Library of synthetic 5' secondary structures to manipulate mRNA stability in *Escherichia coli*

Trent A. Carrier et al., Biotechnology Progress 15 (1999), 58-64

Outline

1. Introduction
2. Objective
3. Methods
4. Results
5. Summary
6. Take-Home-Lesson
7. Discussion & Questions

1. Introduction

Gene expression can be regulated at the stages of:

- **Transcription**
- **Translation**
- **Capping**
- **Polyadenylation**
- **Splicing**
- **Stability of mRNA**

} **specific in eukarya**

1. Introduction

- **mRNA stability is a very important regulation process after transcription**

- **The limited lifetime of mRNA enables a cell to alter protein synthesis rapidly in response to its changing needs**

- **In prokaryotic cells mRNAs can survive from seconds to more than an hour; mean-time is 2.4 min**

- **In eukaryotic cells mRNA lifetimes range from several minutes to days**

1. Introduction

The lifetime of mRNA depends on the structure of the mRNA

In eukaryots:

- **poly(A) tail by polyadenylation is added at the 3'-end of mRNA**
- **5'-cap at the 5'-end of the mRNA is added**

→ save the eukaryotic mRNA from degradation by exonucleases
→ longer lifetime of mRNA

In eukaryotes and prokaryotes mRNAs are stabilized by the sequences at the 5' and 3' untranslated regions (UTRs), which form stem-loops

→ protect the mRNA ends from specific enonucleases
→ longer lifetime of mRNA

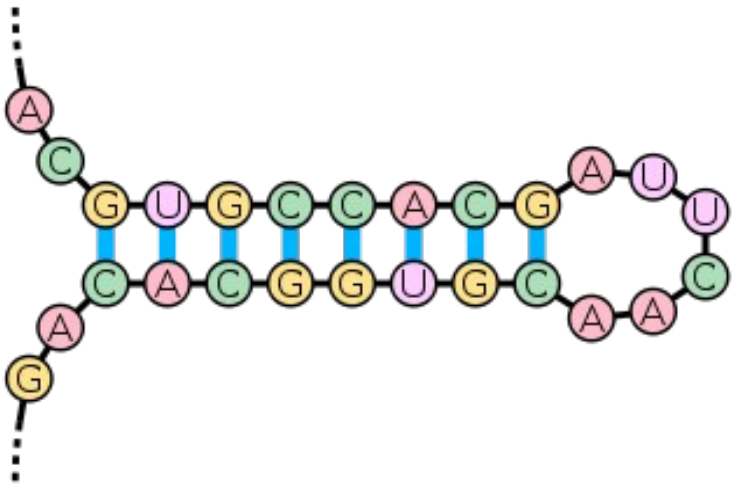
1. Introduction

- Stem-loops occur when two regions of the same strand with palindromic nucleotide sequence form a double helix that ends in an unpaired loop

- The formation of a stem-loop structure depends on the stability of the resulting helix and loop regions

- Large loops with no secondary structure of their own are unstable

- A well known RNA structure with stem-loops is tRNA



source: http://en.wikipedia.org/wiki/Stem-loop

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2. Objective

Analyzing the stabilizing effects of rationally designed synthetic stem-loop structures at the 5'-UTR of mRNA from the ozo1 lacZ-fusion gene in *E.coli*

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3. Methods

- mRNA of the lacZ-fusion gene ozo1 from E.coli was used for testing the stability effects of 5' secondary structures

  → ozo1 was arranged in the non hairpin plasmid pTC 40

- To create hairpins DNA cassettes were introduced into the plasmid:

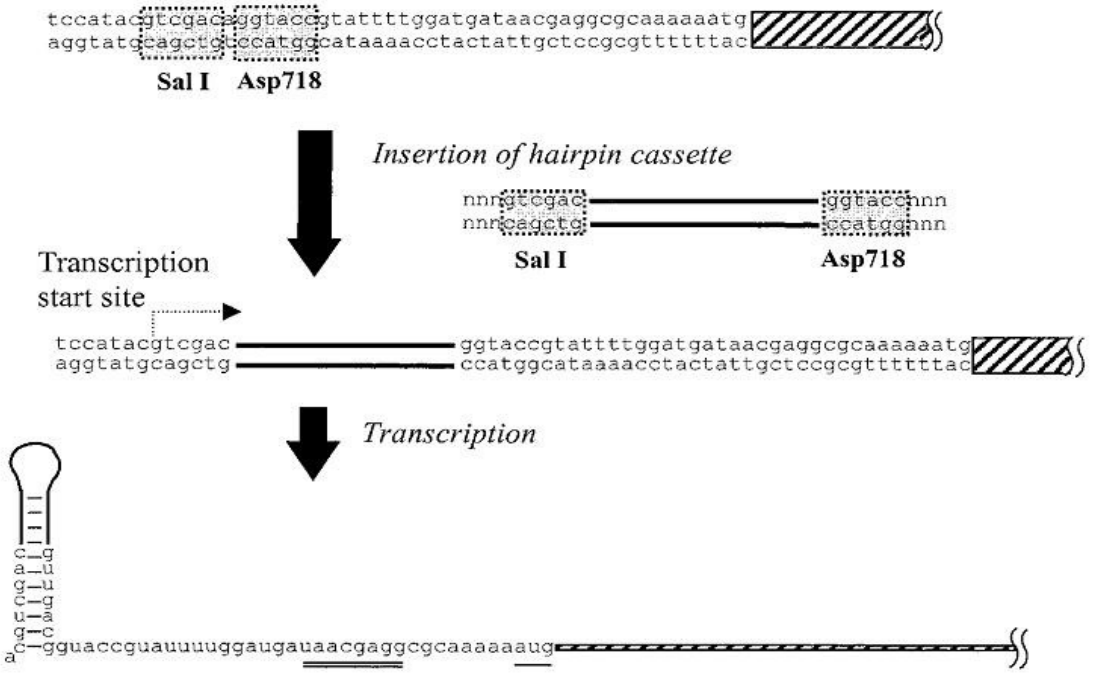
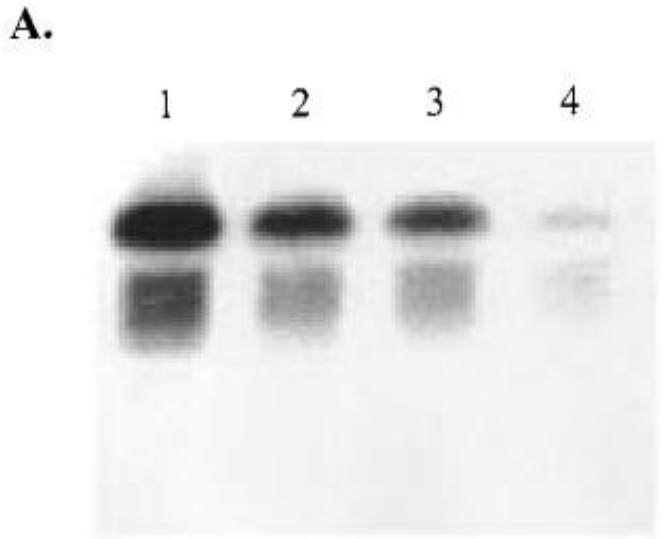


Figure 1. Strategy for inserting synthetic DNA cassettes into the 5′ untranslated region of a gene. Double underlining indicates the Shine–Delgarno region, and single underlining indicates translation start codon.

3. Methods

- To compare the stabilities of the mRNAs the half-life times of them were measured by Northern blot analysis

- Representative example observed for all products:

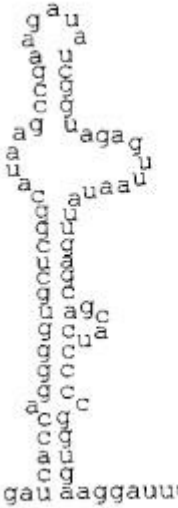


lane 1: 0 min, lane 2: 5 min, lane 3: 10 min, lane 4: 20 min

3. Methods

- **The half-life time of pOZO1 served as a first control for measured half-life times**
  - **→ hairpin of pOZO1 resulted from a gene-fusion of ozo1 with the 5' UTR of ompA 180 of *E.coli***

pOZO1



gau aaggauuuaa

$t_{1/2}$ = 14.7 min (+/- 0.1)

- **The second reference was the half-life time of the 5'-end of the mRNA of the plasmid pTC 40 without a DNA cassette introduction (nonhairpin control)**

pTC40

acgucgacagguaccguauuuu

$t_{1/2}$ = 2.6 min (+/- 0.2)

