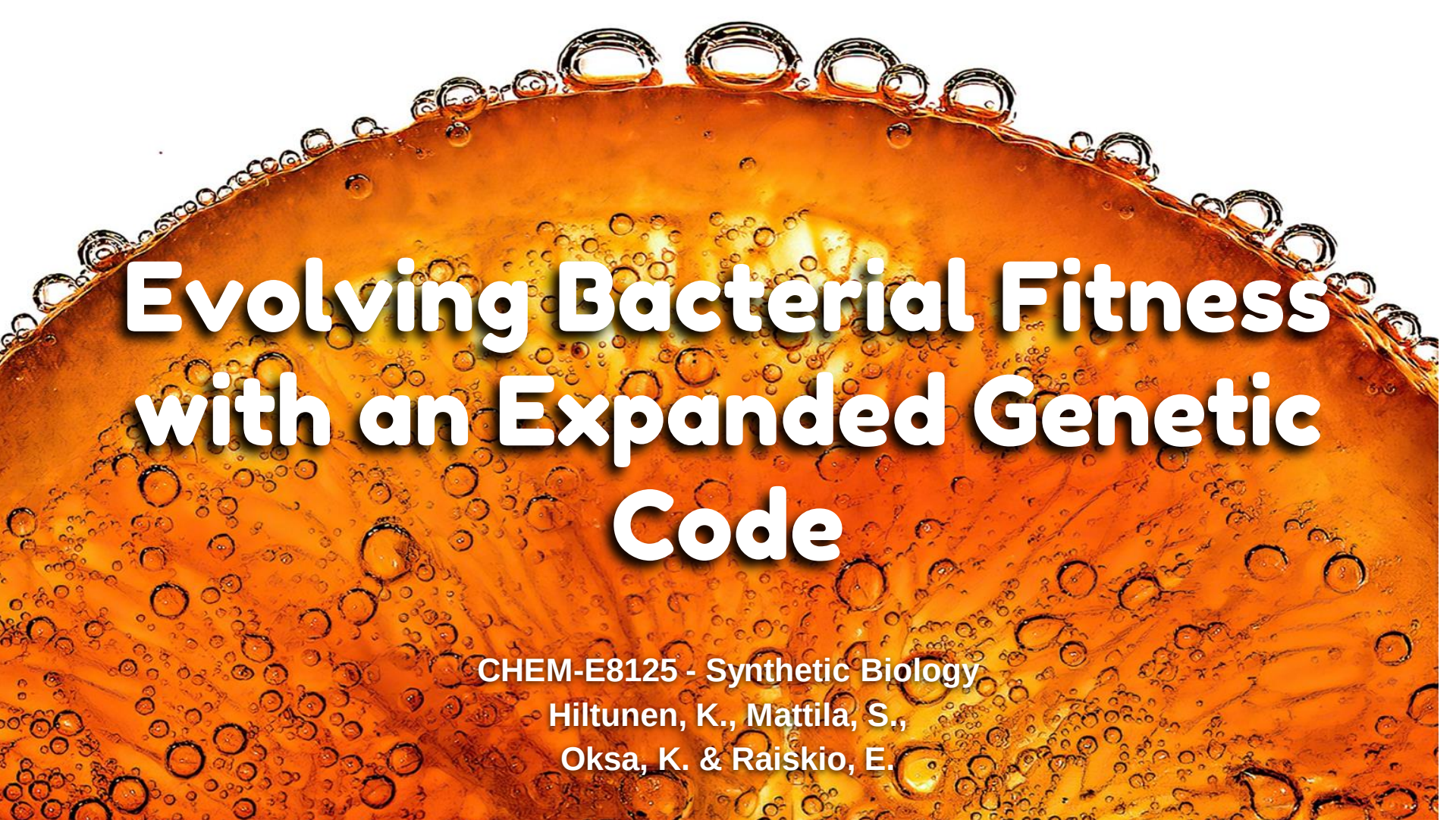

Evolving Bacterial Fitness with an Expanded Genetic Code

CHEM-E8125 - Synthetic Biology

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Introduction

- Evolution has largely confined the proteins to be formed out of 20 amino acids.
- Alternative codon tables, such as the mitochondrial genomes, have most likely evolved from standard codon table
  - supports the hypothesis that canonical **genetic code can evolve**.
- Raises questions:
  - Codon assignment and re-assignment, how does that happen?
  - How does a cell adapt to an expanded genetic code?

