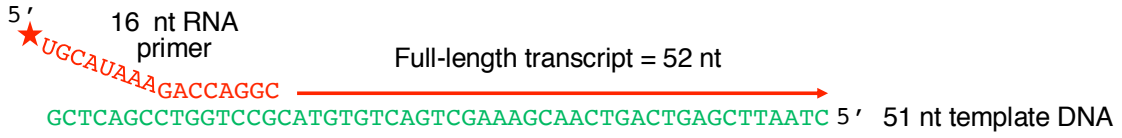
SUPPLEMENTAL INFORMATION

Table S1. DNA and RNA oligonucleotides used in the study, related to Figures 1-5			
A.	**DNA Oligonucleotides**	**Sequence**	**Related to figure/s**
1)	Standard Template DNA	CTAATTCGAGTCAGTCAACGAAAGCTGACTGTGTACGCCTGGTCCGACTCG	1C, E, F,2A, B, C, 3A, 4A, D, 5B
2)	Non-Temp DNA (16nt)	TACTGACTCGAATTAG	2A
3)	Non-Temp DNA (25nt)	TCTTTCGTTGACTGACTCGAATTAG	2C
4)	Non-Temp DNA (28nt)	GCAGCTTTCGTTGACTGACTCGAATTAG	2A, B, C, 3A, 4A, D, 5B
5)	Non-Temp DNA (31nt)	GAGTCAGCTTTCGTTGACTGACTCGAATTAG	2C
6)	Non-Temp DNA (36nt)	CACACAGTCAGCTTTCGTTGACTGACTCGAATTAG	2A
7)	T-Less Temp DNA	CAAAAACGAGACAGACAACGAAAGCAGACAGAGAACGCCAGGACCGACACG	3B, 4B, 4C, 5C, S2
8)	T-Less Non-Temp DNA (27nt bp + 5' 1nt unpaired)	GCTGCTTTCGTTGTCTGTCTCGTTTTTG	3B, 4B, 4C, 5C, S2
9)	T-Less Non-Temp DNA (27nt bp + 5' 6nt unpaired)	AAAAAGCTGCTTTCGTTGTCTGTCTCGTTTTTG	4B
10)	T-Less Non-Temp DNA (27nt bp + 5' 11nt unpaired)	AAAAAAAAAAGCTGCTTTCGTTGTCTGTCTCGTTTTTG	4B
11)	T-Less Non-Temp DNA (27nt bp + 5' 16nt unpaired)	AAAAAAAAAAAAAAAGCTGCTTTCGTTGTCTGTCTCGTTTTTG	4B
12)	T-Less Non-Temp DNA (27nt bp + 5' 21nt unpaired)	AAAAAAAAAAAAAAAAAAAAGCTGCTTTCGTTGTCTGTCTCGTTTTTG	4B
13)	T-Less Non-Temp DNA (27nt bp + 5' 26nt unpaired)	AAAAAAAAAAAAAAAAAAAAAAAAAGCTGCTTTCGTTGTCTGTCTCGTTTTTG	4B
14)	T-Less Non-Temp DNA (22nt bp + 3' 5nt unpaired)	GCTGCTTTCGTTGTCTGTCTCGTAAAAA	4C
15)	T-Less Non-Temp DNA (17nt bp + 3' 10nt unpaired)	GCTGCTTTCGTTGTCTGTAAAAAAAAAA	4C
16)	T-Less Non-Temp DNA (12nt bp + 3' 15nt unpaired)	GCTGCTTTCGTTGAAAAAAAAAAAAAAA	4C
17)	Template DNA (CHG Methylation)	CTAATTCGAGTCAGTCAACGAAAG/iMe-dC/TGACTGTGTACGCCTGGTCCGACTCG	4D
18)	Template DNA (CHH Methylation)	CTAATTCGAGTCAGT/iMe-dC/AACGAAAGCTGACTGTGTACGCCTGGTCCGACTCG	4D
19)	Template DNA (CG Methylation)	CTAATTCGAGTCAGTCAA/iMe-dC/GAAAGCTGACTGTGTACGCCTGGTCCGACTCG	4D
20)	Template DNA (CG and CHG Methylation)	CTAATTCGAGTCAGTCAA/iMe-dC/GAAAG/iMe-dC/TGACTGTGTACGCCTGGTCCGACTCG	4D
21)	Template DNA (CG, CHG and CHH Methylation)	CTAATTCGAGTCAGT/iMe-dC/AA/iMe-dC/GAAAG/iMe-dC/TGACTGTGTACGCCTGGTCCGACTCG	4D

