T-hairpin structure found in the RNA element involved in piRNA biogenesis

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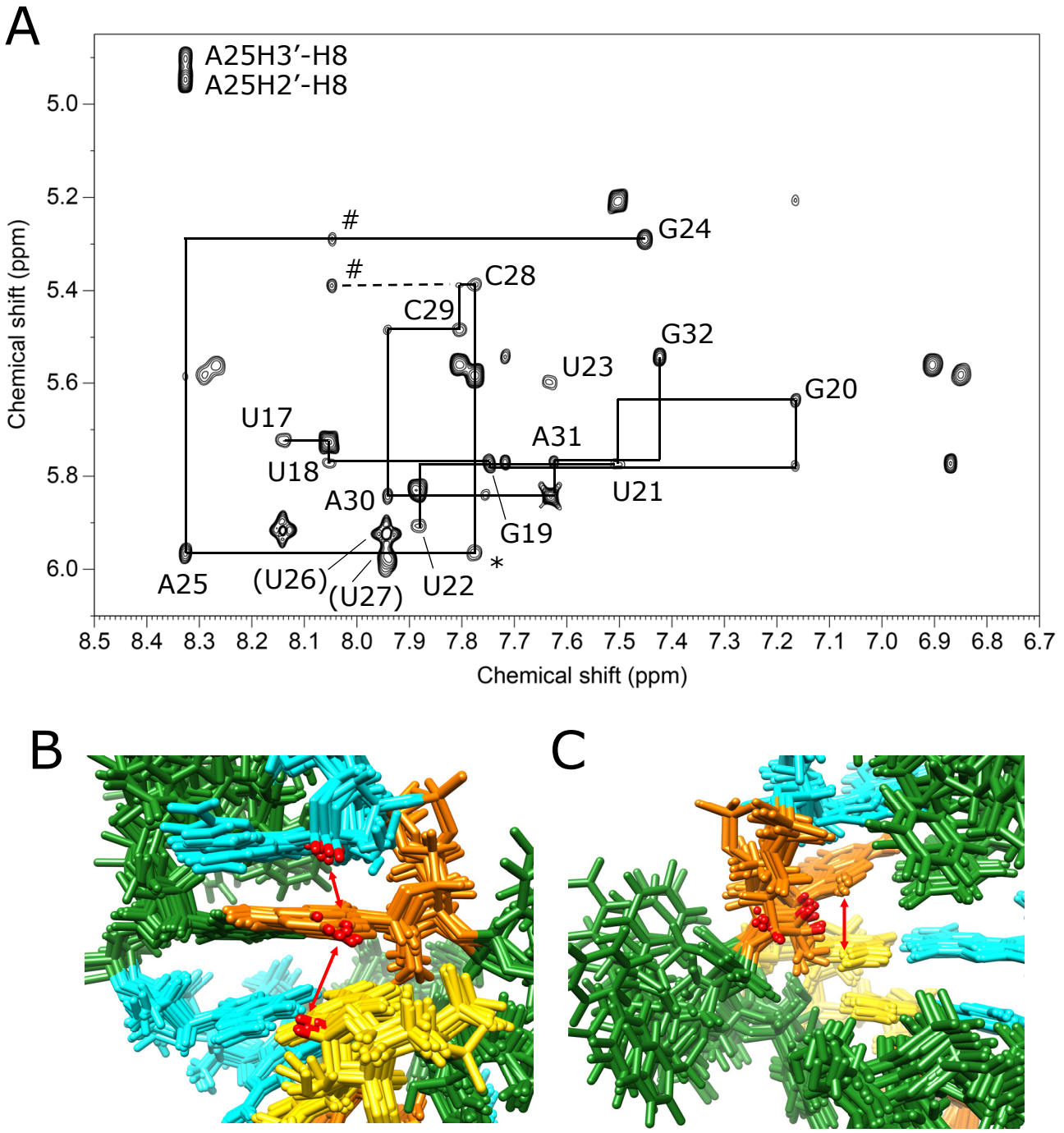
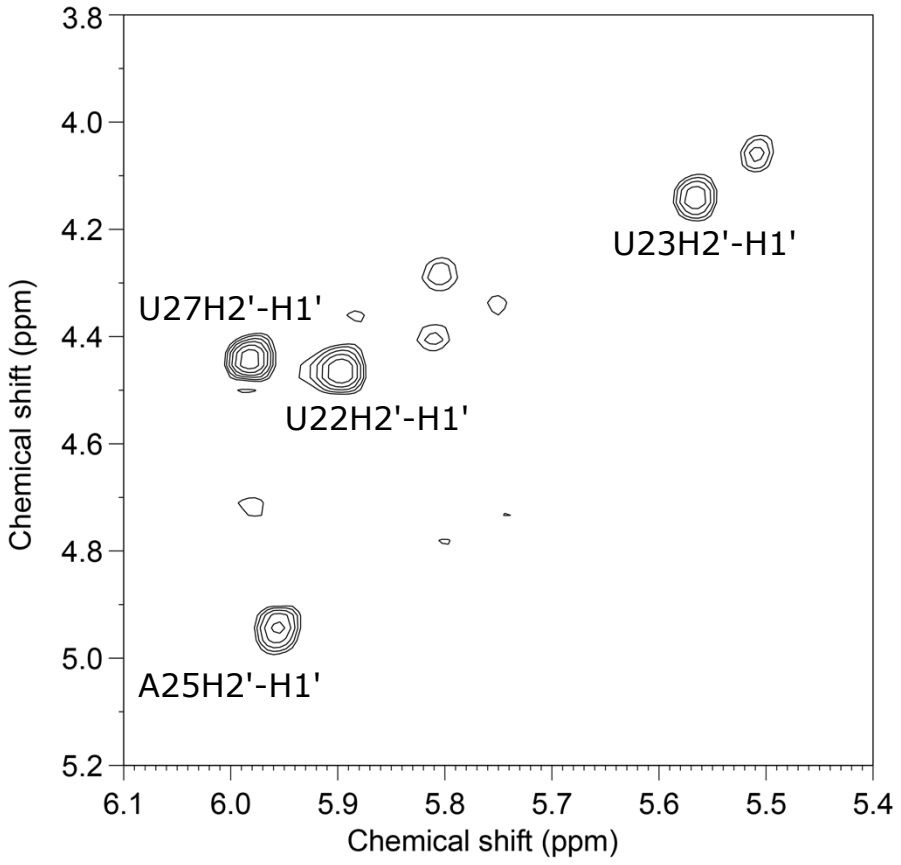
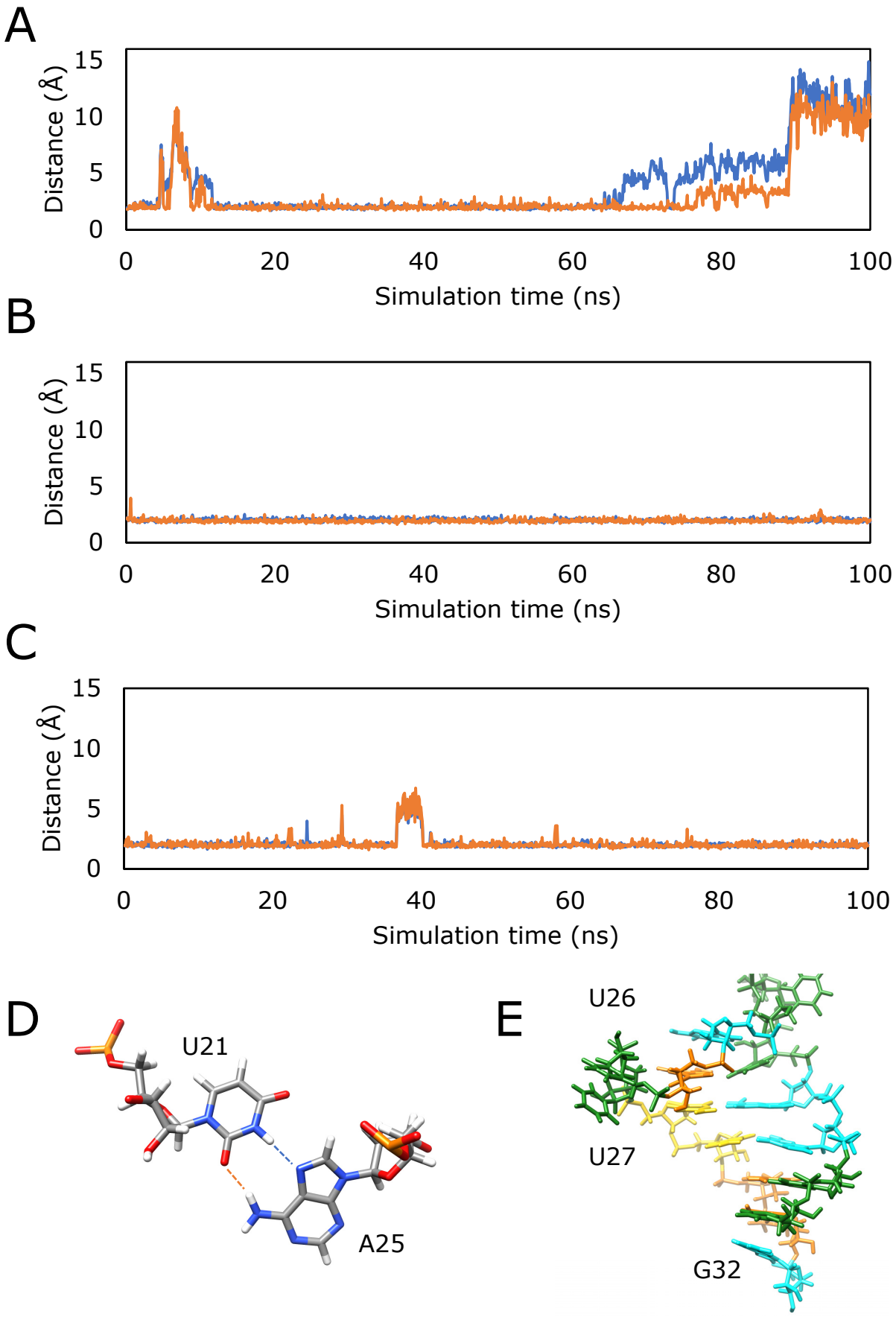