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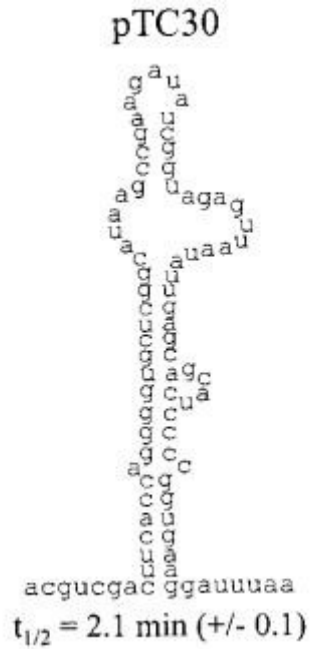
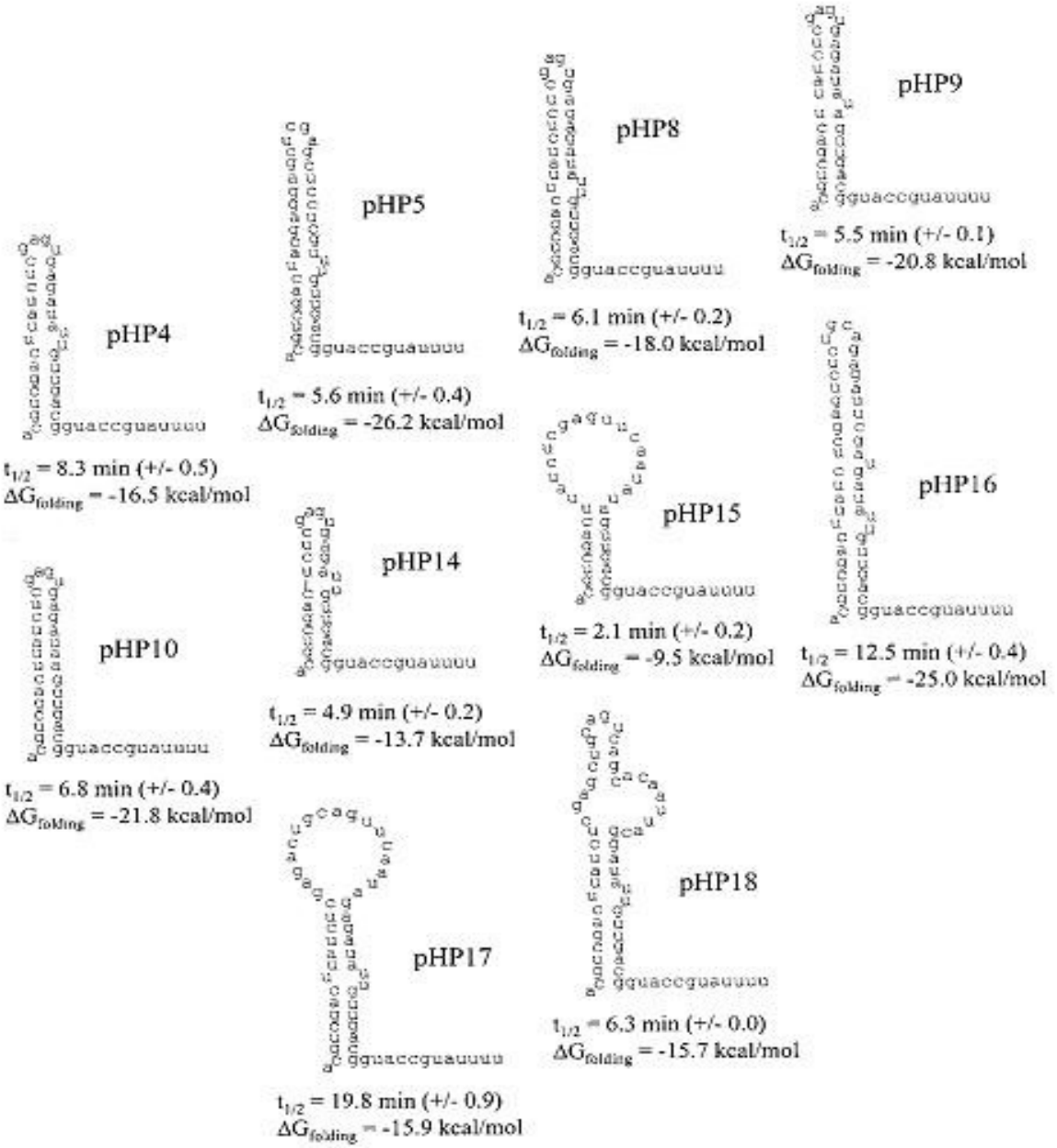
4. Results

- At first additional nucleotids were added at the 5' end of pOZO1
  - → The new mRNA with seven unpaired nucleotids at the 5' end is less stable than the pOZO1 mRNA
    - → Additional unpaired nucleotids at the 5' end of mRNA have destabilizing effects

- The results provided a guide for positioning the desired 5' secondary strucutures in mRNA.