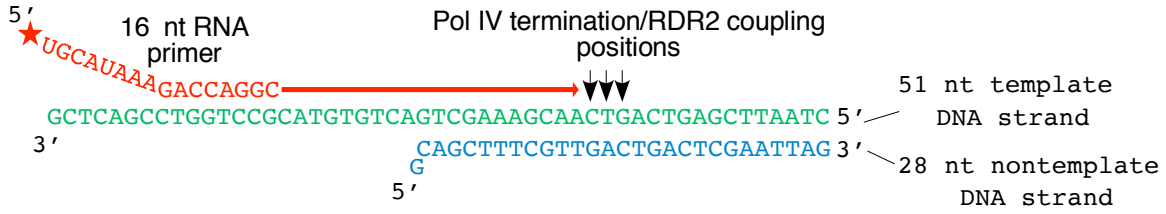
22)	Non-Temp DNA (CHG Methylation)	G/iMe-dC/AGCTTTCGTTGACTGACTCGAATTAG	4D
23)	Non-Temp DNA (CHH Methylation)	GCAG/iMe-dC/TTTCGTTGACTGACTCGAATTAG	4D
24)	Non-Temp DNA (CG Methylation)	GCAGCTTT/iMe-dC/GTTGACTGACTCGAATTAG	4D
25)	Non-Temp DNA (CG and CHG Methylation)	G/iMe-dC/AGCTTT/iMe-dC/GTTGACTGACTCGAATTAG	4D
26)	Non-Temp DNA (CG, CHG and CHH Methylation)	G/iMe-dC/AG/iMe-dC/TTT/iMe-dC/GTTGACTGACTCGAATTAG	4D
27)	T-Less DNA Hairpin (30 bp)	GTGTCTGCTTTCGTTGTCTGTCTCGTTTTTGTCCTTTCAAAAACGAGACAGACAACGAAAGCAGACAGAGAACGCCAGGACCGACACG	4E
28)	T-Less DNA Hairpin (27 bp)	GCTGCTTTCGTTGTCTGTCTCGTTTTTGTCCTTTCAAAAACGAGACAGACAACGAAAGCAGACAGAGAACGCCAGGACCGACACG	4E
29)	T-Less DNA Hairpin (24 bp)	TCTTTCGTTGTCTGTCTCGTTTTTGTCCTTTCAAAAACGAGACAGACAACGAAAGCAGACAGAGAACGCCAGGACCGACACG	4E
30)	Bubble Non-Template DNA	GGATACTTACAGCCATATCAGTTACGCCTACTCCATTCCATCCCGGGTTCGTCCAAGTCGACTACTGGATCCTAGGCAGG	4F
31)	Bubble Template DNA	CCTGCCTAGGATCCAGTAGTCGACTTGGACGAACCCGGGATGGAATGGAGTATTCGCCGTGTCCATGGCTGTAAGTATCC	4F
B. **RNA Oligonucleotides**			
32)	RNA Primer (Standard Template)	rUrGrCrArUrArArArGrArCrCrArGrGrC	1C, E, F, 2 A, B, C, 3A, 4A, 4D, 5B
33)	RNA Primer (T-Less Template)	rUrGrCrArUrArArArGrUrCrCrUrGrGrC	3B, 4B, 4C, 4E, 5C, S2
34)	RNA Primer (8nt bp + 8nt 5' unpaired)	rUrUrUrUrUrUrUrUrGrArCrCrArGrGrC	4A
35)	RNA Primer (8nt bp + 12nt 5' unpaired)	rUrUrUrUrUrUrUrUrUrUrUrUrGrArCrCrArGrGrC	4A
36)	RNA Primer (8nt bp + 16nt 5' unpaired)	rUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrGrArCrCrArGrGrC	4A
37)	RNA Primer (8nt bp + 20nt 5' unpaired)	rUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrUrGrArCrCrArGrGrC	4A
38)	RNA Primer (Bubble Template)	rUrUrUrUrUrUrUrUrGrGrArCrArCrGrG	4F
39)	First Strand RNA (40 nt)	rUrGrCrArUrArArArGrUrCrCrUrGrGrCrGrUrUrCrUrCrUrGrUrCrUrGrCrUrUrUrCrGrUrUrGrUrCrU	S1
40)	Second Strand RNA (40 nt)	rArGrArCrArArCrGrArArArGrCrArGrArCrArGrArGrArArCrGrCrCrArGrGrArCrUrUrUrArUrGrCrA	S1
41)	RDR2 Transcription template	rUrArCrArArGrCrGrArArUrGrArGrUrCrArUrUrCrArUrCrCrUrArArGrUrCrCrArArCrArUrA	1D

