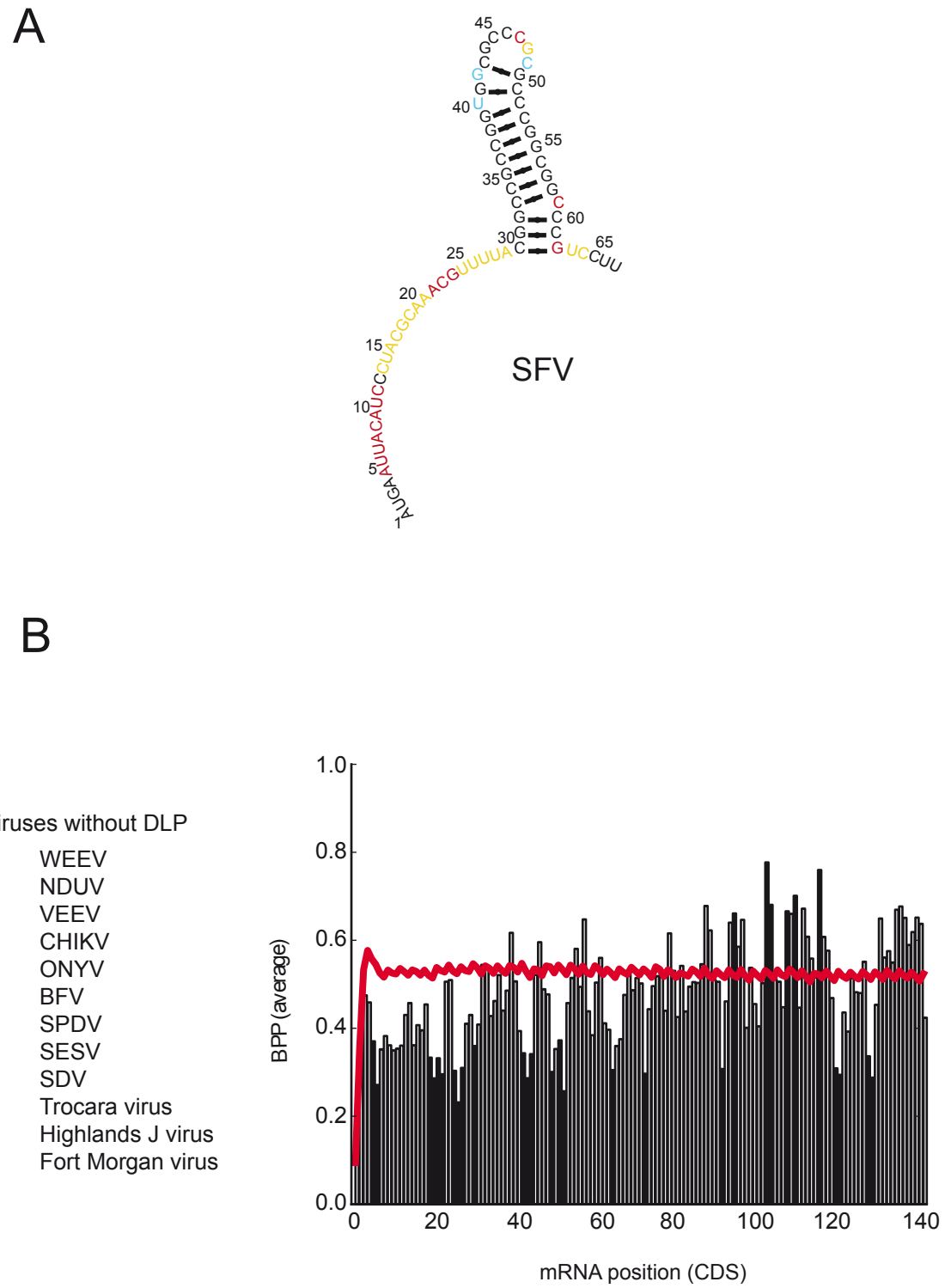

Figure S1. Alternative structure of SFV DLP and BBP distribution in Alphavirus lacking DLPs. (A) Alternative conformation of SFV DLP. (B) Plot of averaged BBP distribution for the first 140 nucleotides of the CDS mRNA of Alphavirus lacking DLPs. Black solid bars represent values that differed significantly ($p < 0.01$, U test) from equivalent positions in whole mouse mRNA transcriptome (red line)