Introduction

- Expanding the standard set of proteinogenic amino acids can help us understand the evolution of the genetic code
- Change the underlying translational machinery to create orthogonal translation systems (OTS)
    - allows the incorporation of non-canonical amino acids (ncAAs)
- Drew etal 2018: UAG will code for both the stop of translation and for the ncAA 3 -nitro-L-tyrosine
    - an engineered β-lactamase (bla) was utilised (dependent on the OTS incorporation)

OTS = orthogonal translation system
ncAA = non-canonical amino acid

Introduction

20 AA → 21 AA

		2. nucleotide			
		U	C	A	G
1. nucleotide	U	UUU (Phe/F)	UCU (Ser/S)	UAU (Tyr/Y)	UGU (Cys/C)
		UUC (Phe/F)	UCC (Ser/S)	UAC (Tyr/Y)	UGC (Cys/C)
		UUA (Leu/L)	UCA (Ser/S)	UAA Ochre (Stop)	UGA Opal (Stop)
		UUG (Leu/L)	UCG (Ser/S)	UAG Ambre (Stop)	UGG (Trp/W)
	C	CUU (Leu/L)	CCU (Pro/P)	CAU (His/H)	CGU (Arg/R)
		CUC (Leu/L)	CCC (Pro/P)	CAC (His/H)	CGC (Arg/R)
		CUA (Leu/L)	CCA (Pro/P)	CAA (Gln/Q)	CGA (Arg/R)
		CUG (Leu/L)	CCG (Pro/P)	CAG (Gln/Q)	CGG (Arg/R)
	A	AUU (Ile/I)	ACU (Thr/T)	AAU (Asn/N)	AGU (Ser/S)
		AUC (Ile/I)	ACC (Thr/T)	AAC (Asn/N)	AGC (Ser/S)
		AUA (Ile/I)	ACA (Thr/T)	AAA (Lys/K)	AGA (Arg/R)
		AUG (Met/M) (Start)	ACG (Thr/T)	AAG (Lys/K)	AGG (Arg/R)
	G	GUU (Val/V)	GCU (Ala/A)	GAU (Asp/D)	GGU (Gly/G)
		GUC (Val/V)	GCC (Ala/A)	GAC (Asp/D)	GGC (Gly/G)
		GUA (Val/V)	GCA (Ala/A)	GAA (Glu/E)	GGA (Gly/G)
		GUG (Val/V)	GCG (Ala/A)	GAG (Glu/E)	GGG (Gly/G)

polar
nonpolar
basic
acidic

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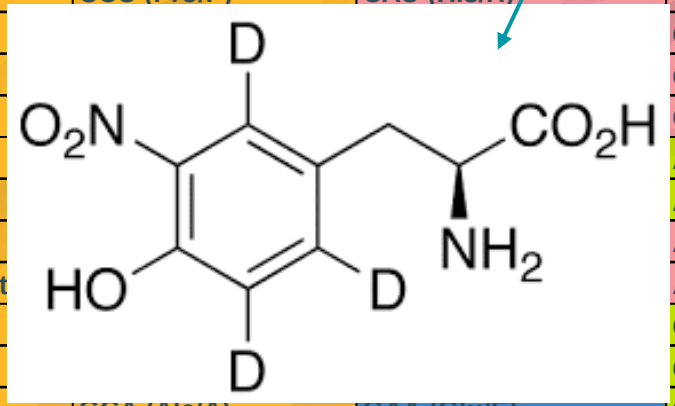
		UUG (Leu/L)	UCG (Ser/S)	UAG Stop / 3nY	UGG (Trp/W)
	C	CUU (Leu/L)	CCU (Pro/P)	CAU (His/H)	CGU (Arg/R)
		CUC (Leu/L)			CGC (Arg/R)
		CUA (Leu/L)			CGA (Arg/R)
		CUG (Leu/L)			CGG (Arg/R)
	A	AUU (Ile/I)			AGU (Ser/S)
		AUC (Ile/I)			AGC (Ser/S)
		AUA (Ile/I)			AGA (Arg/R)
		AUG (Met/M) (Start			AGG (Arg/R)
	G	GUU (Val/V)			GGU (Gly/G)
		GUC (Val/V)			GGC (Gly/G)
		GUA (Val/V)	GCA (Ala/A)	GAA (Glu/E)	GGA (Gly/G)
		GUG (Val/V)	GCG (Ala/A)	GAG (Glu/E)	GGG (Gly/G)



