Developments in synthetic biology

The old and the new in the reprogramming of the genetic code

Technical Journal Club 16/02/2021

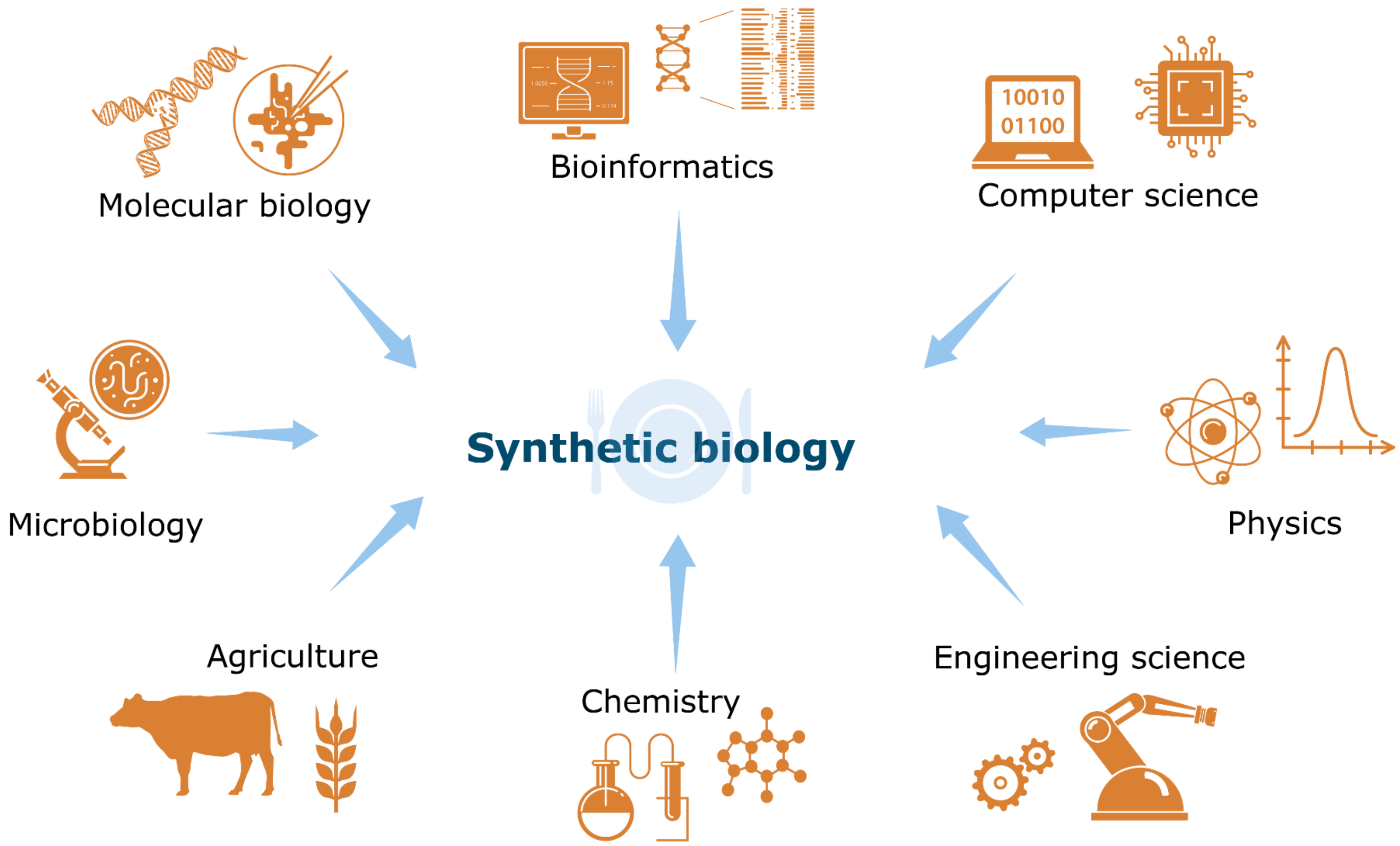
Stefano Sellitto

USZ Universitäts Spital Zürich

University of Zurich UZH

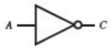
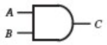
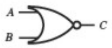
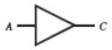
Synthetic biology: an old and new field

