SUPPLEMENTARY DATA

Coordination Among Tertiary Base Pairs Results in an Efficient Frameshift-Stimulating RNA Pseudoknot

Yu-Ting Chen[1,†], Kai-Chun Chang[1,†], Hao-Teng Hu[2], Yi-Lan Chen[3], You-Hsin Lin[1], Chiung-Fang Hsu[1], Cheng-Fu Chang[1], Kung-Yao Chang[2] and Jin-Der Wen[1,3,4,*]

[1] Institute of Molecular and Cellular Biology, National Taiwan University, Taipei 10617, Taiwan.
[2] Institute of Biochemistry, National Chung-Hsing University, Taichung 40227, Taiwan.
[3] Genome and Systems Biology Degree Program, National Taiwan University and Academia Sinica, Taipei, Taiwan.
[4] Department of Life Science, National Taiwan University, Taipei 10617, Taiwan.

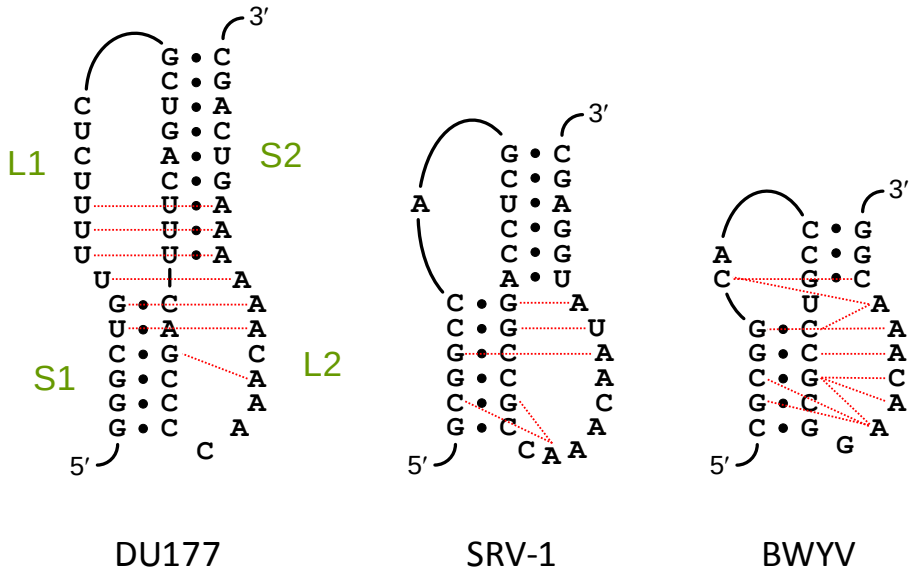
* To whom correspondence should be addressed. Tel: +886-2-33662486; Fax: +886-2-33662478; Email: jdwen@ntu.edu.tw.

† These authors contributed equally to this work.