Supplemental Figure S1. Sequential assignment of *tj*-17-32 and characteristic NOEs
A: The NOESY spectrum measured at 298 K was shown. The intra-residual NOE for H8/H6-H1' were indicated by the residue label. U23, U26 and U27 did not show any inter-residual NOEs in this region. For U26 and U27, position of the H5-H6 signals were indicated by the residue labels in parentheses. The inter-residual NOE between A25 and C28 is indicated by asterisk. The inter-residual NOEs for A25H2-G24H1' and A25H2-C28H1' are indicated by sharp symbols. B, C: Superposition of the ten lowest energy structures. B: H2 of A25, H1' of G24 and C28 are indicated by red. The red arrows indicate the proton pairs which show characteristic NOEs. C: H2' and H3' of A25 indicated by red did not show any inter-residual NOEs and are structurally isolated from other residues. The red arrows indicate the proton pair of A25H8 and C28H42 which shows an NOE.

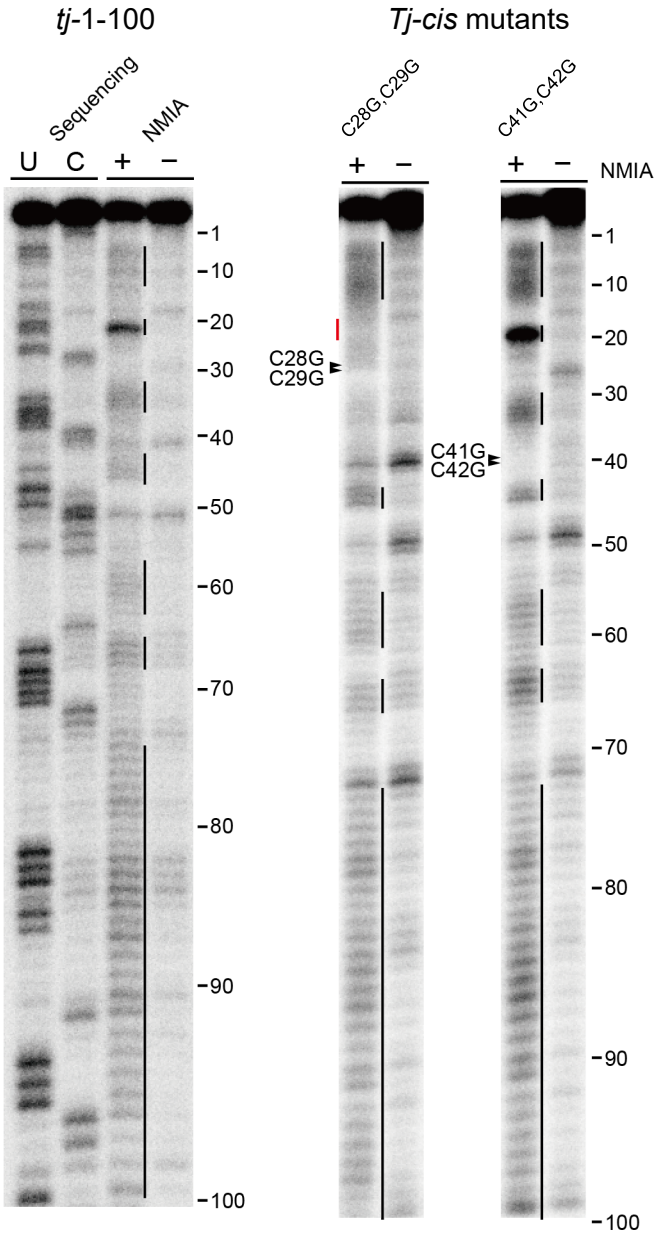


Supplemental Figure S2. HOHAHA spectrum of *tj*-17-32
The regions for H2'-H1' were shown. For A25 and U27, the signals were intense, indicating the formation of the C2'-endo form. For U22 and U23, the signals are rather broad and the possibility of the conformational mixture was considered. Thus, for the structural calculation, A25 and U27 were fixed to the C2'-*endo* form, and sugar packers of U22 and U23 were not fixed.