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Purpose

To study whether directed evolution would help in

- retention of OTSs
- stabilise non-canonical amino acid encoding codon in the genome
- survive greater selection pressure
- and increase the fitness of ncAA ‘addicted’ cells vs. control cells

OTS = orthogonal translation system
ncAA = non-canonical amino acid

Methods and approaches

Expansion of canonical genetic code requires few things from the organism's genome:

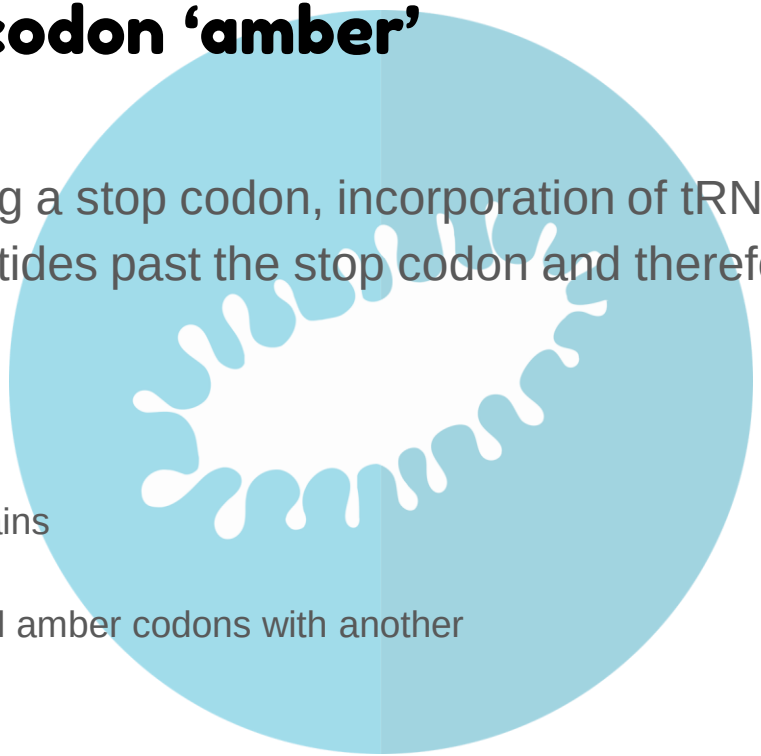
- Codons that can be repurposed to encode a non-canonical amino acid (ncAA)
  - Most commonly the amber codon (UAG)
- Changes to the translational machinery
  - Orthogonal translation systems (OTSs) formed of aminoacyl-tRNA synthase (aaRS) / suppressor tRNA pairs
- Dependance on the ncAA
  - Enforcing agent needed to enhance the stability of transformed trait
  - Often an antimicrobial agent/resistance feature pair

Considerations & Planning

The UAG stop codon ‘amber’

- Due to amber being a stop codon, incorporation of tRNAs might lead to lengthening of peptides past the stop codon and therefore can decrease fitness

- Solutions:
  - UAG-deficient strains
  - Bacteriophages
  - Replacement of all amber codons with another



