Triplex Structures in an RNA Pseudoknot Enhance Mechanical Stability and Increase Efficiency of –1 Ribosomal Frameshifting

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One amino acid in a protein is coded by three nucleotides in a messenger RNA

0 frame:  

Start codon  Stop codon
Ribosomal reading frames

+1 frame: AUG

0 frame: AUG

−1 frame: AUG

Intrinsic frameshift error: $<5 \times 10^{-5}$
Structures in mRNA coding regions have to be unfolded for translation

Structure of prokaryotic ribosome

Three components for programmed $-1$ ribosomal frameshifting

0 frame: $\text{start codon} \quad \text{slippery sequence} \quad \text{single strand spacer (5-10 nucleotides)} \quad \text{pseudoknot}

$X \ Y \ Y \ Z$

$-1$ frame: $\text{start codon} \quad XXX \ YYY \ Z$

$X$ is any nucleotide, $Y$ is A or U, and $Z$ is usually not G
Pseudoknot structure stimulates –1 ribosomal frameshifting

Drawing is not to scale
Programmed –1 ribosomal frameshifting is utilized by many viruses

- Human immunodeficiency virus (HIV) (–1 frameshifting efficiency: <10%).

- Severe Acute Respiratory Syndrome coronavirus (SARS-Cov) (–1 frameshifting efficiency: ~15%).

- Novel anti-virus therapy may be developed by targeting –1 frameshifting.
Choosing a model pseudoknot to study 
–1 ribosomal frameshifting

• High resolution three dimensional structure
• High efficiency (>40%) in stimulating –1 frameshifting
NMR structure of human telomerase RNA pseudoknot (ΔU177)

Base triples in human telomerase RNA pseudoknot (ΔU177)

Major groove base triples

U·A·U

C⁺·G·C
Human telomerase RNA pseudoknot (ΔU177) as a model system to study –1 frameshifting

- Are pseudoknot base triples required for high efficiency –1 ribosomal frameshifting?
- How do base triples affect mechanical stability of RNA pseudoknot?
The construct for bulk frameshifting assay

In vitro translation in rabbit reticulocyte lysate (Promega)

start codon

0 frame stop codon
The construct for bulk frameshifting assay

In vitro translation in rabbit reticulocyte lysate (Promega)

0 frame: Smaller protein

–1 frame: Larger fusion protein
Pseudoknot mutants based on $\Delta U177$:

Mutations in loops

<table>
<thead>
<tr>
<th>Pseudoknot</th>
<th>Number of base triples disrupted</th>
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<tbody>
<tr>
<td>$\Delta U177$</td>
<td>0</td>
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<tr>
<td>TeloWT (with U177)</td>
<td>0</td>
</tr>
<tr>
<td>100C115C174G</td>
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Pseudoknot mutants based on ΔU177:
Mutations in stem 2

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Pseudoknot mutants based on ∆U177: Mutations in stem 2 and loop 1

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Diagram showing stem-loop structures with mutations indicated.
Frameshifting efficiency measured by SDS-PAGE

Pseudoknot base triples are required for high-efficiency $-1$ frameshifting
Pseudoknot base triples are required for high efficiency –1 frameshifting.
Bulk studies:

• Pseudoknot **base triples** are required for high efficiency (>40%) –1 ribosomal frameshifting.

Single-molecule studies:

• How does RNA pseudoknot **unfold** and **fold**?
• How do **base triples** affect **mechanical stability** of pseudoknot?
• Is there correlation between pseudoknot **mechanical stability** and –1 ribosomal frameshifting efficiency?
Bulk thermal unfolding pathway

Stem 1 hairpin

Folding intermediate

Native pseudoknot

High temperature

Medium temperature

Low temperature

Optical trap behaves as a linear Hooke spring

\[ F = -k \Delta X \]

1 kcal/mol corresponds to ~7 pN•nm or 1.7 \( k_B T \) (298 K)

Single-molecule mechanical (un)folding of RNA

- Mechanical force is a unique physical variable which is applied locally to RNA structures.
- RNA folding and unfolding can be studied in near physiological solution and at room temperature.
- Kinetic intermediates can be directly revealed.
Optical tweezers for single-molecule manipulation of RNA pseudoknots

