# Selection of the initiator tRNA in the ribosomal P-site and regulation of ribosome heterogeneity in *Escherichia coli*

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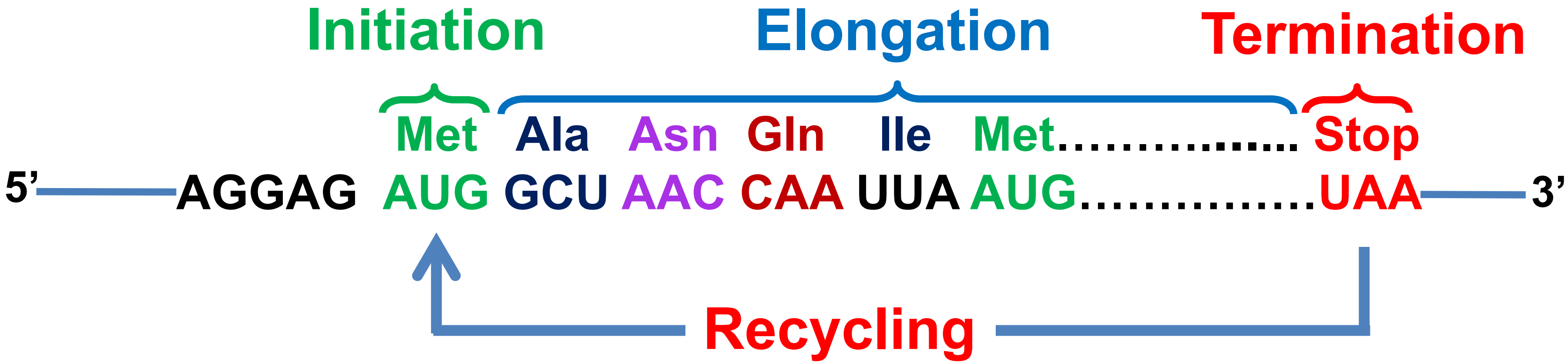
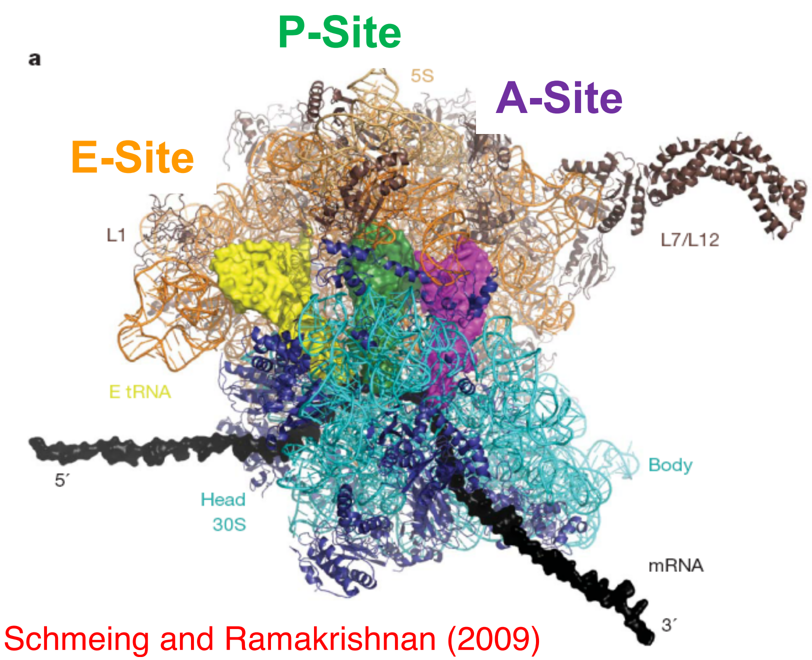
# Translation of mRNA



# Participation of initiator and elongator tRNAs in protein synthesis

tRNA $\xrightarrow[\text{1}]{\text{aaRS}}$ aa-tRNA $\xrightarrow[\text{2}]{\text{EFTu.GTP}}$ EFTu.GTP aa-tRNA $\xrightarrow[\text{3}]{}$ Ribosomal A site

$\text{tRNA}^{\text{fMet}}$ $\xrightarrow[\text{1}]{\text{MetRS}}$ $\text{Met-tRNA}^{\text{fMet}}$ $\xrightarrow[\text{2}]{\text{Formylase}}$ $\text{fMet-tRNA}^{\text{fMet}}$ $\xrightarrow[\text{3}]{\text{IF1, IF2, IF3}}$ Ribosomal P site




Schmeing and Ramakrishnan (2009)

# Participation of initiator and elongator tRNAs in protein synthesis

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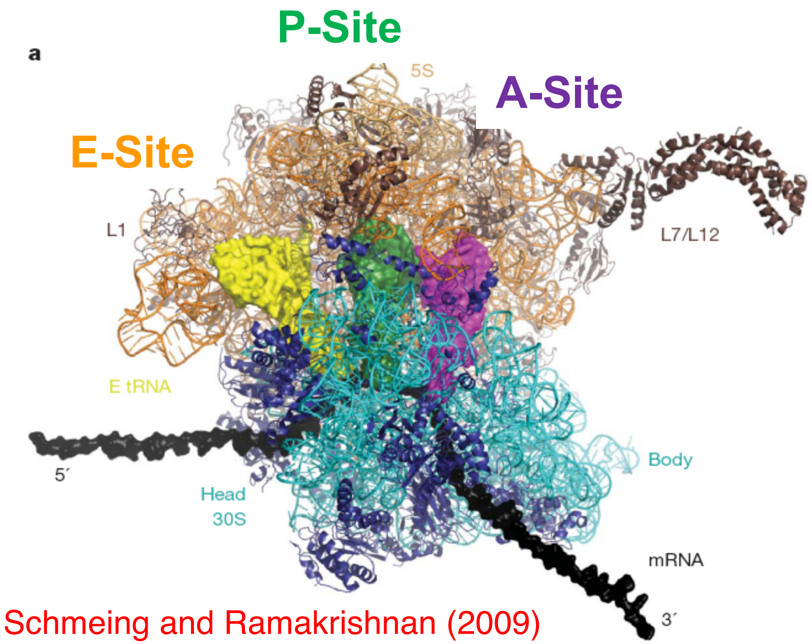
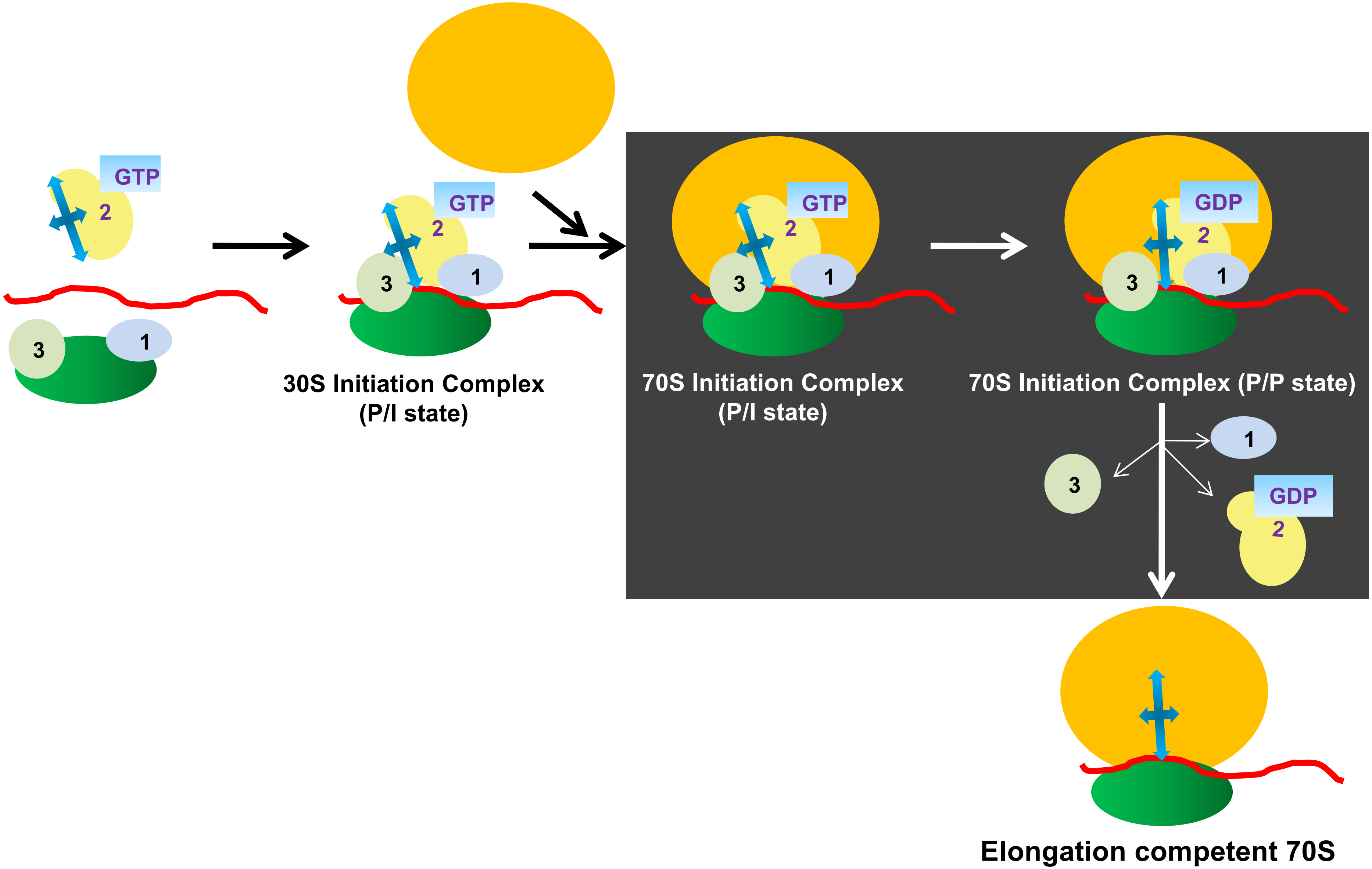


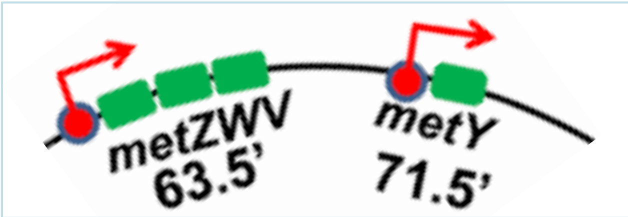

Schmeing and Ramakrishnan (2009)

**How is the initiator tRNA selected on the ribosomes?**

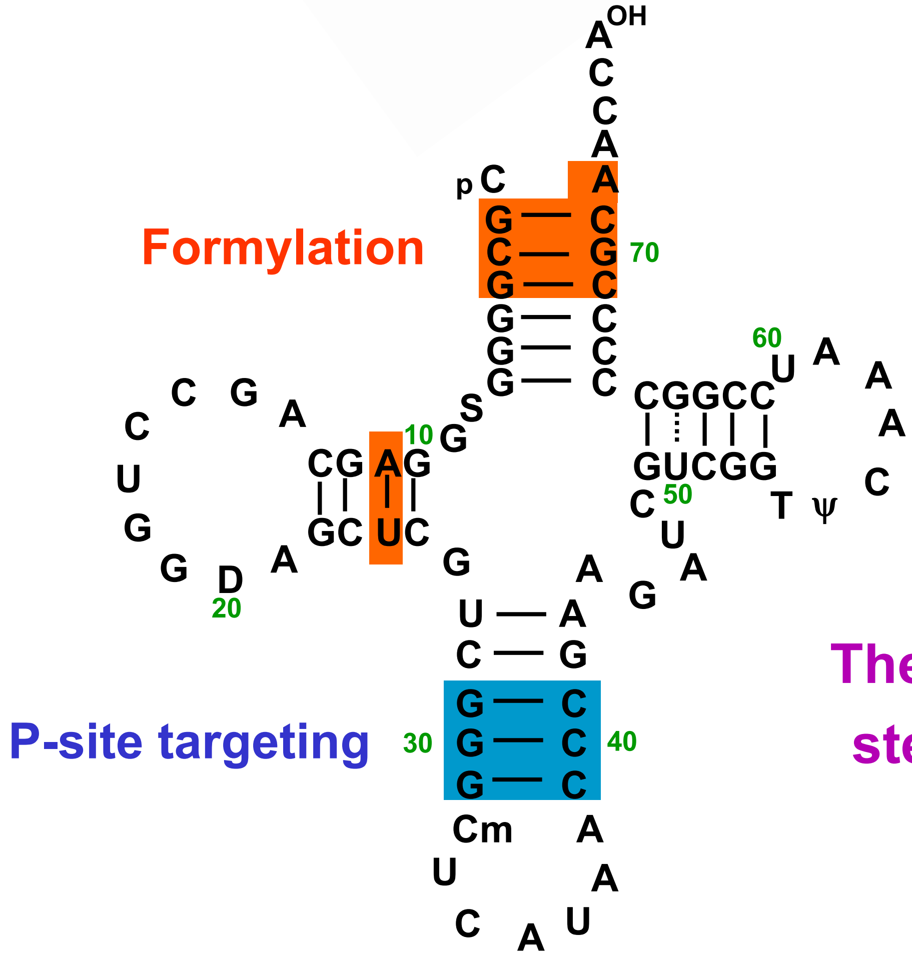
# Initiation in prokaryotes



# Initiator tRNA




***E. coli*** **has four genes for initiator tRNAs**

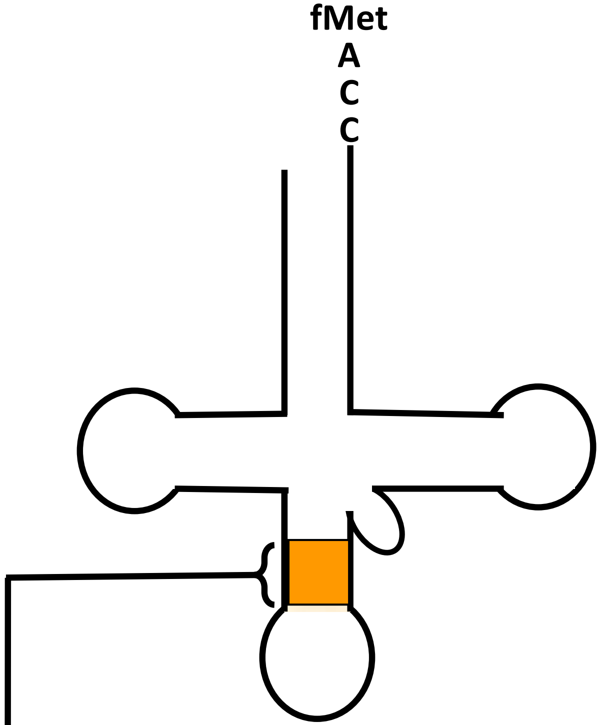



**Formylation is absent in eukaryotes and archaea**

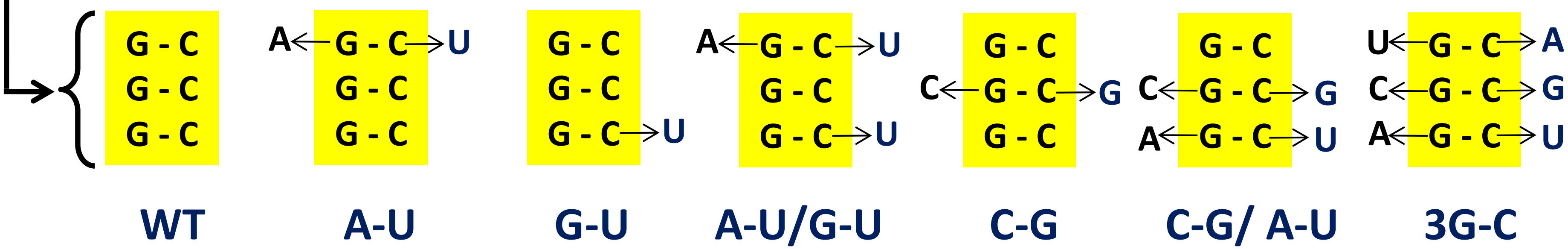
**The 3GC pairs in the anticodon stem are conserved in all the three domains of life**

# Unconventional initiator tRNAs

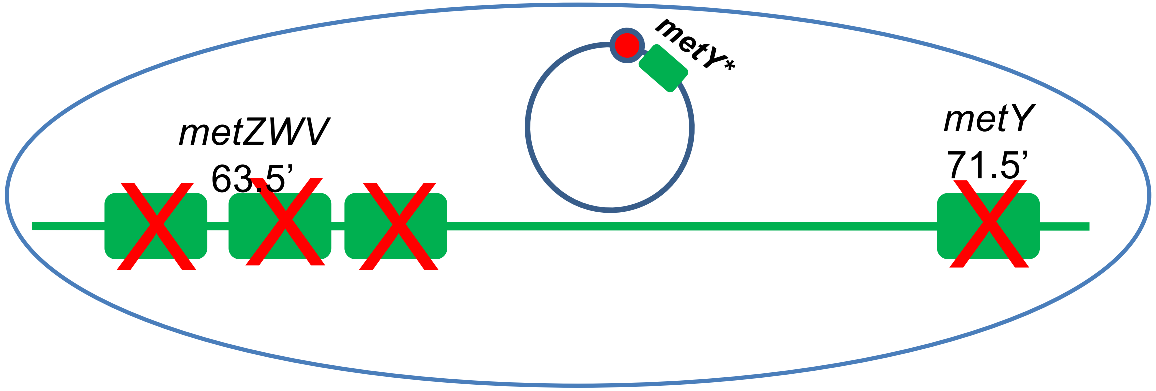
Laasya

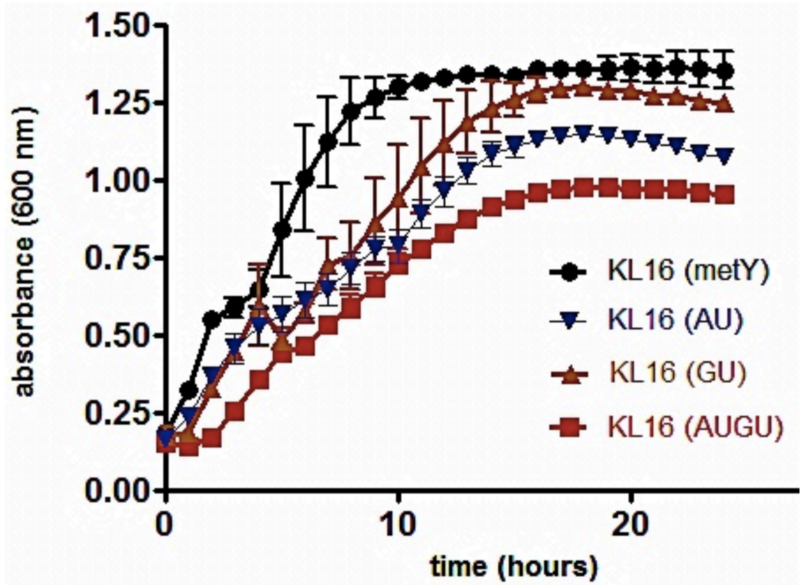


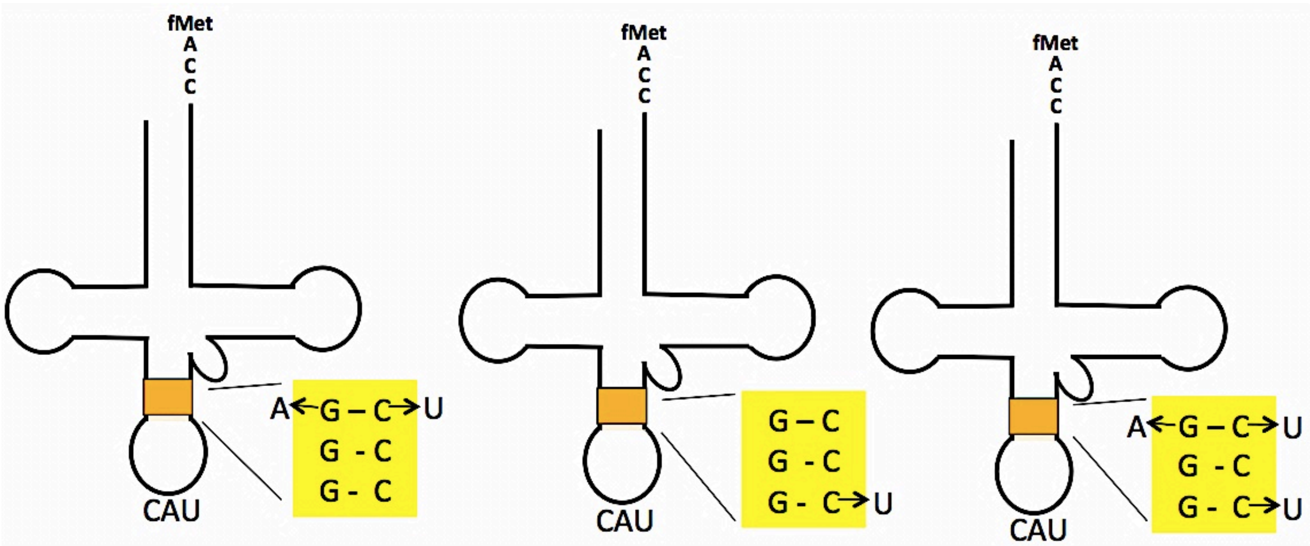

```
E. coli          CGCGG----GGGCTCATAACCC----CAACCA
M. synoviae      CGCGG----GGGCTCATAACCC----CAACCA
M. mobile        CGCGG----AGGCTCATAACCT----CAACCA
M. pulmonis      CGCGG----GGGCTCATAATCC----CAACCA
M. pneumoniae    CGCGG----AGGCTCATAATCT----CAACCA
R. leguminosarum CGCGG----AGGCTCATAACCT----CAACCA
```