Elimination of genes involved in ncAA use

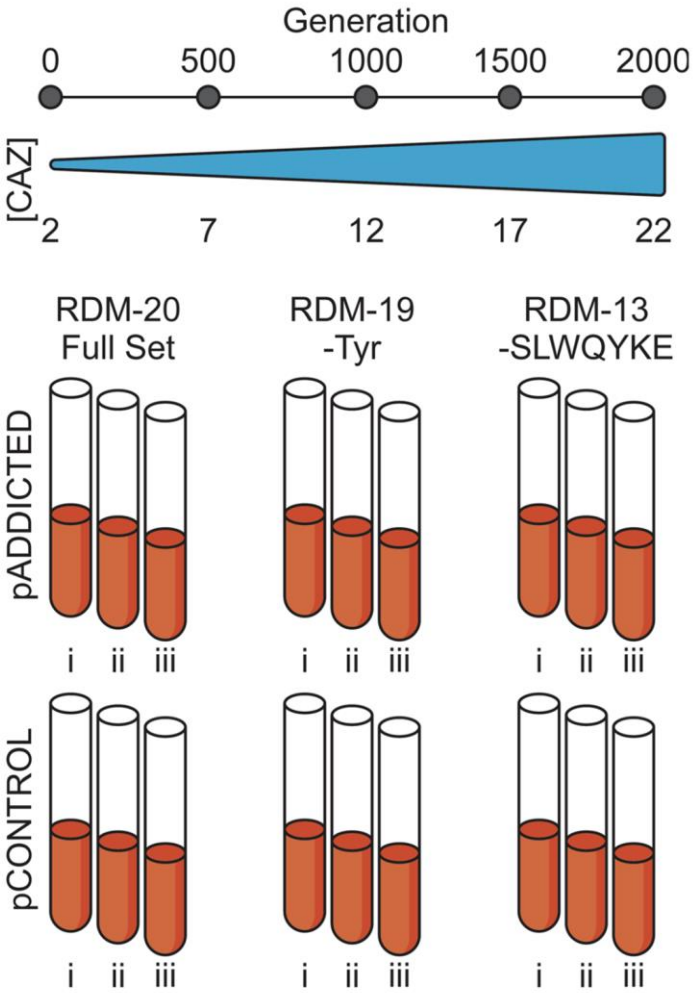
- Mutations, including new genes, tend to be eliminated due to decreased fitness
  - Therefore, organism needs to dependent on the ncAA ‘addicted’ for it to remain in the genome
  - Constant selection pressure needs to be present, for example a protein conferring resistance or increased fitness

ncAA = non-canonical amino acid

Procedure

1. Gene encoding a β-lactamase with 3nY-encoding amber codon was transformed to E. coli strain MG1655 via plasmid pADDICTED
2. Control: a plasmid with the β-lactamase with P instead of 3nY was constructed
3. Transformants grown in three different depletion media to decrease the chance of amber mutating to canonical AA encoding codon
4. Sequencing of a single cell line each time transfering to a new media
5. Survivability and fitness of the cells was recorded