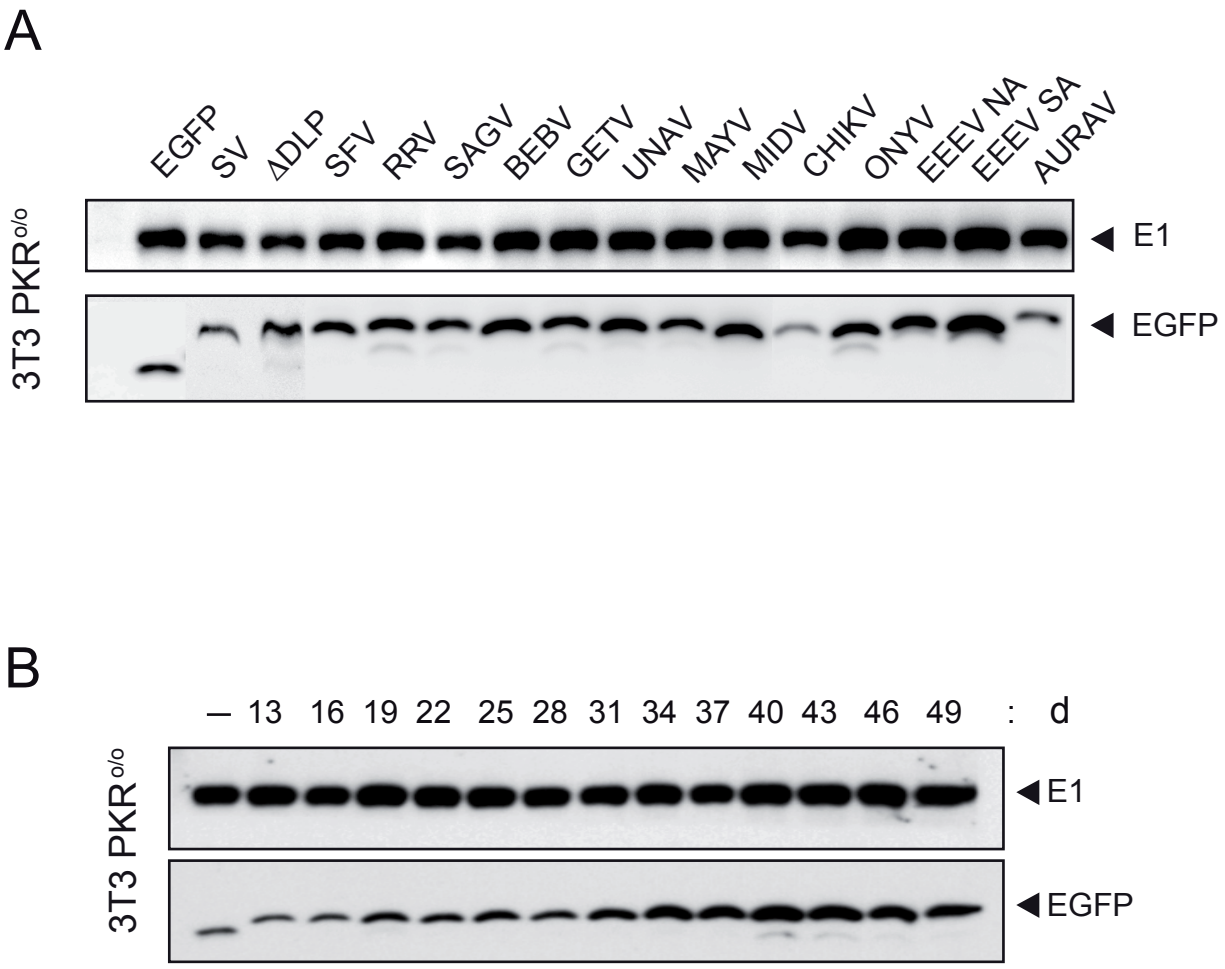

Figure S2. Activity of DLPs in PKR$^{o/o}$ cells.
(A) Activity of Alphavirus DLPs in PKR$^{o/o}$ cells. (B) Effect of DLP-AUG distance (d) on translation of EGFP in PKR$^{o/o}$ cells. Cells were infected with the indicated virus and analyzed as described in materials and methods and in Figures 2 and 3.

A

B

