# Evolving Bacterial Fitness with an Expanded Genetic Code

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# Introduction and aims

# Introduction

- Canonical genetic code has the ability to evolve, since alternative mitochondrial genomes are evolved from the standard codon table
- The full adaptation process of a cell to an expanded genetic code is not yet presented
- Tack et al. researched how the genetic code can be expanded with the development of **orthogonal translation systems** (OTS) and how this can be used to improve bacterial fitness
  - Active OTS have been found to cause fitness deficits in cells since any protein terminated by an amber stop codon can be unnaturally extended
  - Previous research on expanded genetic code has been performed with bacteriophages (so the fitness of the host organism is irrelevant) or with strains that do not contain amber codons

# Main aim

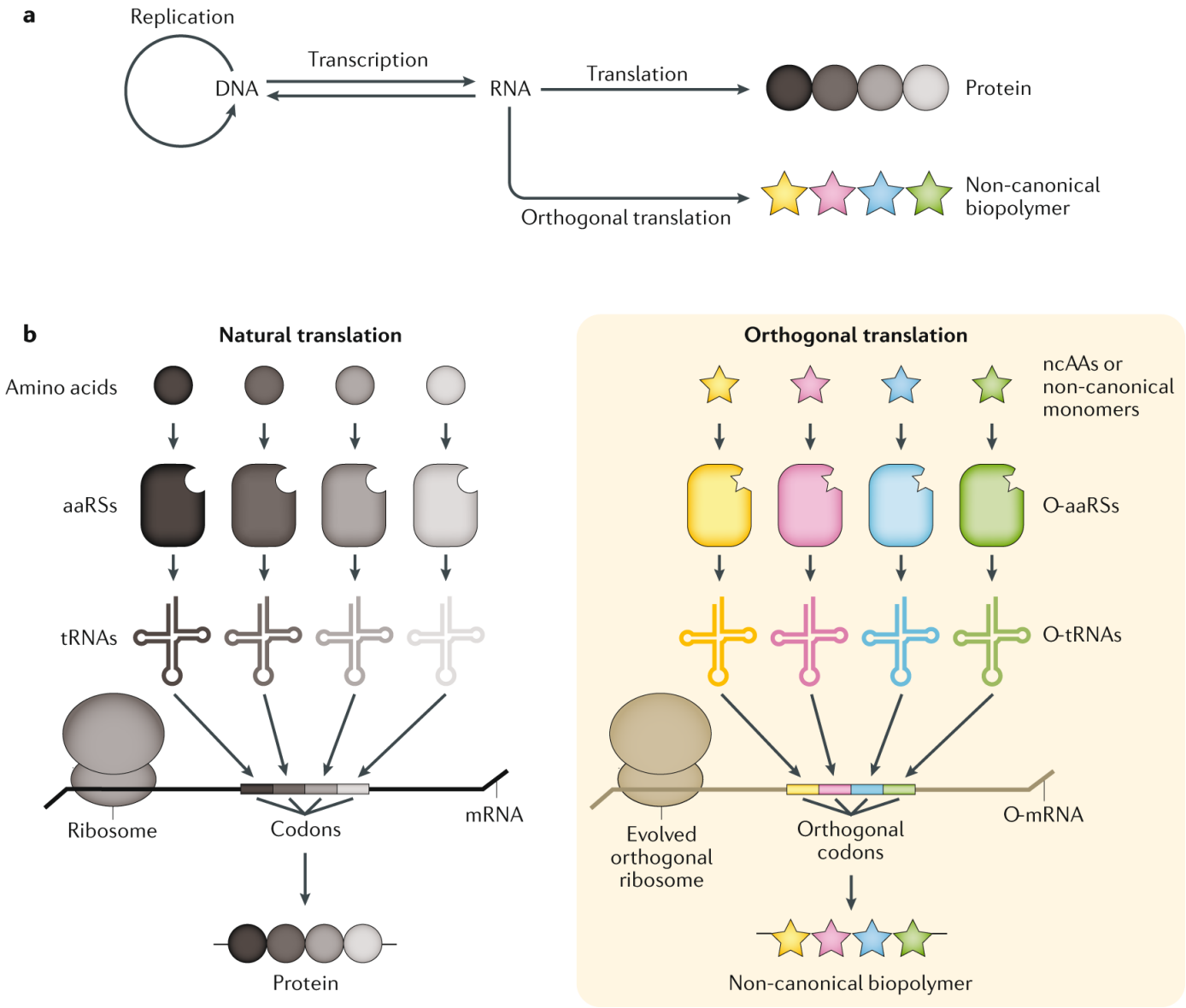
- Expanding the bacterial genome to accept the noncanonical amino acid (ncAA) 3-nitro-L-tyrosine (3nY), and explore its utility in the proteome
- Studying long-term adaptation and evolution of *E. coli* addicted to 3nY and creating a hybrid approach to expand amino acid genetic codes
- Preserve an active OTS in an unmodified organism
- Improving bacterial fitness: engineered essential gene for a ß-lactamase *(bla)* used to tackle fitness issues and study long term evolution