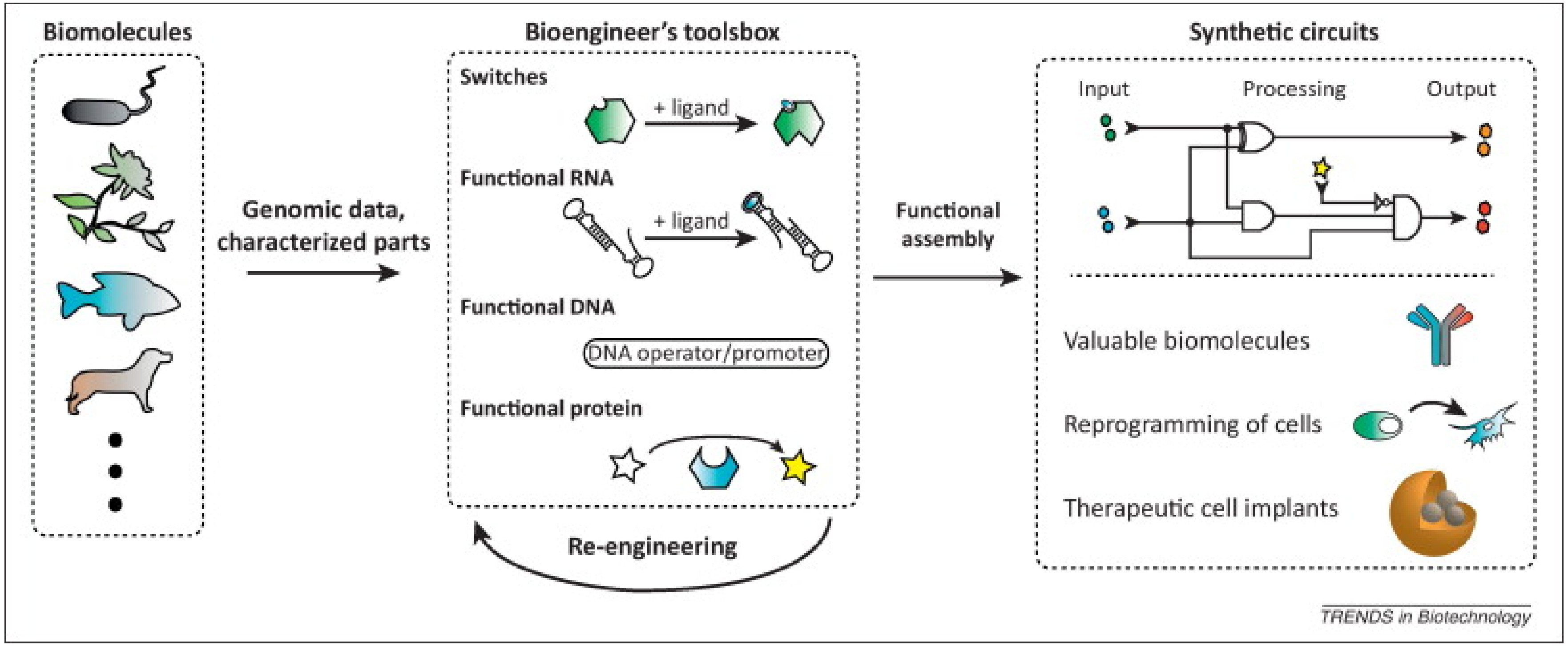
Synthetic biology is a multidiscplinar area of research



