$S\pi th\sigma\eta$ 3.0

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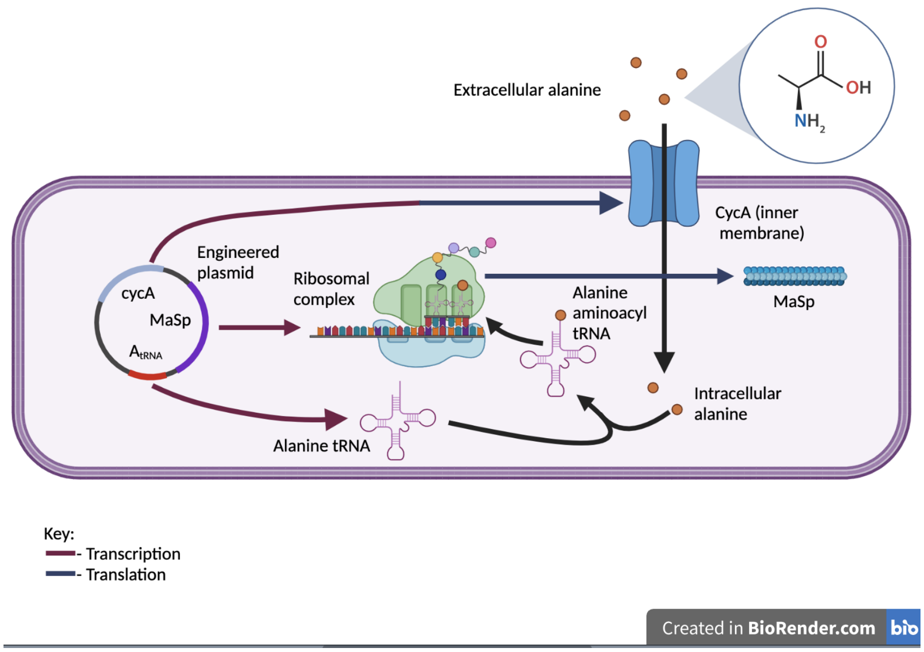

1 Quantities modelled

- A_{intra} - Intracellular alanine
- tRNA - Alanine tRNA
- A-tRNA - Alanyl aminoacyl tRNA
- Synthetase - Alanyl-tRNA synthetase catalysing binding of alanine to tRNA
- MaSp - Recombinant spider silk protein construct i.e. target protein for synthesis

- CycA - Channel protein which enables alanine uptake into the cell via active transport

2 Parameters

- N_p = No. plasmids per cell = 20
  - Derived from medium copy number of plasmid backbone
- L_{RNA} = Nucleotide base pair length of the mRNA transcript of a gene (or a tRNA itself)
  - $L_{cycA} = 1413$ bp [1]
  - $L_{tRNA} = 76$ bp [2]
  - $L_{synth} = 2628$ bp [3]
- Base transcription rate $k_0 = 45$ base pairs per second[4]
- k$_{transc}$ - Rate constant for transcription = $\frac{N_p k_0}{L_{RNA}}$
  - $k_{transc}^{cycA} = 0.6369\ s^{-1}$
  - $k_{transc}^{tRNA} = 11.84\ s^{-1}$
  - $k_{transc}^{synth} = 0.3424\ s^{-1}$
- k$_{deg}$ - Rate constant for degradation
  - Assume all degradation steps follow first-order kinetics, therefore
    * $k_{deg} = \frac{ln(2)}{\tau_{1/2}}$
  - Protein
    * Mean $\tau_{1/2} = 20$ hours [5]
    * $k_{deg}^{CycA} = k_{deg}^{MaSp} = k_{deg}^{Synth} = 9.6 \text{ x } 10^{-6}\ s^{-1}$
  - tRNA
    * tRNA degradation is only non-negligible for the case of amino acid starvation [6]
    * $\tau_{1/2} = 10$ minutes
    * $k_{deg}^{tRNA} = 1.2 \text{ x } 10^{-3}\ s^{-1}$
  - Here we assume alanyl-tRNA complexes are used up sufficiently quickly that they are unlikely to be degraded (by a tRNA nuclease)
- N_k– No. alanine residues present within a particular protein k
  - $N_{CycA} = 47$ residues [1]
  - $N_{MaSp} = 51$ residues [7]
  - $N_{synth} = 91$ residues [3]