Figure S1 (related to Figures 1-4). Template, primer and nontemplate strand sequences

A. Standard template



B. Standard template annealed to nontemplate strand



C. T-less template annealed to nontemplate strand

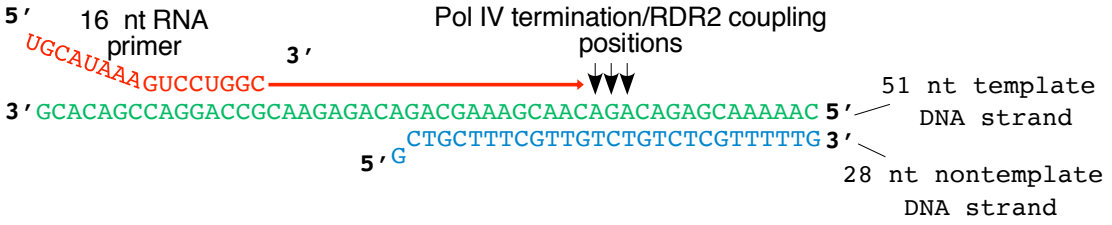


Figure S2 (related to Figure 3A). RDR2 transcripts have triphosphate groups.
Pol IV and RDR2 transcripts generated using the T-less template were tested for sensitivity to Terminator™ exonuclease, which degrades RNAs with 5' monophosphate groups but not RNAs with 5' triphosphate groups. End-labeled primer RNA was used to initiate transcription and label Pol IV transcripts in lanes 1-6. Alternatively, incorporation of α-^{32}P-ATP was used to body label RDR2 transcripts (lanes 7-12). Pol IV isolated from a *rdr2* mutant was tested in the reactions of lane 1-3 and 7. All other reactions had Pol IV-RDR2. Reactions of lanes 11 and 12 underwent treatment with RNA pyrophosphohydrolase (RppH, New England Biolabs) which converts 5' triphosphates into monophosphates. Reactions of lanes 3, 6,10-12 were heated at 75°C, 15 min to melt dsRNA duplexes into ssRNA. Reactions of lanes 2, 3, 5, 6, 9, 10, and 12 were subjected to treatment with Terminator exonuclease. The white line indicates editing of the original image to crop out a marker lane.

The results show that Pol IV transcripts (without RDR2) are sensitive to Terminator when single-stranded, consistent with their 5' monophosphate ends. In contrast, RDR2 transcripts are Terminator-insensitive unless first treated with RppH and heat-treated to melt duplex RNA into ssRNA. These results support the results of the capping assay in Figure 3, as both assays indicate that RDR2 transcripts have 5' triphosphate groups.

