Genomically Recoded Organisms Expand Biological Functions
基因组重构物种扩展生物功能

全源

Content

Background

The advantage of genetic code conservation ?

- 1 Permit organisms to share beneficial traits through horizontal gene transfer .

- 2 Enable the accurate expression of heterologous genes in nonnative organisms .

Background

The disadvantage of genetic code conservation ?

- 1 The common genetic code also allows viruses to hijack host translation machinery and compromise cell viability.
- 2 Additionally, genetically modified organisms (GMOs) can release functional DNA into the environment
- 3 Furthermore, biotechnology has been limited by the 20 amino acids of the canonical genetic code, which use all 64 possible triplet codons, limiting efforts to expand the chemical properties of proteins by means of nonstandard amino acids (NSAAs).

Background

What can wo do? *Changing the genetic code !*

- 1 Could solve above challenges .
- 2 Reveal new principles that explain how genetic information is conserved, encoded, and exchanged .
- 3 Furthermore, could provide dedicated codons to improve the purity and yield of NSAA-containing proteins.

Abstract

We replaced all known UAG stop codons in Escherichia coli MG1655 with synonymous UAA codons, which permitted t he deletion of release factor 1 and reassignment of UAG translation function.

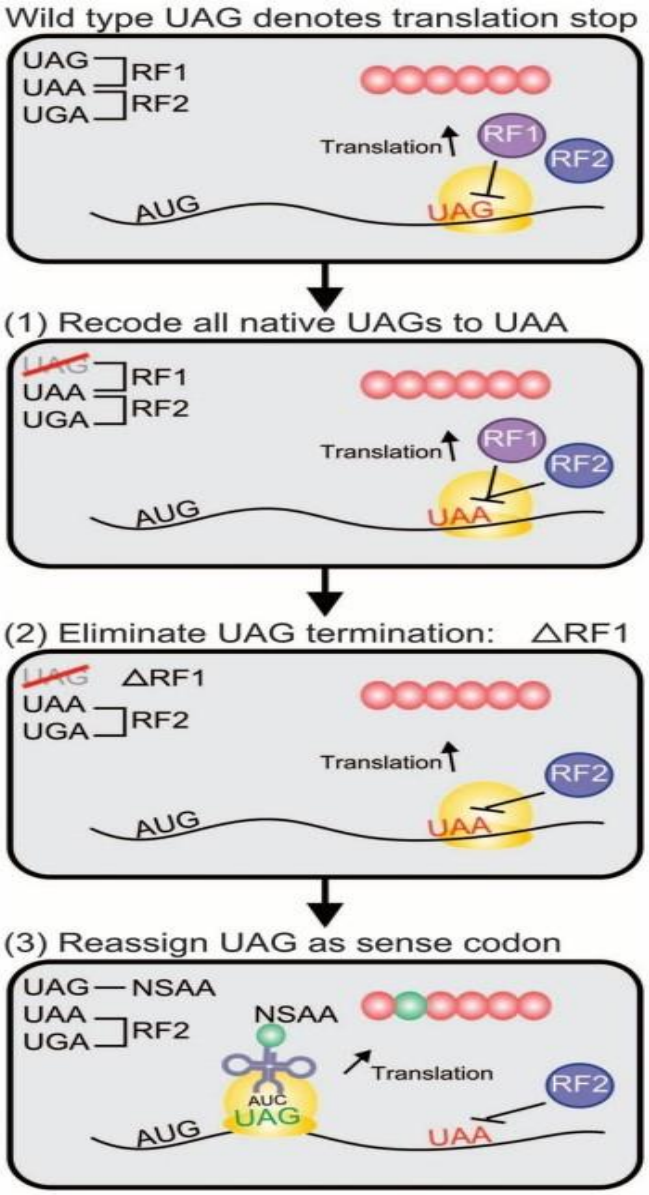
This GRO (Genomically Recoded Organism) exhibited improved properties f or incorporation of nonstandard amino acids that expand the chemical diversity of proteins in vivo.