- Other rate constants
  - k_{cat} - Rate constant for alanine uptake through CycA (determined using ML tool DLKCat [8])
    - $k_{cat} = 0.9953\ s^{-1}$
  - k_{synth} = Rate constant for rate-determining aminoacylation step = $2.0s^{-1}$ [9]
- U_0 - Base alanine uptake rate
  - $U_0 = 1.17$ mmol/30 seconds/kg wet weight of *E. coli* [10]
  - *E. coli* wet cell weight = 10^{-12} g/cell [11]
  - $\therefore U_0 = 23500$ molecules/cell/second
- Initial values of quantities (if non-zero)
  - Equilibrium population of alanine tRNAs per cell = $[D]_0 = 4000$ [12]
  - Equilibrium population of alanyl-tRNA synthetases per cell = $[I]_0 = 6695$ [12]
    - Uses known equilibrium rate constant (i.e. turnover rate) for the synthetase from [9]

3 Governing Equations

3.1 Alanine import

$$\frac{d[A_{intra}]}{dt} = U_0 + k_{cat}[CycA] \tag{1}$$

3.2 Aminoacylation

If tRNA availability is the limiting factor:

$$\frac{d[A - tRNA]}{dt} = [tRNA] \tag{2}$$

$$\frac{d[A_{intra}]}{dt} = -[tRNA] \tag{3}$$

$$\frac{d[tRNA]}{dt} = -[tRNA] \tag{4}$$

If intracellular alanine availability is the limiting factor:

$$\frac{d[A - tRNA]}{dt} = [A_{intra}] \tag{5}$$

$$\frac{d[tRNA]}{dt} = -[A_{intra}] \tag{6}$$

$$\frac{d[A_{intra}]}{dt} = -[A_{intra}] \tag{7}$$

If synthetase activity is the limiting factor:

$$\frac{d[A-tRNA]}{dt} = k_{synth}[Synthetase] \tag{8}$$

$$\frac{d[tRNA]}{dt} = -k_{synth}[Synthetase] \tag{9}$$

$$\frac{d[A_{intra}]}{dt} = -k_{synth}[Synthetase] \tag{10}$$

3.3 Transcription + translation

Demand = Alanyl tRNA demand for translation of CycA and alanyl-tRNA synthetase

$$Demand = k_{transc}^{CycA} N_{CycA} + k_{transc}^{Synth} N_{Synth} \tag{11}$$

If [A-tRNA] $\geq$ Demand:

$$\frac{d[CycA]}{dt} = k_{transc}^{CycA} \tag{12}$$

$$\frac{d[Synthetase]}{dt} = k_{transc}^{Synth} \tag{13}$$

$$\frac{d[MaSp]}{dt} = \frac{[A-tRNA] - Demand}{N_{MaSp}} \tag{14}$$

Else:

$$\frac{d[CycA]}{dt} = \frac{[A-tRNA]}{Demand} k_{transc}^{CycA} \tag{15}$$

$$\frac{d[Synthetase]}{dt} = \frac{[A-tRNA]}{Demand} k_{transc}^{Synth} \tag{16}$$

3.4 Degradation

$$\frac{d[CycA]}{dt} = -k_{deg}^{CycA}[CycA] \tag{17}$$

$$\frac{d[Synthetase]}{dt} = -k_{deg}^{Synth}[Synthetase] \tag{18}$$

$$\frac{d[MaSp]}{dt} = -k_{deg}^{MaSp}[MaSp] \tag{19}$$

If alanine availability is the limiting factor for aminoacylation (i.e. under amino acid starvation conditions):

$$\frac{d[tRNA]}{dt} = -k_{deg}^{tRNA}[tRNA] \tag{20}$$

References

[1] https://biocyc.org/gene?orgid=ECOLI&id=EG12504#

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[3] https://www.uniprot.org/uniprotkb/P00957/entry#sequences

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[5] https://bionumbers.hms.harvard.edu/bionumber.aspx?s=n&v=2&id=111930

[6] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5314770/

[7] Link registry page

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[9] https://doi.org/10.1016/j.jmb.2006.06.015

[10] https://www.sciencedirect.com/science/article/pii/S0021925818945072

[11] https://ecmdb.ca/e_coli_stats

[12] https://doi.org/10.1128/jb.158.3.769-776.1984