected ~600 bp full-length primer extended product when analyzed on 6% polyacrylamide-8M urea gels (Fig. 1E). Second, chemical addition of a 3’-phosphate group on the antisense primer prevented primer extension (Fig. 1E). Together the data confirmed that baculovirus rD-elp1 can initiate dsRNA synthesis using either primer-dependent or primer-independent mechanisms, similar to other known cellular RdRPs. Using the specific activity of the αUTP or the primer to estimate the relative moles of product produced per mol of template (cpm [PSL units]/mm²/mg protein (AU units)): 32P incorporation measured on the FujiFilm FLA 5100; protein amount determined by Western blot analysis using a flag tagged protein standard measured on the FujiFilm LAS-3000; unprimed dsRNA synthesis produced at least five times more RNA than the primed reaction. In both instances the relative ratio indicated less than one mole of product was produced per mol of template, consistent with a single transcription initiation event. The difference in product yield between the unprimed and primed synthesis reactions could reflect the efficiency of the primer-template annealing step, i.e., unusable primer-template complexes are formed. However, we cannot exclude the possibility that the differences also arise at the transcription initiation step.

An rD-elp1 Deletion Made in E. coli Has RdRP Activity. To strengthen the argument that baculovirus rD-elp1 RdRP activity was not due to an Sf9-associated viral RdRP or an unidentified protein contaminant, and to initiate studies to identify domains in the protein responsible for RdRP activity, we prepared full length soluble rD-elp1 protein in E. coli. E. coli lacks the RNA silencing machinery and has not been shown to have an RdRP related gene or RdRP activity. N-terminal His6−T7-tagged rD-elp1, isolated on nickel agarose resin, was of the expected size on Coomassie blue-stained gels, ~142 kDa, and reacted with antibody to the T7 peptide sequence fused to amino terminus of D-elp1 (Fig. 2, D-elp1 lanes) (22). Similar to rD-elp1 produced in baculovirus, E. coli rD-elp1 (Fig. 2A) was active in both primer-independent (Fig. 2B and C) and primer-dependent dsRNA synthesis, as shown by the analysis of the reaction products on 6% polyacrylamide-8M urea gels (Fig.
D-elp1 interacts with Dcr-2 and is involved in RNAi. Knockdown of D-elp1 inhibited dsRNA-mediated gene silencing to the same extent as Dcr-2 depletion (Fig. 3 A and B). As mentioned above, inhibition of siRNA-mediated silencing of a GFP reporter was similarly affected by the knockdown of D-elp1 (Fig. S3B). Although we do not have antibody to endogenous D-elp1, S2 cells expressing V5-tagged D-elp1 and a GFP marker selectively lost D-elp1 expression when knocked down in parallel cultures, suggesting the endogenous protein was also depleted under the treatment conditions used.

D-elp1 is the largest of the three subunits in the core elongator complex, and D-elp3 has been shown to have histone acetyl transferase activity that facilitates pol II transcription through chromatinized templates in vitro (16, 23, 24). We wanted to know if D-elp1 depletion alone was responsible for the effects on RNA silencing or if the three subunits of the core elongator complex were equally involved, suggesting a role for the elongator complex itself. Unlike the results with D-elp1, the knockdown of either D-elp2 or D-elp3 RNA had no affect on RNAi, indicating the role of D-elp1 is relatively specific and outside the context of the elongator complex (Fig. 3 A and B). In support of this interpretation, the Elp1-related proteins are found predominately in the cytoplasmic compartment in cells examined from yeast to man (23, 25, 26) and ectopically expressed D-elp1 in S2 cells was localized mainly in the cytoplasmic compartment, in agreement with previous observations (Fig. S3A, Upper). However, the RNA pol II CTD, which has been shown to bind the core elongator complex, was observed only in the nuclear compartment in S2 cells (Fig. S3A, Lower).

The cytoplasmic location of D-elp1 and its role in RNAi suggested it might be interacting with components of the RISC. RdRP has been shown to form a complex with Dicer in S. pombe and Tetrahymena thermophila (27, 28). To test this, V5-tagged Ago-2, Dcr-2 and Dcr-1 were individually coexpressed with Flag-tagged D-elp1 or the empty vector in S2 cells and subjected to either V5 or Flag antibody pulldown and blot analysis with the counter tag antibody. Pulldowns were performed in the presence of Ribonuclease A to eliminate possible RNA-mediated interactions. Dcr-2 showed extremely robust interaction with D-elp1, using either V5 or Flag antibody in the pulldown reaction (Fig. 3C), while the interactions observed with Ago2 and Dcr-1 were evident but not as intense (Fig. 3D).

