Supplementary Material

In previous work, we demonstrated that $3' \rightarrow 5'$ RNA decay followed by scavenger decapping of the resulting cap was very active in our translation extracts (1). In contrast, direct mRNA decapping occurred at very low levels in the translation extracts, and thus $5' \rightarrow 3'$ decay played a very minor role in mRNA decay compared to the $3' \rightarrow 5'$ RNA decay pathway. To rule out that the mutations in blocks 1 or 3 led to differential RNA decapping of RNA that might contribute to the differences in translation observed, we examined the rate of decay of 5' monophosphate RNAs (the products of RNA decapping) and compared the amounts of decapped RNAs derived from wild type or mutant SL mRNAs that accumulated during translation in the extracts (see Materials and Methods). 5' monophosphate RNAs were significantly less stable than TMG-capped RNAs in the extracts indicating that decapped RNAs are not likely to accumulate in the extracts (Supplementary Figure 2A). In addition, comparison of the wild-type and mutant RNAs at different time points during translation did not show any differences in the levels of decapped or 5' monophosphate RNAs for these RNAs during the translation reactions (Supplementary Figure 2B). Overall, these data suggest that differential mRNA decapping of wild-type vs mutant SL 1 and 3 is not an explanation for the reduction in translation observed for the mutations in the SLs.

References

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Supplementary Figures

Supplementary Figure 1. elF4G protein alignment. Identical residues are shaded grey and similar

residues are white with black shading. Ascaris is Ascaris suum (ACX37244), Brugia is Brugia malayi

(XP_001895525, parasitic nematode), C. elegans is *Caenorhabditis elegans* (NP_001022259), bee is *Apis mellifera* (XP_393239), human is *Homo sapiens* eIF4GI (Q04637), and wheat is *Triticum aestivum* (Q03387). Boxed and arrowed regions indicate domains in the human eIF4GI.

Supplemental Figure 2. 5' monophosphate RNAs decay faster than capped RNAs and levels of uncapped RNAs are not different between the WT SL and SL Mut-3. A). Decay of monophosphate mRNA compared with capped RNAs during Ascaris cell-free translation. GMP RNAs were primed with GMP and thus contain a 5'-monophosphate. RNAs were analyzed as described in Figure 1 C. B). Assay for presence of decapped, 5'-monophosphate RNAs during translation. RNAs were isolated at the illustrated time points and treated with Terminator enzyme. Terminator enzyme degrades only 5'-monophosphate RNAs (not 5' capped, triphosphate, or diphosphate RNAs)(Epicentre, Madison, WI). The plots illustrate the amount of remaining RNA during translation that is resistant to terminator enzyme (e.g., capped RNA). Note that WT SL and the Mut-3 SL RNAs do not show significant differences in the degree of uncapped RNAs.

Supplemental Figure 3. Spliced leader sequences required for efficient translation. A). Multiple nucleotides within block 1 of the SL contribute to translation of TMG-capped RNAs. **B).** Multiple nucleotides within block 3 of the SL contribute to translation of TMG-capped RNAs. **Raw data for spacing analysis normalized in Figure 3. C).** Insertion mutations were designed with compensating deletions after the SL (e.g., SL-G+2N and -2N) to account for changes in the length of the 5' UTR on translation. The actual decrease in translation from the insertions (SL-G+XN) is greater when deletion mutations (-XN) are considered. These data were used to present normalized values in Figure 6 by dividing the translation level of the "SL-G+XN" value by the "-XN" value

Supplementary Figure 4. Mutations in block 3 of the spliced leader results in increased flexibility in both blocks 1 and 3 suggesting residues within these blocks interact. A). TMG-capped 64 nucleotide WT SL and GGGU MUT 3 RNAs were analyzed using SHAPE (3, 4). Mutations in nucleotides 10 - 13 (Block 3) caused increased flexibility in those nucleotides as well as near the cap, nucleotides 2 - 4 (Block 1). Marked in red are the nucleotides in blocks 1 and 3. B). Quantitation of

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accessibility changes illustrated in A. Gels illustrated in A were subjected to phosphoimager analysis. Experiments were carried out as described in the Materials and Methods.

