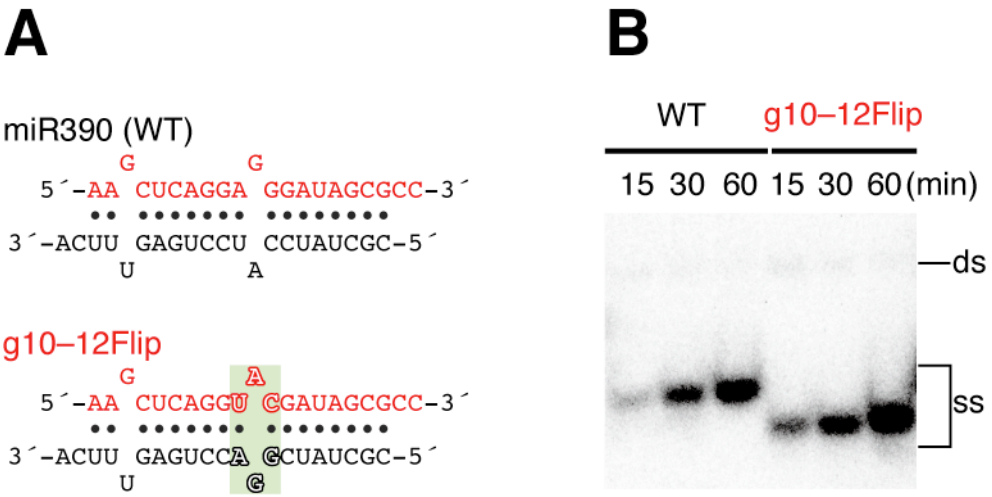
Endo et al., supplementary Fig S1



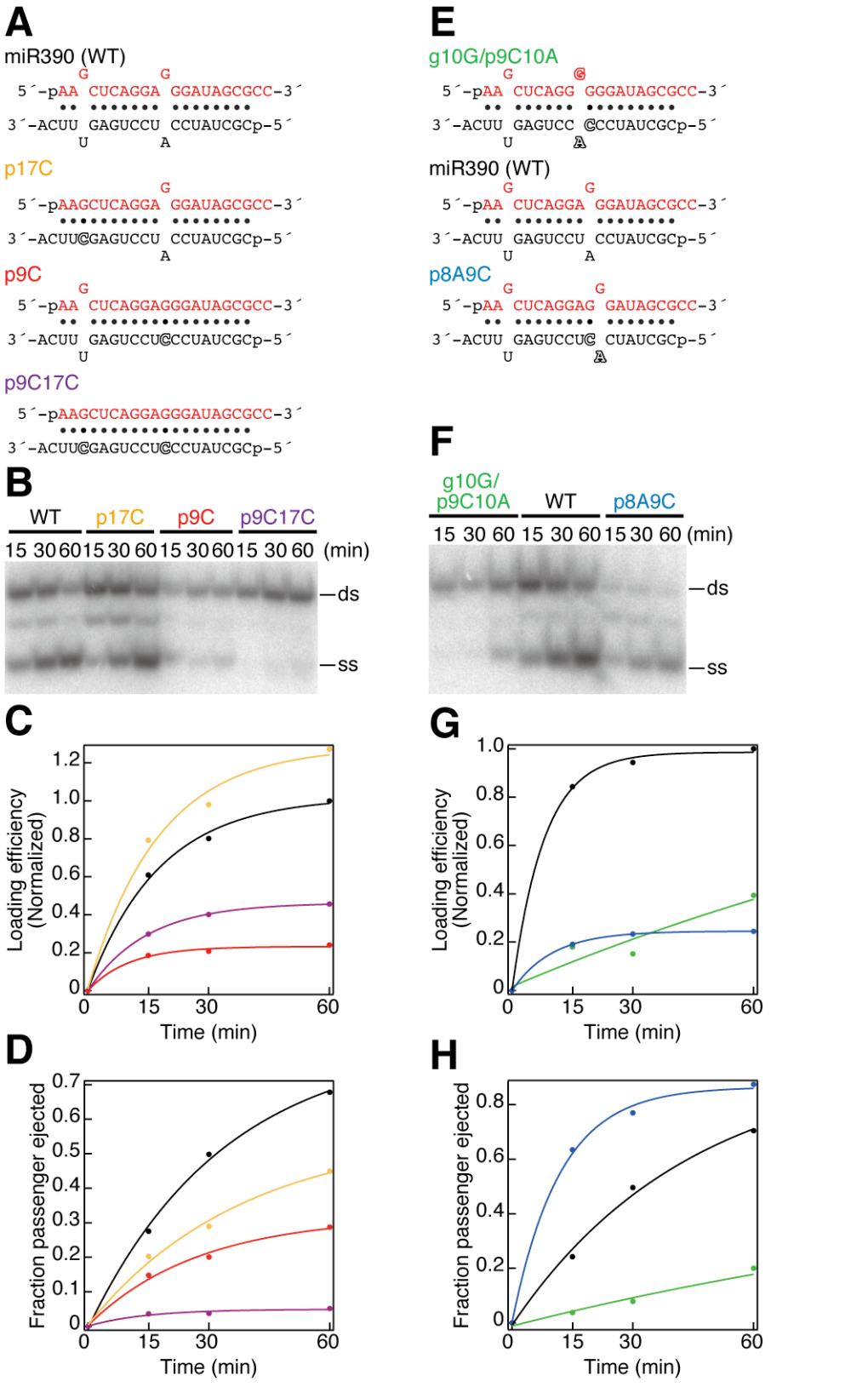
supplementary Fig S1. AGO2 does not recognize the central region of miR390/miR390* duplex for RISC assembly.