Given that D-elp1 interacts strongly with Dcr-2, it was formally possible that loss of D-elp1 was affecting the stability of Dcr-2 resulting in the inhibition of RNAi. However, depletion of D-elp1 protein in transfected S2 cells did not reduce Dcr-2 protein expression or affect Dcr-2 RNA levels (Fig. 3A). Ago2 RNA levels were also unaffected by D-elp1 depletion. In contrast to the RNAi pathway, micro RNA regulation was unaffected since targeting of the nautilus gene transcript by mirR-3 was normal in D-elp1 depleted
S2 cells [Ravulapalli et al. (2008) 49th Annual Drosophila Research Conference Abst. no. 111] (Fig. S3C).

D-elp1 Has a Role in Transposition Suppression. The *Drosophila* genome harbors a variety of retrotransposons and mobile elements that are silenced post transcriptionally through the RNAi pathway (11, 12, 29–31). We looked at the impact of D-elp1 depletion on transposition suppression and the production of the corresponding endo siRNA for representative transposons highly expressed in S2 cells. Previous studies have shown that a reduction in the RISC components Ago2 and Dcr-2, but not Dcr-1, result in the increased steady state levels of several transposon RNAs, including 297, mdg1, and the non-LTR transposon TART-B (11, 12, 32) accompanied by a decrease in the corresponding transposon endo-siRNAs. We measured the RNA levels for 297, mdg1, and the telomeric non-LTR transposon, Het-A, after treating S2 cells with dsRNA to D-elp1, using Dcr-2 depletion as the positive control (32). We also determined the endo siRNA levels for 297 and mdg1. Just as with Dcr-2 depletion, knockdown of D-elp1 resulted in a similar increase in RNA levels for the mdg1, 297, and HetA transposons and a dramatic decrease in the corresponding endo-siRNAs for mdg1 and 297 (Fig. 4 A and B) (33). By contrast knockdown of D-elp2 or D-elp3 had no effect on transposon RNA levels (Fig. 4A). This demonstrates that D-elp1 is specifically involved in the post transcriptional regulation of transposon RNAs and their regulatory endo siRNAs.

The fact that D-elp1 knockdown and transposon siRNA depletion were correlated, and that D-elp1 has RdRP activity, suggested a pathway in which transposon RNAs are converted into dsRNA by D-elp1 and processed into endo siRNAs by Dcr-2. This interpretation was strengthened with the observation that transposon antisense RNA levels for mdg1 and 297 decreased dramatically in S2 cells treated with D-elp1 dsRNA (Fig. 4C). This result establishes a direct link among D-elp1 depletion, a decrease in transposon antisense RNA levels, and a decline in transposon endo siRNA abundance, presumably due to a decrease in the levels of transposon dsRNA available for Dcr-2 cleavage. Importantly, Dcr-2 depletion, which also reduces endo siRNA production and increases transposon RNA levels, did not result in a reduction in the transposon antisense transcripts (Fig. 4C).

Two different P-element insertions in the D-elp1 gene are both late larval lethals, indicative of maternally loaded D-elp1 protein: P-element insertion [l (3) 4,629] (34), and a Gypsy P-element insertion targeted to the coding region, obtained from the Harvard Exelixis collection (pBac [PB] aCG10350 c90296). When either P-element insertion was placed over a deficiency that removes D-elp1 [Df (3R) Exel6276, Bloomington 7743] or over one another, the phenotype was identical i.e., late larval lethal. In the D-elp1 null, the third instar larvae never form pupae but wander in the food for several days. The null larvae are viable and therefore can be analyzed for transposon RNA levels. Similar to the D-elp1 knockdown in S2 cells, RNAs for mdg1, 297 and Het-A transposons were increased substantially in D-elp1 null larvae while the corresponding transposon antisense RNA transcripts were reduced (Fig. 4 D and E). Based upon these results with representative transposon RNAs, we conclude D-elp1 plays an important role in transposon regulation in S2 cells and during *Drosophila* development.

Elp-1–Related Proteins Are Conserved in All Eukaryotes. Amino acid sequence comparisons (Fig. S4) show both *S. pombe* iki-3 and human elp-1 proteins have ~45–50% similarity to D-elp1 across the entire protein, while the *S. cerevisiae* and *C. elegans* proteins have significant regions of nonhomology. There are no conserved amino acid domains between the elp1 family members and the canonical cellular RdRPs, particularly the highly conserved DXDGD amino acid sequence required for activity of the cellular RdRPs (35). We have recently produced recombinant *S. pombe* iki-3, *C. elegans* elp-1, and human IKAP and all three have RdRP activity, suggesting there is a conservation of an alternative RdRP pathway in most eukaryotes involving the elp1-related proteins. Recombinant *S. cerevisiae* elp-1 was not active in our assay, consistent with the lack of the RNAi machinery in budding yeast. However, we cannot exclude the possibility that other factors may explain the inactivity of *S. cerevisiae* elp-1. Both *S. pombe* and *C. elegans* have classical cellular RdRPs, Rdp-1 and rrf1, respectively, as well as elp-1 homologs with RdRP activity indicating that the two enzyme activities are not mutually exclusive and may have nonoverlapping functions. A recently published genetic interaction screen in *S. pombe* indicates that the double null for Dicer and iki3, the elp-1 homolog in *S. pombe*, is synthetically sick/lethal indicating there is genetic cross talk between the RNAi and elongator functional modules, in support of our findings (36). We speculate that the
functional conservation among these proteins points to a common role in transposon regulation and genome defense.