https://www.wur.nl/en/show-longread/Synthetic-biology-longread.htm

Synthetic biology creates new biological circuits

Gate	Operation	Symbol	Expression	Truth Table
Inverter (INV,NOT)	Invert signal (complement)		$C = \overline{A}$	A C 0 1 1 0
AND gate	AND logic		$C = A \cdot B$	A B C 0 0 0 0 1 0 1 0 0 1 1 1
NAND gate	Inverted AND logic		$C = \overline{A \cdot B}$	A B C 0 0 1 0 1 1 1 0 1 1 1 0
OR gate	OR logic		$C = A + B$	A B C 0 0 0 0 1 1 1 0 1 1 1 1
NOR gate	Inverted OR logic		$C = \overline{A + B}$	A B C 0 0 1 0 1 0 1 0 0 1 1 0
XOR gate	Exclusive OR logic		$C = A \oplus B$ $= A \cdot \overline{B} + \overline{A} \cdot B$	A B C 0 0 0 0 1 1 1 0 1 1 1 0
Buffer	Increase output signal current		$C = A$	A C 0 0 1 1

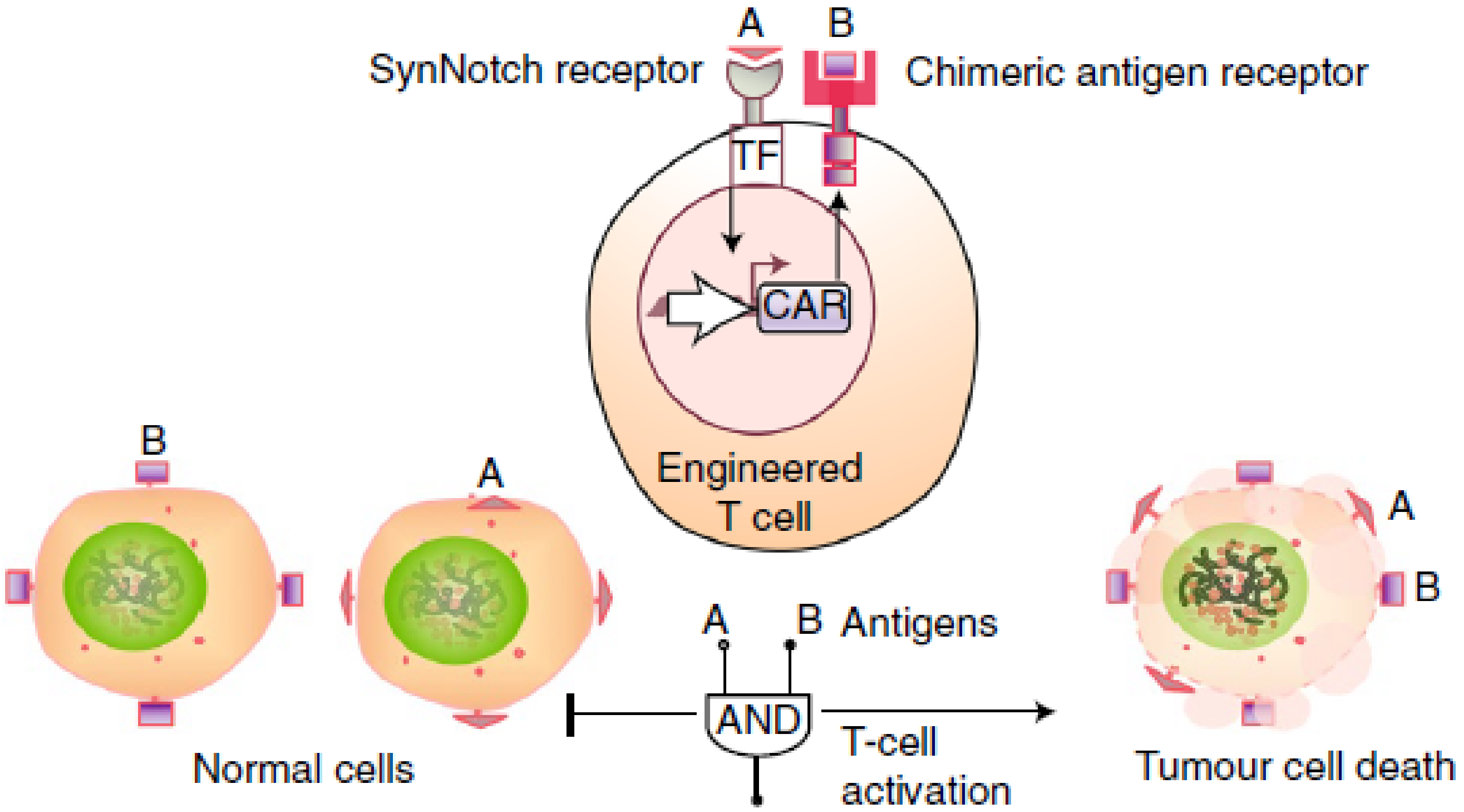


https://mechatronics.colostate.edu/files/tables/6-3.jpg

Ausländer and Fussenegger 2013

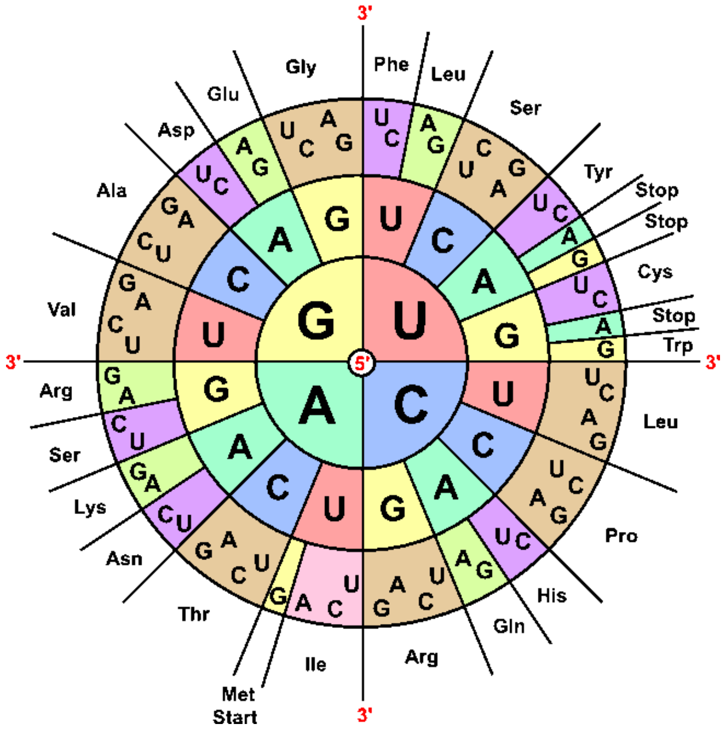
For example…

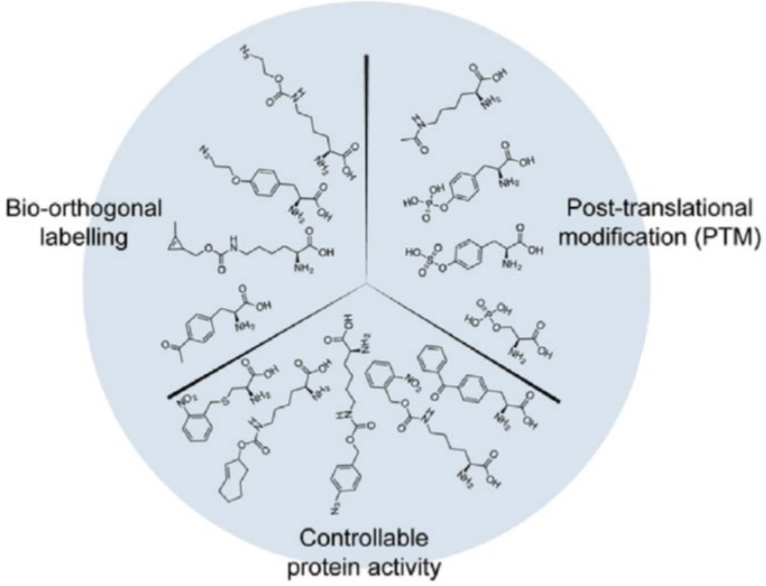
CAR-T is a well known example of synthetic gentic circuitry with therapeutic applications