The GRO also exhibited increased resistance to T7 bacteriophage, demonstrating that new genetic codes could enable increased viral resistance.

Why we selected UAG as first target ?

- UAG is the rarest codon in Escherichia coli MG1655 (321 known instances).
- Prior studies demonstrated the feasibility of amino acid incorporation at UAG.
- A rich collection of translation machinery capable of incorporating NSAAs has been developed for UAG

F i g . 1 . Engineering a GRO with a reassigned UAG codon.



Wild-type E. coli MG1655 has 321 known UAG codons that are decoded as translation stops by RF1 (for UAG and UAA).

(1) Remove codons: converted all known UAG codons to UAA, relieving dependence on RF1 for termination.

(2) Eliminate natural codon function: abolished UAG translational termination by deleting RF1 , creating a blank codon.

(3) Expand the genetic code: introduced an orthogonal aminoacyl–tRNA synthetase (aaRS) and t RNA to reassign UAG as a dedicated sense codon capable of incorporating nonstandard amino acids (NSAAs) with new chemical properties.

Strain*	Essential codons changed†	Total codons changed‡	Previously essential codon functions manipulated§	Expected (obs.) UAG translation function‖
EcNR2	0	0	None	Stop
C0.B¶	0	0	*prfB*#	Stop
C0.B¶.ΔA::S	0	0	*prfB*,# Δ*prfA*::*spec*^{R}	None (stop¶)
C7	7	7	None	Stop
C7.ΔA::S	7	7	Δ*prfA*::*spec*^{R}	None (sup)
C13	7	13	None	Stop
C13.ΔA::S	7	13	Δ*prfA*::*spec*^{R}	None (sup)
C321	7	321	None	Stop
C321.ΔA::S	7	321	Δ*prfA*::*spec*^{R}	None (nc)
C321.ΔA::T	7	321	Δ*prfA*::*tolC*	None (nc)
C321.ΔA	7	321	Δ*prfA*	None (nc)

Table 1. Recoded strains and their genotypes.

:: :insertion D:deletion

† Out of a total of 7. ‡ Out of a t otal of 321.

§ prfA encodes RF1, terminating UAG and UAA; prfB encodes RF2, terminating UGA and UAA.

prfB# is an RF2 variant exhibiting enhanced UAA termination and weak UAG termination .

||Observed translation function: Stop, expected UAG termination.

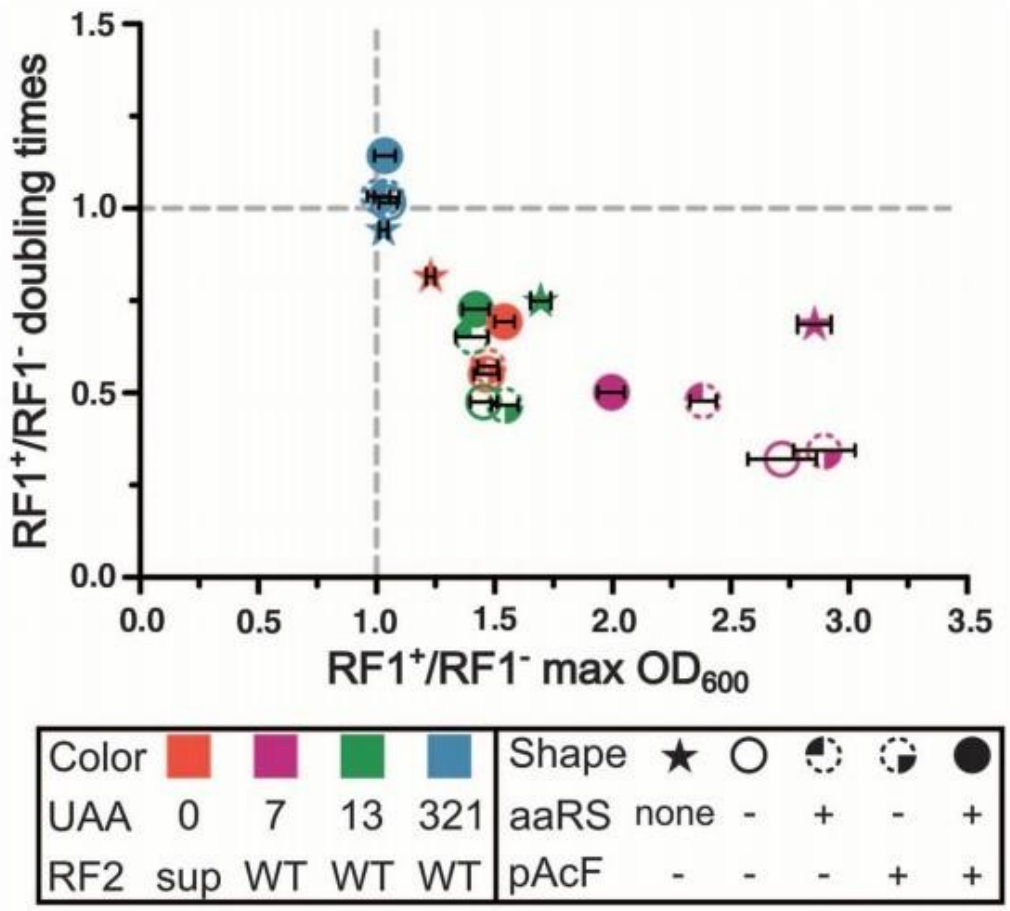
stop¶, weak UAG termination from RF2 variant.