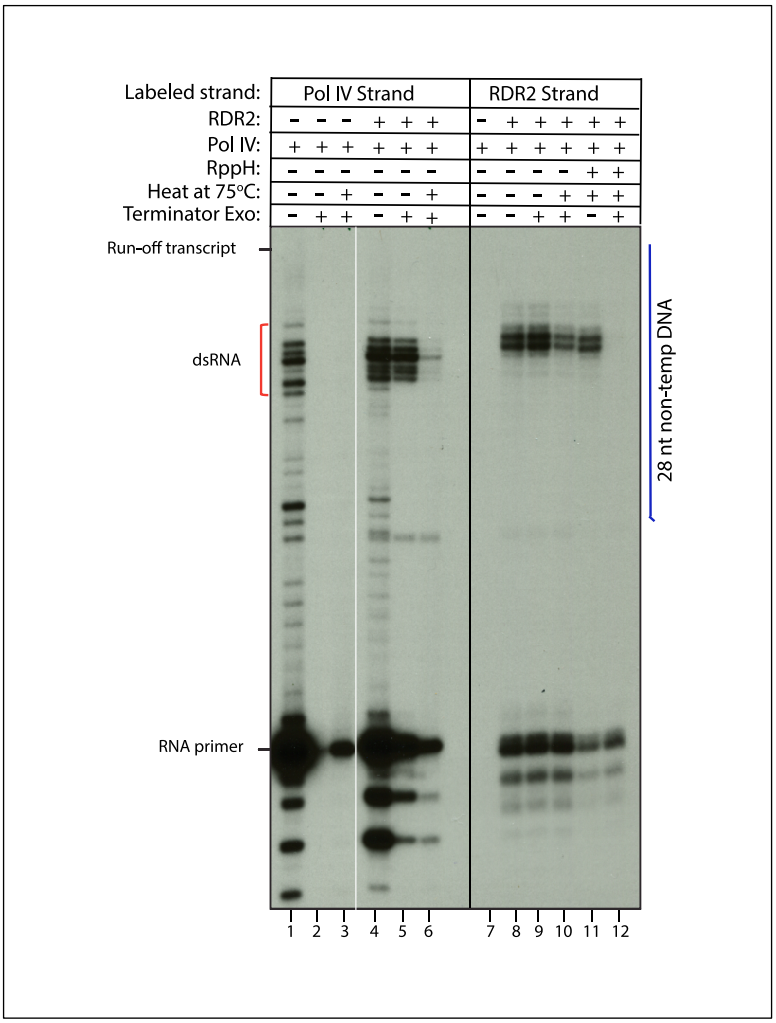
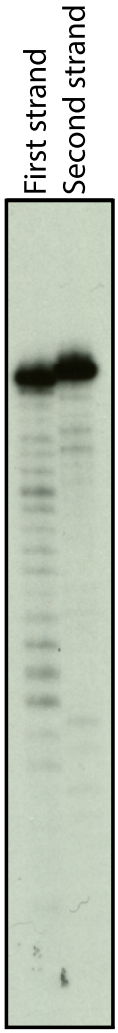


Figure S3 (related to Figure 3B). Pol IV and RDR2-transcribed strands have different electrophoretic mobilities.

40 nt synthetic RNAs corresponding to a primer-initiated first strand transcript of the T-less DNA template and its 40 nt reverse complement were end-labeled and subjected to denaturing PAGE and autoradiography. The Table shows the molecular weights of the two oligoribonucleotides. The mass difference is approximately the average mass of a single ribonucleotide.



Oligo	Length (nt)	Mol wt.
First strand	40	12839.4
Second strand	40	13179
Difference in mol wt.		339.6

Figure S4 (related to Figure 5D) Predicted M13MP18 (+) strand folding. This image, generated using using UNAFold (http://unafold.rna.albany.edu/) shows the potential for extensive secondary structure in single-stranded M13 DNA, enabling Pol IV termination and coupling to RDR2 activity.