# Initiator tRNA mutants that sustain *E. coli*



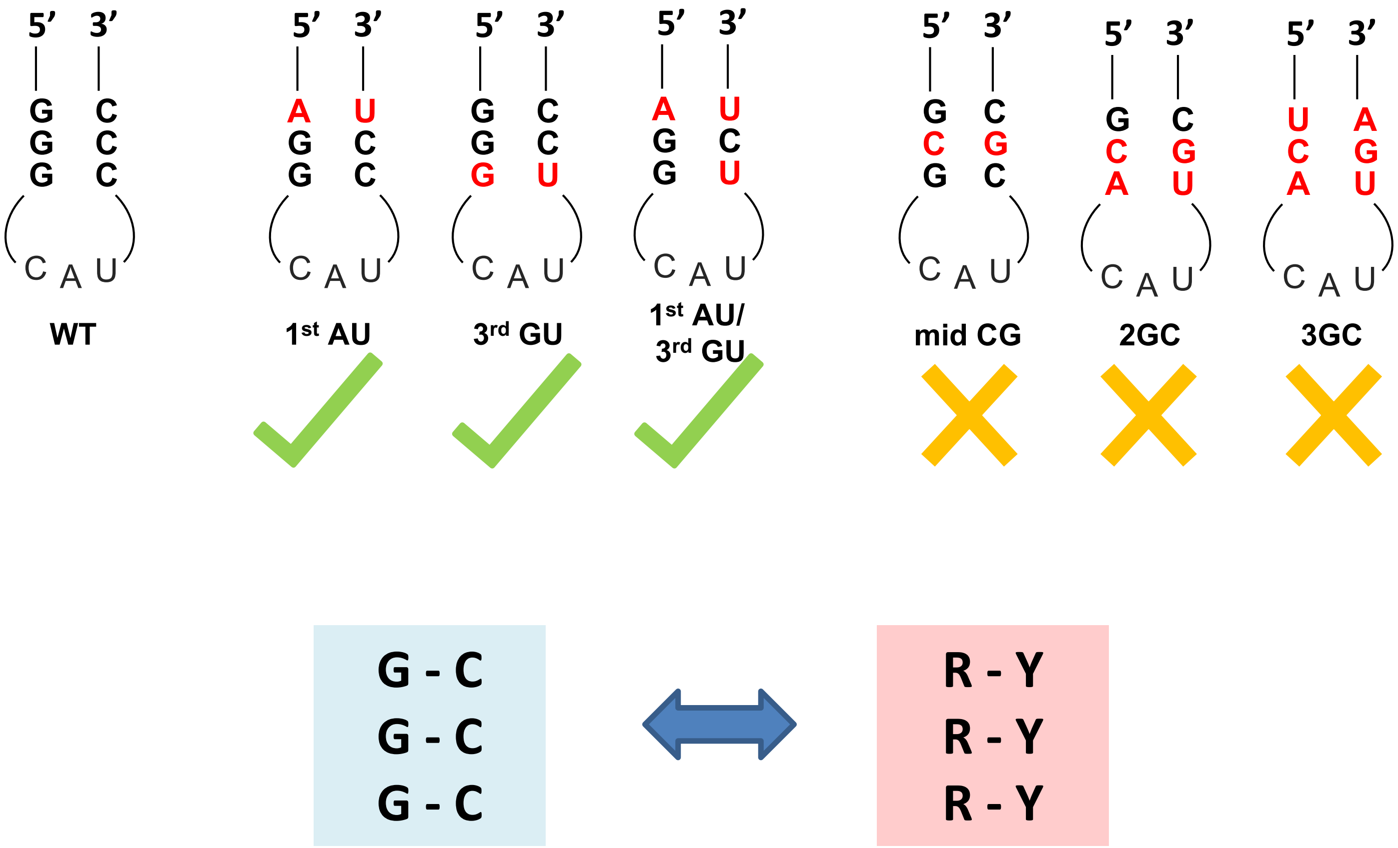




Laasya

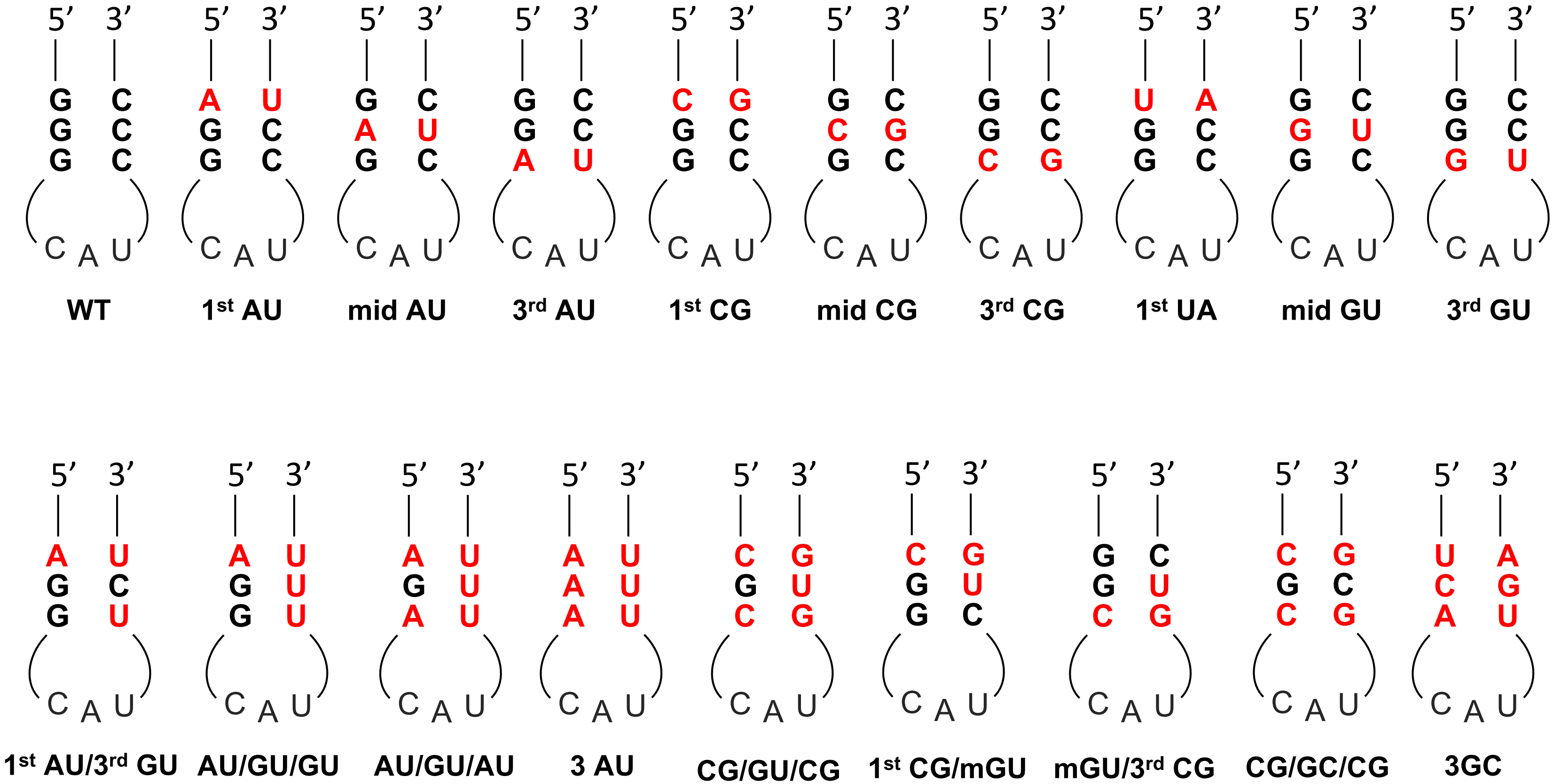
Samhita, L. Shetty, S. and Varshney, U. (2012) Unconventional initiator tRNAs sustain *Escherichia coli.*
***Proc. Natl. Acad. Sci. USA***. 109, 13058-13063

# Functionality of i-tRNA variants in *E. coli*

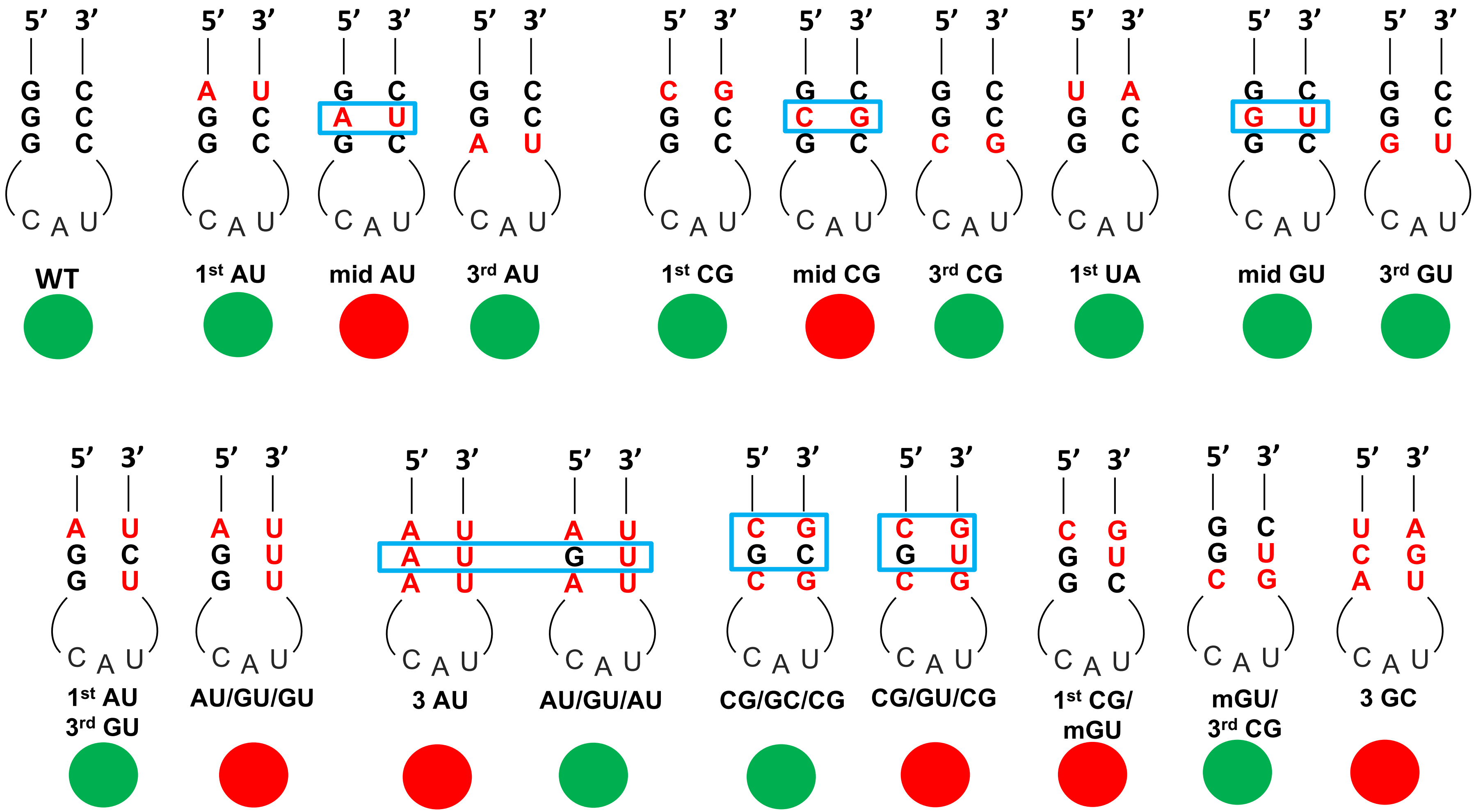



**3 Purine- 3 Pyrimidine rule?**

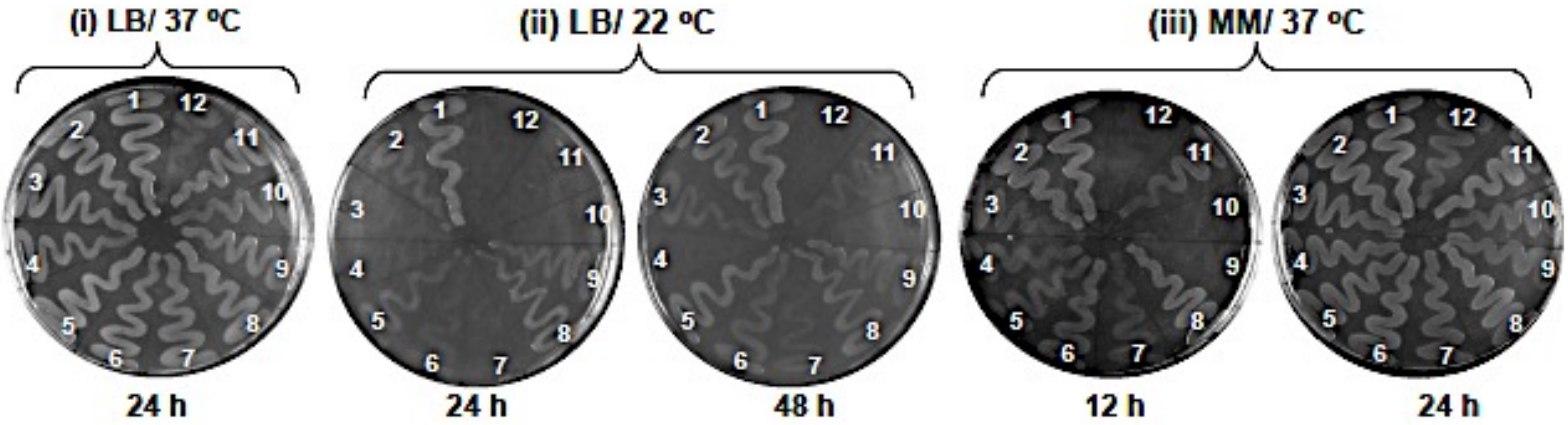
# Mutations in the anticodon stem

# Mutations in the anticodon stem



# Why are the 3GC pairs retained?

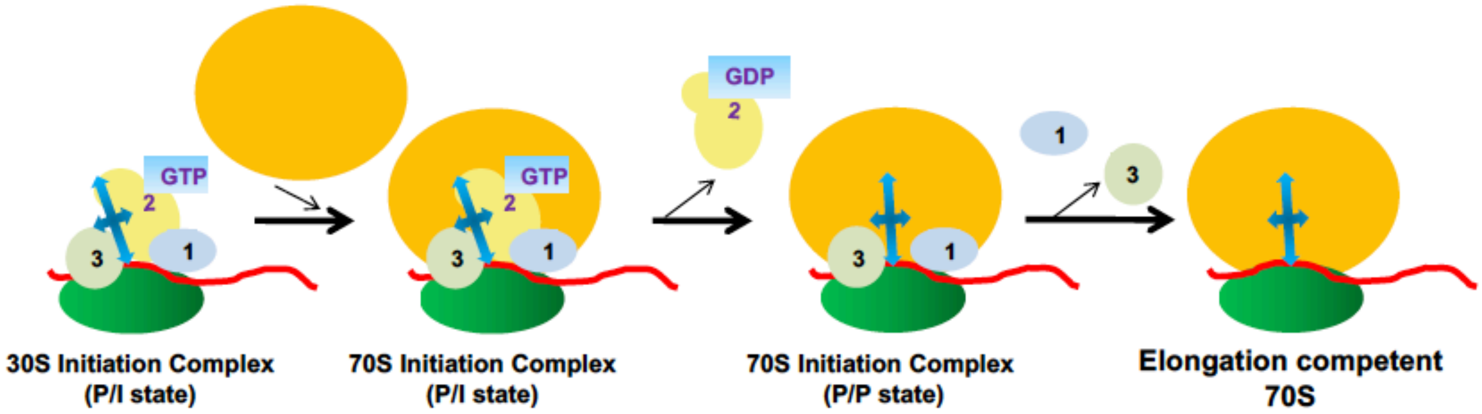


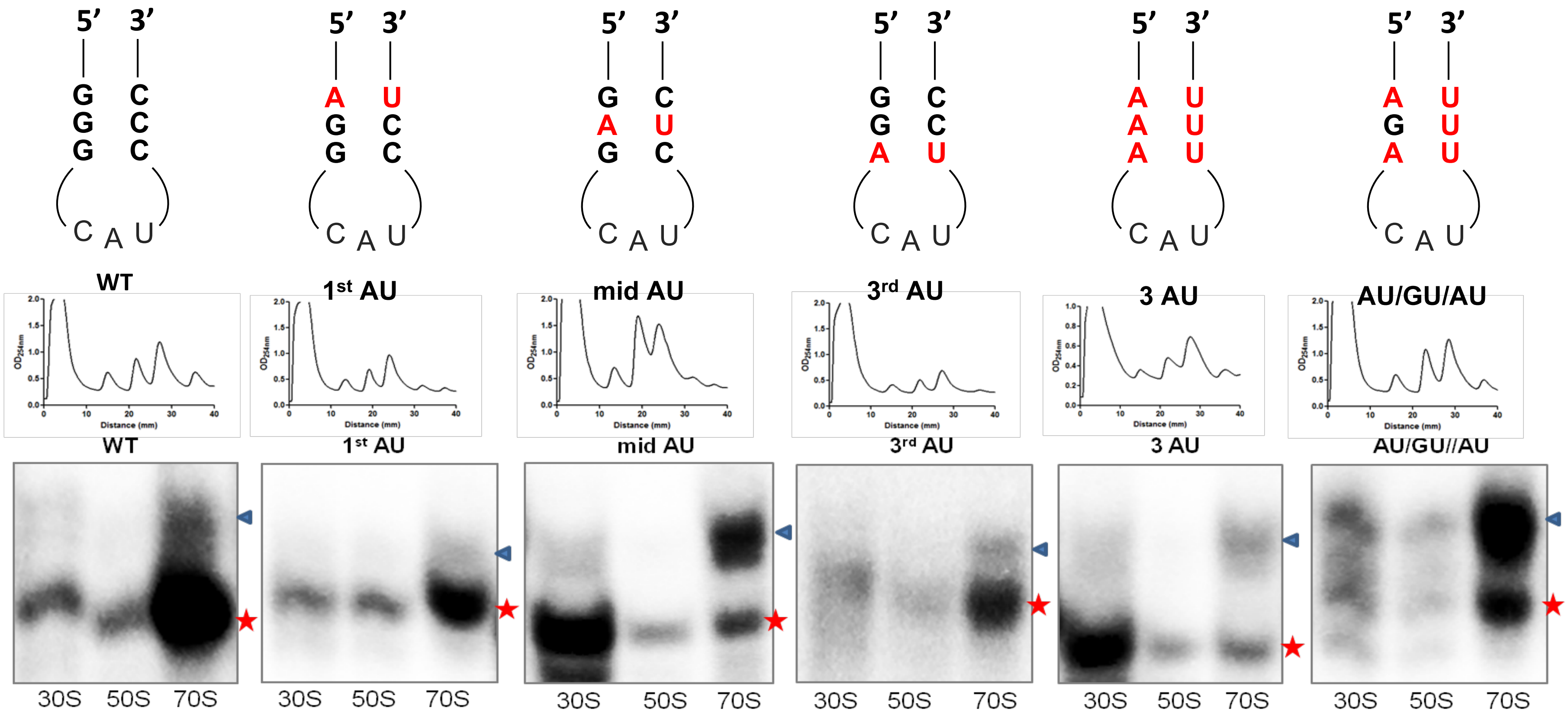

**Sectors:**

KL16 Δ*metZWV*Δ*metY*

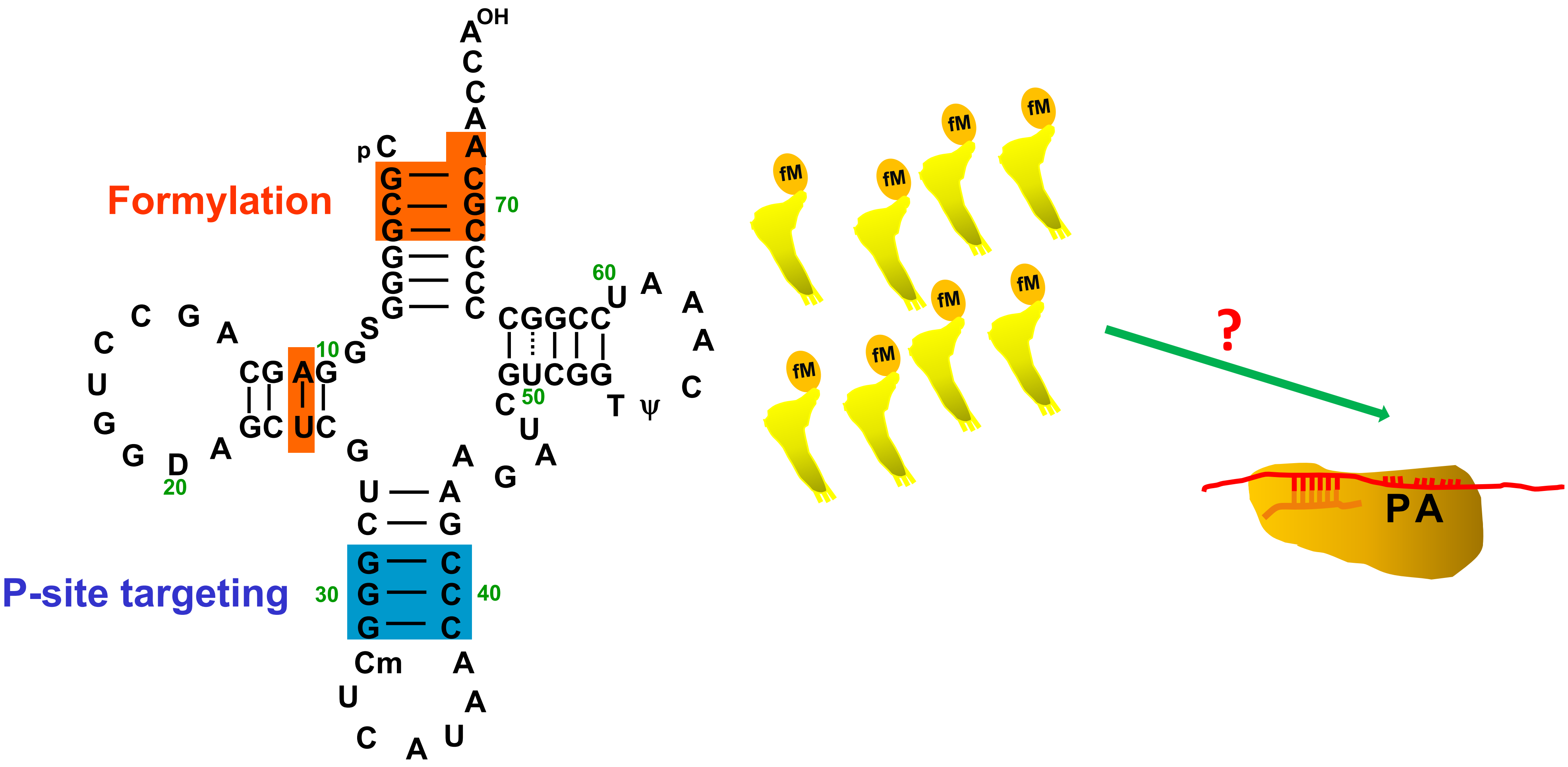
1. p*metY*
2. 1st AU
3. 3rd AU
4. 1st CG
5. 3rd CG
6. 1st UA
7. mid GU
8. 3rd GU
9. au/GC/gu
10. au/gu/au
11. GC/gu/cg
12. cg/GC/cg