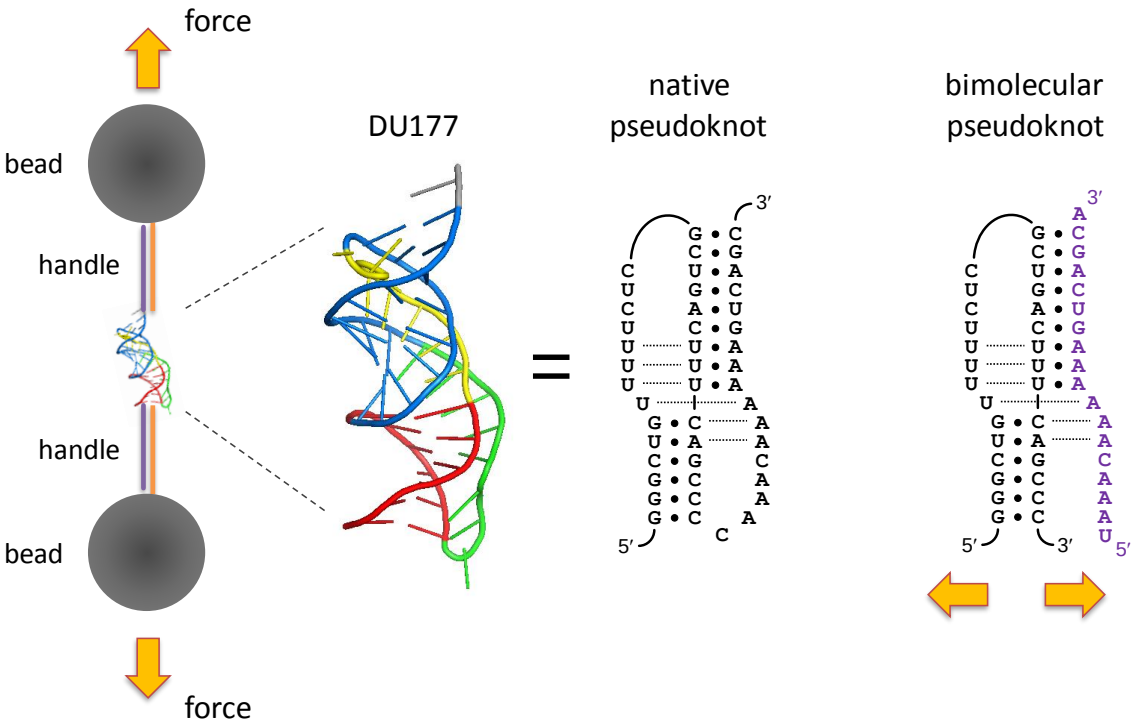
Present Address: You-Hsin Lin, Department of Cell Pharmacology, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan.

SUPPLEMENTARY METHODS

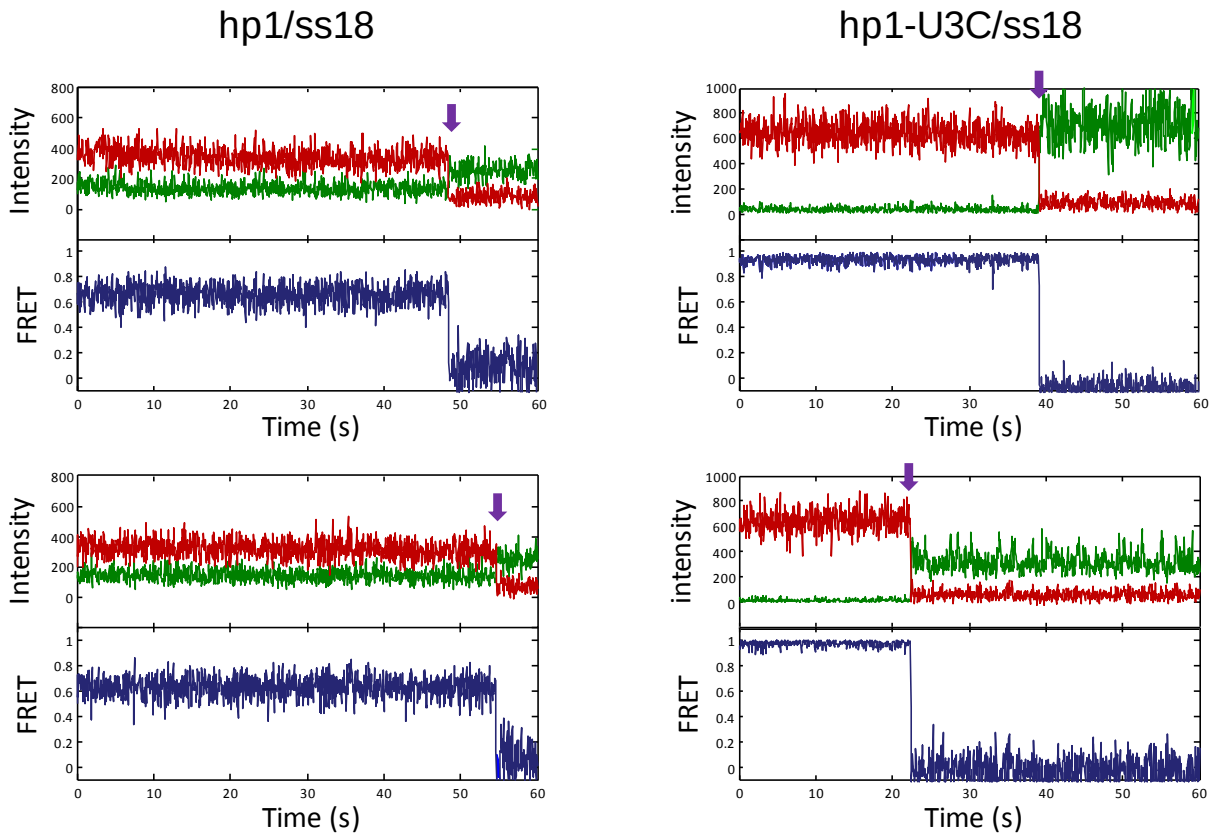
Setup of TIRF microscope. We built an objective-type total internal reflection fluorescence (TIRF) microscope on the IX71 platform (Olympus) for smFRET measurements, per methods described in the literature (1,2). The beam from a 532-nm green laser (75 mW, CL532-075-L, CrystaLaser) was attenuated by a half-wave plate and a beam splitter before expanded 10× through a pair of convex lenses. A 2-inch iris was installed to cut the blur rim of the expanded Gaussian beam, which was focused on the back focal plane of a 100× oil immersion objective (UAPON 100XOTIRF, NA = 1.49, Olympus) by a 2-inch diameter, 300-mm focal-length convex lens. The lens position was adjusted to tune the reflection angle to optimize the signal-to-noise ratio of fluorescence. In the microscope, the emitted light was separated from the excitation beam by a dichroic mirror (FF562-Di03-25x36, Semrock) and then passed through a dual-band emission filter (FF01-577/690-25, Semrock). A home-build dual view system, containing another dichroic mirror (FF650-Di01-25x36, Semrock), was used to separate the signals from donor and acceptor dyes, which were imaged on an EMCCD camera (iXon DU-897D, Andor).