(A) The structure of wild-type miR390/miR390* and its variants used in (B). The mutated nucleotides are in outlined letters.

(B) AGO2-RISC assembly using wild-type miR390 and its variant shown in (A). The experiment was performed as in Fig 1A. AGO2-RISC assembly was not affected by swapping the central 3 nt of miR390/miR390*.

supplementary Fig S2. The central G-A mismatch is perfectly conserved among monocot and eudicot miR390 family.

The sequences of monocot and eudicot miR390 were collected from miRbase (version 19) [1]. Experimentally validated 35 sequences were used. miR390* strands were determined from the sequences of pre-miR390. The graphical representation was made using WebLogo [2]. The sequence conservation at each position is shown as the overall height of the stacked symbols, while the relative frequency of each nucleotide at that position was shown as the height of symbols within the stack.


A
miR390 (WT)
5´-pAA CUCAGGA GGAUAGCGCC-3´
3´-ACUU GAGUCCU CCUAUCGCp-5´
p17C
5´-pAAGCUCAGGA GGAUAGCGCC-3´
3´-ACUUGGAGUCCU CCUAUCGCp-5´
p9C
5´-pAA CUCAGGAGGGAUAGCGCC-3´
3´-ACUU GAGUCCUGCCUAUCGCp-5´
p9C17C
5´-pAAGCUCAGGAGGGAUAGCGCC-3´
3´-ACUUGGAGUCCUGCCUAUCGCp-5´
B
WT
p17C
p9C
p9C17C
15 30 60 15 30 60 15 30 60 15 30 60 (min)
ds
ss
C
Loading efficiency (Normalized)
Time (min)
D
Fraction passenger ejected
Time (min)
E
g10G/p9C10A
5´-pAA CUCAGG GGGAUAGCGCC-3´
3´-ACUU GAGUCC GCCUAUCGCp-5´
miR390 (WT)
5´-pAA CUCAGGA GGAUAGCGCC-3´
3´-ACUU GAGUCCU CCUAUCGCp-5´
p8A9C
5´-pAA CUCAGGAG GAUAGCGCC-3´
3´-ACUU GAGUCCUG CUAUCGCp-5´
F
g10G/ p9C10A
WT
p8A9C
15 30 60 15 30 60 15 30 60 (min)
ds
ss
G
Loading efficiency (Normalized)
Time (min)
H
Fraction passenger ejected
Time (min)

supplementary Fig S3. The conserved G-A mismatch at position 11 is required for the AGO7–miR390 interaction.

(A) The structure of wild-type miR390/miR390* and its variants used in (B). The mutated nucleotides are in outlined letters.