# Transition of i-tRNAs from 30S to 70S



# Role of Formylation



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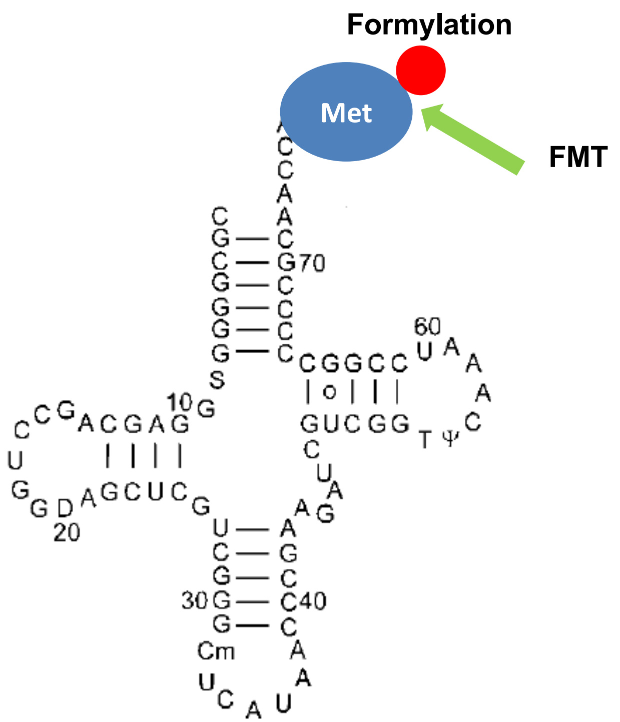
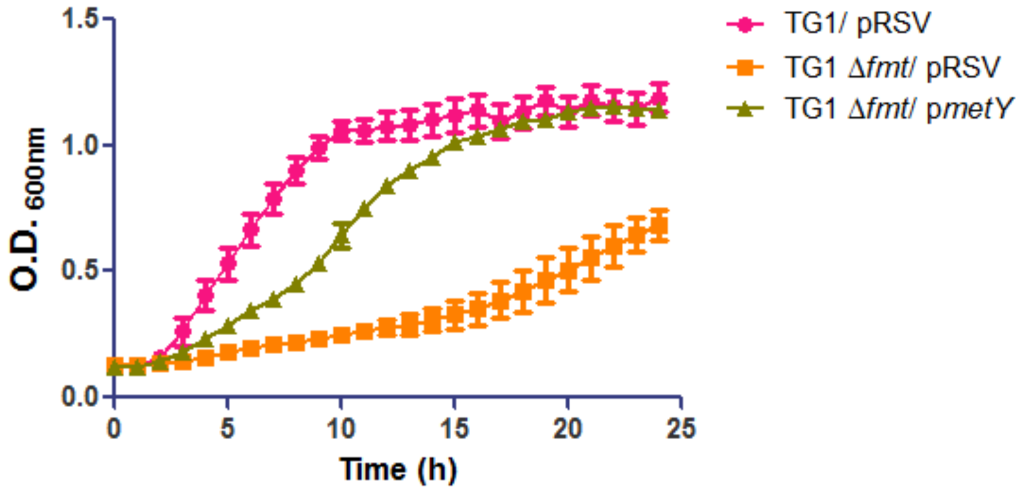
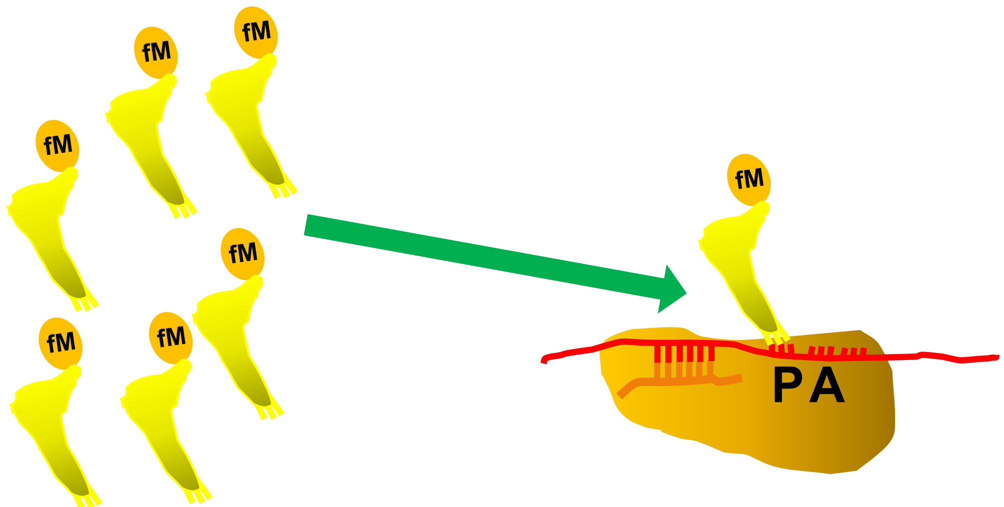
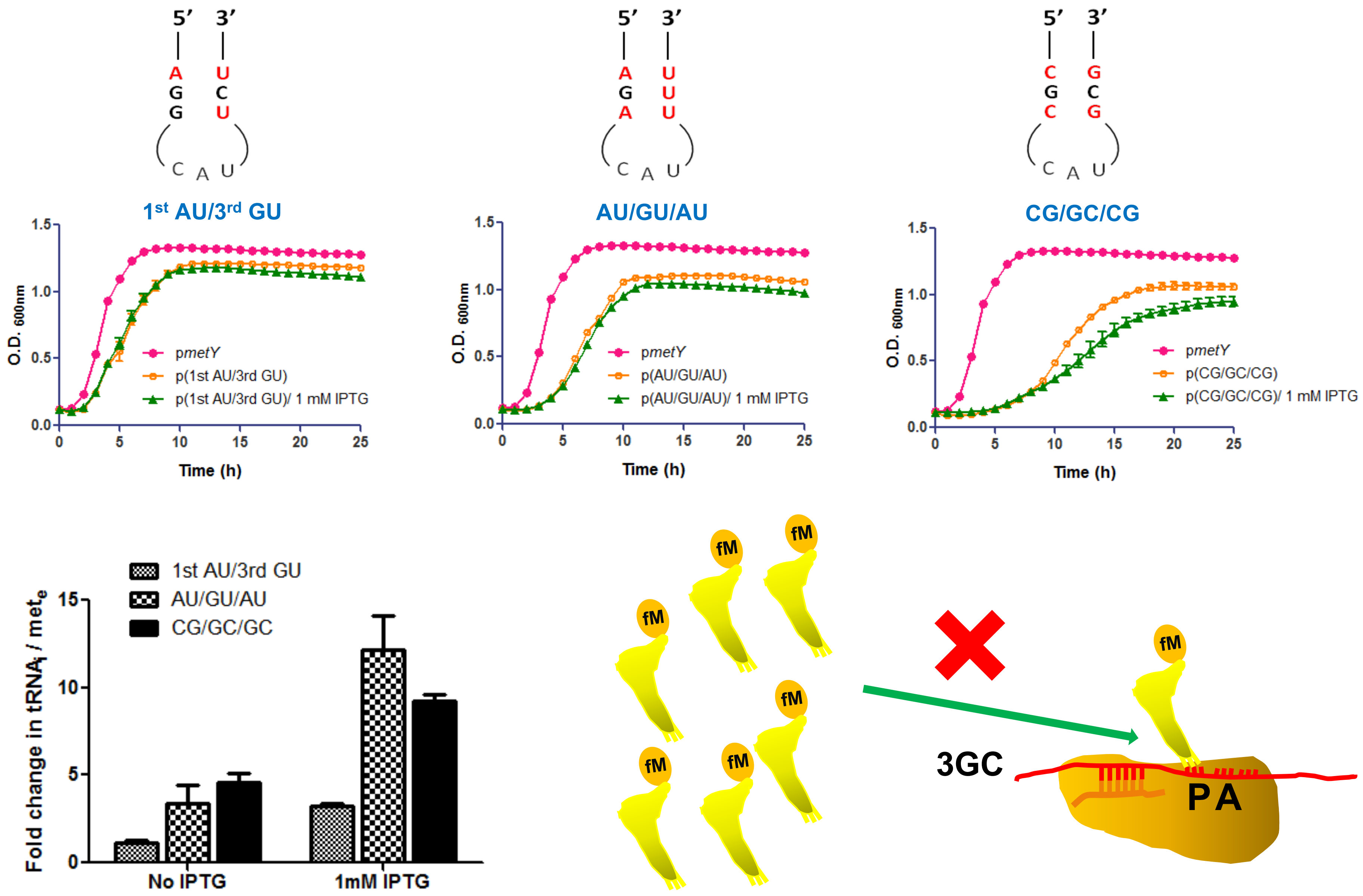


# Effect of overexpression of 3GC mutant tRNAs



# Formylation/3GC: Functions

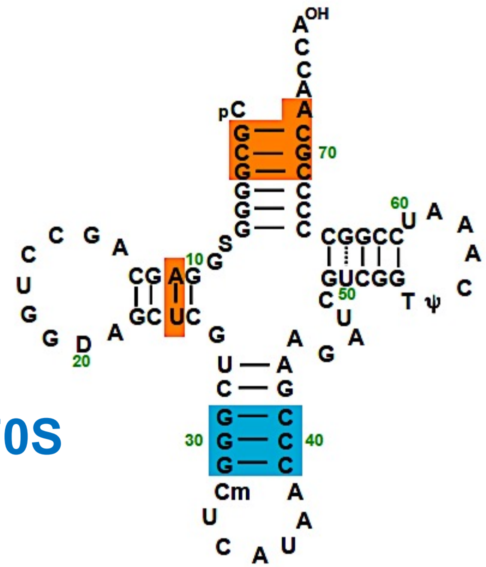
**(i) Formylation:**

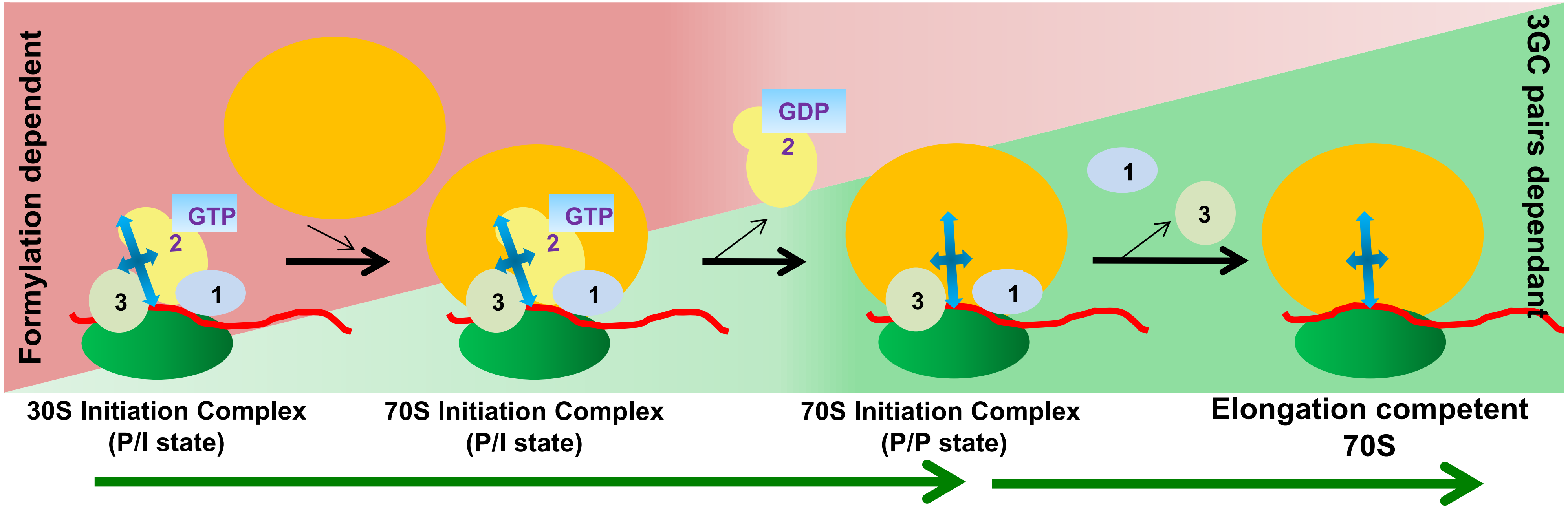
**(a) Facilitates the initial binding of the i-tRNA**

**(ii) The 3GC base pairs:**

**(a) i-tRNA binding**

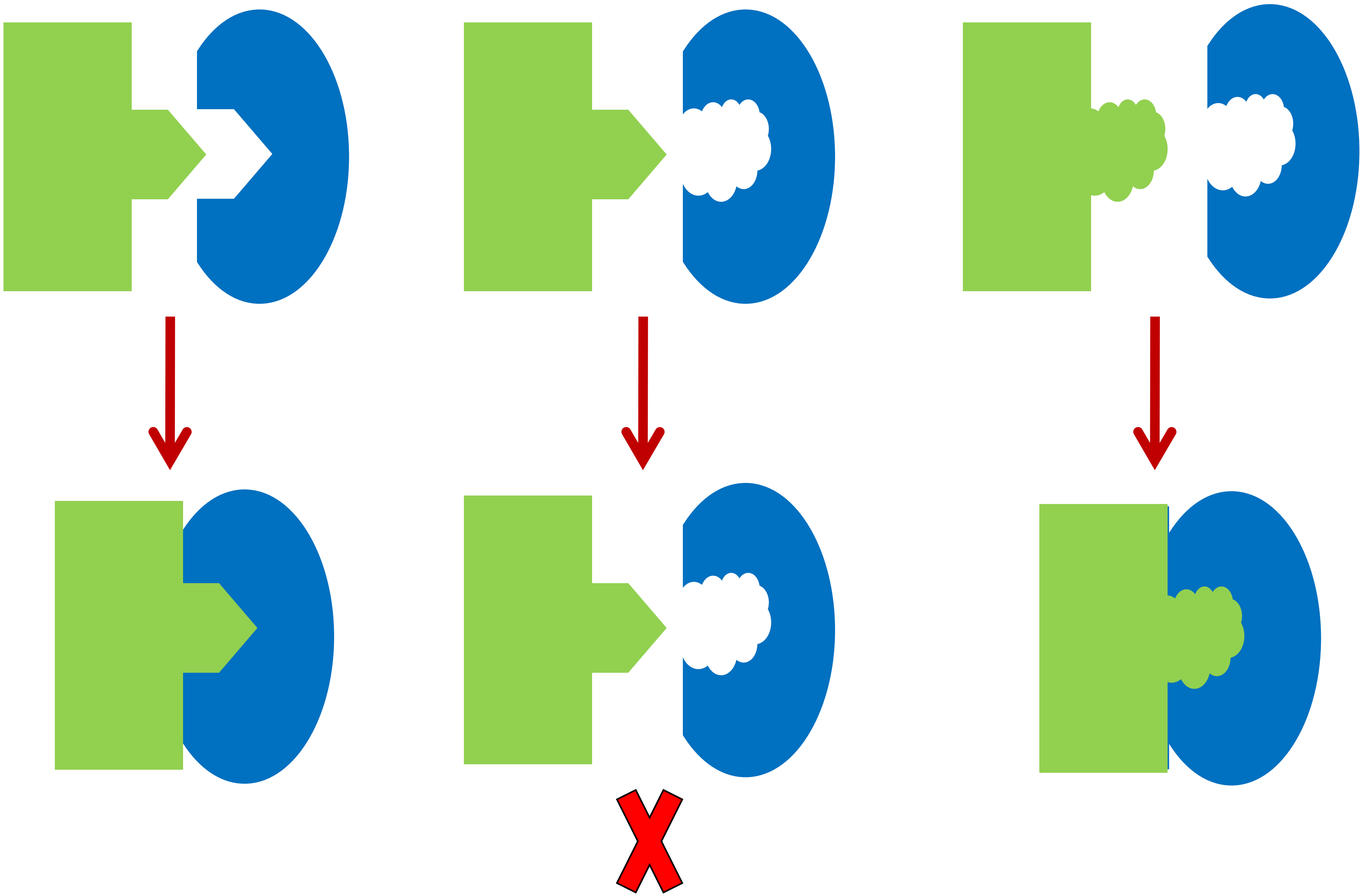
**(b) i-tRNA transitions from 30S to elongation competent 70S**



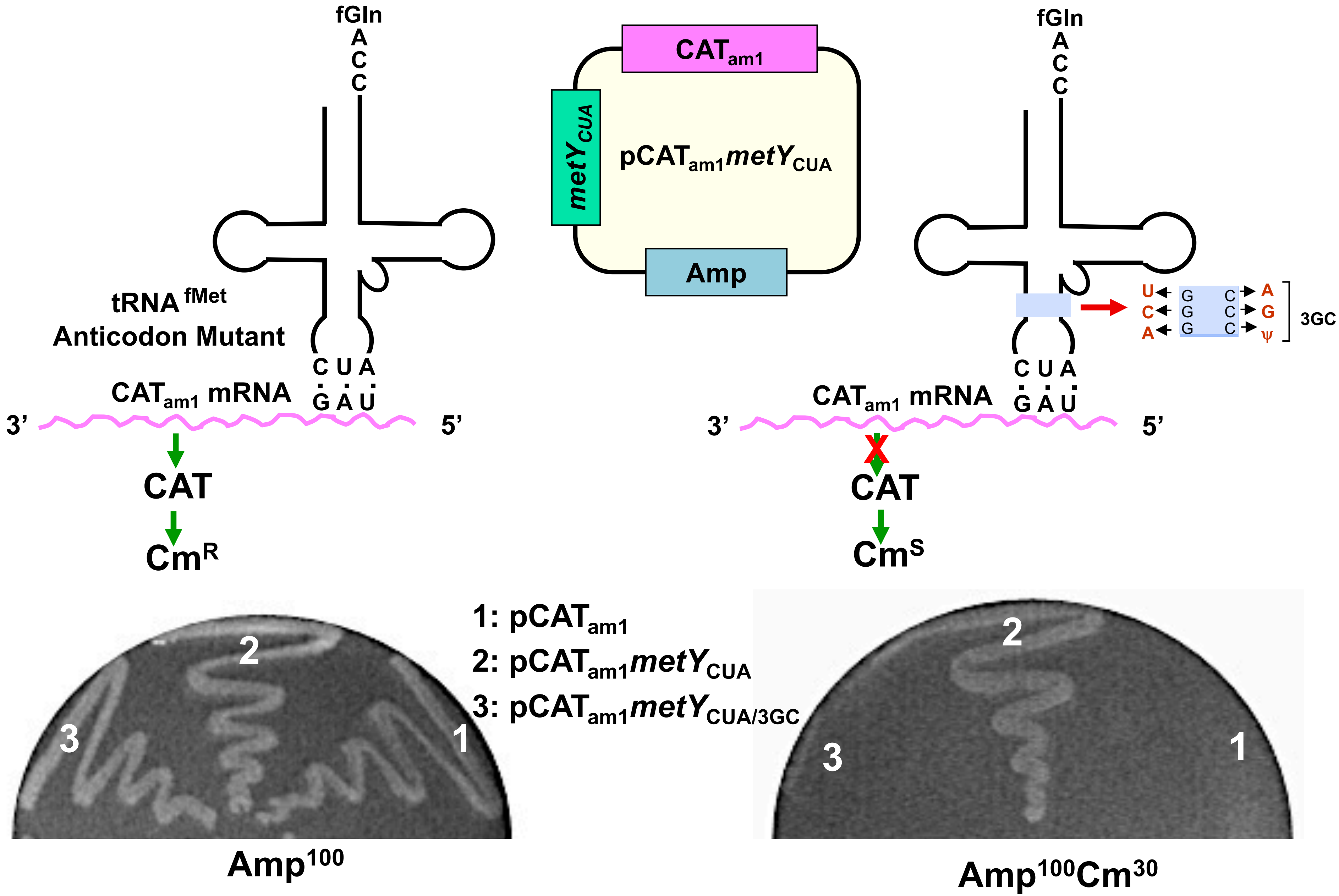



Shetty, S., Shah, R., Chembazhi, U. V., Sah, S. and **Varshney, U.** (2017) **Nucleic Acids Res.** 45, 2040-2050.