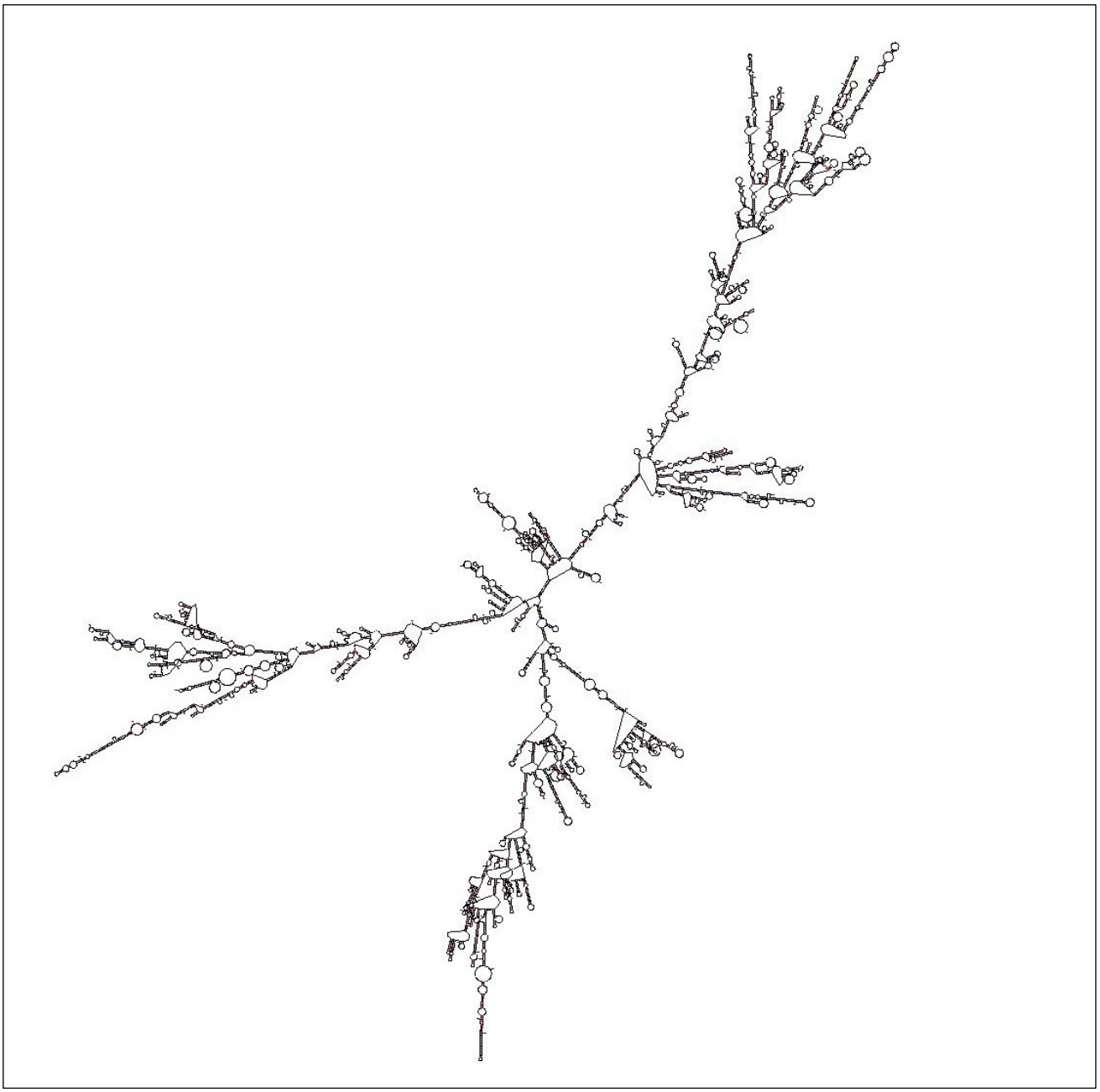


Figure S5 (related to Figure 5E). RDR2 is needed for second strand transcripts of siRNA precursors generated from M13 DNA

Second strand transcripts are depleted in the absence of RDR2. The plots show transcript abundance (reads per million) vs. transcript length for first strand (top panel, salmon) or second strand (bottom panel, blue) transcripts, comparing transcripts made by Pol IV-RDR2 complexes (left) to transcripts of Pol IV isolated from *rdr2* mutant plants (right).