Supplementary Figure 5. m⁷GTP-sepharose treatment of Ascaris extracts reduces elF4G and elF4E. Ascaris translation extract was treated with m⁷GTP-sepharose and the amount of various proteins removed from the extract determined by Western Blotting. Antibodies to the Ascaris proteins were generated to full-length elf4E-3, a truncated form of elF4G, and peptides derived from the N-terminus of Ascaris elF4E-1 and elF4E-4 (Davis et al, unpublished).

Supplementary Figure 6. SL affects translation at the initiation step. A). Translation time course for WT SL and GGGT Mut-3 RNA. B). Translation time course for WT SL and GGGT Mut-3 RNA illustrating the linear phase of translation. C). Sucrose gradients of Ascaris Extract with WT SL and GGGT Mut-3 RNA. Experiments were carried out as described in the Materials and Methods. Supplementary Figure 7. The SL stem loop does not increase Ascaris elF4E/4G's affinity for TMG-capped RNAs. A). Ascaris elF4E/G complex has the same affinity for the TMG-capped wild-type SL, stem-loop mutant, and compensatory stem-loop mutant. The figures illustrate the surface plasmon resonanse responses and residuals obtained for binding of varying concentrations of elF4E/G to different immobilized RNAs. The burnt orange lines show best fit used for analysis. These data were used to calculate values shown in Table 1. Concentrations of elF4E/G used were 1.73 nM, 3.45 nM, 6.9 nM, 13.8 nM, 27.6 nM. B). Translation inhibition assay using trimethylguanosine cap analog. Note that the cap analog does not differentially affect translation of the mRNAs. C). Ultraviolet light crosslinking of recombinant Ascaris elF4E-3 to universally labeled RNAs. Experiments were performed as previously described (2).

Supplementary Figure 8. The C. elegans SL2 and hybrid SLs between SL1 and C. elegans SL2
variants support translation of a TMG-capped RNA. A). Analysis of translation of C. elegans SL2, hybrid spliced leaders, and other SL variants.
B). Translation of Trichinella spliced leaders.
Experiments were carried out as described in Figure 1. Blue sequences represent SL1 nucleotides, orange SL2 nucleotides, and green and purple nucleotides are variant in alternate SL2-like leaders