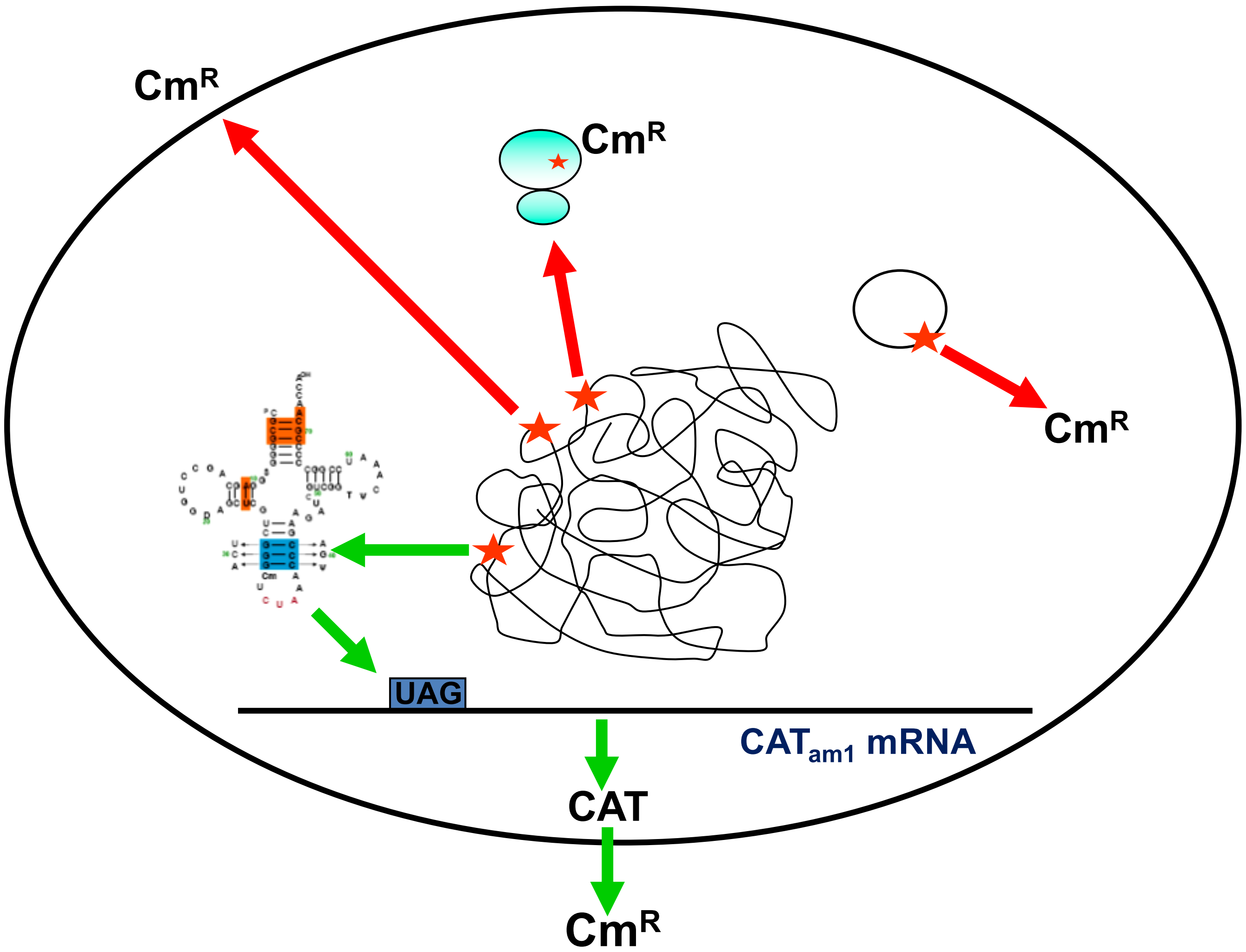
# Suppressor Analysis

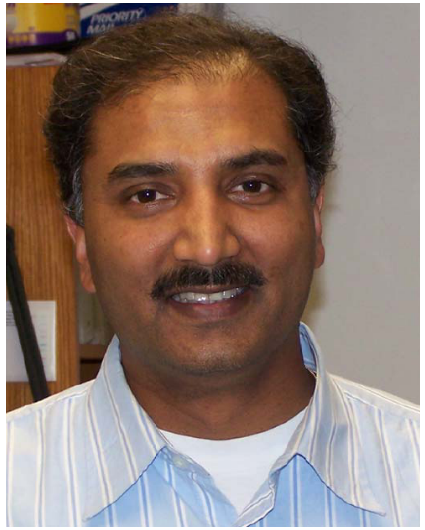


# *In vivo* assay system



# MNNG Mutagenesis/Genetic Screen



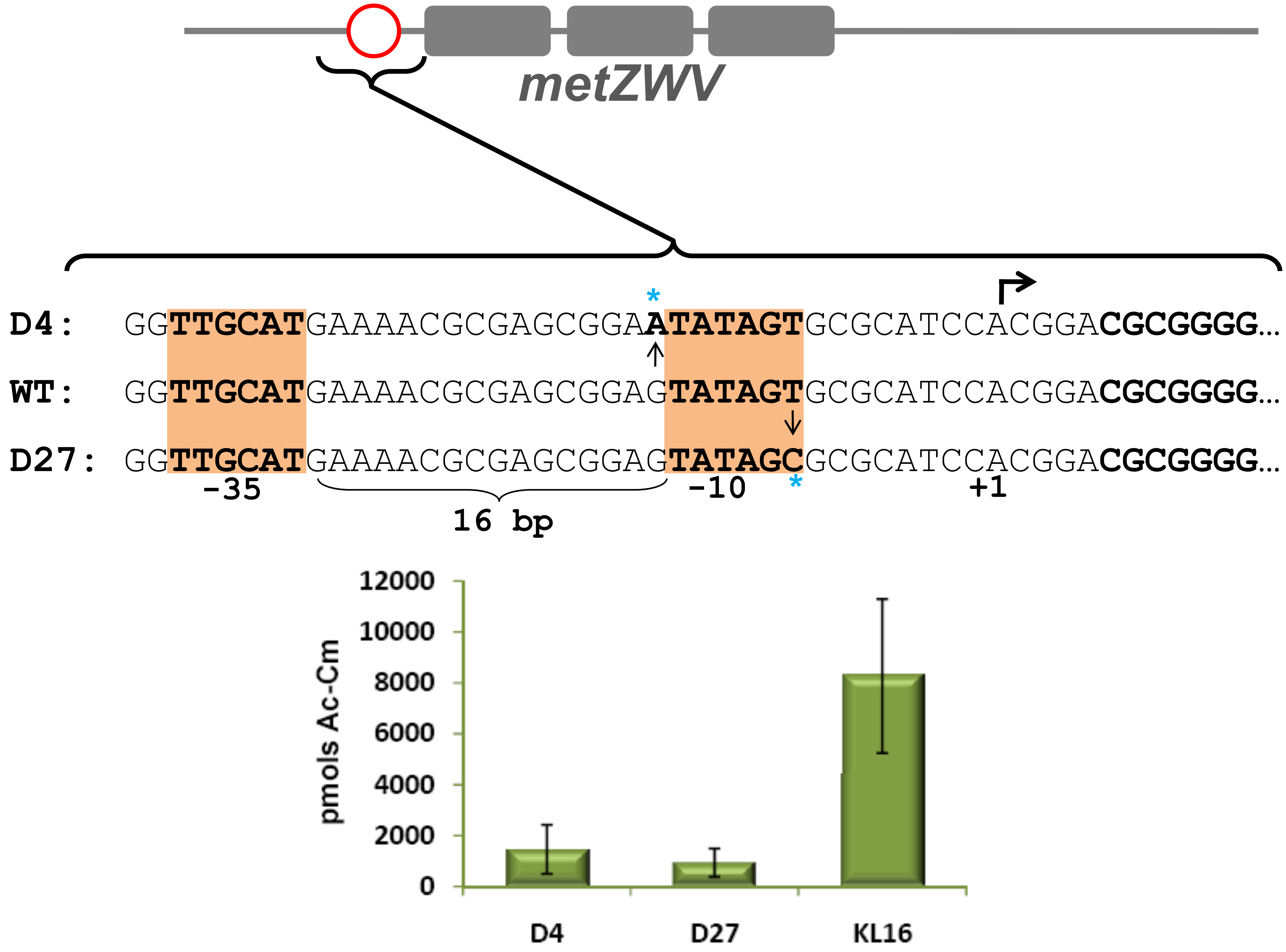



Dinesh

1. A18
2. A26
3. A30
4. A48
5. B2
6. B21
7. B22
8. B29
9. B33
10. C5
11. D1
12. D4
13. D27
14. D29
15. D35

Suman

# Characterization of D4 and D27



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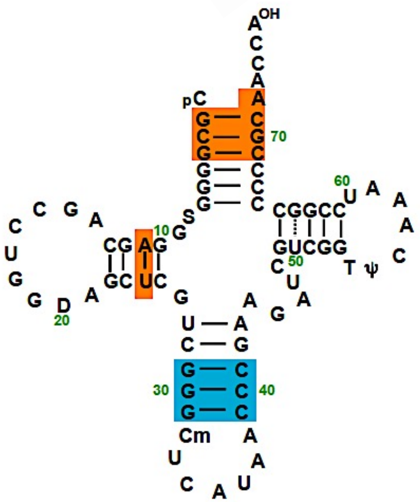
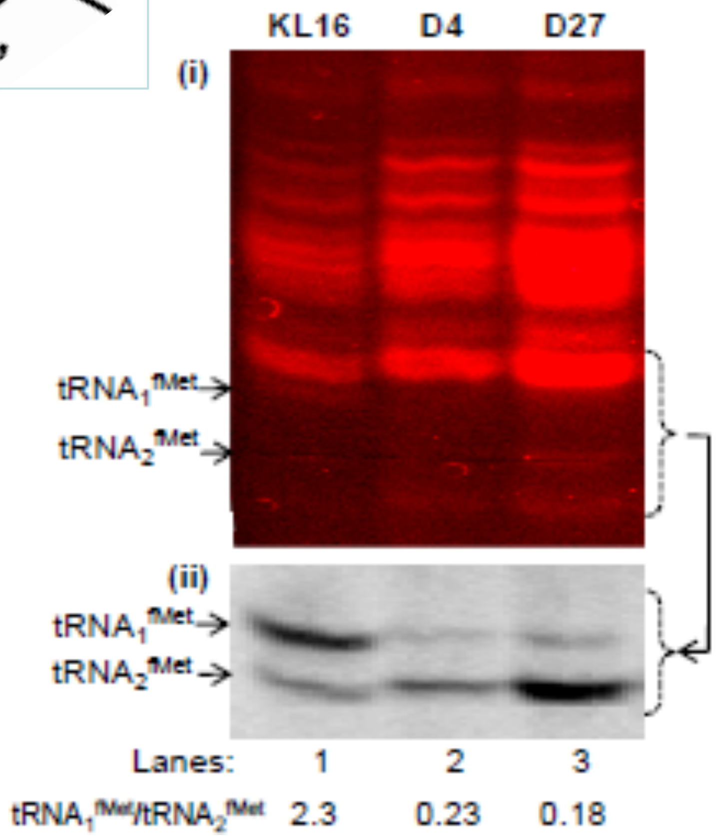
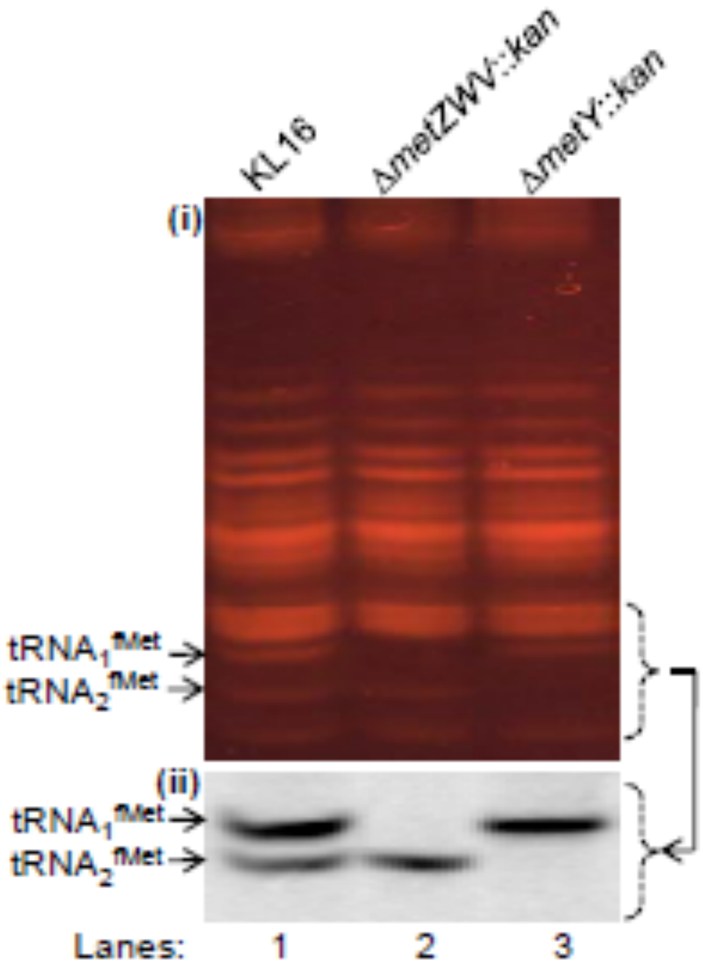
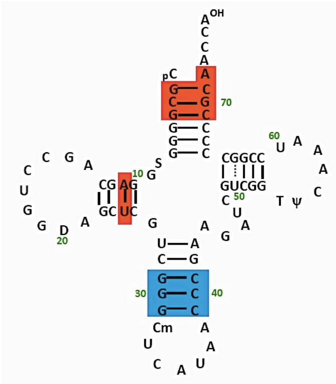


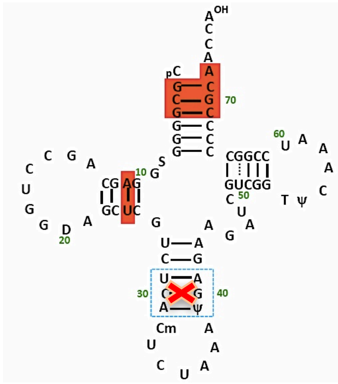


Suman

# D4/D27: Summary



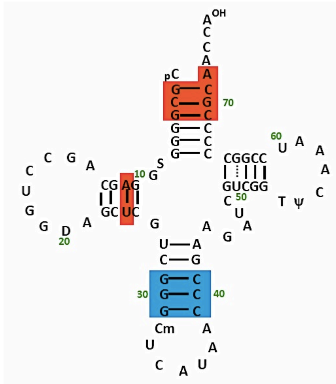


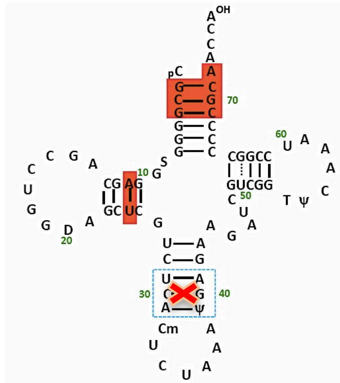
| *metZWV* | | Chromosomally encoded tRNA$^{fMet}$ | Initiation with tRNA$^{fMet}$/3GC |
|---|---|---|---|
| **Wild-type** | | High | No |
| **D4/D27** | | Low | Yes |

Kapoor, S., Das, G. and Varshney, U. (2011) Crucial contribution of the multiple copies of the initiator tRNA genes in the fidelity of tRNA$^{fMet}$ selection on the ribosomal P-site in *Escherichia coli*. **Nucleic Acids Res.** 39, 202–212.

Suman

# D4/D27: Summary





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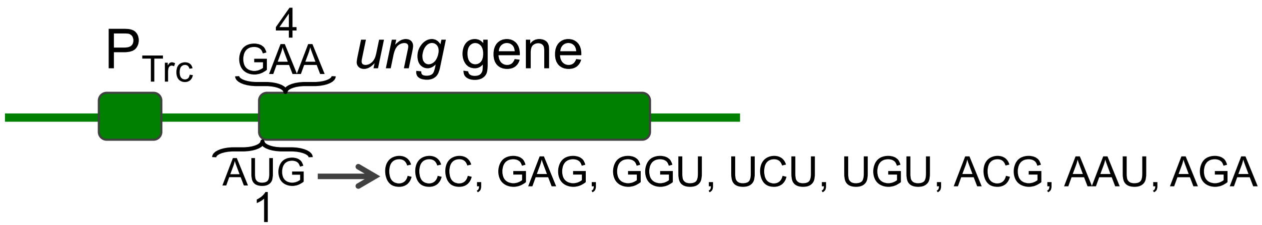
|---|---|---|
| **Wild-type** | High | No |
| **D4/D27** | Low | Yes |

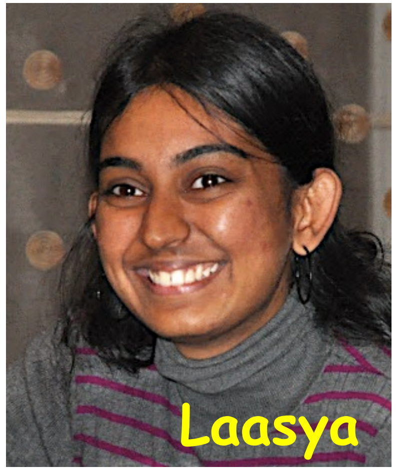
There is a competition between the 3GC tRNA and the chromosomally encoded tRNA$^{fMet}$ for their binding to the ribosomal P-site.

Kapoor, S., Das, G. and Varshney, U. (2011) Crucial contribution of the multiple copies of the initiator tRNA genes in the fidelity of tRNA$^{fMet}$ selection on the ribosomal P-site in *Escherichia coli*. **Nucleic Acids Res.** 39, 202–212.