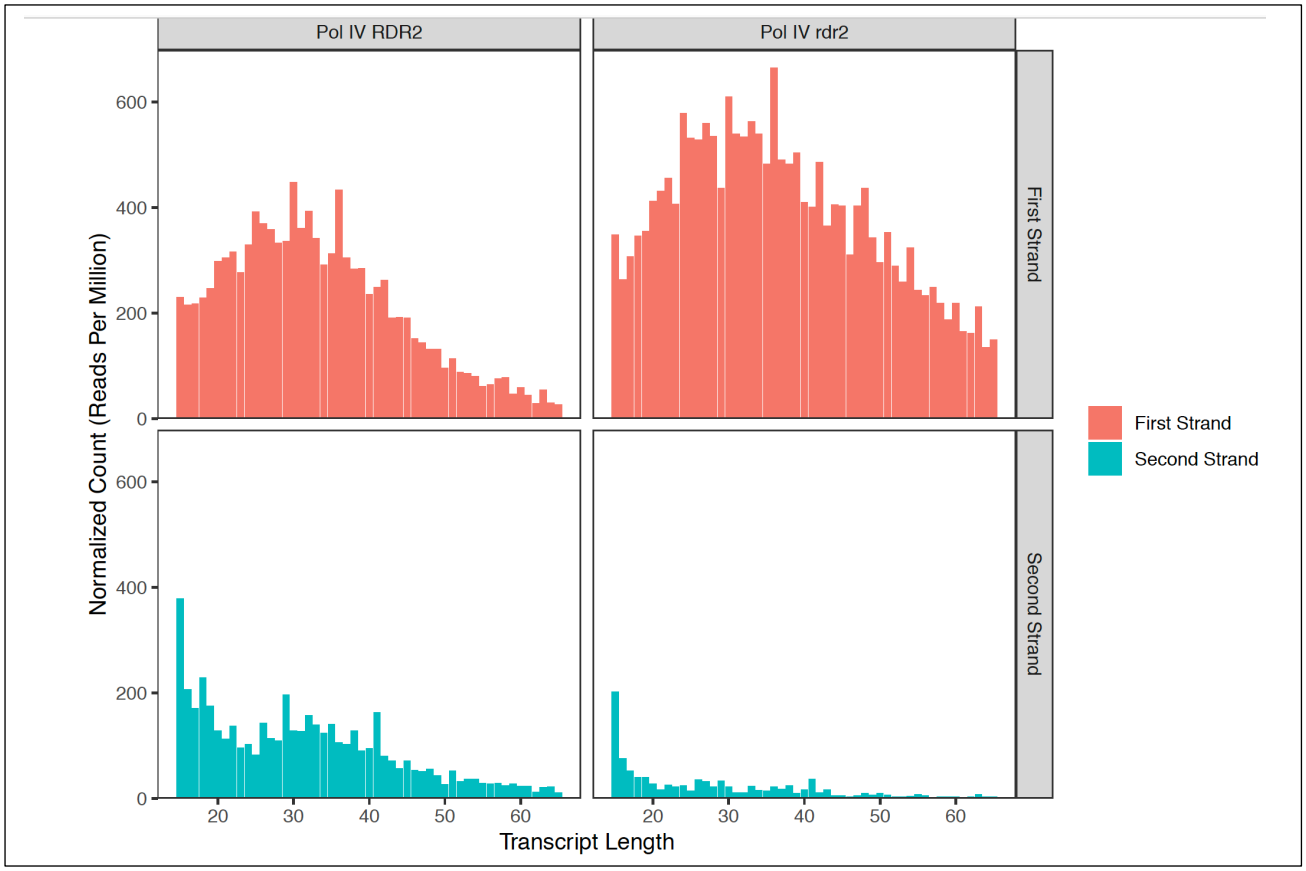


Figure S6 (related to Figure 6). Features of template-mismatched nucleotides at RNA 3' ends.

A. Number of 3' untemplated nucleotides. The histograms show the frequency of Pol IV or RDR2 transcripts having 0, 1, 2 or 3 template-mismatched nucleotides at their 3' ends.

B. Nucleotide identities of 3' untemplated nucleotides. The histograms show the frequency of Pol IV or RDR2 transcripts with single 3' terminal nucleotides (A, U, G or C) mismatched to the DNA template as well as the various sequence combinations for those rare transcripts with two mismatched nucleotides.