Sedlmayer, Aubel, and Fussenegger 2018

Expansion of the genetic code





https://commons.wikimedia.org/wiki/File:Codons_aminoacids_table.png

Huang and Liu 2018

de la Torre and Chin 2020

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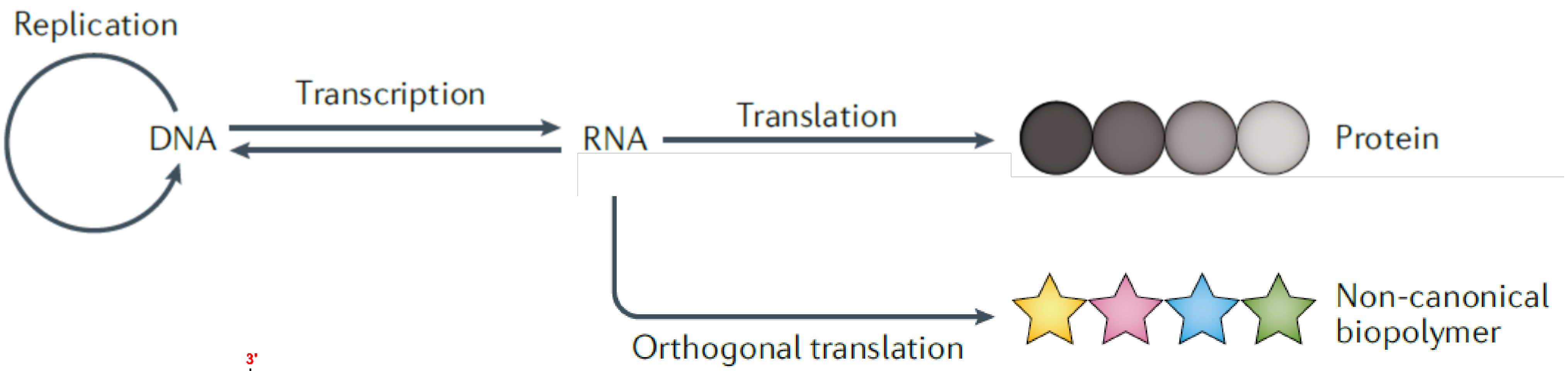