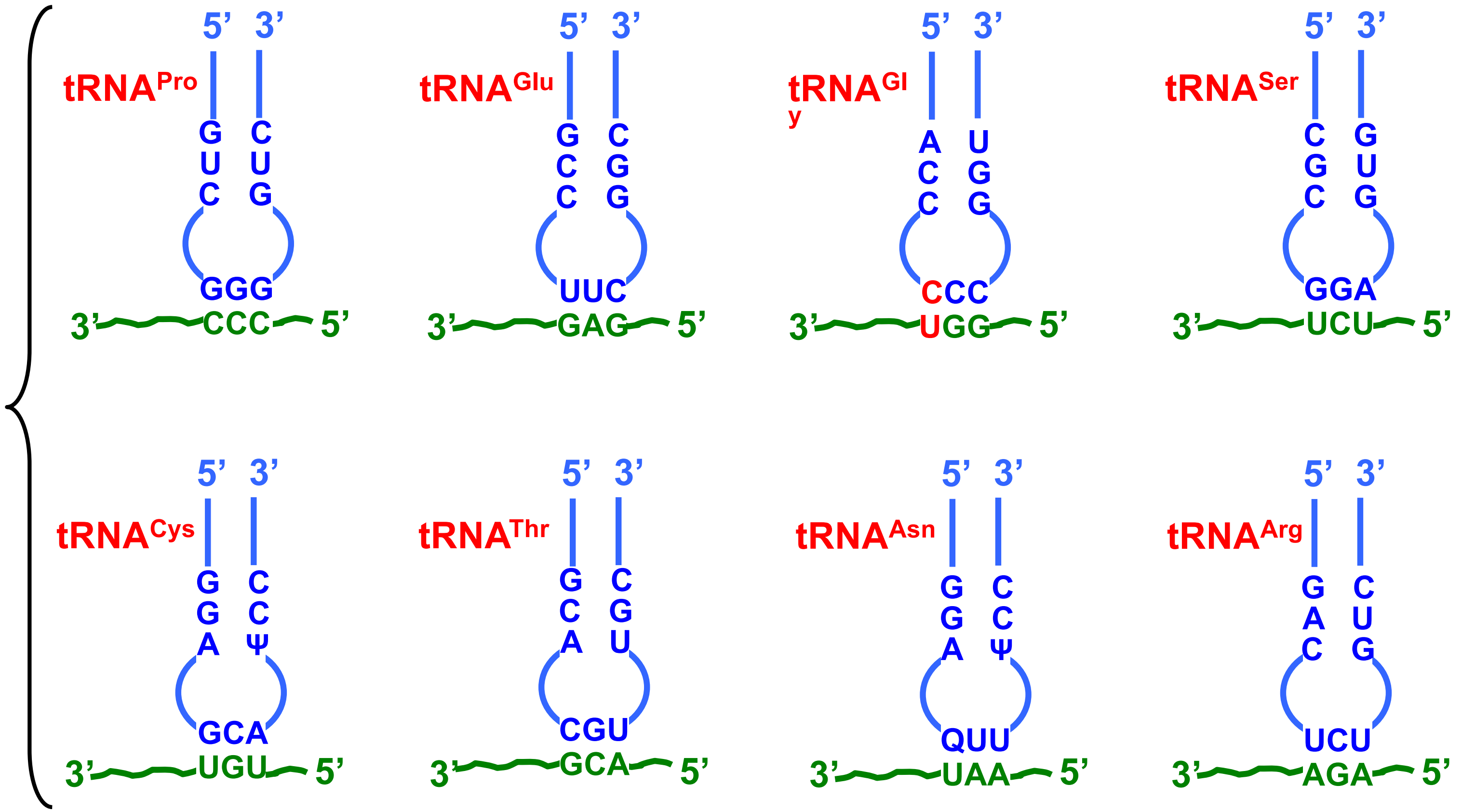
# What about initiation with elongator tRNAs?’

(A)



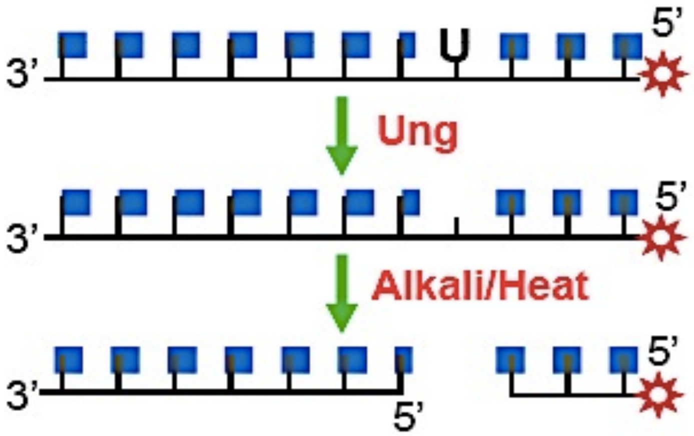



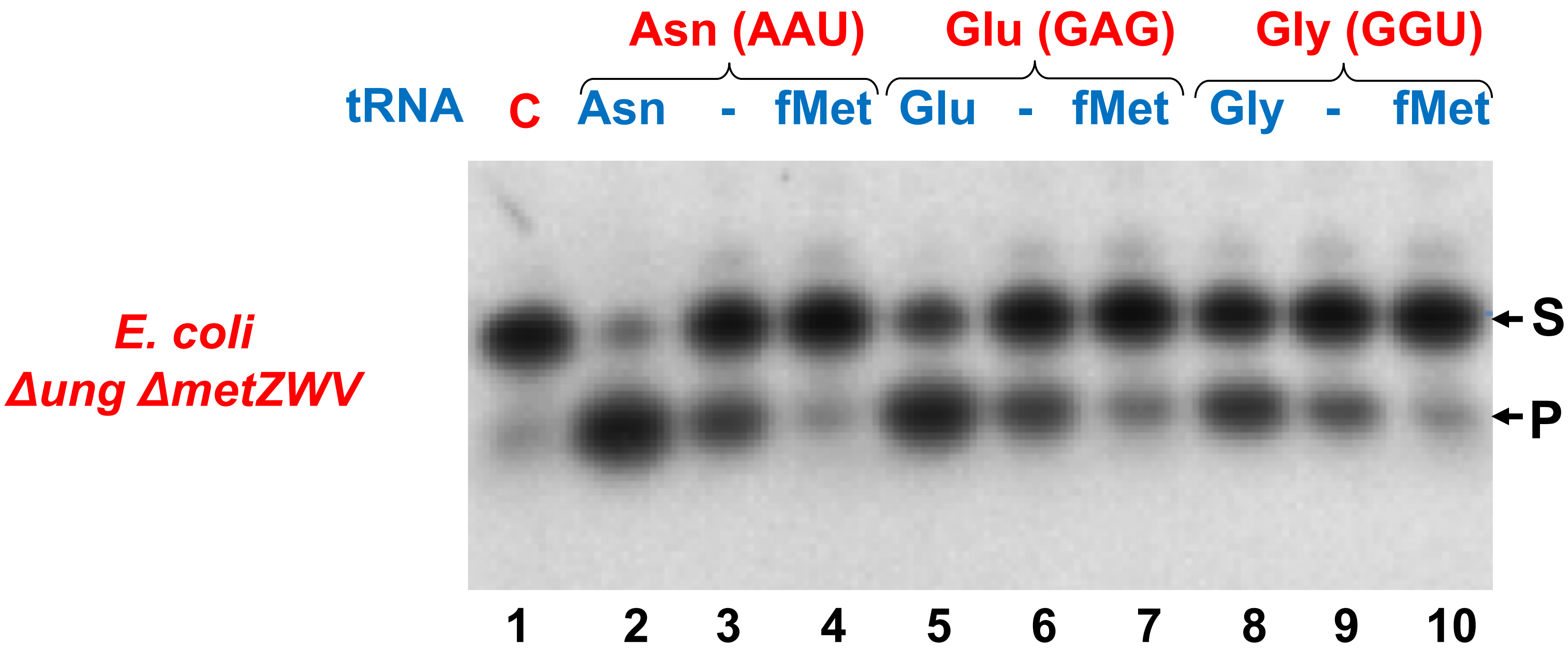

(B)

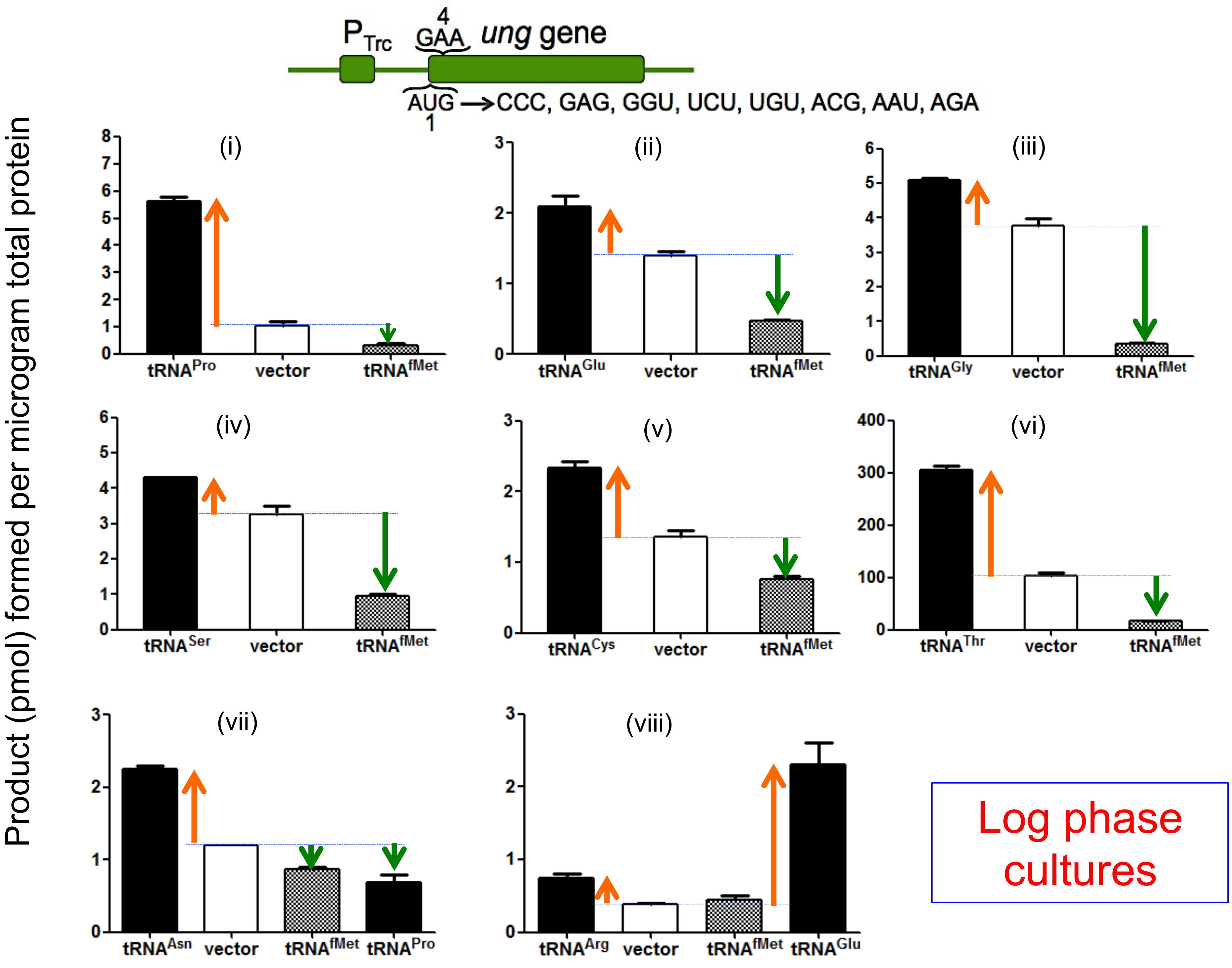



Samhita, L., Virumäe, K., Remme, R. and Varshney, U. (2013) Initiation with elongator tRNAs. **J. Bacteriol.** 195, 4202-4209.

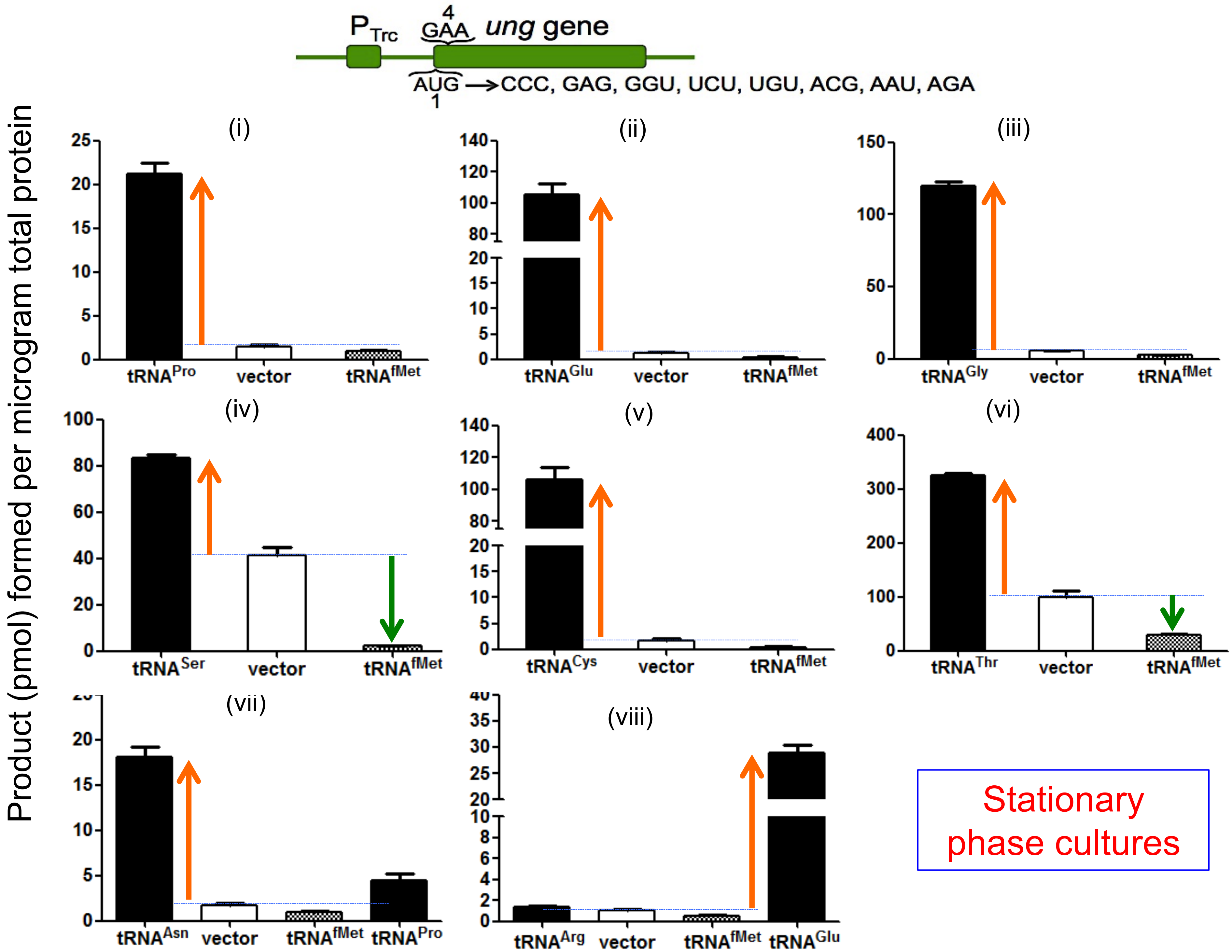
# Assays for initiation with elongator tRNAs





P Trc
4
GAA
ung gene
AUG ⟶ CCC, GAG, GGU, UCU, UGU, ACG, AAU, AGA
1
Product (pmol) formed per microgram total protein
(i)
tRNAPro
vector
tRNAfMet
(ii)
tRNAGlu
vector
tRNAfMet
(iii)
tRNAGly
vector
tRNAfMet
(iv)
tRNASer
vector
tRNAfMet
(v)
tRNACys
vector
tRNAfMet
(vi)
tRNAThr
vector
tRNAfMet
(vii)
tRNAAsn
vector
tRNAfMet
tRNAPro
(viii)
tRNAArg
vector
tRNAfMet
tRNAGlu
Log phase cultures



P Trc
4
GAA
ung gene
AUG → CCC, GAG, GGU, UCU, UGU, ACG, AAU, AGA
1
Product (pmol) formed per microgram total protein
(i)
tRNAPro
vector
tRNAfMet
(ii)
tRNAGlu
vector
tRNAfMet
(iii)
tRNAGly
vector
tRNAfMet
(iv)
tRNASer
vector
tRNAfMet
(v)
tRNACys
vector
tRNAfMet
(vi)
tRNAThr
vector
tRNAfMet
(vii)
tRNAAsn
vector
tRNAfMet
tRNAPro
(viii)
tRNAArg
vector
tRNAfMet
tRNAGlu
Stationary phase cultures

# Are there physiological conditions that change tRNA$^{fMet}$ levels in the cell?

- **Multiple copies of tRNA$^{fMet}$ genes** —*metZ*—*metW*—*metV*— 63.5' —*metY*— 71.5'

- ***metY* down-regulation:**
  - **(a) cAMP-CAP/ArgR complex**

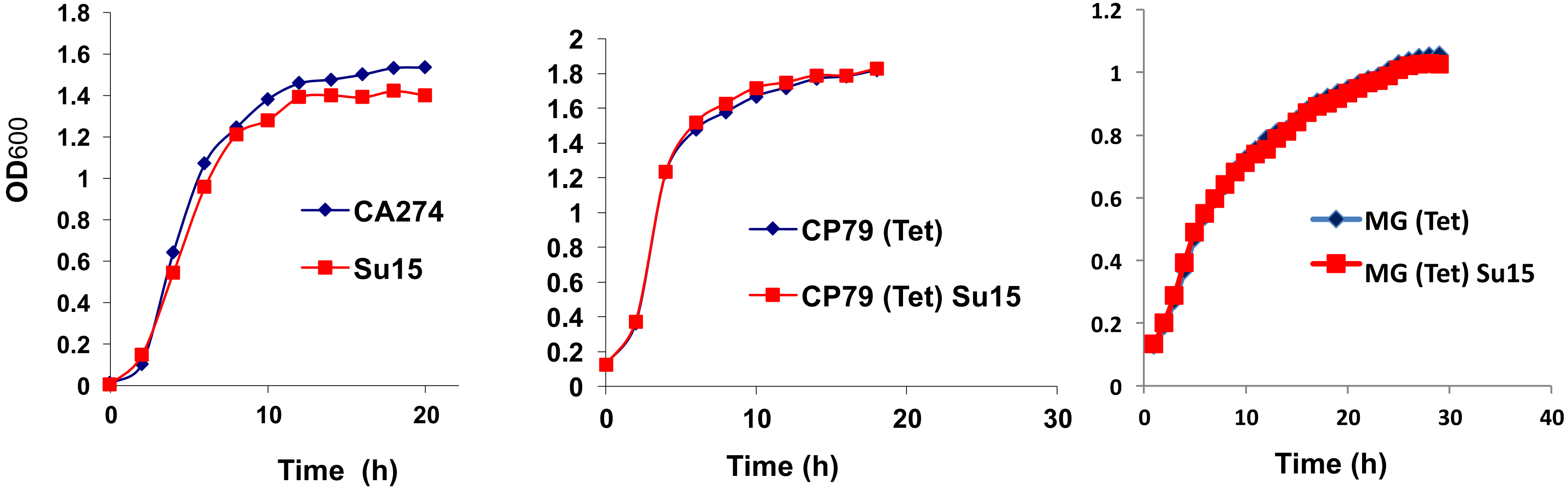
- ***metZWV* down-regulation:**
  - **(a) A G + C-rich sequence (5'-GCGCATCCAC-3')**
  - **(b) by ppGpp (stringent response)**

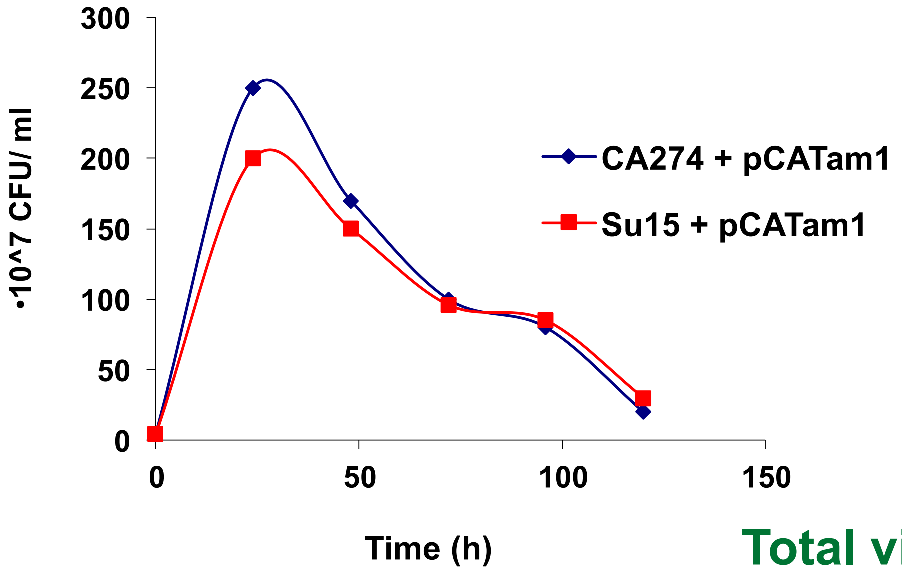
- ***metZWV* and *metY* down-regulation:**
  - **(a) initiator tRNA-targeting by enterotoxin, VapC**

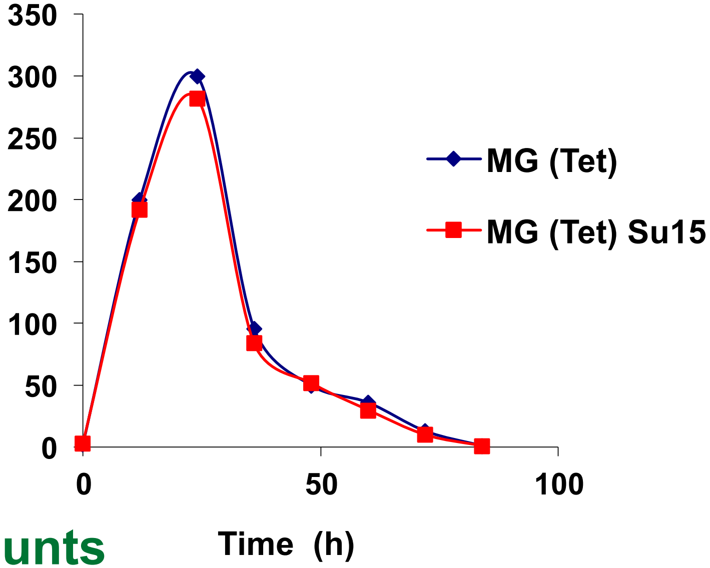
**Proteome plasticity/remodeling?**

Could changes in initiator tRNA contents confer fitness advantages?