sup, strong selection for UAG suppressor mutations.

nc, weak near-cognate suppression in the absence of all other UAG translation function.

Experiment

Fig. 2. Effects of UAG reassignment at natural UAG codons.



horizontal axis: Ratios of maximum cell densities
Vertical axis: Doubling times

@ Strains that do not rely on RF1 are expected to have a RF1+/RF1– ratio at (1, 1).

@ RF1– strains exhibitings lower growth are below the horizontal gray line,

@ RF1– strains exhibiting lower maximum cell density are to the right of the vertical gray line.

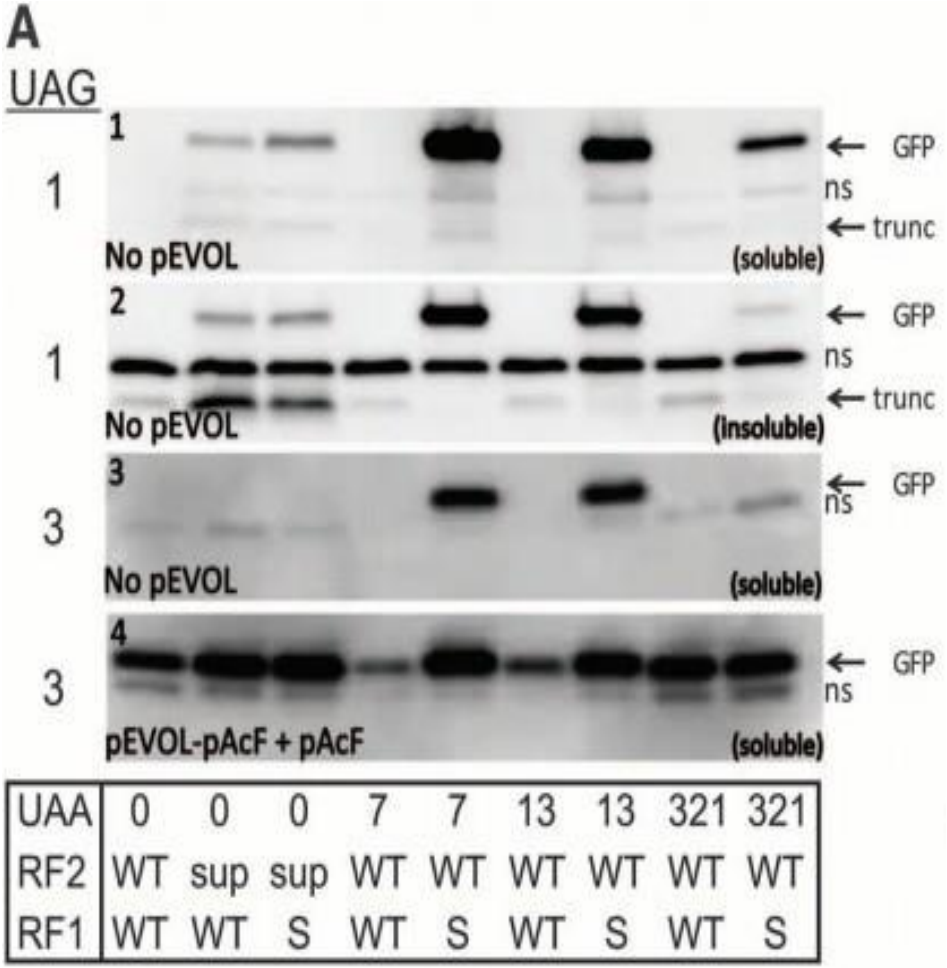
Results

Fig. 2. Effects of UAG reassignment at natural UAG codons.

- Together, these results indicate that only the complete removal of all instances of the UAG codon overcomes these deleterious effects; therefore, it may be the only scalable strategy for sustained NSAA translation and for complet reassignment of additional codons.

Experiment

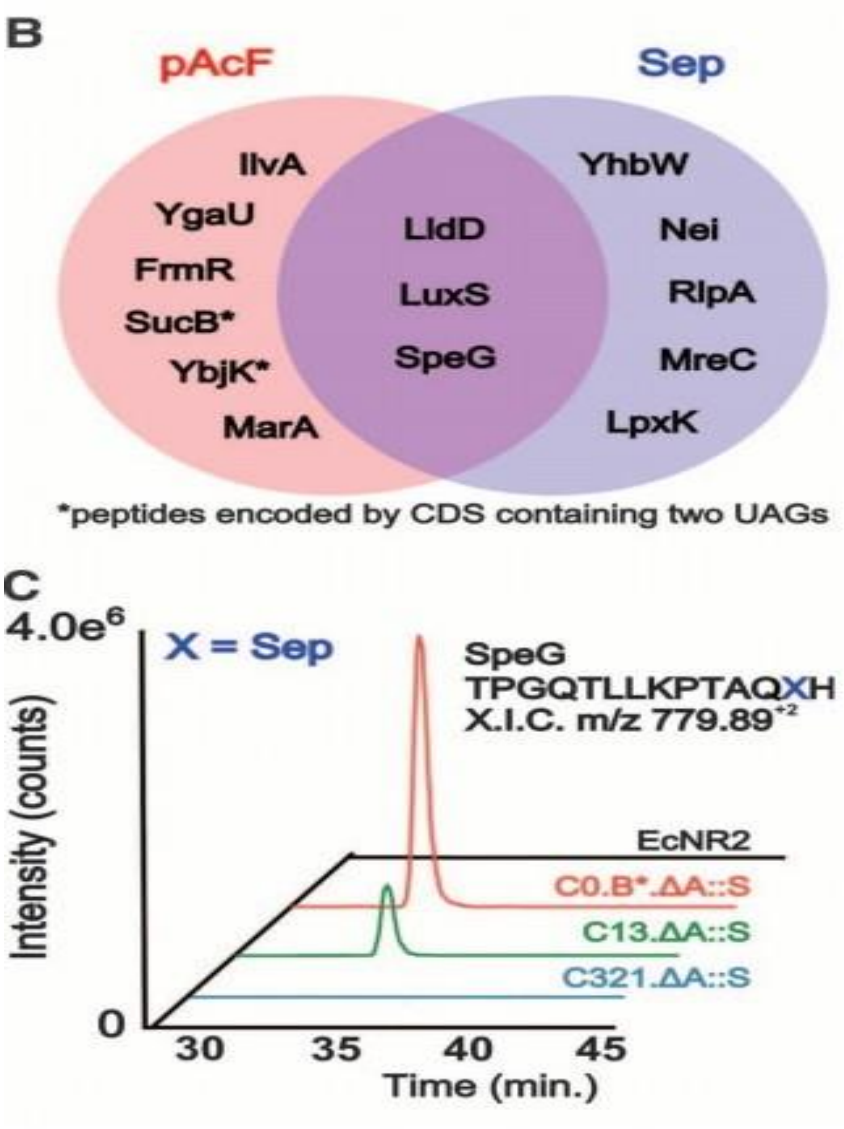
Fig. 3. NSAA incorporation in GROs.