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Ascaris Brugia C.elegans Bee Human Wheat	1 1 1 1 1	
Ascaris Brugia C.elegans Bee Human Wheat	64 34 37 1 101 1	PABP Binding Site TAH SNHMPQAAYRSA RENLPNPSMQ FAPGEV HV ERPQQCTPQ ITTIHPTRYAQQPEAQQPE PPTSQMATTT SAPPHYNPANYNANYSTESAYSTFQ 161 SVLG
Ascaris Brugia C.elegans Bee Human Wheat	162 74 100 1 201 1	PQAAYYQPAAVTPQMFTSFYQMQAPEFLQQRVGWAYPOTAMLVPWQTQPPVHQKEKKILRIVD 224
Ascaris Brugia C.elegans Bee Human Wheat	225 113 150 20 301 12	P D T K E V T N EK E I SA S M CAPL D E H M S G D S Q G G R R
Ascaris Brugia C.elegans Bee Human Wheat	317 173 246 22 401 12	W SPWEH SAP SAM ST STEPPAAHVEEMPAEVPERSTVILEEK PEREVAE EAKEQKIEEALVEKDEKDKPKNIDKSESEQAPEQWGISPQPSESAAV409
Ascaris Brugia C.elegans Bee Human Wheat	410 206 315 22 501 12	
Ascaris Brugia C.elegans Bee Human Wheat	494 276 389 95 601 87	
Ascaris Brugia C.elegans Bee Human Wheat	584 365 465 181 701 176	OR KRGP I SRPSTDRPAREPVKLHRSENAWKHEPSSNLDKNOQUYKDIRGLLNKTTPSTEDALCADFLSFKVYQNKEQMSEVTS DR KRGP I SRPSTDRPNREPVKLHKAENAWKPESSNLDKNOQUYKDIRGLNKTTPSTEDALCADFLSFKVYQNKEQMSEVTS ORDMHNKRPPVVRSTERVORVTLPSSKDAWKPOROKTAE
Ascaris Brugia C.elegans Bee Human Wheat	667 446 558 263 800 250	IIFDKAVEEPKFCPLYSDLCKKQVVEESQESGKSEFRSGILTRCQQTFETKRQEEINKKRAEAEAETDERROKELKLEVMEMEAKERRRM 756 IIFDKAVEEPNFCALYSDLCKMQVVKESKESKDKSKFRSALLTRCQNTFEEKRMSEINNKKIEMEKETDEKKKRELKTELMEMEARERRRM 536 IVFDKAVEEPNFCALYSDLCKMQVAKESKESKDKSKFRSALLTRCQNTFEEKRMSEINNKKIEMEKETDEKKKRELKTELMEMEARERRRM 536 IVFDKAVEEPNFCALYSDLCKMQVAKESKESKDKSKFRSALLTRCQNTFEEKRMSEINNKKIEMEKETDEKKKRELKTELMEMEARERRRM 536 IVFDKAVEEPNFSVAVANMCRCLMALKVPTEKPTVTVNFRKLLINRCQKEFERNVVNEVARSKIKETEEDFVKKEMLAEERQKFR-RRK 5895 DOMAIN-2 LIFEKATSEPNFSVAVANMCRCLMALKVPTEKPTVTVNFRKLLINRCQKEFERNVDVNEVARSKIKETEEERRKTELEEARDIARRS 895 DOMAIN-2
Ascaris Brugia C.elegans Bee Human Wheat	757 537 645 363 896 337	EGNIGFIGQUFRHELIVPRILNWCIIHLIKNHSEAELQ-GGSDEESIECAVRMLETVGKIADRQGLSTSRQDSQGAQSEFNLSVEFT 842 LGNIGFIGQUFRHELIVPRILNWCIVHLIKNHSESPDGDEESIECAVKMLESVGKIADRQCQQQASYQSGLSDPHAQDHPQEAQKHEEFNTNLYFE 632 FGOTTFMOTUYRNQLISTKIVQTGTFELFNSTKDQDIKEOVDESSEHEGQUIETVGWLDKSKDSTVFDQWFQ 721 RONTFMOTUYRNQLISTKIVQTGTFELFNSTKDQDIKEOVDESSEHEGQUIETVGWLDK
Ascaris Brugia C.elegans Bee Human Wheat	843 633 722 428 961 408	HLSEIAPKVSNRVRFLILNLIELKNNNWNPRKSADSGPKTIEGVHSEARKEELQNKLQREOYEKKRGPYEGRPSLDR919 HLNEVSTKVSNRVRFLILNLIELROWKWVPR-TSESGPKILGEVHDEARREEMANRLQREOYANKKR-YEGRTSLDK
Ascaris Brugia C.elegans Bee Human Wheat	920 708 799 513 057 486	-RSQR PTHIGROSQDGR-YG-RGGDSSRDQKARAAGAASMVASTASRKNQSUN SMDOPOSLGARRPQFSSG SAG 990 -RNQRPVLIGROSQDGRQYGSRVGEPSRDQKARAAGAANUKANTASRKNQTUN SVDOPOSLGARRPPFAGGAIG 780 -NIRK SVPVSRNSLDRNRGMQHPEQKRAAAAANUKLASS-SVQPKNISUSSMOD-NTLGKSKKEWHSGASG 887 -PRIDGGWSGPVWRTRPQVSVETAKLKNKPPPMDDLQGSRNVYWWKTPSSGGSKAINSNKFACLENVSTLDQDKRITSPQLS594 LSKGSRPIDISRLIKLIKPGSIDSNDLFAPGGELSMGZGSSGSGGAKPSDAASEAARPATSTLNRFSALQQAVPTESIDDRRVVQRSSLSRERGEKA 1154 DOMAIN - 2 -FSVNRPGTGGMMPGMPGSRKMPGMPGLDNDNWEVQRSRSMPGGDLRNOGPLINKVPSINKPSLEPQGTGAL 562