# Growth Curves

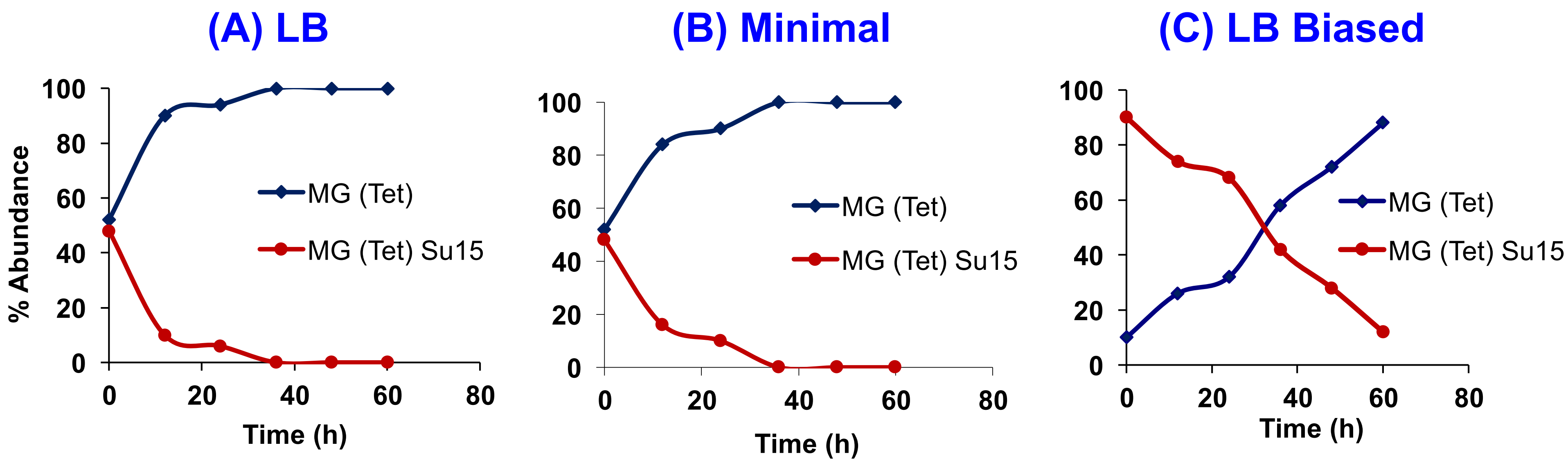


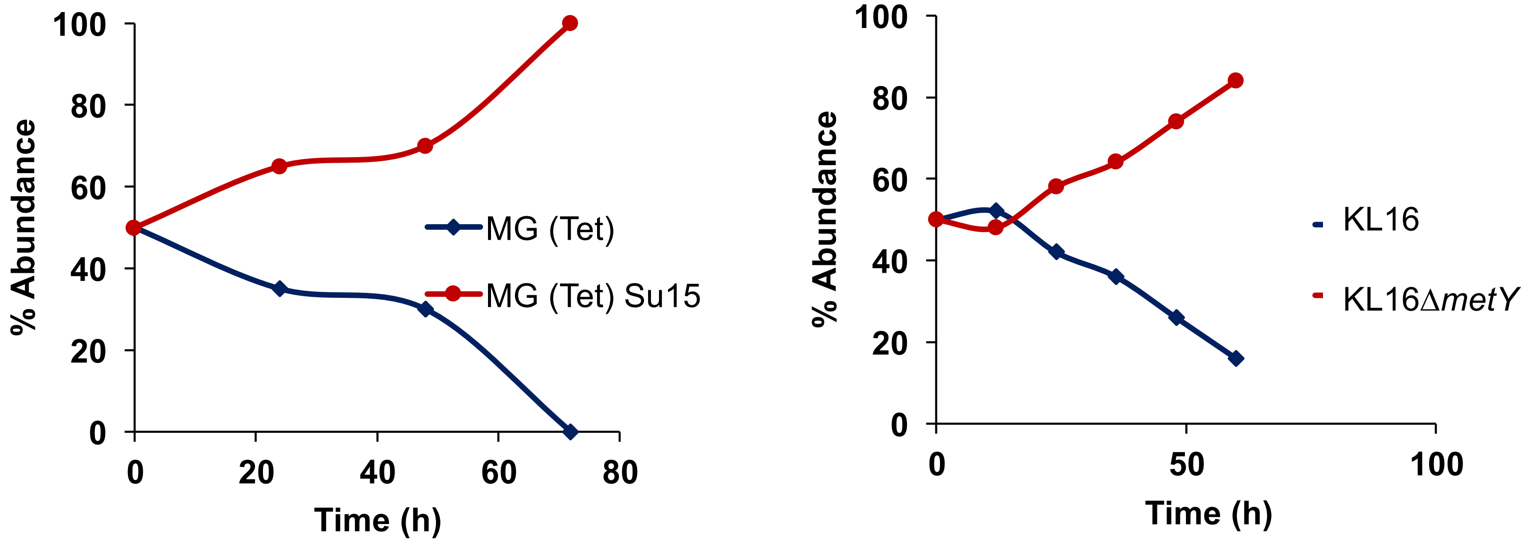




Total viable counts

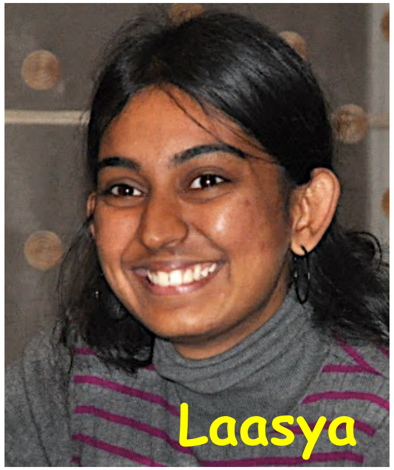
# *E. coli* MG1655: Growth in mixed cultures



# Competitions in 0.1% glycerol

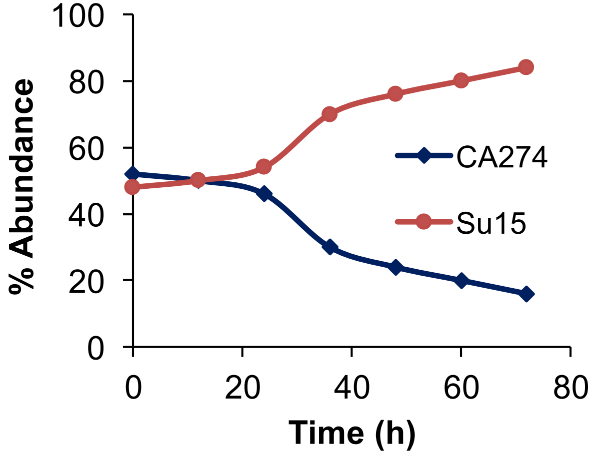


# Dependence on a single amino acid concentration in the growth medium

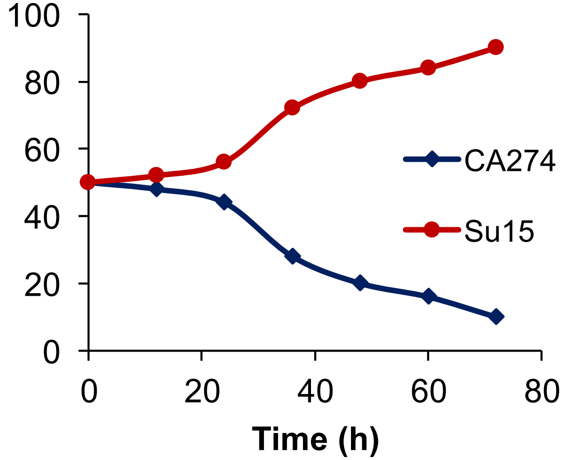



***E. coli* CA274 (Trp⁻)**

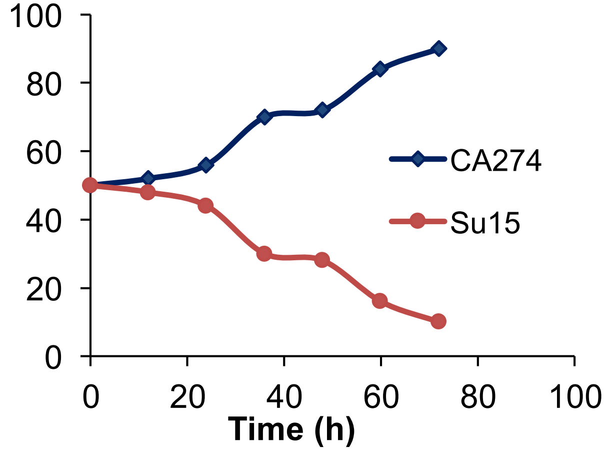
**(A) Minimal+Trp (0.05 µg/ml)**




**(B) Minimal+Trp (0.1 µg/ml)**




**(C) Minimal+Trp (0.4 µg/ml)**





Samhita, L., Nanjundiah, V. and Varshney, U. (2014) How many initiator tRNA genes does *Escherichia coli* need? **J. Bacteriol.** 196, 2607-2615.

# *E. coli* genomes with a different copy number of initiator tRNA genes

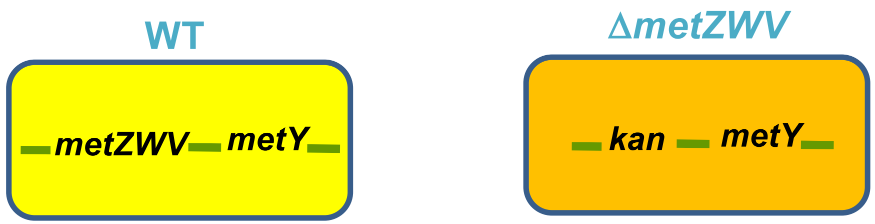
– *metZ* – *metW* – *metV* –   – *metY* –

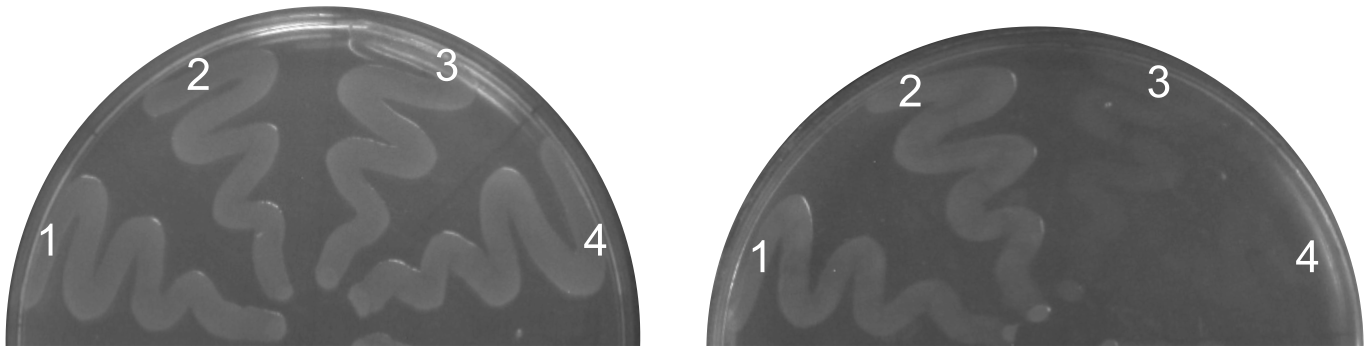
63.5'   71.5'

**Extra-intestinal pathogenic *E. coli* strain (UTI, 1980's, France)**
***E. coli* IAI39: Has two initiator tRNA genes in *metZ* locus, 3 in all**

**Human commensal gut isolate, colonizes the intestine but non infective, possibly a substrain of O157**
***E. coli* HS: Has four initiator tRNA genes in *metZ* locus, 5 in all**

# Cold sensitive phenotypes of D4/D27 and Δ*metZWV*




37 °C

22 °C

1,2 WT

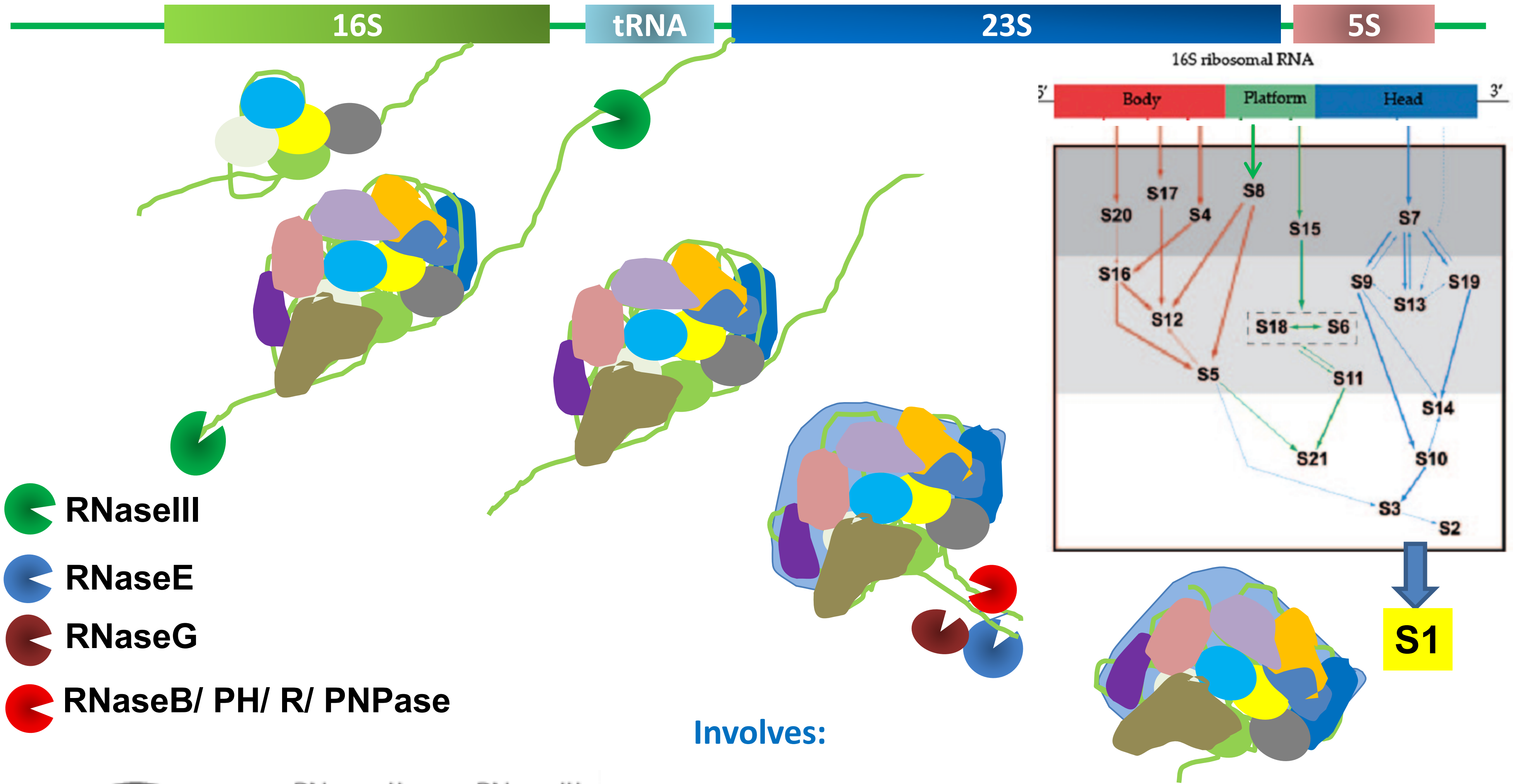
3,4 Δ*metZWV*



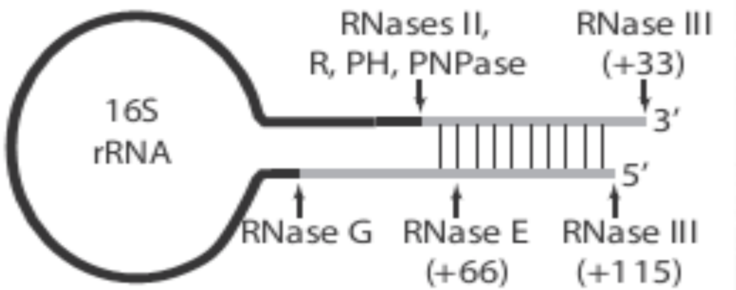
**Is i-tRNA involved in ribosome biogenesis?**

Kapoor, S., Das, G. and Varshney, U. (2011) Crucial contribution of the multiple copies of the initiator tRNA genes in the fidelity of tRNA$^{fMet}$ selection on the ribosomal P-site in *Escherichia coli*. **Nucleic Acids Res.** 39, 202–212.

# 30S Ribosome maturation




Mature 30S

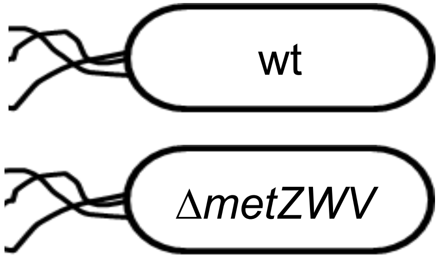


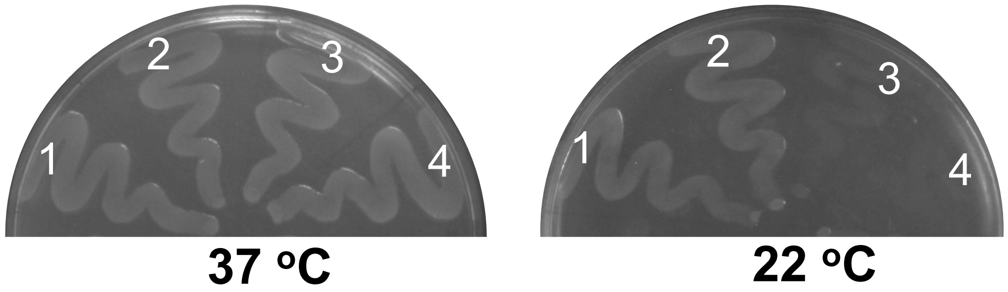

**Involves:**

- Folding of rRNA
- Coordinated binding of r-proteins
- Processing of rRNAs
- Modifications of specific sites

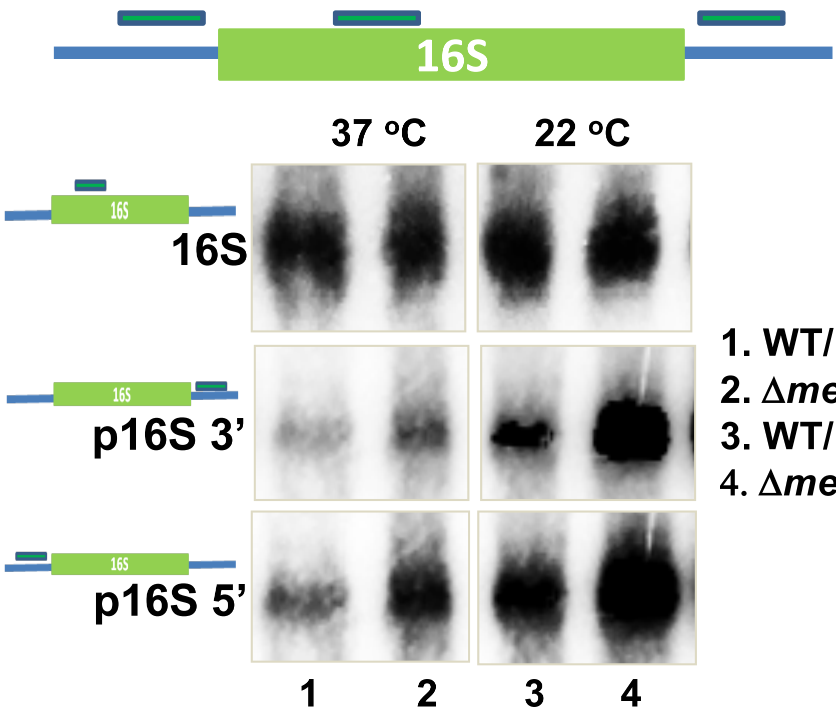
# Decrease in i-tRNA levels leads to increased biogenesis 'defect'