Western blots demonstrate that :
In the presence of NSAAs, the RF1+ strains efficiently read through variants containing three UAGs, demonstrating that the episomal pEVOL translation system(which expresses an aaRS and tRNA that incorporate a NSAA at UAG codons)is extremely active and strongly outcompetes RF1.

Experiment

Fig. 3. NSAA incorporation in GROs.



@Venn diagram representing NSAA-containing peptides detected by mass spectrometry (质谱分析法) in C0.B* .△A::S.

@No Sep-containing peptides were observed for EcNR2, illustrating that RF1 removal is necessary for NSAA incorporation by the episomal phosphoserine system.

@By contrast, we observed NSAA-containing peptides in unrecoded(C0.B*. △ A::S) and partially recoded (C13. △ A : : S) strains, and not the GRO (C321. △ A::S)

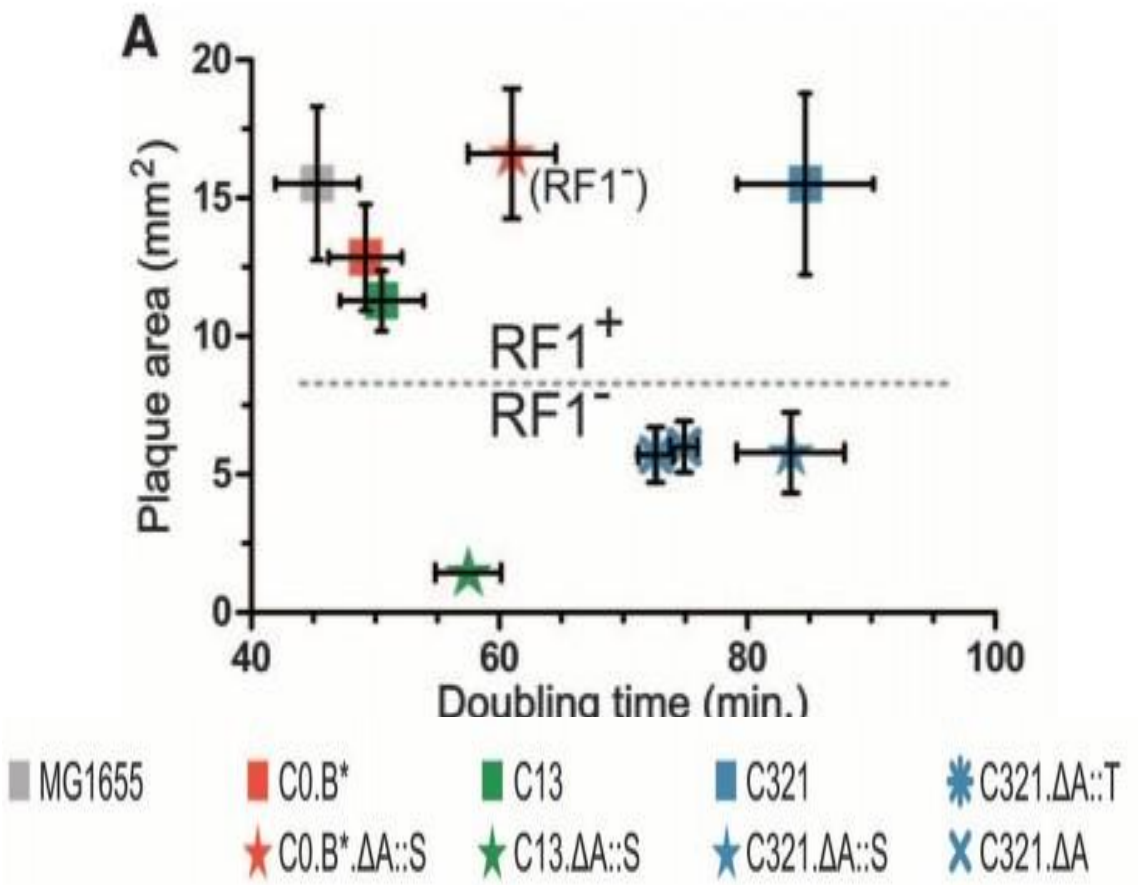
Results

Incorporation of nonstandard amino acids

- Such undesired incorporation of NSAAs (or natural amino acids) likely underlies the fitness impairments observed f or C0.B*.△A::S, C7.△A::S, and C13.△A::S. In contrast to the other RF1– strains, C321.△A::S demonstrated equivalent fitness to its RF1+ precursor (Fig. 2) and efficiently expressed all GFP variants without incorporating NSAAs at unintended sites (Figs. 2 and 3).