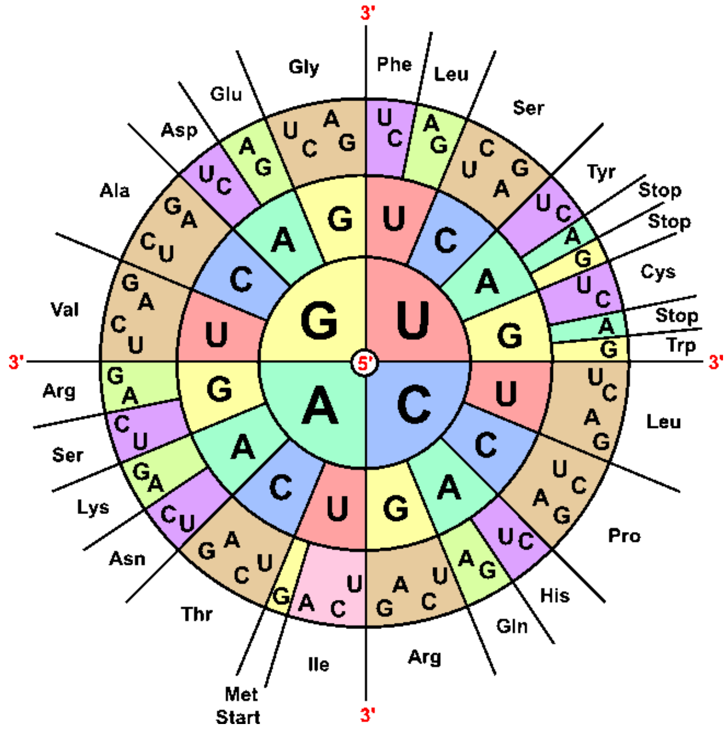

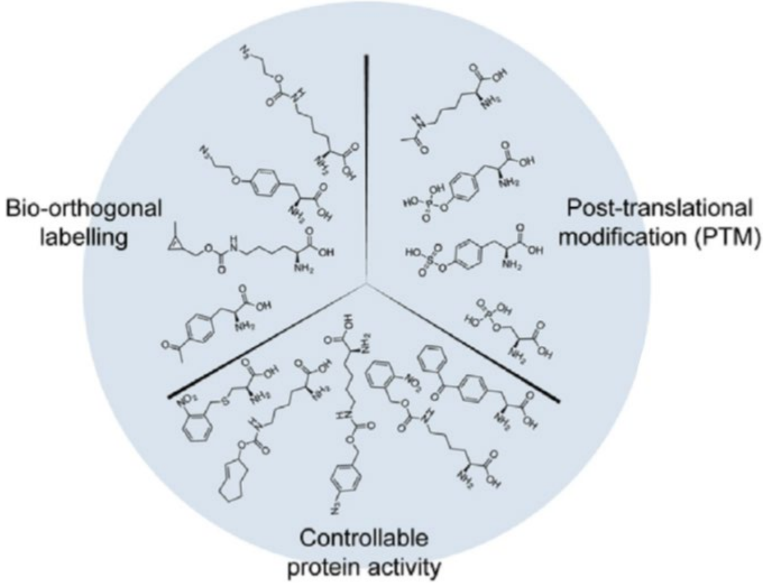

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Why incorporate non-canonical amino acids into a protein?

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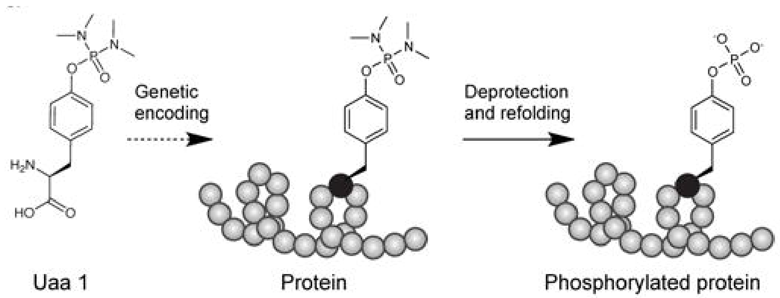

BRIEF COMMUNICATION
PUBLISHED ONLINE: 12 JUNE 2017 | DOI: 10.1038/NCHEMBIO.2406
nature chemical biology

Site-specific incorporation of phosphotyrosine using an expanded genetic code

Christian Hoppmann[1], Allison Wong[2], Bing Yang[1], Shuwei Li[3], Tony Hunter[4], Kevan M Shokat[2] & Lei Wang[1*]

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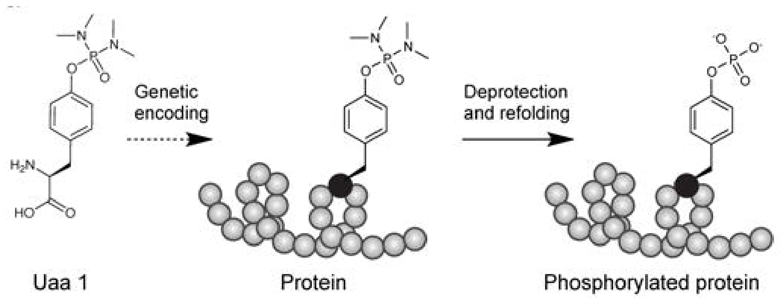