*E. coli* strains

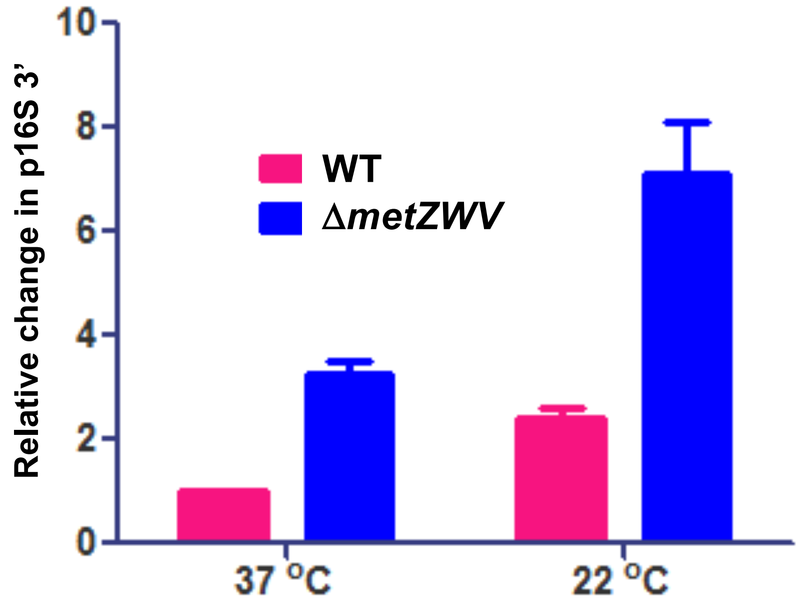


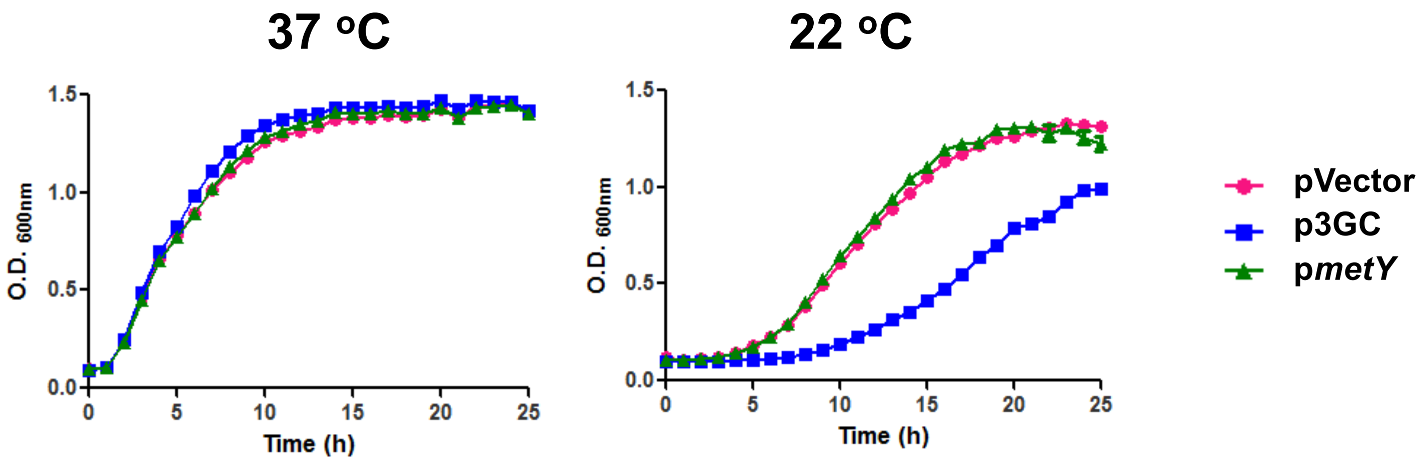

1,2 WT
3,4 Δ*metZWV*

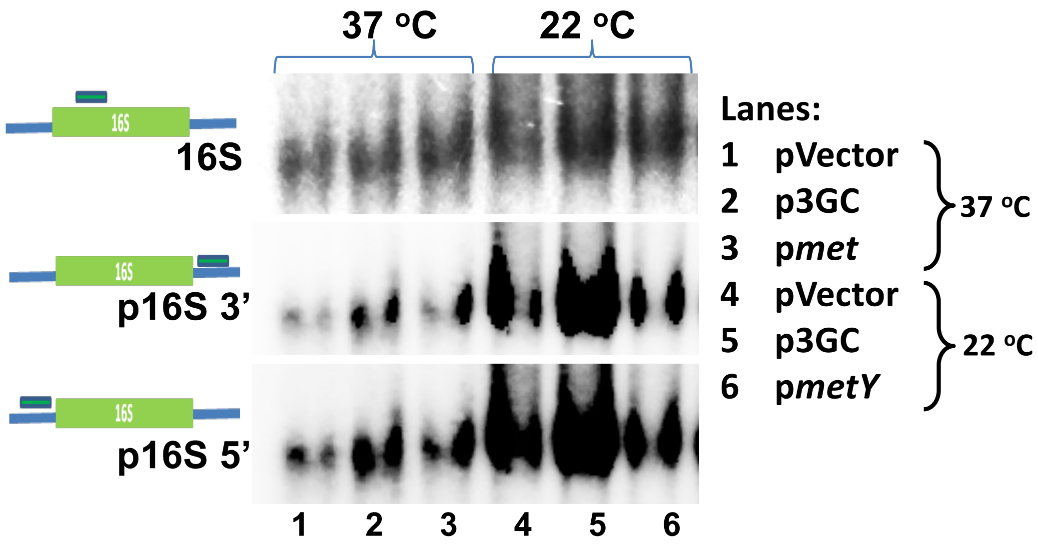


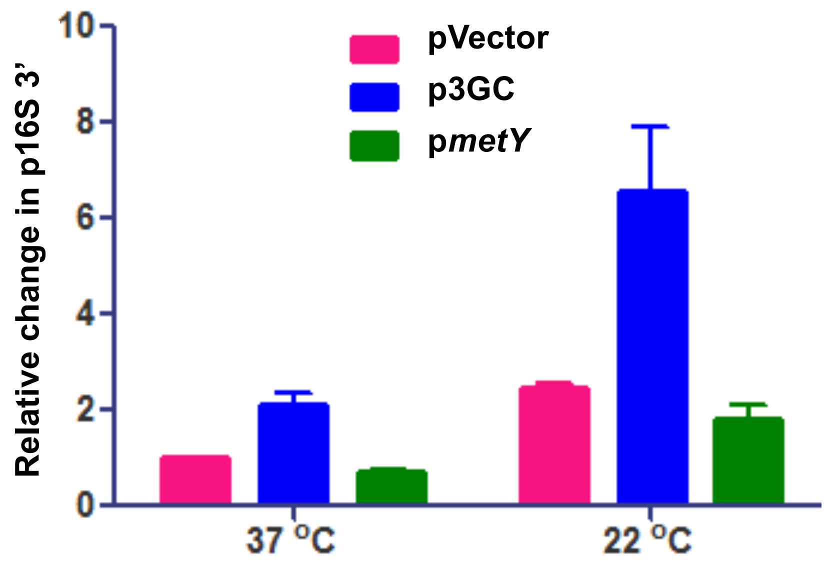

1. WT/ 37 °C
2. Δ*metZWV*/ 37 °C
3. WT/ 22 °C
4. Δ*metZWV*/ 22 °C



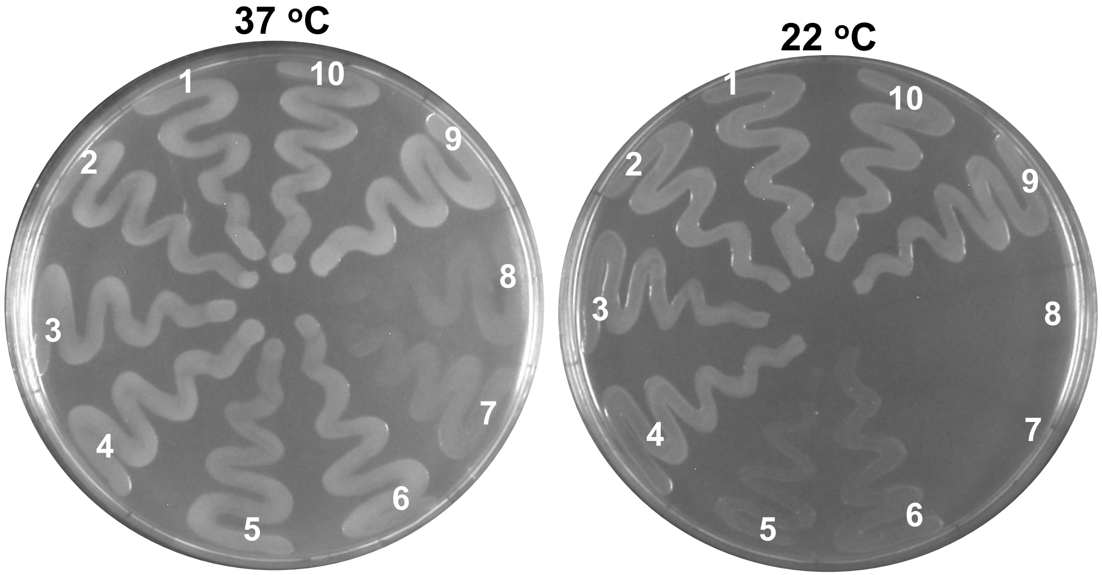
# Overexpression of 3GC mutant tRNA leads to biogenesis 'defect'



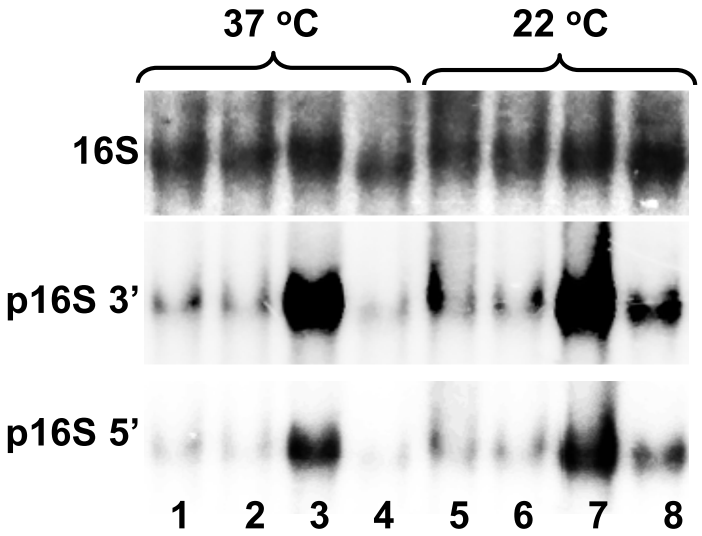



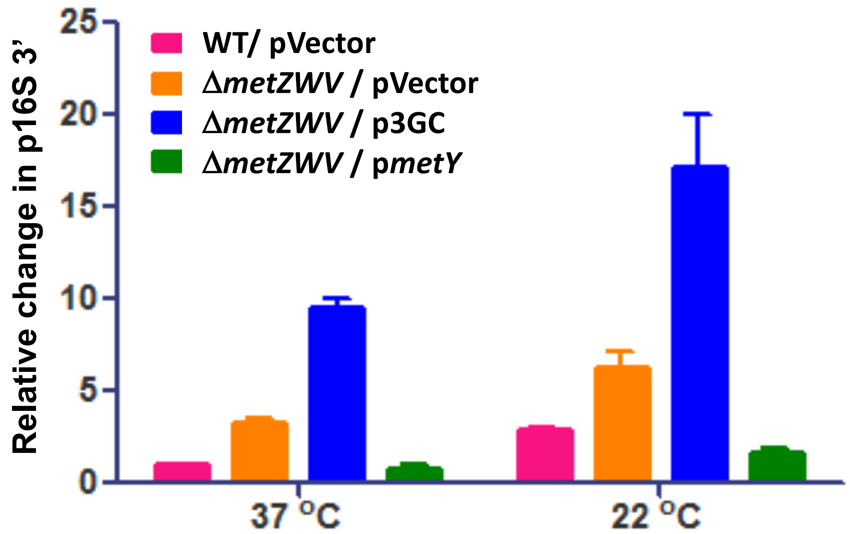
# Effect of the 3GC mutant tRNA is synergistic with the low levels of cellular i-tRNA




1,2 WT/ pVector
3,4 WT/ p3GC
5,6 Δ*metZWV* /pVector
7,8 Δ*metZWV* /p3GC
9, 10 Δ*metZWV* /p*metY*



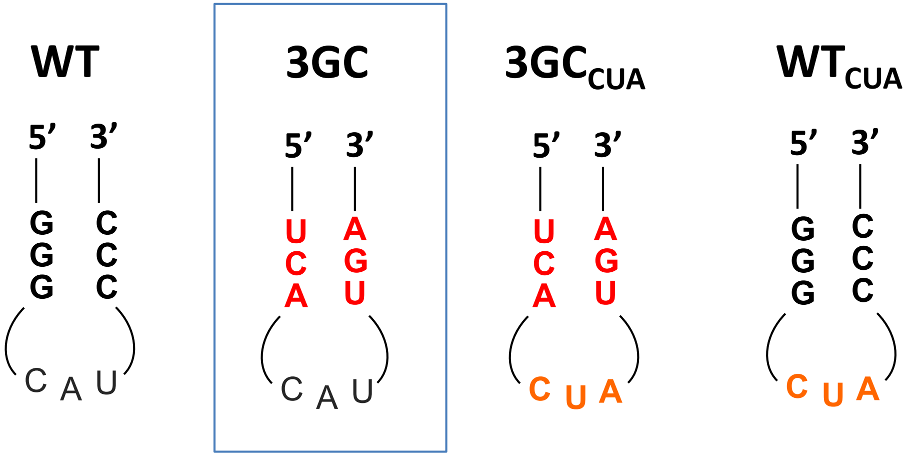

1,5 WT/ pVector
2,6 Δ*metZWV* / pVector
3,7 Δ*metZWV* / p3GC
4,8 Δ*metZWV* / p*metY*



# 3GC mutant tRNA requires CAU anticodon to confer cold sensitivity

Sunil


WT



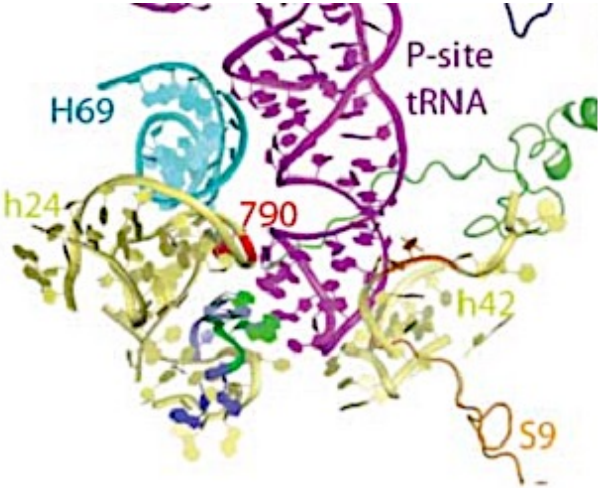


37 °C/9 hr | 22 °C/14 hr | 22 °C/24 hr

| | |
|---|---|
| 1,2 | WT/ pVector |
| 3,4 | WT/ p3GC |
| 5 | WT/ p3GC$_{CUA}$ |
| 6 | WT/ p*metY*$_{CUA}$ |

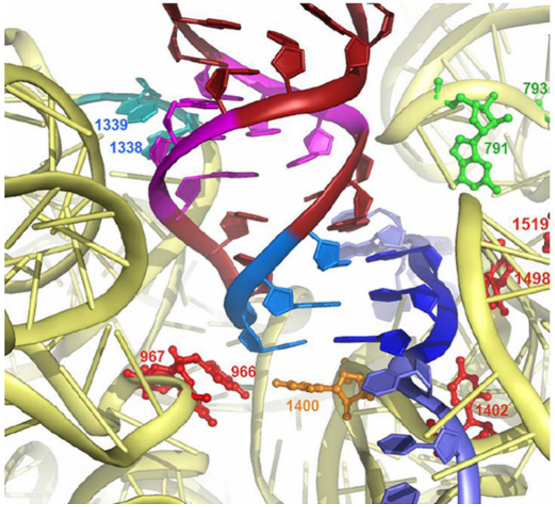
# Genetic interactions with S9, RsmB/D and RbfA

## S9 tail




Selmer et al., 2006

## RsmB/D



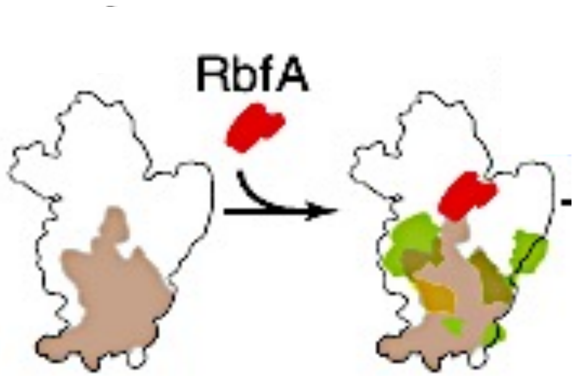

Das et al., 2008

**Cold sensitivity**

**Affect binding of initiator tRNA**

Arora, S., Bhamidimarri, S. P., Bhattacharyya, M., Govindan, A., Weber, M. H. W., Vishveshwara, S. and **Varshney, U.** (2013) Distinctive contributions of the ribosomal P-site elements m²G966, m⁵C967 and the C-terminal tail of the S9 protein in the fidelity of initiation of translation in *Escherichia coli*. **Nucleic Acids Res.** 41, 4963-4975.

## RbfA

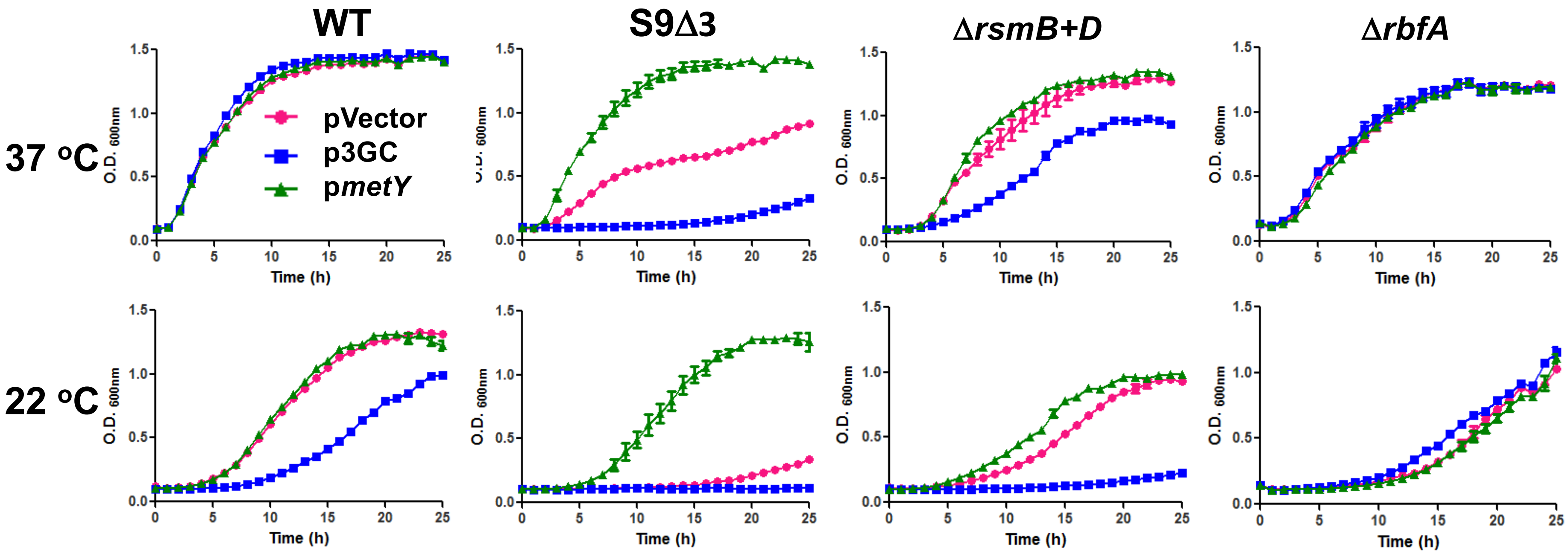


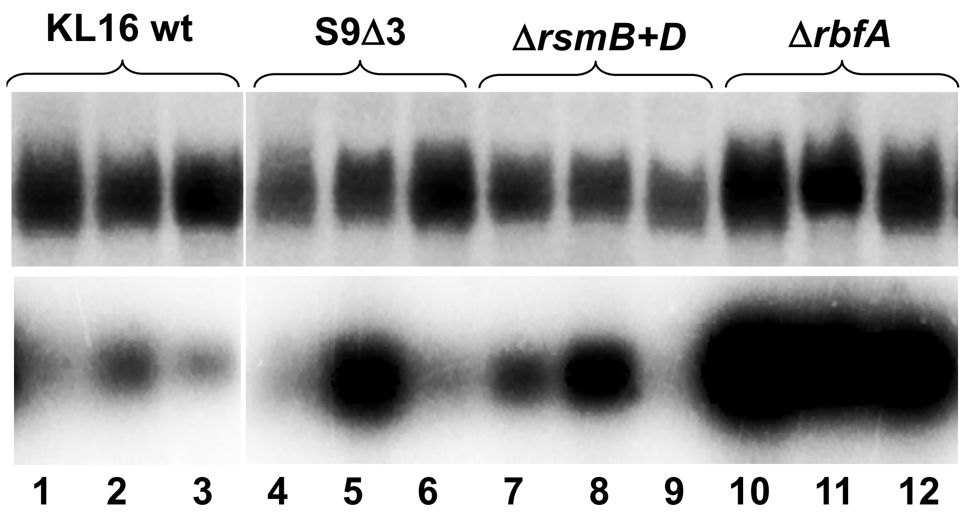

Soper et al., 2013

**Cold sensitivity**

**Affects early ribosome biogenesis**

# Genetic interactions with S9, RsmB/D and RbfA



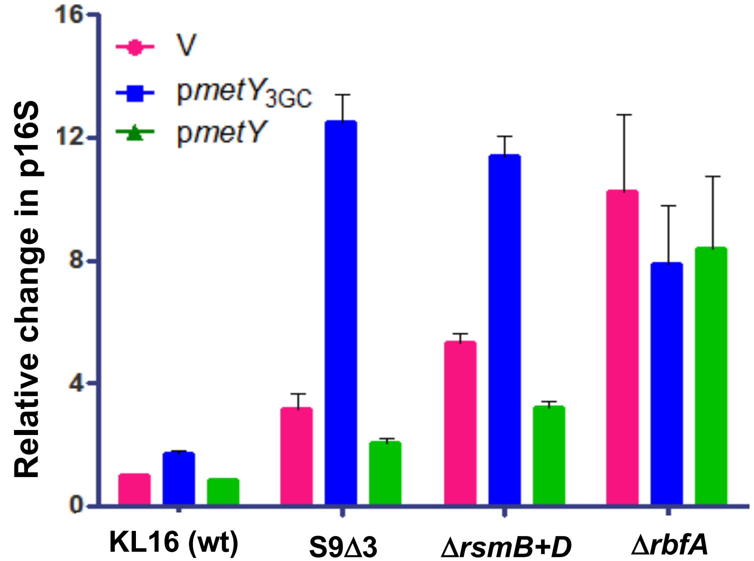




Lanes/bars:

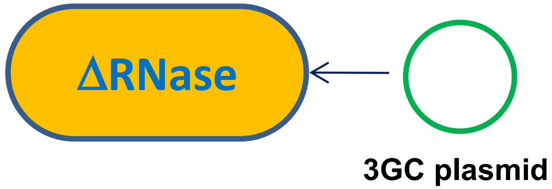
1,4,7,10: vector

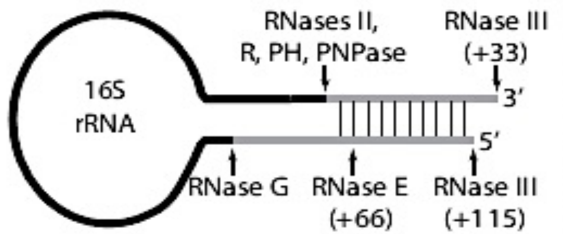
2,5,8,11: p*metY*$_{3GC}$

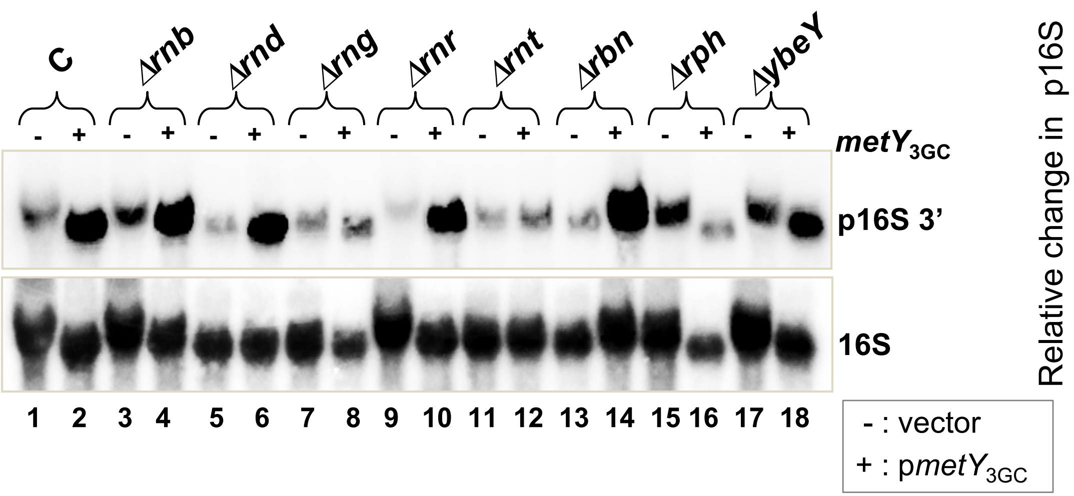
3,6,9,12: p*metY*

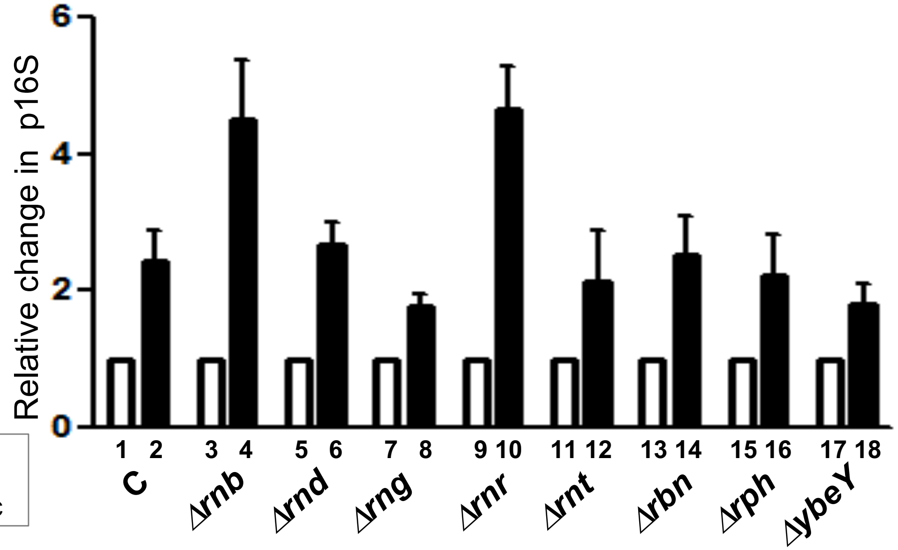


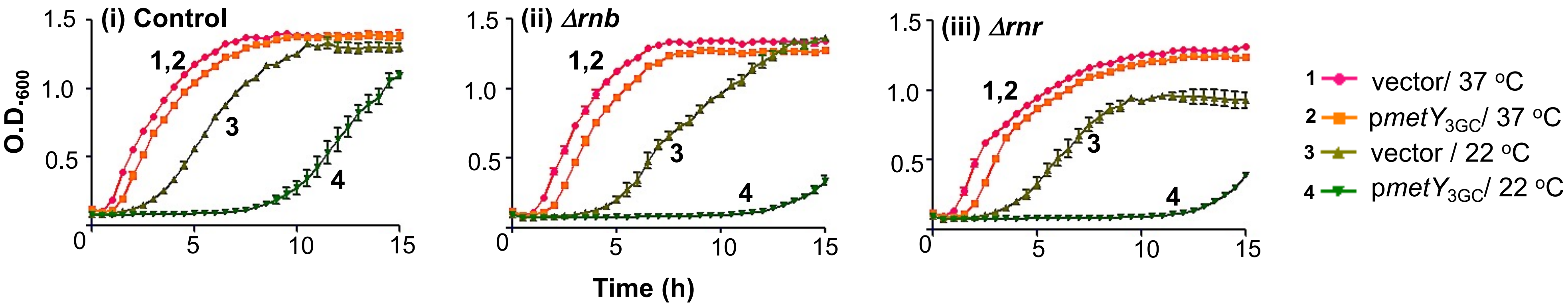
# Effect of deletion of RNases



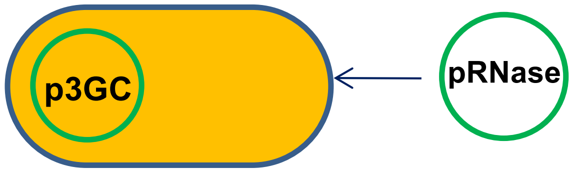



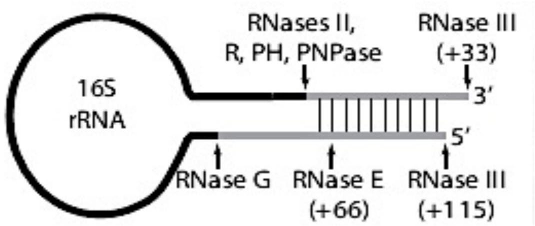



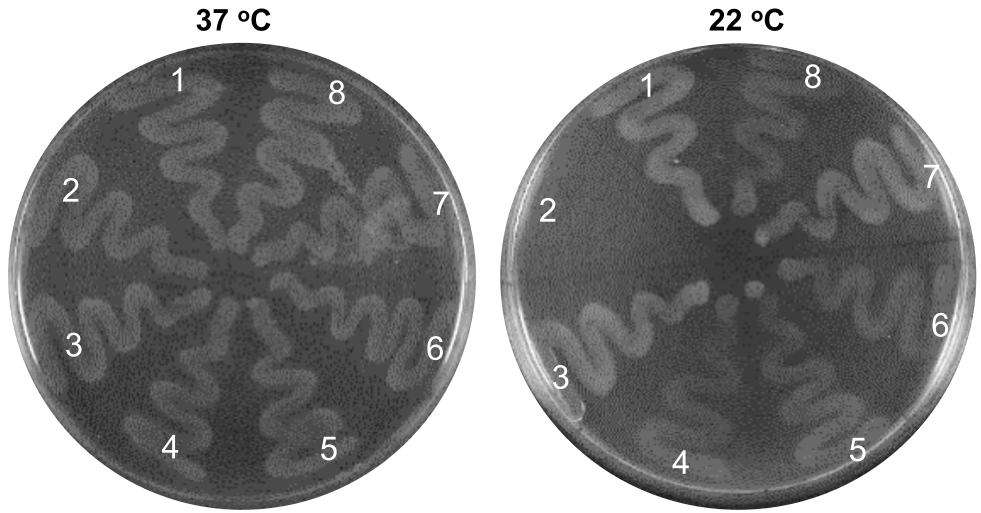



# Effect of overexpression of RNases

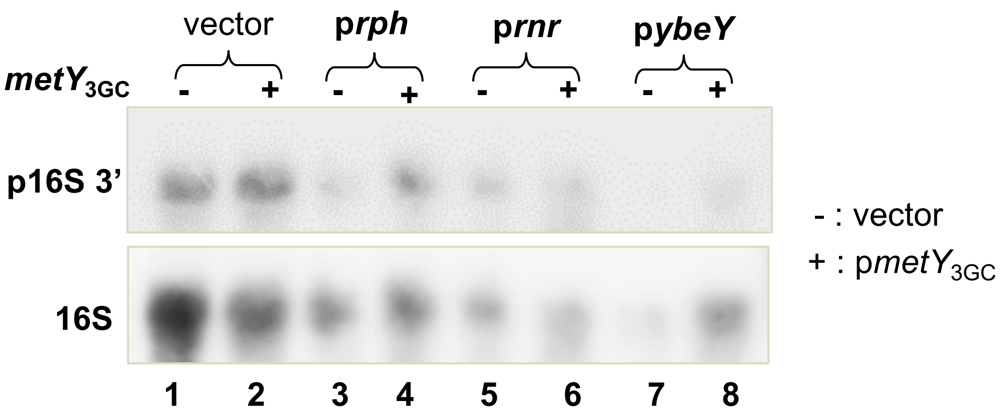


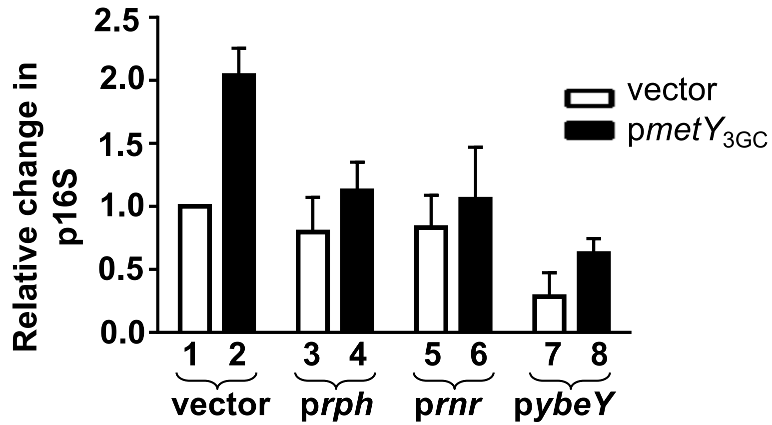



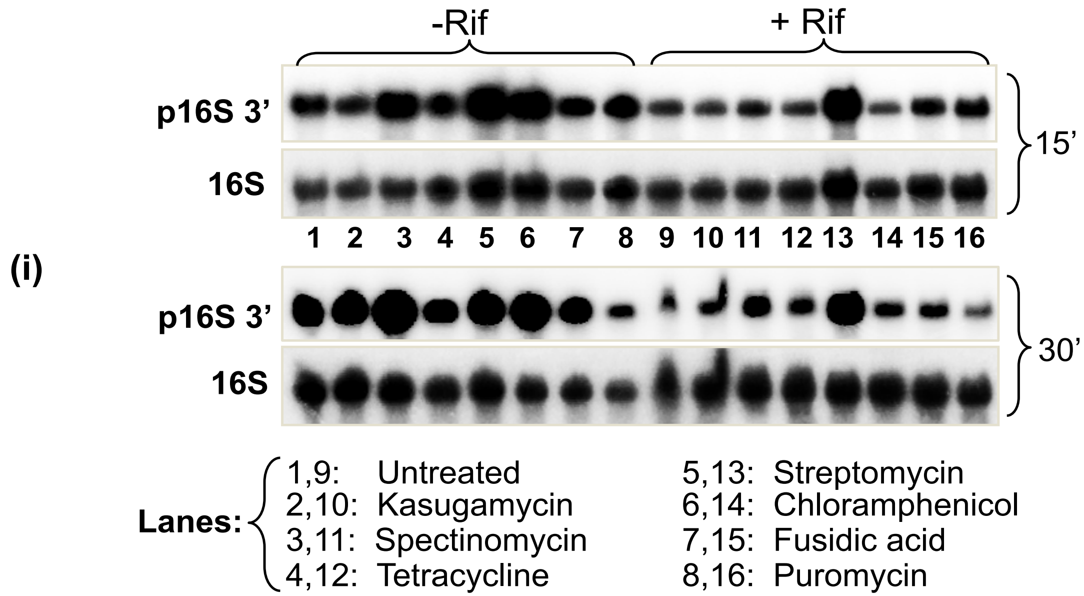

**Sectors:**
***E. coli* (wt) +**

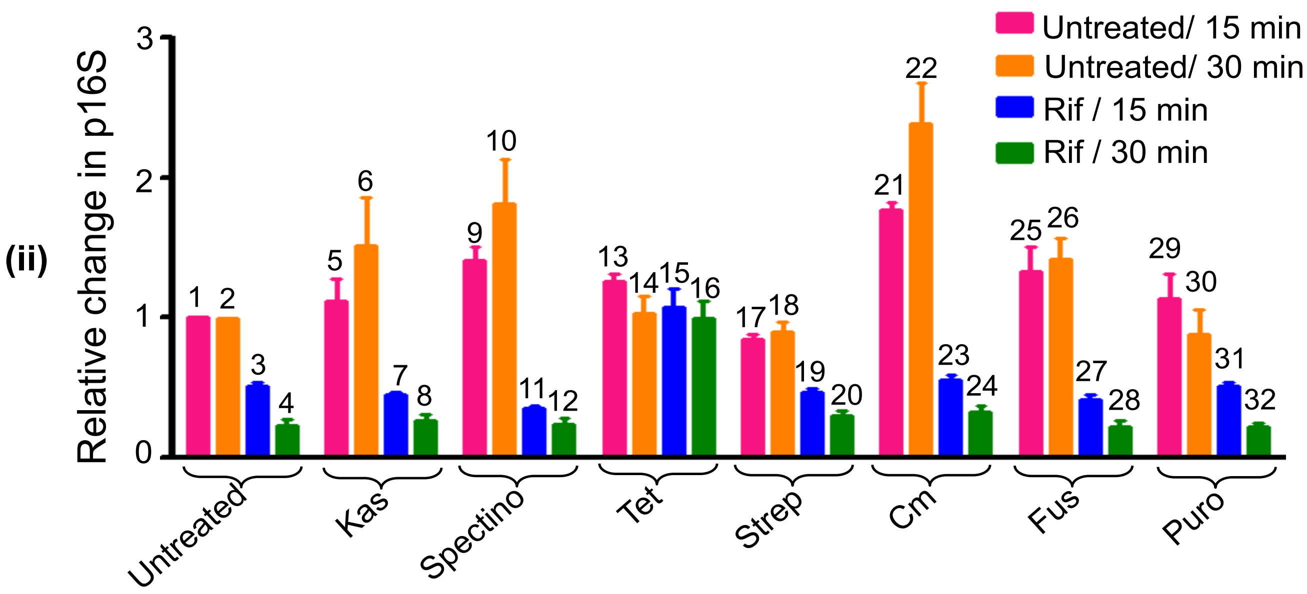
1: vector + vector
2: p*metY*$_{3GC}$ + vector
3: vector + p*rph*
4: p*metY*$_{3GC}$ + p*rph*
5: vector + p*rnr*
6: p*metY*$_{3GC}$ + p*rnr*
7: vector + p*ybeY*
8: p*metY*$_{3GC}$ + p*ybeY*



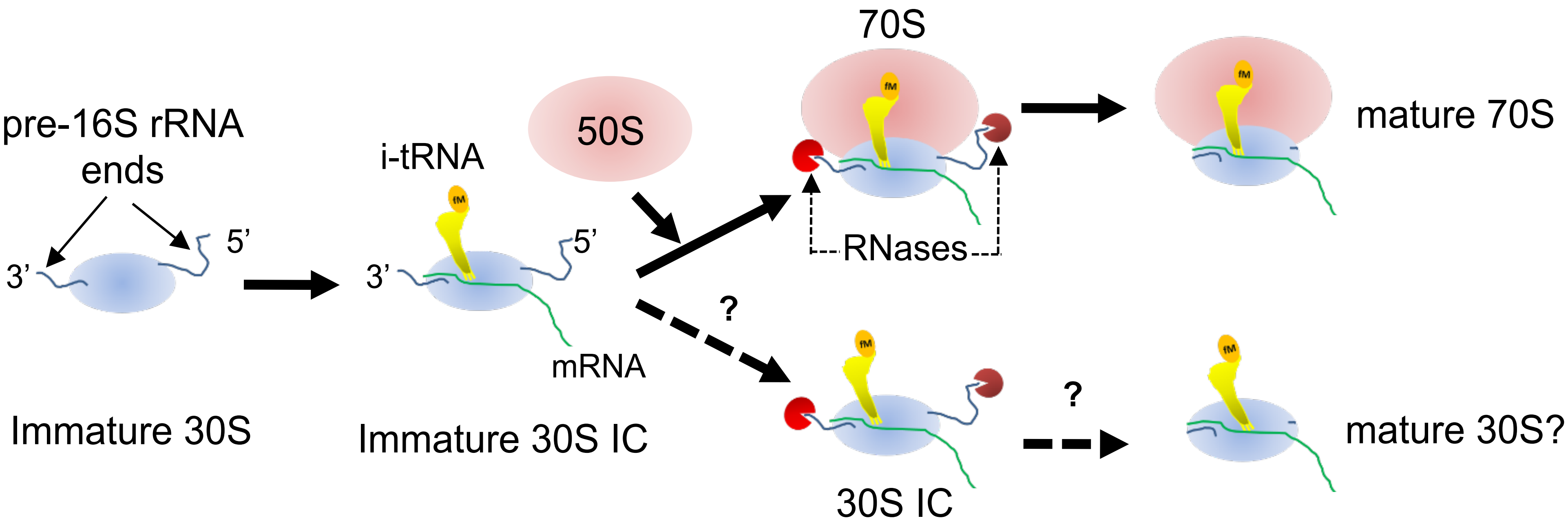



# 16S rRNA processing



# Role of i-tRNA in 30S biogenesis




Shetty, S. and Varshney, U. (2016) An evolutionarily conserved element in initiator tRNAs prompts ultimate steps in ribosome maturation. **Proc. Natl. Acad. Sci. (USA)** 113, E6126-E6134

# Summary

Formylation of i-tRNA:

(a) Facilitates its initial binding to ribosome

The 3GC base pairs:

(a) i-tRNA binding

(b) i-tRNA transitions from 30S to elongation competent 70S

(c) Ribosome maturation

Levels of i-tRNA

(a) High level of i-tRNA avoids initiation with elongator tRNAs

(b) Low level of i-tRNA responsible for ribosome heterogeneity

(c) Low level of i-tRnA may lead to proteome diversity

(d) Fitness under stressful conditions

# ACKNOWLEDGMENTS...

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