Elongator genes are essential in metazoans and the D-elp1 (CG10350) loss-of-function is a late larval lethal, as discussed above (16, 34). In C. elegans, RNAi knockdown of elp-1 is embryonic lethal (white body), and the recent study using IKBKAP 2/−/− mouse embryos reports loss of IKBKAP is an embryonic lethal as well (11). In the mouse IKBKAP 2/−/−, the phenotype could be rescued by the human gene. Just D-elp1 itself has been reported to be involved in Drosophila immunodefence (flybase.org) and is upregulated along with Dcr-2, Ago-2 and Ars-2 in S2 cells depleted for the UPF proteins that are involved in nonsense-mediated decay (32). This reinforces the interpretation that D-elp1 has separate functions outside the elongator complex and is involved with components of the RNAi machinery.

Mutations in the human elp-1 gene, also known as the IKBKAP gene that encodes the IKAP protein, are correlated with a recessive hereditary neuropathy common in the Ashkenazi Jewish population, called familial dysautonomia (FD), or the Riley-Day syndrome (16). The most prominent mutation, an intronic noncoding point mutation (T to C) in intron 20 that disrupts splicing and results in the variable skipping of exon 20, is found in all FD patients. However, the molecular basis for the disease has not been determined and has been attributed to defects in tRNA modification, defects in transcription, and also disruption of the cytoskeleton and cell motility (17). Thus, elp-1/RD-related IFAK lacks various reduced RdRP activity, or fails to interact with components of the RISC, particularly Dcr-2, our findings suggest this could affect the RNA silencing pathway and the normal level of post transcriptional gene regulation, thus providing a possible alternative explanation for neuronal cell death. Deletion studies are in progress to determine if either the D-elp1 or human IKAP FD deletions have RdRP activity and can interact with Dcr-2.

In summary, we have shown that Drosophila RNA polymerase II elongator complex Subunit I, D-elp1, is noncanonical RdRP that plays a role in both dsRNA and siRNA mediated RNAi, as well as transposon suppression by modulating the levels of transposon dsRNA and the corresponding endo siRNAs in the later instance. This outcome highlights the multifunctional role of elongator in RNAi gene defense and transcription. Although earlier reports concluded transposon and systemic RNAi did not occur in Drosophila, a recent study has clearly shown that antiviral immunity in flies requires systemic RNAi spreading that involves a dsRNA uptake pathway. Furthermore, protection against viral infection could be established by pretreatment with very low doses of viral dsRNA, similar to immunity in plants and C. elegans (6, 38). This means dsRNA has to spread in the absence of viral infection. Earlier studies have also shown that low levels of injected dsRNA induced RNAi in adult flies (39, 40). The role identified here for D-elp1 as an RdRP involved in transposon regulation suggests spreading via the production of either unprimed or siRNA primed dsRNA synthesis could mediate this process. Production of dsRNA from aberrant RNAs through a similar mechanism may also complement and facilitate Ago-2 mediated RNAi in higher eukaryotes under certain conditions of cellular stress.

Materials and Methods

Flag tagged recombinant D-elp1 and Dcr-2 were produced in Sf9 cells using the Bac-to-Bac baculovirus system according to the manufacturers (Invitrogen). D-elp1 containing an amino terminal 6-His and a carboxyl terminal Flag tag in Pet 28 was expressed in E. coli using either Rosetta BL21-Lys5 cells (Novagen) or BL21 CodonPlus (DE3)-RIPL (Stratagene) (22). The recombinant D-elp1 proteins were purified as described in the SI Text and stored in 10% glycerol at −80 °C. The enzymatic assays and western blot analysis were performed as described previously (32, 33). 52 cell transfection, Western analysis protocols, and the luciferase assay are described in SI Text. Flag tagged D-elp1 and VS tagged Ago2, Dicer-1 and Dcr-2 cDNAs were cloned into pET/V5-His (Invitrogen) for expression in S2 cells. RNA expression levels for transponds 297, mdg1, and Het-A were measured by quantitative RT-PCR in S2 cells using the primers and protocol described previously (12, 32).

SI Text including full methods, Tables S1–S3, and any associated references are available in the online version of the paper.

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