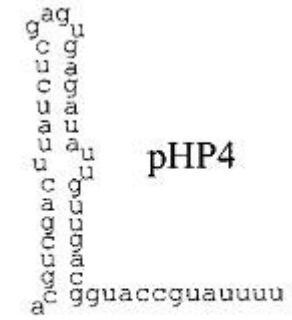
4. Results


pHP4
gguaccguauuuu
$t_{1/2}$ = 8.3 min (+/- 0.5)
$\Delta G_{folding}$ = -16.5 kcal/mol
pHP5
gguaccguauuuu
$t_{1/2}$ = 5.6 min (+/- 0.4)
$\Delta G_{folding}$ = -26.2 kcal/mol
pHP8
gguaccguauuuu
$t_{1/2}$ = 6.1 min (+/- 0.2)
$\Delta G_{folding}$ = -18.0 kcal/mol
pHP9
gguaccguauuuu
$t_{1/2}$ = 5.5 min (+/- 0.1)
$\Delta G_{folding}$ = -20.8 kcal/mol
pHP10
gguaccguauuuu
$t_{1/2}$ = 6.8 min (+/- 0.4)
$\Delta G_{folding}$ = -21.8 kcal/mol
pHP14
gguaccguauuuu
$t_{1/2}$ = 4.9 min (+/- 0.2)
$\Delta G_{folding}$ = -13.7 kcal/mol
pHP15
gguaccguauuuu
$t_{1/2}$ = 2.1 min (+/- 0.2)
$\Delta G_{folding}$ = -9.5 kcal/mol
pHP16
gguaccguauuuu
$t_{1/2}$ = 12.5 min (+/- 0.4)
$\Delta G_{folding}$ = -25.0 kcal/mol
pHP17
gguaccguauuuu
$t_{1/2}$ = 19.8 min (+/- 0.9)
$\Delta G_{folding}$ = -15.9 kcal/mol
pHP18
gguaccguauuuu
$t_{1/2}$ = 6.3 min (+/- 0.0)
$\Delta G_{folding}$ = -15.7 kcal/mol

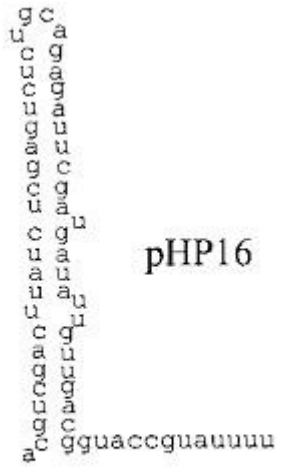
4. Results

- The first trend: The mRNA stability depends on the hairpin strength

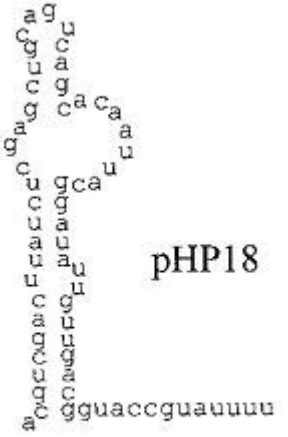
- Example: The stabilities of pHP4, pHP16 and pHP18 show a dependence on $\Delta G_{folding}$



$t_{1/2}$ = 8.3 min (+/- 0.5)
$\Delta G_{folding}$ = -16.5 kcal/mol



$t_{1/2}$ = 12.5 min (+/- 0.4)
$\Delta G_{folding}$ = -25.0 kcal/mol



$t_{1/2}$ = 6.3 min (+/- 0.0)
$\Delta G_{folding}$ = -15.7 kcal/mol

4. Results

- If there's a correlation between folding energy and half-life time there must be a mathematic dependence (semilog dependence)

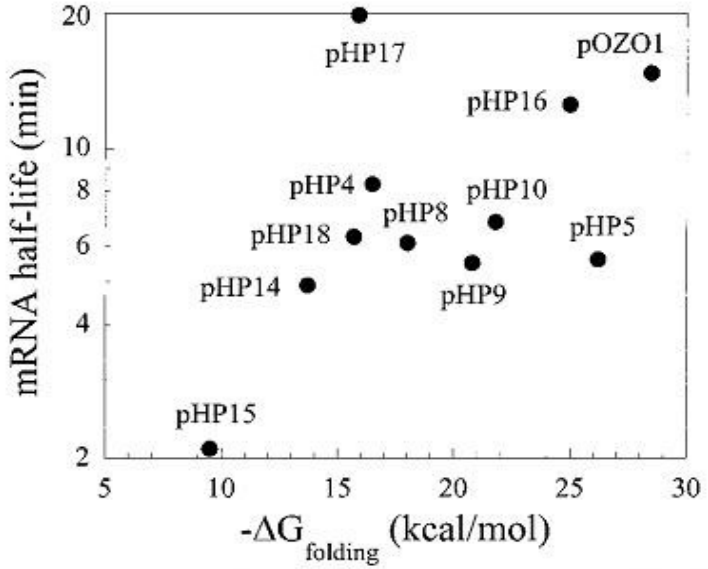


Figure 5. Comparison of free energies of folding and measured mRNA stabilities for library constructs.