# Terminology

# Orthogonal translation systems (OTSs)

- Allow for the expansion of proteinogenic amino acids by modifying the translational machinery of the organism
- Comprised of aminoacyl-tRNA synthetase (aaRS)/suppressor tRNA pairs
  - **Aminoacyl-tRNA synthetase**: an enzyme that connects an amino acid to its corresponding tRNA
  - **Suppressor tRNA**: tRNA with a mutation which allows it to recognize a stop codon and insert an amino acid in its place (1)
- Do not significantly interact with the host translational machinery or have interference with the occupied parts of the genetic code
  - Allows the incorporation of noncanonical amino acids by suppressing the amber stop codon (UAG)

- **Noncanonical amino acids** (ncAAs): amino acids beyond the usual 20 amino acids
  - Usually synthesized in a laboratory
  - Different side chain structures and backbone conformations
  - Not integrated into proteins naturally during ribosomal synthesis
- The **amber stop codon** is a nonsense codon, leading to a premature stop codon in the translated mRNA
  - Amber stop codons are possible in bacteria that contain amber suppressor mutations
  - Can be recognized by tRNA molecules carrying unnatural amino acids which have been designed to recognize the amber stop codon


a
Replication
DNA
Transcription
RNA
Translation
Protein
Orthogonal translation
Non-canonical biopolymer
b
Natural translation
Amino acids
aaRSs
tRNAs
Ribosome
Codons
mRNA
Protein
Orthogonal translation
ncAAs or non-canonical monomers
O-aaRSs
O-tRNAs
Evolved orthogonal ribosome
Orthogonal codons
O-mRNA
Non-canonical biopolymer