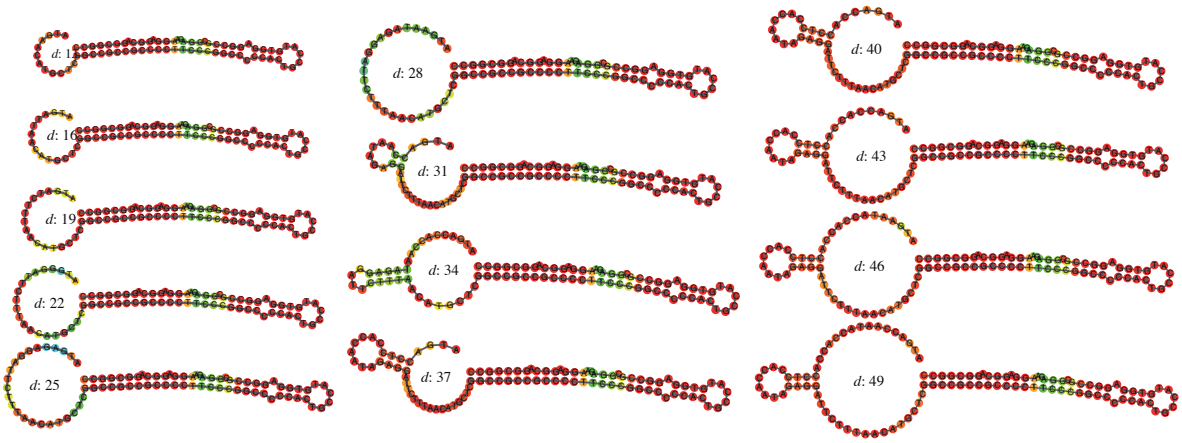

Figure S3. Sequences and folded structures of SV DLP with changes in AUG-DLP spacing. (A) Initiation codon is underlined and triplet insertions are in bold, whereas the region encompassing the DLP is marked in green. (B) Predicted folded structures using the MFE of the centroid structure. Colours represent the entropy according to RNAfold.