Ascaris Brugia C.elegans Bee Human Wheat	991 781 868 595 155 563	G G Q Q TERNA A A R SM I G V R G A G R G SLA SR D N SQ P SS R B P SE S R B Q S SN D ER V A A LA A A SA M TH SS SS G G L K R SG Y A SA A G G G G SS SA L SS TG D D K ST G SD 1090 G G Q M D R S I L S R G T L G - R G A G R G S LA SR D N SQ P SS R P SE S R K - S R D E R SA A TA A A SA V TH SA SS NA L R R - G Y A G I A A G G G G SS SA L SS TG D D K ST G SD 1090 G G Q M D R S I L S R G T L G - R G A G R G S LA SR D N SQ P S S R P SE S R K - S R D E R SA A TA A A SA V TH SA SS NA L R R - G Y A G I A A G G G - S S P P S S I V D D C I K D G SQ G G N T B SE S Q N S N T D K I S N D G R K S T V D E K Q A A LA A A K E I SA M SI S S R A L R R - G Y A G I A A G G S S P P S S I V D D C I K D G SQ G G N T B SE S Q N S N T D K I S N D G R K S T V D E K SQ Q N V S M P P P S L K S SQ S ST S S H K P S N T D K I M K D Y L K N T I L K Y S L A I S S G G N T G G P R E Y G R D Y N P SY D G R SS S N G S T G S L N S S G S S N A S N Y D P P S L K S SQ S ST S S H K P S T H E F L K T I M K D Y L K N T I L K Y S L A I G 94 G D R G D R L R S E R G G D R G D R L D R A T P A T K R IS F S K E Y B E R S R E G P S Q P G G L R K M A S L T E O R D R G R D A Y K R E A L P P V S P L K A A L S E E E L E K K G K A I I E E Y L 1254 G D R G D R L E R S E R G G D R G D R J D R A T P A T K R IS F S K E Y B E R S R E G P S Q P G G L R K M A S L T E O R D R G R D A Y K R E A L P P V S P L K A A L S E E E L E K K G K A I I E E Y L 1254 I G K SA L L G T G G P P S R P S L A S P T P L P A Q T T A S P K P S S M T P A S Y P I P A K X I Y P A G L Q K K A S L L E Y F 635
Ascaris Brugia C.elegans Bee Human Wheat	1091 876 937 695 255 636	H GD H EH D R D D D A A RAMK EK QA F SML V GD L N DY FAD N V D V EQVY T EV MEV C ET TA V R V Y FR L I M Q Y G I EK V SQA 1163 G GR V S EV D EK E I F G S L Y R EL H EY F SD A S N I ELL F Q S I M EI M I D E R T M N MD L R R V R QA FR L L M K I G V EK T N GD 947
Ascaris Brugia C.elegans 1 Bee Human Wheat	164 948 000 780 354 672	V TN PHRRY V GOVICR CLQADQIK QDA H HGIADFCTY V V ENELWEDN
Ascaris Brugia C.elegans Bee Human Wheat	249 033 086 880 1451 742	NADTRKADA LLYV LRRIVEIEYEREKRISSWGMAFDEIKDLRTDALIVALKGCHUTSGENLYELUN - 1315 DADSRKPYA LLVSVLKRLVEIEHDRDGQVSSTGMAFDEIKDLHSDSLVDELKSCQLSFGKNLYQLLLN 1100 KGEGNKKFDLLVQVLKQUIELELEVTGAAEGISWEFSD
Ascaris Brugia C.elegans Bee Human Wheat	316 1101 156 980 548 789	1315 1000 1156 SU SOVNIISSEAFLTWIKKSPEPAQREWHGVATMALUSEFTGUQEADDASSVEDVSTSVSQDRC 1042 1599 Supplemental Figure 1, Wallace et al. 788







Supplemental Figure 2, Wallace et al.

Supplemental Figure 3, Wallace et al.



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В







Supplemental Figure 5, Wallace et al.



Supplemental Figure 6, Wallace et al.





Supplemental Figure 8, Wallace et al.