3nY = 3-nitro-L-tyrosine
P = phenylalanine
AA = amino acid

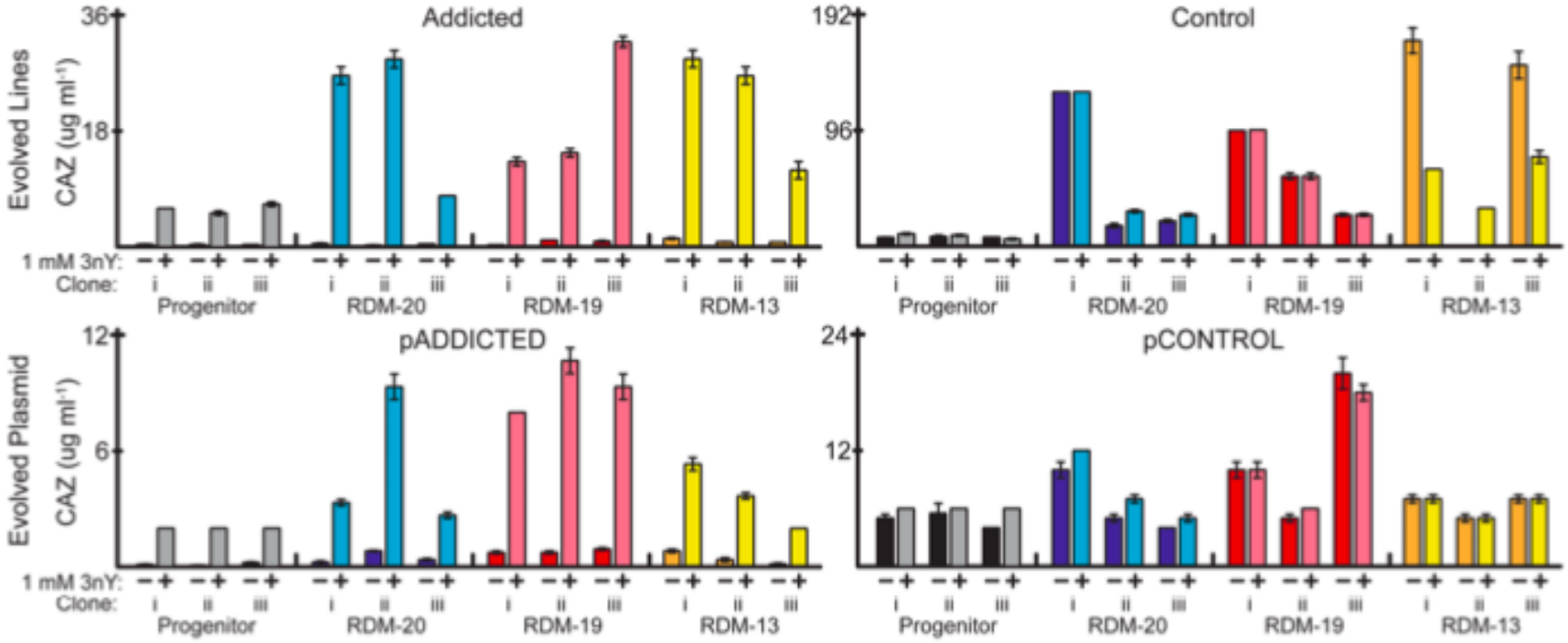


- 2,000 generations
- Three replicates
- Sequential transfer to -Y and to -SLWQYKE rich defined media to suppress amber-specific point mutation chance
- Cephalosporin antibiotic **ceftazidime** as a selection factor, fitness burden and OTS enforcer

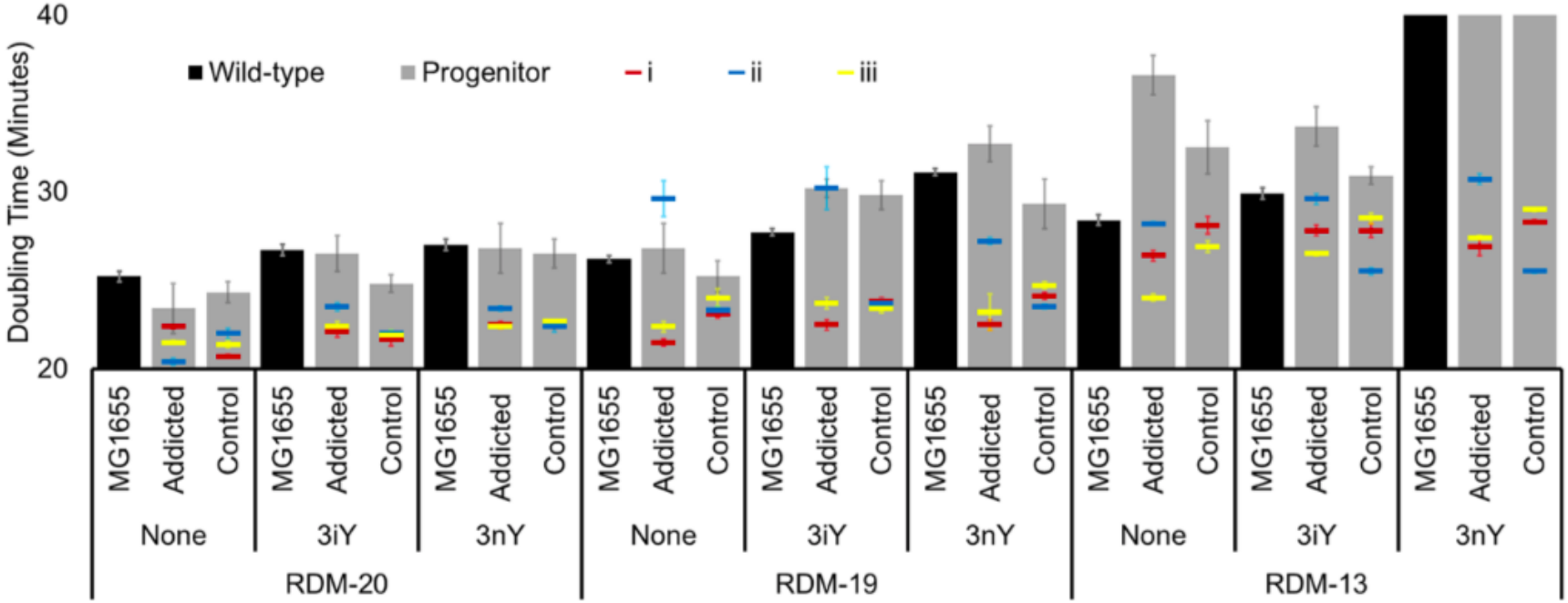
What was achieved

- Successful ncAA (3nY) incorporation
- Active OTS in the lineages that had ncAA addiction
- All lineages overcame the fitness burden associated with 3nY toxicity through the mutations
  - hypothesis: aromatic amino acid transporter deactivation reduced the fitness burden imposed by 3nY
  - the encoding of OTS on a plasmid on its own did not have a profound effect on fitness
- Increased resistance to the antibiotic ceftazidime (CAZ)