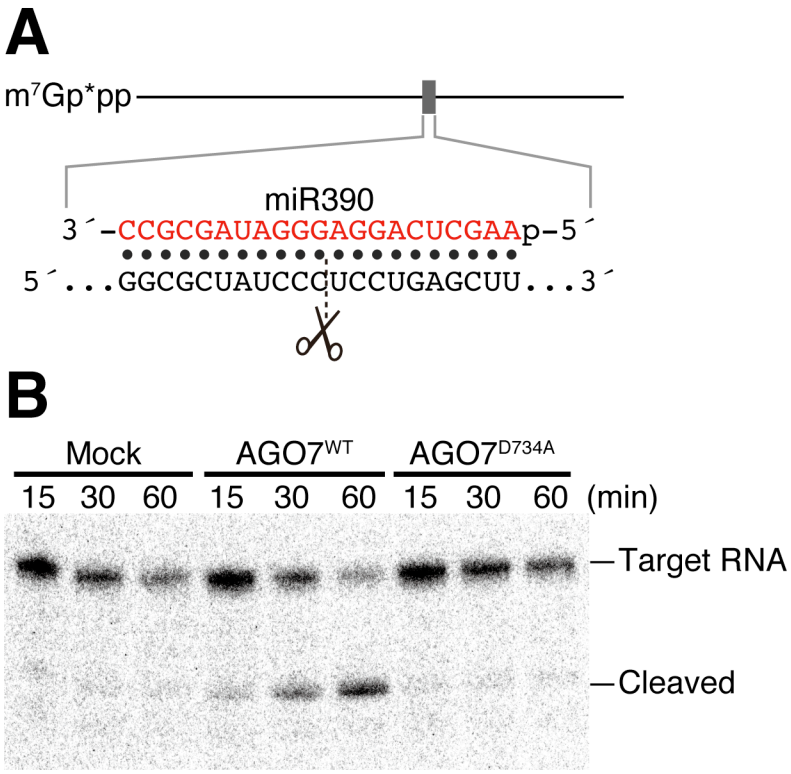
(B) AGO7-RISC assembly using miR390 variants shown in (A). The experiment was performed as in Fig 1A. The central mismatch of miR390/miR390* duplex is required for efficient AGO7-RISC assembly.

(C, D) Quantification of duplex loading and passenger-ejection efficiency in (B).

(E) The structure of wild-type miR390/miR390* and its variants used in (F). The mutated nucleotides are in outlined letters.

(F) AGO7-RISC assembly using miR390 variants shown in (E). The experiment was performed as in Fig 1A. The G-A mismatch at position 11 of miR390/miR390* duplex is important for AGO7-RISC assembly.

(G, H) Quantification of duplex loading and passenger-ejection efficiency in (F).



supplementary Fig S4. AGO7 has slicer activity

(A) The base-pairing configuration between miR390 and the target sequence.

(B) Target cleavage by AGO7 in the BY-2 lysate. BY-2 lysate expressing $AGO7^{WT}$ or $AGO7^{D734A}$ were incubated with miR390/miR390* duplex and cap-labeled target mRNA shown in (A). Cleaved RNAs were analyzed by denaturing 8% polyacrylamide gel. $AGO7^{WT}$ but not $AGO7^{D734A}$ cleaved the target mRNA.

Supplementary Methods

Plasmid constructions

The following constructs used in this study have been described previously: pCRHA-AGO1L [3], pCRHA-AGO7 [3], pCRHA-AGO2 [3], pBYL2 [4], and pAFW (*Drosophila* Gateway vector collection).

pBYL-AGO1

A PCR product containing the AGO1 ORF was amplified with pCRHA-AGO1L as a template using oligo 1 (5′-GCTGGCGCGCCATGGTGAGAAAGAGAAGAACGG-3′) and oligo 2 (5′-GCTGGCGCGCCTCAGCAGTAGAACATGACACG-3′), digested with AscI, and used to replace the corresponding region of pBYL2.

pBYL-AGO7

A PCR product containing the AGO7 ORF was amplified with pCRHA-AGO7 as a template using oligo 3 (5′-GCTGGCGCGCCATGGAAGAAAAAACTCATCATCATCATCAC-3′) and oligo 4 (5′-GCTGGCGCGCCTCAGCAGTAAAACATGAGATTCTTGAC-3′), digested with AscI, and used to replace the corresponding region of pBYL2.

pBYL-AGO2

A PCR product containing the AGO2 ORF was amplified with pCRHA-AGO2 as a template using oligo 5 (5′-GCTGGCGCGCCATGGAGAGAGGTGGTTATCGAGGAGG-3′) and oligo 6 (5′-GCTGGCGCGCCTCAGACGAAGAACATAACATTCTCAAG-3′), digested with AscI, and used to replace the corresponding region of pBYL2.

pBYL-3×FLAGAGO1

A PCR product containing a 3×FLAG-tag sequence was amplified with pAFW as a template using oligo 7 (5′-CTGGCGCGCCATGGACTACAAAGACCATGAC-3′) and oligo 8 (5′-CTTCTCTTTCTCACCTTGTCATCGTCATCCTTGTAATC-3′). Another PCR product containing the AtAGO1 ORF was amplified with pBYL-AGO1 as a template using oligo 9