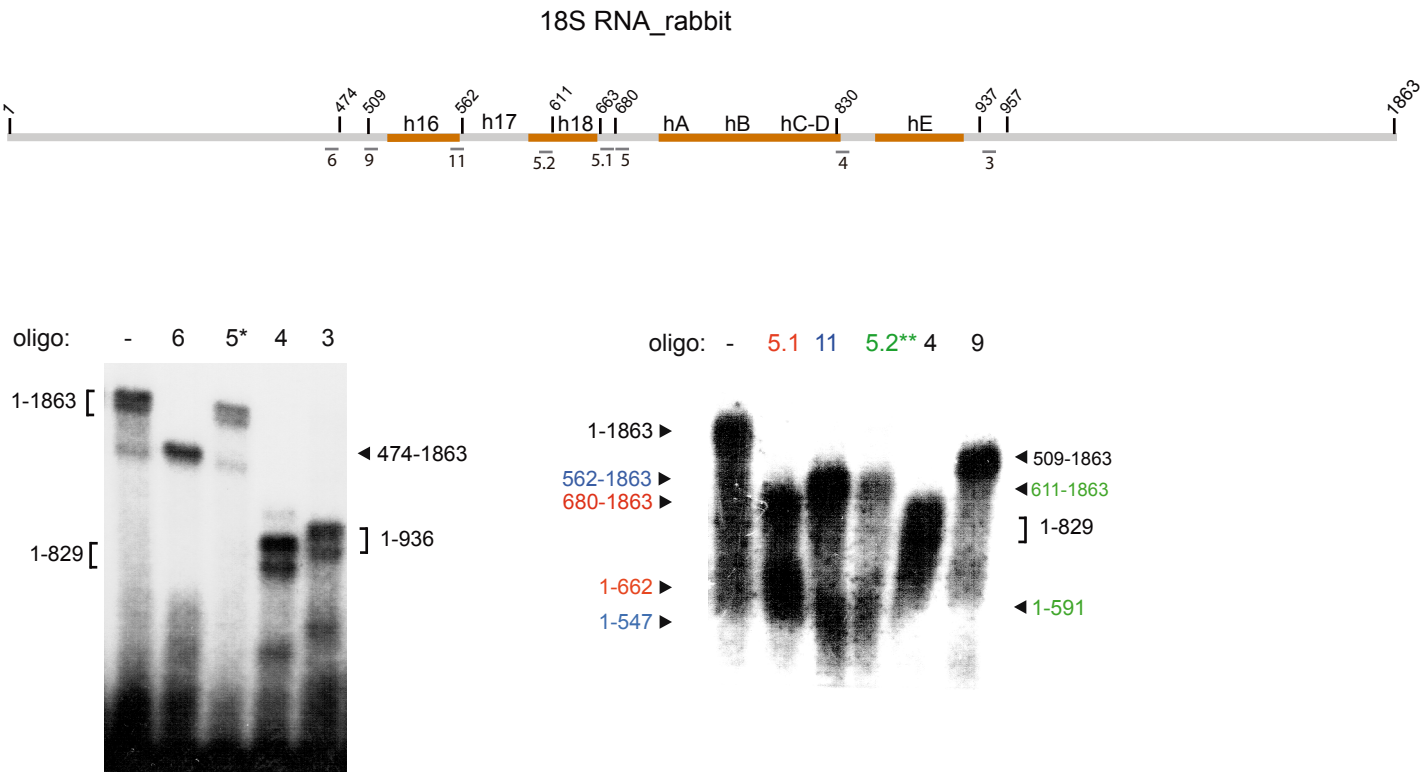

Figure S4. RNAse H mapping of mRNA-18S rRNA contacts (extended information of Figure 5C).
The complete set of oligonucleotides used in RNAse H experiments is shown on 18S rRNA (upper).
Helices of 18S rRNA involved in crosslinking with SV DLP U1 mRNA are shown (similar to Figure 5C).
RNAse H assays with the indicated oligonucleotides from two independent experiments (lower).
For clarity, RNA fragments generated by the corresponding oligonucleotide are in the same colour (right).
(*) oligonucleotide 5 failed in this assay, whereas oligonucleotide 5.2 hybridized only partially (**).