200 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.3, 22 °C
Three classes of unfolding trajectories

- Low-force 1-step unfolding
- High-force 1-step unfolding
- The second step of 2-step unfolding

The second step of 2-step unfolding

Pulling rate: 100 nm/s
Three classes of unfolding trajectories

The second step of 2-step unfolding

Low-force 1-step unfolding

High-force 1-step unfolding

The second step of 2-step unfolding

Low-force 1-step unfolding

CCC mutant

Pulling rate: 100 nm/s
NMR structure of stem 1 hairpin

Isolated stem 1 and stem 2 hairpins for single-molecule studies

Isolated stem 1 hairpin (29 nt)

Isolated stem 2 hairpin (30 nt)
Analysis of the second step of two-step unfolding forces

Which stem is disrupted during the second step of low force two-step unfolding?
Stem 1 is disrupted during the second step of low force two-step unfolding
Single-molecule mechanical (un)folding pathway

Stem 1 hairpin

Native pseudoknot

Hopping (10-20 pN)

Folding (3 pN)

Unfolding (50 pN)

Unfolding (10-20 pN)

Unfolding (15-20 pN)

Folding (<10 pN)

Single strand
Analysis of one-step unfolding forces to map structural features of the folding intermediate state

Are base triples and stem 2 formed at the folding intermediate state?
Base triples only affect mechanical stability of native pseudoknots
Position of base triple matters

Diagram showing the position of base triple matters with corresponding unfolding force values.

Unfolding force (pN)

- 101C: 17.9 ± 1.7
- 102C: 45.4 ± 1.9

Counts

- 101C: 18.0 ± 2.2
- 102C: 49.8 ± 1.9

Delta U177

- 17.8 ± 1.6
- 49.8 ± 1.9
At the intermediate state, **base triples are NOT formed and stem 2 is partially formed**
Hopping of stem 1 hairpin and Folding intermediate of ΔU177 observed at constant force

Single Strand ↩ Stem 1 Hairpin ↔ Folding Intermediate
Hopping of stem 1 hairpin and Folding intermediate of ΔU177 observed at constant force

Folding Intermediate ↔ Stem 1 Hairpin → Native Pseudoknot
Similar mechanical stability observed for UAU, C+GC, and UGC base triples
Structures of major groove base triples

U·A·U  C⁺·G·C  U·G·C
New folding intermediate structures appear for pseudoknots with mutations in stem 2.
At the new intermediate state, **base triples** are NOT formed and **stem 2** is fully formed.

Folding intermediate  New folding intermediate  Native pseudoknot
Multiple folding intermediate structures appear for TeloWT.
NMR reveals similar structures but different dynamics for \textbf{TeloWT} and \textbf{ΔU177}

\begin{center}
\includegraphics[width=\textwidth]{image.png}
\end{center}

Kim, Zhang, Zhou, Theimer, Peterson, and Feigon, \textit{(2008)} \textit{J. Mol. Biol.}
• Triplex structures significantly enhance mechanical stability of native pseudoknots.

• Triplex structures do not form in the folding intermediate states.

• Is there correlation between native pseudoknot mechanical stability and −1 ribosomal frameshifting efficiency?
Correlation between native pseudoknot mechanical stability and frameshifting efficiency

\[ y = (0.19 \pm 0.27) \exp((0.11 \pm 0.03)x) \]

\[ R^2 = 0.84 \]

- \[ x = 0 \text{ pN}, \quad y = 0.2 \% \]
- \[ x = 57 \text{ pN}, \quad y = 100 \% \]
Unwinding of mRNA pseudoknot by ribosome: Rotation is needed

Frameshifting is enhanced by stabilizing both stem 1 and stem 2.
Conclusions

- RNA pseudoknot unfolding and folding pathways were revealed by single-molecule and mutational studies.
- Both major groove and minor groove base triples of a pseudoknot contribute to mechanical stability and −1 frameshifting.
- Mechanical unfolding may mimic helicase activity of ribosome on mRNA.
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