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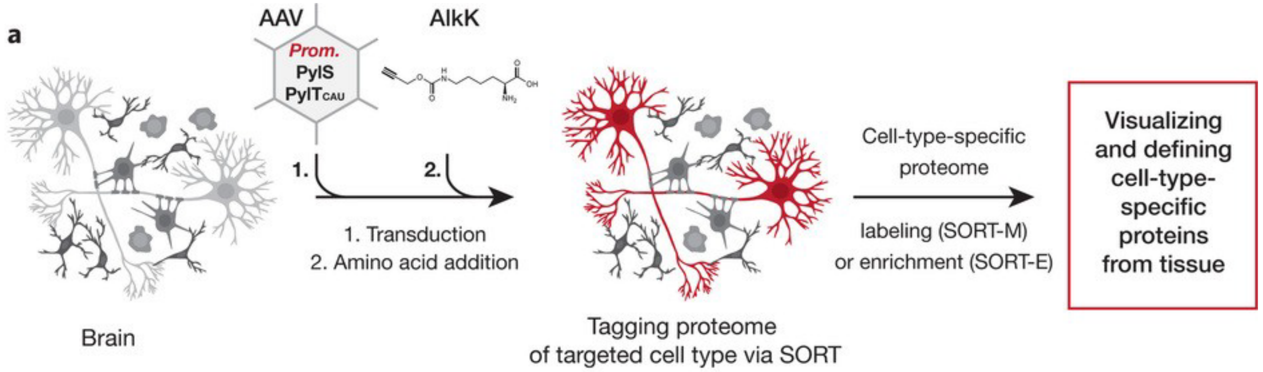

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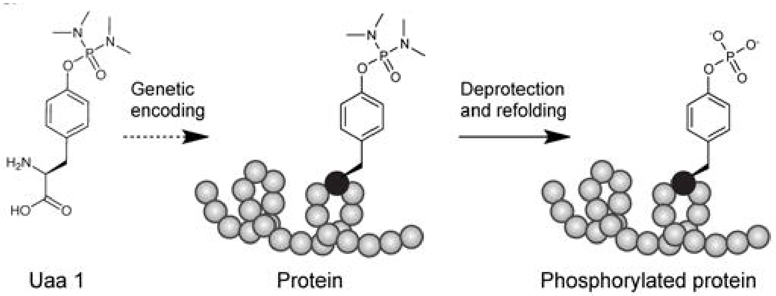

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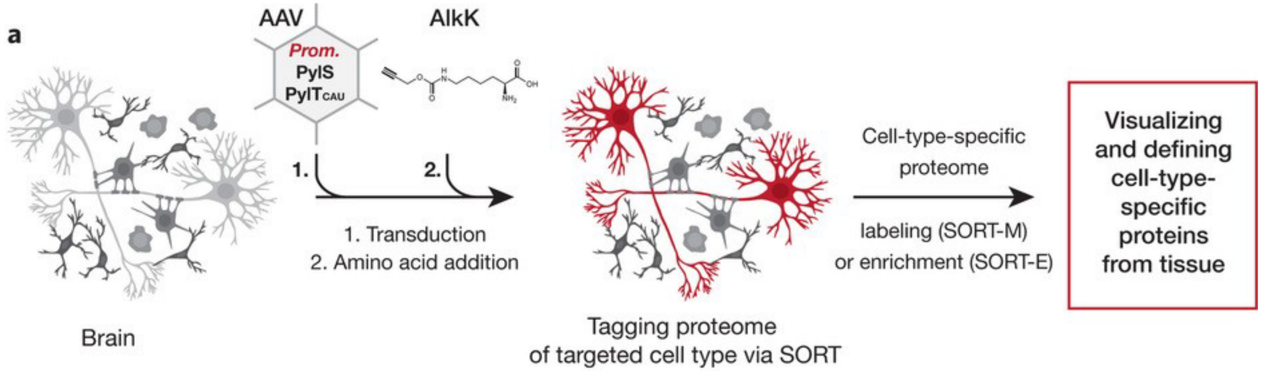