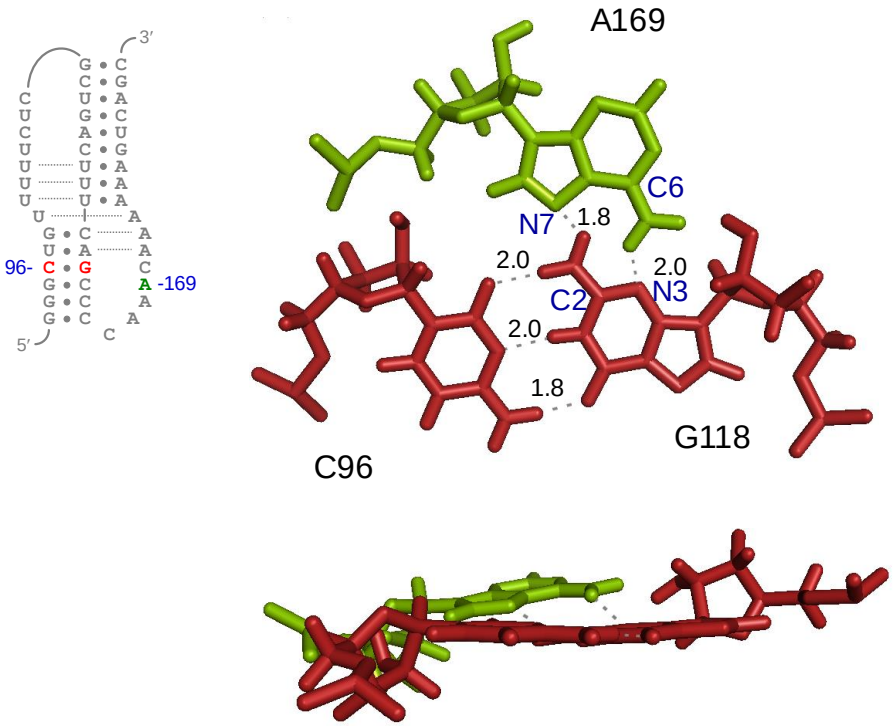
Supplementary Figure S1. Sequence and structural comparison among some of the frameshift-stimulating RNA pseudoknots. DU177 is similar to the retroviral *gag-pro* [such as SRV-1 (3)] and luteoviral P1-P2 [such as BWYV(4)] pseudoknots, in which stem S1 is G/C-rich and mostly 5–6 bp long, and most nucleotides in loop L2 are adenosines appearing in stretches. A rich non-Watson-Crick hydrogen bonds (red dotted lines) form in this region. On the other hand, DU177 has a long loop L1 forming multiple base triple interactions with stem S2, whereas the viral loop L1 usually contains only 1 or 2 nucleotides and thus the tertiary base pair interactions between L1 and S2 are generally limited.