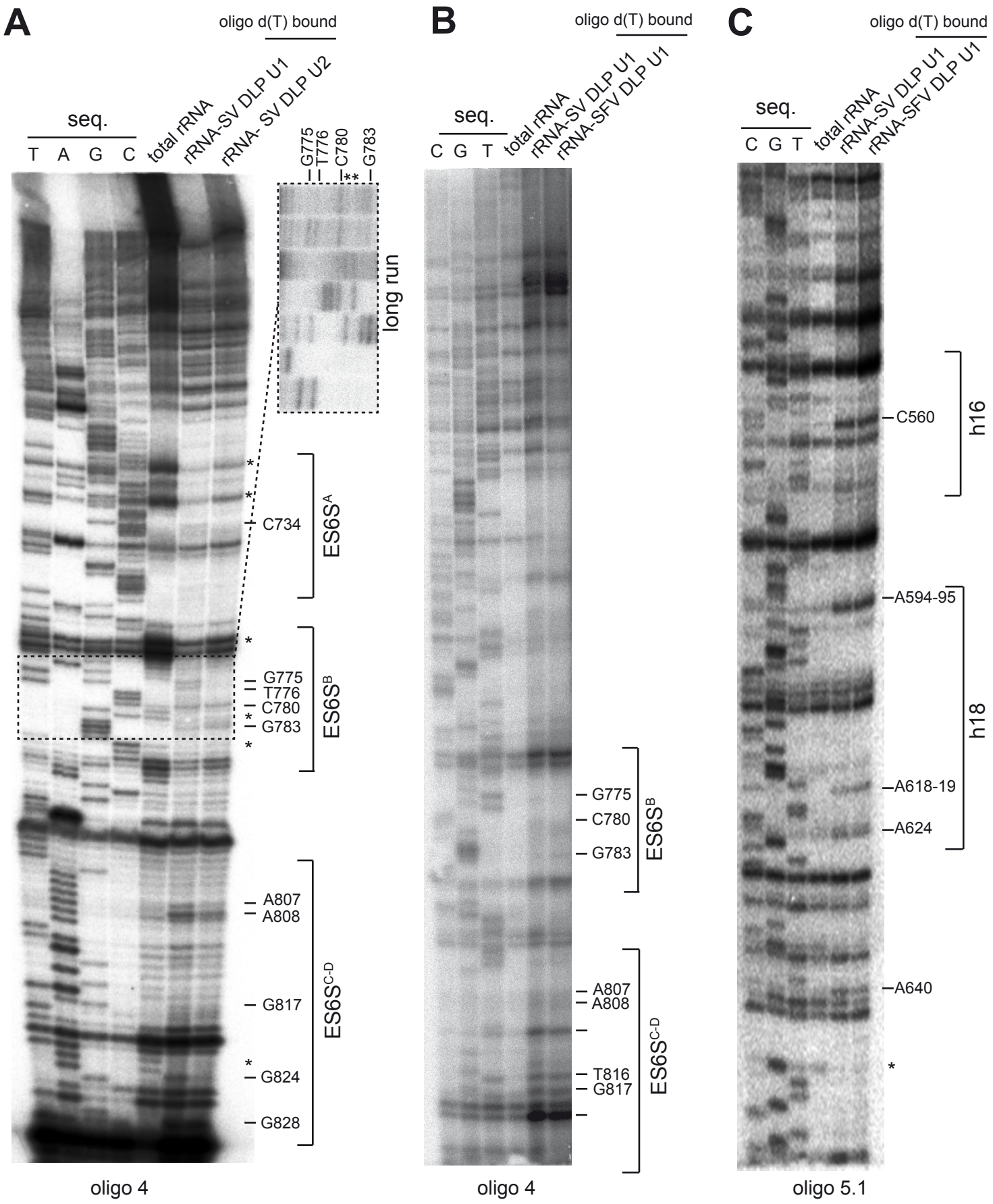

Figure S5. Contacts of DLP mRNA with 18S rRNA in 48S complex identified by UV crosslinking followed by primer extension analysis. (A) A representative acrylamide-urea gel of primer extension arrests induced by crosslinking with SV-DLP U1 and U2 mRNAs analyzed using oligo 4 (a long run of gel shows ES6SB region, inset).(B) The same analysis using SV-DLP U1 and SFV-DLP U1 mRNAs and analyzed with oligo 4. (C) The same analysis using SV-DLP U1 and SFV-DLP U1 mRNAs analyzed with oligo 5. 1. All mRNAs used were poly-adenylated to recover rRNA-mRNA complexes by oligo d(T) beads as described in materials and methods.The corresponding regions in 18S rRNA are indicated. Note that an arrest at a given position is caused by the crosslinking adducts generated immediately upstream. *denotes a significant reduction of extension arrest in rRNA-DLP mRNA complexes.