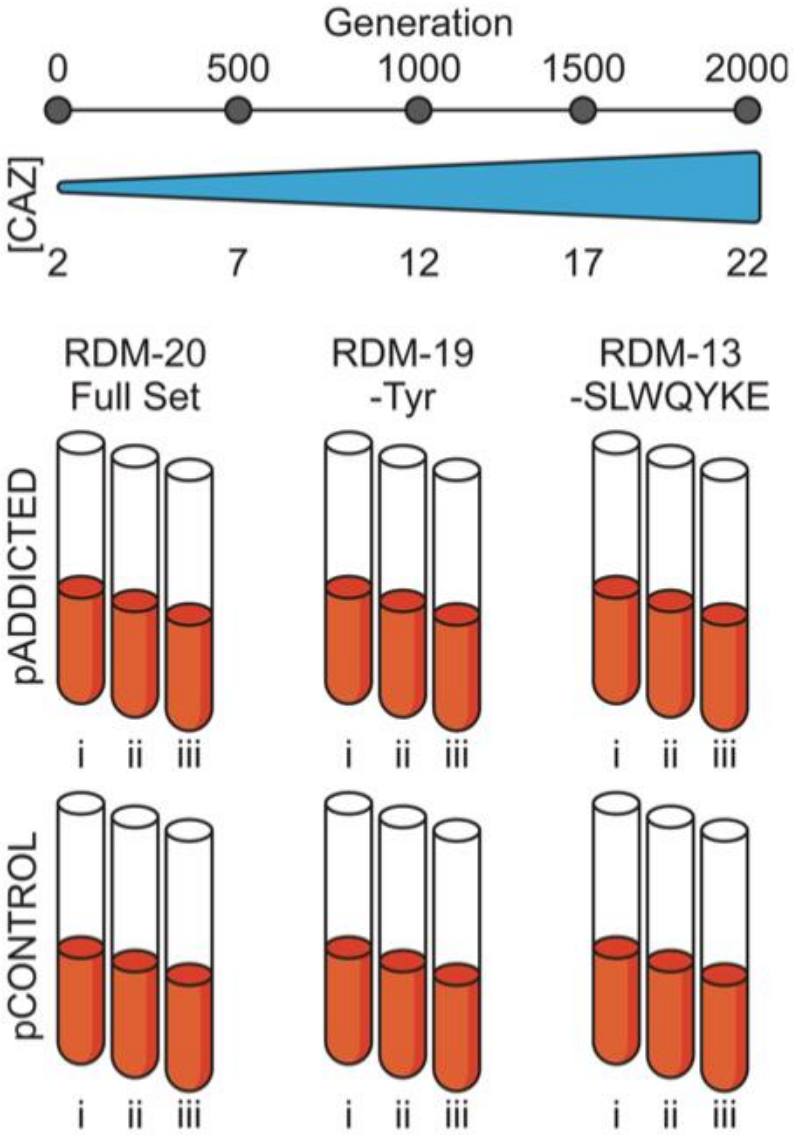
# Methods and approaches

# Experimental set-up

- Assembly of the OTS compatible with 3nY
  - Aim: incorporation of 3nY to the genome
  - Parts of the system
    - *Methanocaldococcus jannaschii* tyrosyl-aaRS variant previously engineered to be specific for 3-iodo-L-tyrosine (3iY) and also compatible with 3nY
    - Corresponding tyrosyl-tRNA with an anticodon complementary to the UAG amber stop codon
- The OTS was assembled to enable addiction via a ß-lactamase variant ($bla_{\text{TEM-1.B9}}$)
  - Dependent on the incorporation of 3nY at amino acid position 162
- The ß-lactamase variant was further engineered to use ceftazidime (CAZ) as a substrate → $bla_{\text{Addicted}}$
  - This variant provided a moderate resistance to CAZ dependent on 3nY concentrations usually used in bacterial cultures
- The new variant allowed the retainment and challenging of 3nY incorporation into the genome by increasing CAZ concentrations in the culture
  - Increased CAZ concentrations provided a fitness burden to the system and enforced the activity of the OTS

# Experimental set-up

- Wild-type *E. coli* strain MG1655
- pADDICTED or pCONTROL evolved in biological triplicates (i, ii, and iii) for 2000 generations
- Three defined media conditions each supplemented with 10 mM 3nY
  1. media (RDM-20): all 20 canonical amino acids
  2. media (RDM-19): no tyrosine
  3. media (RDM-13): lacked seven amino acids (serine, leucine, tryptophan, glutamine, tyrosine, lysine, and glutamate)
- CAZ concentration increased during evolution
  - Fitness burden, enforcement of OTS activity



# Test methods

- Growth rates as doubling times measured to evaluate cellular fitness both before and after evolution
  - Different conditions for measuring doubling times:
    - In absence of CAZ
    - Amino acid environments: without ncAA, with 3nY, and with 3iY
- **Minimum inhibitory concentration** (MIC):
  values of the lowest concentration at which no bacterial growth observed
- GFP assays: absorbance and fluorescence