Supplementary Figure S2. Experimental setup for optical tweezers. An RNA structure of interest was flanked by two handles of RNA/DNA duplexes, which were immobilized on the surface of two polystyrene beads (2.1 μm in diameter) through digoxigenin/anti-digoxigenin antibody (top) and biotin/streptavidin (bottom) interactions. The top bead was trapped by laser beams and the bottom bead was fixed on a micropipette. The trap was moved at a rate of 100 nm/s upwards to unfold the structure or downwards to let it refold. When pulled, the native pseudoknot shall experience the tension mainly along the axis of the two stems. By contrast, the opening end of the hairpin component is to be unzipped directly when a bimolecular pseudoknot is pulled (right panel).



Supplementary Figure S3. Representative time traces of smFRET for bimolecular pseudoknots. Two from each of the hp1/ss18 (left) and hp1-U3C/ss18 (right) complexes. Shown are fluorescence intensity of Dy547 (green) and Dy647 (red) and the corresponding FRET (blue). Arrows indicate photo-bleaching of the Dy647 dyes.



Supplementary Figure S4. A putative base triple in DU177. A169 (green) in loop L2 is positioned very close to the G118•C96 base pair (red; top panel) and roughly on the same plane (bottom), such that two hydrogen bonds may form between A169 and G118 (dotted lines; distances in Angstrom are indicated).

Supplementary Table S1. Unfolding forces for the regular RNA pseudoknots measured by optical tweezers.

			unfolding force (pN)	
	force range	trace # (percentage)	mean	SD
DU177	low	380 (84%)	14.8	1.3
	high	71 (16%)	42.3	2.1
UUC	low	42 (11%)	15.3	4.6
	high	347 (89%)	39.1	2.3

Supplementary Table S2. Unfolding forces for the bimolecular RNA pseudoknots measured by optical tweezers.

			unfolding force (pN)	
hairpin	annealed oligo	trace #	mean	SD
hp1	-	589	15.5	1.6
	ss11	228	16.9	1.3
	ss13	540	21.0	1.5
	ss18	504	22.3	1.6
	ss18U	264	19.8	1.3
hp1-U3C	-	805	12.7	1.7
	ss18	760	12.6	1.8

SUPPLEMENTARY REFERENCES

1. Roy, R., Hohng, S. and Ha, T. (2008) A practical guide to single-molecule FRET. *Nat. Methods*, **5**, 507-516.
2. Joo, C. and Ha, T. (2008) In Selvin, P. R. and Ha, T. (eds.), *Single-Molecule Techniques : A Laboratory Manual* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
3. Michiels, P.J., Versleijen, A.A., Verlaan, P.W., Pleij, C.W., Hilbers, C.W. and Heus, H.A. (2001) Solution structure of the pseudoknot of SRV-1 RNA, involved in ribosomal frameshifting. *J. Mol. Biol.*, **310**, 1109-1123.
4. Su, L., Chen, L., Egli, M., Berger, J.M. and Rich, A. (1999) Minor groove RNA triplex in the crystal structure of a ribosomal frameshifting viral pseudoknot. *Nat. Struct. Biol.*, **6**, 285-292.