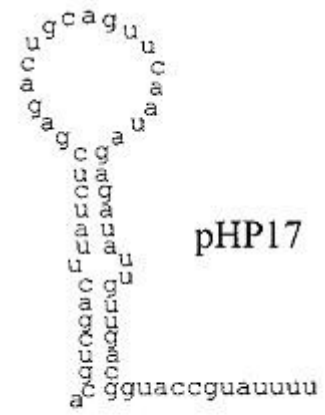
→ The degree of stabilization doesn't correlate with the folding energy

→ The half-life time not only depends on the folding energy

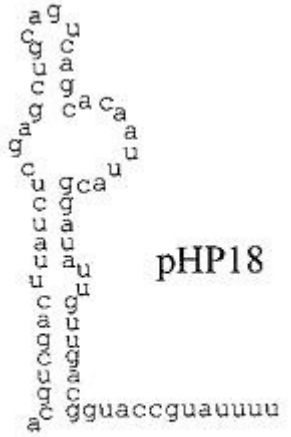
4. Results

- The second trend: The interplay between structure and sequence plays a primary role in determining mRNA stabilization by 5′hairpins

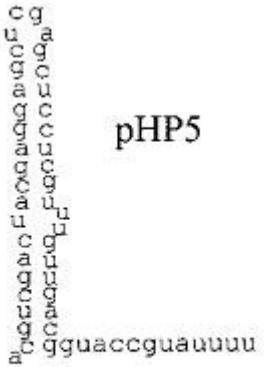
- Example: mRNAs with similar folding energies have very different half-life times (pairs pHP17/pHP18 and pHP5/pHP16)



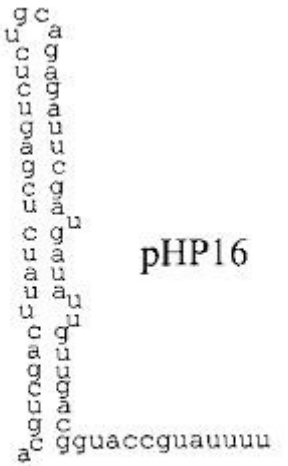
$t_{1/2}$ = 19.8 min (+/- 0.9)
$\Delta G_{folding}$ = -15.9 kcal/mol



$t_{1/2}$ = 6.3 min (+/- 0.0)
$\Delta G_{folding}$ = -15.7 kcal/mol



$t_{1/2}$ = 5.6 min (+/- 0.4)
$\Delta G_{folding}$ = -26.2 kcal/mol



$t_{1/2}$ = 12.5 min (+/- 0.4)
$\Delta G_{folding}$ = -25.0 kcal/mol

4. Results

Possible reasons for the stabilizing effect of 5' stem-loops:

- First assumption:

The presence of a 5′ hairpin structures block the binding of RNase E

→ protecting the mRNA from decay

- Contraindication:

This mechanism would be a competition between RNase E binding and secondary structure formation

→ degree of stabilization would only depends on $\Delta G_{folding}$

(was not observed)

4. Results

- Conclusion:

  mRNA decay is not only influenced by Rnase E but also by additional mechanisms or factors

  → RNA cleavage of the 5′ hairpin by RNase III with following decay by Rnase E is the most likely explanation

- Second assumption:

  The stabilization effect of the hairpins result from the blocking of an unidentified mechanism of mRNA decay and exhibited little dependence on hairpin size

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5. Summary

- Synthetic introduced hairpins can influence mRNA half-lives over an great range

- Some groups of predicted structures having half-lives that showed a strong correlation with hairpin strength while half-lives for another group of predicted structures exhibited little or no dependence on this property

- The details by which mRNA stabilization is achieved remain unclear, but additional mechanisms and/or factors have to be involved

6. Take-Home-Lesson

The introduction of synthetic hairpins can greatly influence the half-life of mRNA, which, coincidentally, does not correlate with the secondary strucutre folding energy and the exact process, by which mRNA stabilization is achieved, still remains unclear.

7. Questions

1. **Wie kann eine 5'-Sekundärstruktur einer mRNA ihre Stabilität beeinflussen?**

2. **Wie kann man den Einfluss einer 5'-Sekundärstruktur auf die Stabilität einer mRNA nachweisen?**