# Results

# Experimental evolution

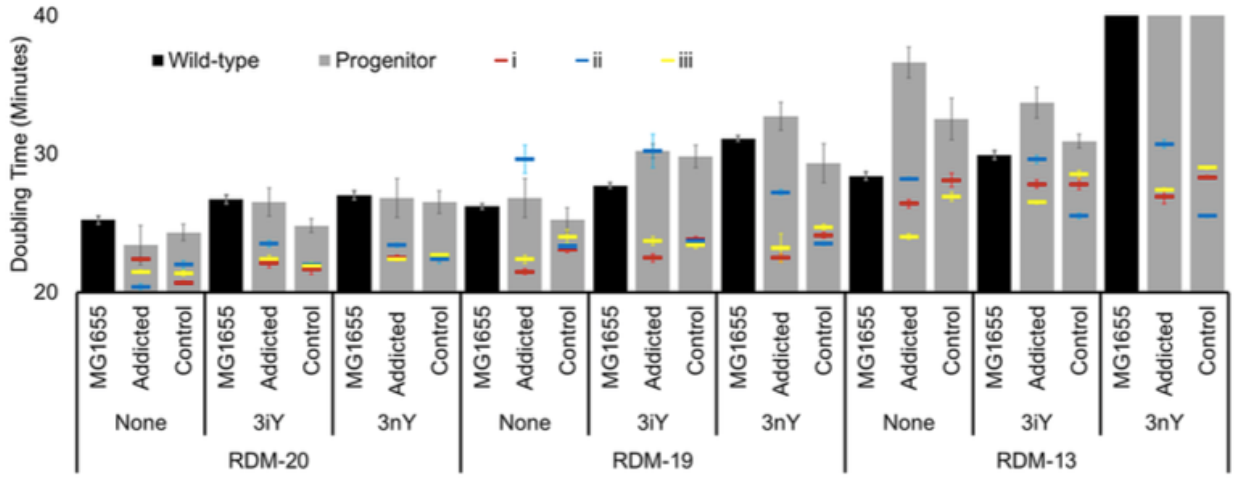
- The research group were able to create a system that incorporates an ncAA for 2 000 generations of evolution
- System evolved to overcome the fitness burden from the expanded genetic code
  - Fitness deficit of the strain is largely repaired through mutations
    - Alleviation of toxicity of an unnatural amino acid
    - Optimization of the composition of the 20 amino acids normally used
    - All lineages overcame the burden of 3nY toxicity
  - Improved fitness allows new amber codons to populate coding sequences
- The group was able to identify the entire complement of genetic mutations that lead to improved fitness in the presence of the 21 amino acid code

# Effect of media composition

- Media lacking tyrosine (RDM-19, RDM-13)
  - Tyrosine permease (*tyrP*) inactivated
- Media also lacking tryptophan (RDM-13)
  - Tryptophan permease *mtr* inactivated
- All amino acids present (RDM-20)
  - Serine permease *sdaC* inactivated
    - Serine most abundant → amino acid pool is better balanced through this deletion with other serine transporters (*sstT*) available for serine uptake

# Growth rates

- Growth rates, or doubling times were used as general indicators of cellular fitness
- Doubling times of wild-type E. coli strain MG1655 compared well to those in rich media, in the absence of 3nY
- Reduced doubling time of the Addicted-19(ii) line in the absence of 3nY
  - Possible preference to new media condition containing 3nY with no in-frame amber codons
  - Resemblance to evolution of *B. subtilis* that could utilize 4-fluoro-tryptophan in place of tryptophan



# Importance and future aspects

# Importance and path forward

- One of the first experiments to describe a hybrid approach to modify a protein genome
- Previous studies include two different approaches to generate expanded genetic codes
  - Engineer components of the organism to function with ncAA
  - Evolve the organism in the presence of ncAA
- Hybrid approach includes engineering of an addiction element *bla,* utilized to preserve an OTS over evolution
- Amberless *E. coli* can now be used to produce fully synthetic genomes with depencende on ncAA
- Further research on recoding of the genetic code during evolution or evolution of biochemically unique organisms

# Thank you!

# Sources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