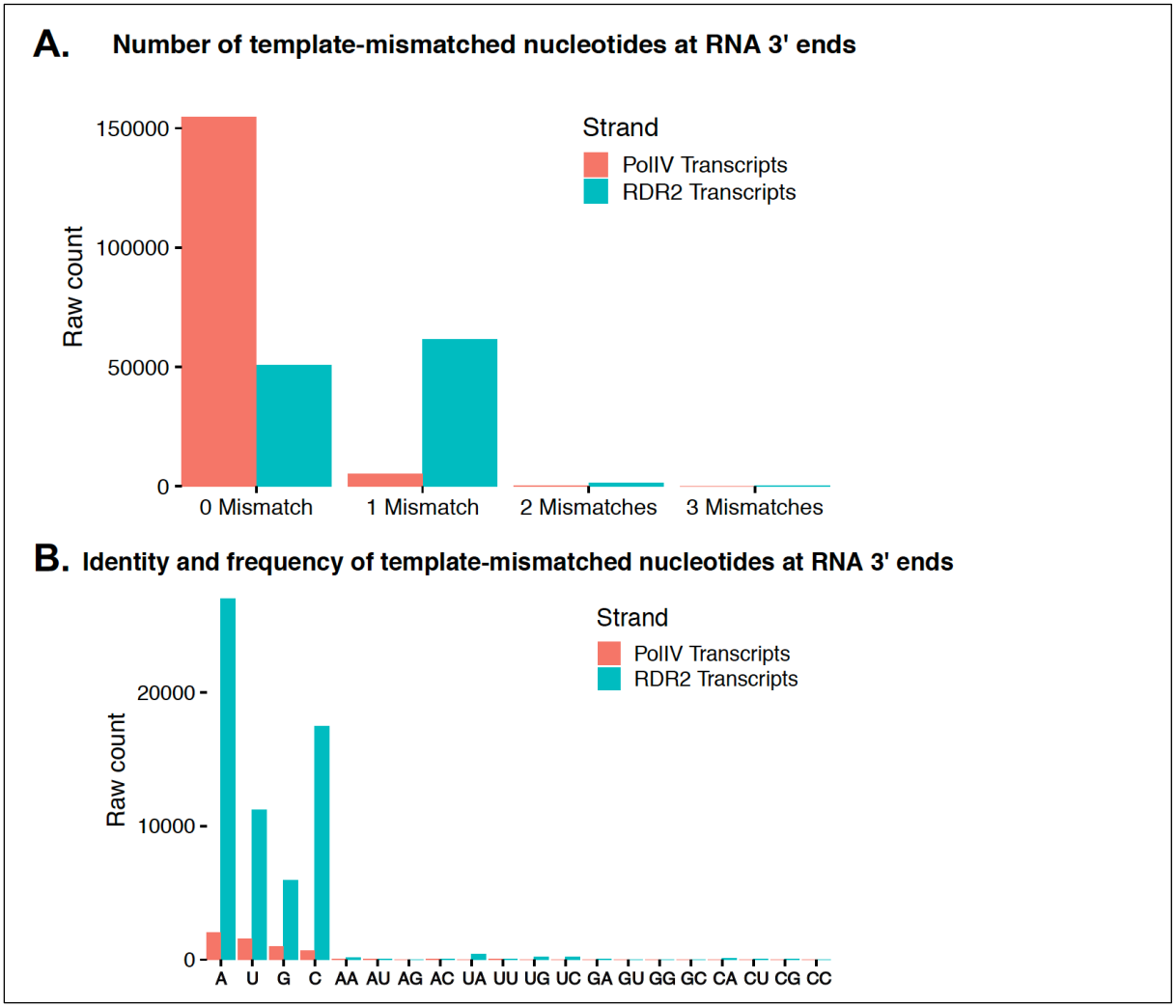
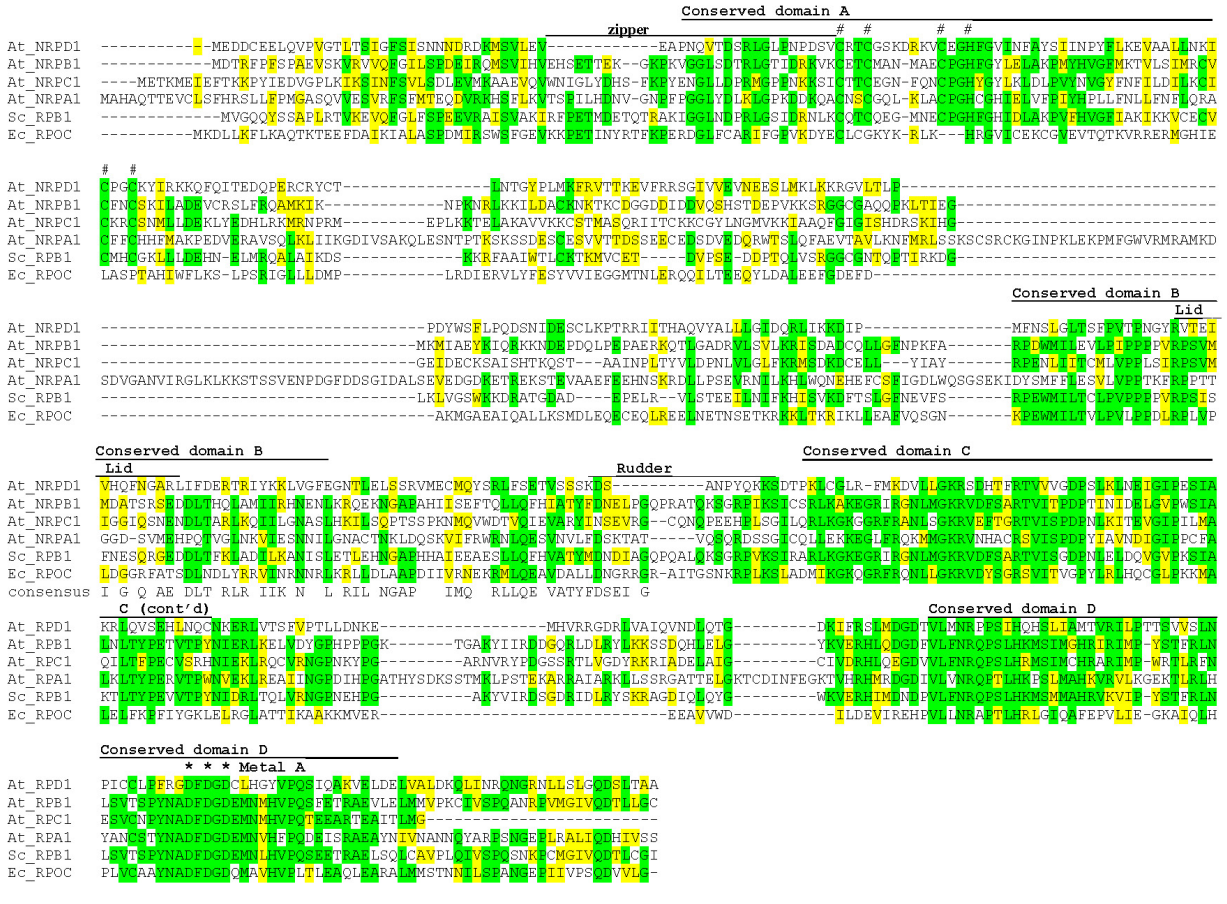


Figure S7 (related to Figure 7 and Discussion). Multiple alignment of the amino terminal ends of multisubunit RNA polymerase largest subunits.
Arabidopsis thaliana NRPD1 (At_NRPD1), Pol I (At_RPA1), Pol II (At_RPB1), Pol III (At_RPC1), budding yeast Pol II (Sc_RPB1) and *E. coli* RNA polymerase (Ec_RPOC) were aligned using CLUSTAL W followed by hand annotation. Identical amino acids are shaded green. Similar amino acids are highlighted in yellow. Positions of RNA polymerase conserved domains A through D, and zipper, rudder and lid loop positions are according to Cramer et al., 2001, as are zinc-coordinating amino acids (denoted by #). Asterisks denote the invariant aspartates of the catalytic center's Metal A site.

It is important to note that precise positions of deletions in the vicinity of the zipper and rudder loops are uncertain and can be shifted, or split into smaller deletions, by alternative alignment algorithms, including CLUSTAL O, MUSCLE, Kalign, webPRANK, or MAFFT.