- Therefore, complete UAG removal is the only strategy that provides a devoted codon for plug-and-play NSAA incorporation without impairing fitness (Figs. 2 and 3).

Experiment

Fig. 4. Bacteriophage T7 infection is attenuated in GROs lacking RF1.

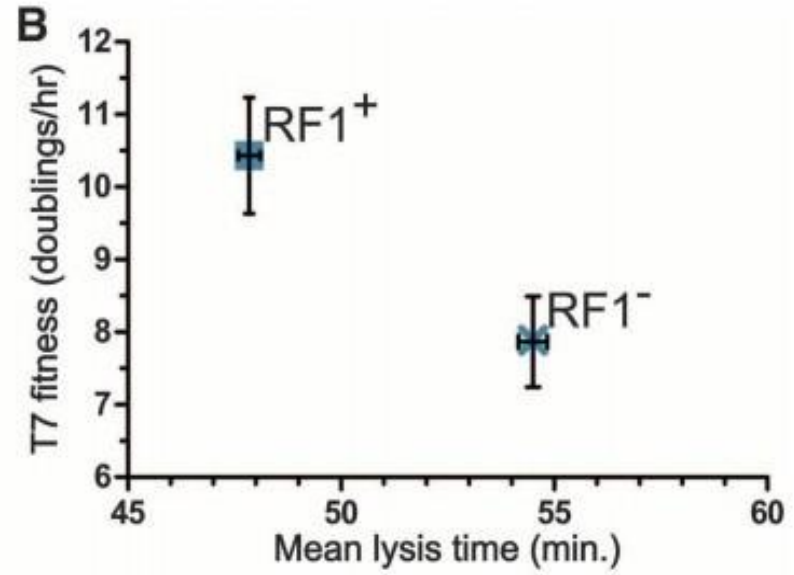


@RF1– hosts produced significantly smaller T7 plaques.

@The only exception was C 0.B *.△A::S, which produced statistically equivalent plaque sizes regardless of whether RF1 was present . Consistent with the observation that the modified RF2 variant could weakly terminate UAG, our results suggest that C0.B*.△A : :S terminates UAG codons well enough to support normal T7 infection.

Experiment

Fig. 4. Bacteriophage T7 infection is attenuated in GROs lacking RF1.



T7 infection is inhibited in RF1– hosts by comparing T7 fitness and lysis time in C321 versus C321.△A

4 Conclusions

Using multiplex genome editing, we removed all instances of the UAG codon and reassigned its function in the genome of a living cell.

- The resulting GRO possesses a devoted UAG sense codon for robust NSAA incorporation that is suitable for industrial protein production.
- GROs also establish the basis for genetic isolation and virus resistance