Supplemental Figure S3. MD analysis of the T-hairpin structure

Distances of U21H3-A25N7 and U21O2-A25H62 during 100 ns of MD simulations were shown as blue and orange, respectively. The lowest energy structure (A), the second lowest energy structure (B) and the third lowest energy structure (C) were used as the initial structures. (D) The U21-A25 base pair of the minimized average structure. (E) A snapshot at 30 ns for the MD simulation with the lowest energy structure as the initial structure.



Supplemental Figure S4. SHAPE analysis of *Tj-cis* RNA mutants

Single stranded region with structural fluctuation were detected by the effect of NMIA. Positions of point mutations were indicated in each pair of lanes. Black bars indicate regions affected by NMIA. Red bars indicate regions affected by point mutations.

Supplemental Table S1. RNA fragments used for NMR measurements

Fragments with stable isotopic labelling and sequence variants were not shown.

Additional G residues for *in vitro* transcription were indicated by lower letter.

RNA	Sequence
transcript	
tj-1-100	gggCAAUGUAUAAGGUAAAUUGGUUUGAUUCCAAGAAUGUUUUCCAAAUAGUCUCCACUCGAAAGAACAUGUUUUCCAAAAAGAGUUUGUUAAAGCGUUUCCA
tj-1-74	gggCAAUGUAUAAGGUAAAUUGGUUUGAUUCCAAGAAUGUUUUCCAAAUAGUCUCCACUCGAAAGAACAUGUUUUCCA
tj-1-50	gggCAAUGUAUAAGGUAAAUUGGUUUGAUUCCAAGAAUGUUUUCCAAAUAGUC
Chemically synthesized	
tj-1-32	CAAUGUAUAAGGUAAAUUGGUUUGAUUCCAAG
tj-2-32	AAUGUAUAAGGUAAAUUGGUUUGAUUCCAAG
tj-6-32	UAUAAGGUAAAUUGGUUUGAUUCCAAG
tj-8-32	UAAGGUAAAUUGGUUUGAUUCCAAG
tj-17-32	UUGGUUUGAUUCCAAG

Lower characters are indicated the stable isotopic labelled residues.

Supplemental Table S2. NMR constraints and structural statistics for *tj*-17-32

Number of experimental restraints	
Distance restraints	189
Intraresidue	41
Sequential	39
Medium range	6
Long range	2
vdw distance	89
Hydrogen bonding	12
Dihedral restraints	98
Planarity for base pairs	5
Heavy-atoms r.m.s. deviation (Å)[a]	
All	1.897 ± 0.945
All (pairwise)	2.247 ± 0.647
Backbone	1.155 ± 0.483
Backbone (pairwise)	1.215 ± 0.308
Stem (17-20, 28-31)	0.283 ± 0.129
Stem (pairwise)	0.356 ± 0.100
r.m.s.d. around the ideal values	
bonds (Å)	0.0027 ± 0.00008
angle (º)	0.7118 ± 0.0070

[a]Averaged r.m.s.d. between an average structure and the 10 converged structures were calculated. The converged structures did not contain experimental distance violation of >0.5 Å or dihedral violation >5º.

Supplemental Table S3. Templates and primer for SHAPE analysis and PCR primers and probes used in the piRNA biogenesis assay

tj cis-element SHAPE template, forward		GAGATAATACGACTCACTATAGGGCAATGTATAAGGTAAATTGG
tj cis-element SHAPE template, reverse		GAACCGGACCGAAGCCCGATTTGGATCCGGCGAACCGGATCGATGGAAACGCTTTAACAAACT
SHAPE reverse transcription primer		GAACCGGACCGAAGCCCG
Primer	Pseudoknot_MT-F	TGGAAAGCGTTTCCACGGTCTC
	Pseudoknot_MT-R	AACAAACTCTTTTTGGAAAACA
	SL3_MT-F	ATCTCTCCACTCGAAAGAACATGT
	SL3_MT-R	AATGGAAAACATTCTTGGAATCA
	T-hairpin_MT-F	ATATAAGTATCGTCTGAATGTTTTCCAAATAGTCTCCA
	T-hairpin_MT-R	ACTTCATGATCATAATCTAGTACAGCTCGTCCATGC
	C28G,C29G MT-F	GGAAGAATGTTTTCCAAATA
	C28G,C29G MT-R	AATCAAACCAATTTACCTTA
	C41G,C42G MT-F	GGAAATAGTCTCCACTCGAA
	C41G,C42G MT-R	AAAACATTCTTGGAATCAAA
Probe	repeat probe	GACCGAGTCAAGTTGAAAACCTATGGACCGAGTCAAGTTGAAAACCTATG
	WT (*Tj*-piRNA)	GGTAATGGGAATGCACTTCTCTTGAA