(5′-CGATGACAAGGTGAGAAAGAGAAGAACGGATGC-3′) and oligo 2. These PCR products were mixed and further amplified using oligo 7 and oligo 2. The PCR product was digested with AscI, and used to replace the corresponding region of pBYL-AGO1.

pBYL-AGO7^{D734A}

Two PCR products were amplified with pBYL-AGO7 as a template using primers oligo 10 (5′-AGATATCTCCACAGCCTTCA-3′) and oligo 11 (5′-TGCGTTACAGCAGCTCCCAT-3′), and oligo 12 (5′-TGGGAGCTGCTGTAACGCAT-3′) and oligo 4. These PCR products were mixed and further amplified using oligo 8 and oligo 4. The PCR product was digested with MscI and AvrII, and used to replace the corresponding region of pBYL-AGO7.

pBYL-3×FLAGAGO7

A PCR product containing a 3×FLAG-tag sequence was amplified with pBYL-3×FLAGAGO1 as a template using oligo 5 and oligo 13 (5′-ATGAGTTTTTTCTTCCTTGTCATCGTCATCCTTGTAATC-3′). Another PCR product containing the AGO7 ORF was amplified with pBYL-AGO7 as a template using oligo 14 (5′-GATGACGATGACAAGGAAGAAAAAACTCATCATCATCATCAC-3′) and oligo 4. These PCR products were mixed and further amplified using oligo 4 and oligo 5. The PCR product was digested with AscI, and used to replace the corresponding region of pBYL-3×FLAGAGO1.

pBYL-3×FLAGAGO7^{D734A}

Two PCR products were amplified with pBYL-AGO7 as a template using primers oligo 8 and oligo 9, and oligo 10 and oligo 4. These PCR products were mixed and further amplified using oligo 8 and oligo 4. The PCR product was digested with MscI and AvrII, and used to replace the corresponding region of pBYL-3×FLAG AGO7.

pBYL-3×FLAGAGO2

A PCR product containing a 3×FLAG-tag sequence was amplified with pBYL-3×FLAGAGO1 as a template using oligo 15 (5′-GTTTTCCCAGTCACGAC-3′)

and oligo 16 (5′-CCACCTCTCTCCTTGTCATCGTCATCCTT-3′). Another PCR product containing the AGO2 ORF was amplified with pBYL-AGO2 as a template using oligo 17 (5′-ACAAGGATGACGATGACAAGGAGAGAGGTGGTTATCGAGGAGG-3′) and oligo 6. These PCR products were mixed and further amplified using oligo 15 and oligo 6. The PCR product was digested with AscI, and used to replace the corresponding region of pBYL-3×FLAGAGO7.

Preparation of mRNAs

Briefly, in vitro transcription and 5′-capping were performed using Ampliscribe T7 High Yield Transcription Kit (Epicentre) and ScriptCap m^7G Capping System (Epicentre), respectively. *3×FLAGAGO1*, *3×FLAGAGO7*, and *3×FLAGAGO7*^{D734A} mRNAs were transcribed from NotI linearized pBYL3×FLAGAGO1, pBYL 3×FLAGAGO7, and pBYL 3×FLAGAGO7^{D734A}, respectively. The target mRNA for cleavage assay were transcribed from a PCR product which was amplified with pGL3-basic (Promega) as a template using oligo 18 (5′-AATTCGAGCTCTAATACGACTCACTATAGTCACATCTCATCTACCTCCC-3′) and oligo 19 (5′-CCCATTTAGGTGACACTATAGATTTACATCGCGTTGAGTGTAGAACGGTTGTATAAAAGGTAAGCTCAGGAGGGATAGCGCCGAAGAGAGGAGTTCATGATCAGTGC-3′).

Supplementary References

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2. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. *Genome Res*, **14:** 1188–190
3. Takeda A, Iwasaki S, Watanabe T, Utsumi M, Watanabe Y (2008) The mechanism selecting the guide strand from small RNA duplexes is different among Argonaute proteins. *Plant Cell Physiol*, **49:** 493–00
4. Mine A, Takeda A, Taniguchi T, Taniguchi H, Kaido M, Mise K, Okuno T (2010) Identification and characterization of the 480-kilodalton template-specific RNA-dependent RNA polymerase complex of *Red clover necrotic mosaic virus*. *J Virol*, **84:** 6070–081