Antibiotic resistance (CAZ MICs)



Growth rates as doubling times



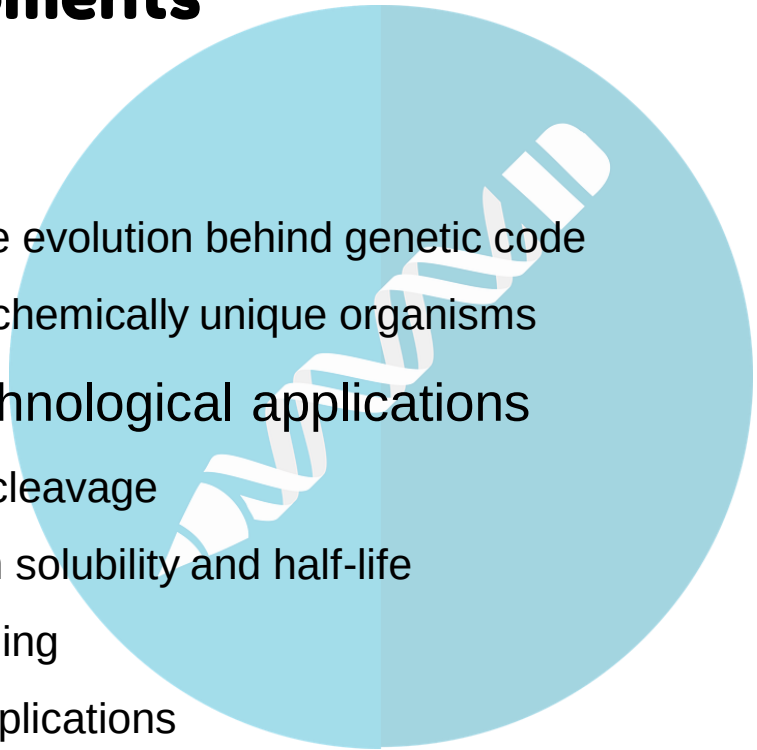
What did not work

- Whereas the wt-MG1655 was capable of growth in all media conditions,
  the progenitor clones transformed with pADDICTED or pCONTROL proved largely incapable of growth in RDM-13 when supplemented with 3nY, even without CAZ
    - However, after 125 generations all lineages were capable of growth in RDM-13

- Without addiction, the OTSs were lost or compromised during evolution

- Four clones had acquired a single in-frame amber codon in protein coding sequences

Future developments

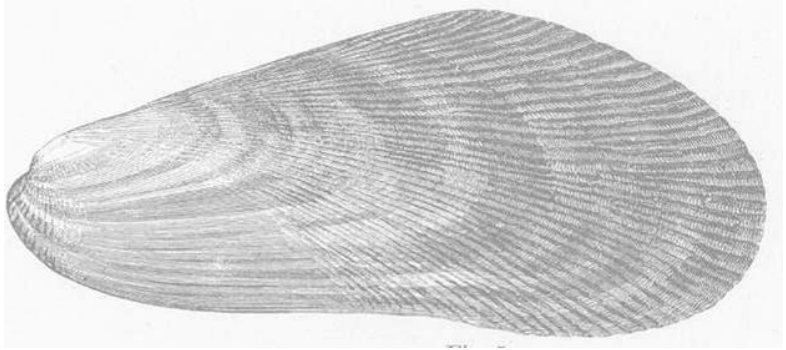


- Importance
  - Recording of the evolution behind genetic code
  - Evolution of biochemically unique organisms
- Promising biotechnological applications
  - Trigger protein cleavage
  - Increase protein solubility and half-life
  - In vivo cell imaging
  - Biomedicinal applications
  - Post-translational modification (PTM) mimicking

Mimicking PTM using ncAA

Example: Healthcare bioadhesives from marine mussel foot protein

- Water-resistant adhesive that can be used to make healthcare materials
  - Adhesive properties depend on PTM hydroxylation of amino acids
  - Normally happens only in ER in eukaryotes
- Addition of hydroxyproline as ncAA into *E. coli* strain
  - Synthesis of functional protein in bacteria
  - Protein production more cost- and time-effective



PTM = post-translational modification
ER = endoplasmic reticulum
ncAA = non-canonical amino acid

Thank you!

Questions?

Paramecium Parlor