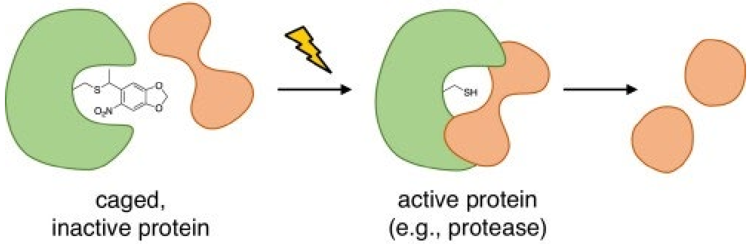

ELSEVIER
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Chemical Biology

Recent advances in the optical control of protein function through genetic code expansion

Taylor Courtney and Alexander Deiters

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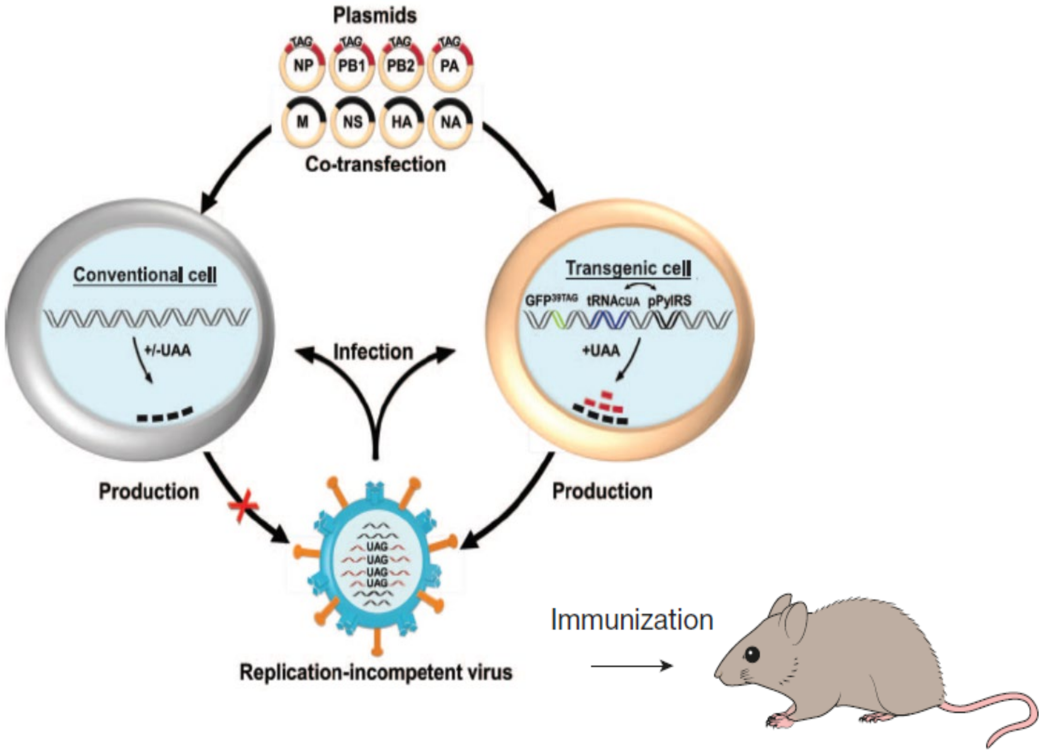

Angewandte Chemie

Vaccine Engineering

Construction of a Live-Attenuated HIV-1 Vaccine through Genetic Code Expansion**

Nanxi Wang, Yue Li, Wei Niu, Ming Sun, Ronald Cerny, Qingsheng Li, and Jiantao Guo**

RESEARCH | REPORTS

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Generation of influenza A viruses as live but replication-incompetent virus vaccines

Longlong Si,* Huan Xu,* Xueying Zhou, Ziwei Zhang, Zhenyu Tian, Yan Wang, Yiming Wu, Bo Zhang, Zhenlan Niu, Chuanling Zhang, Ge Fu, Sulong Xiao, Qing Xia, Lihe Zhang, Demin Zhou†

SCIENCE

Chin 2017

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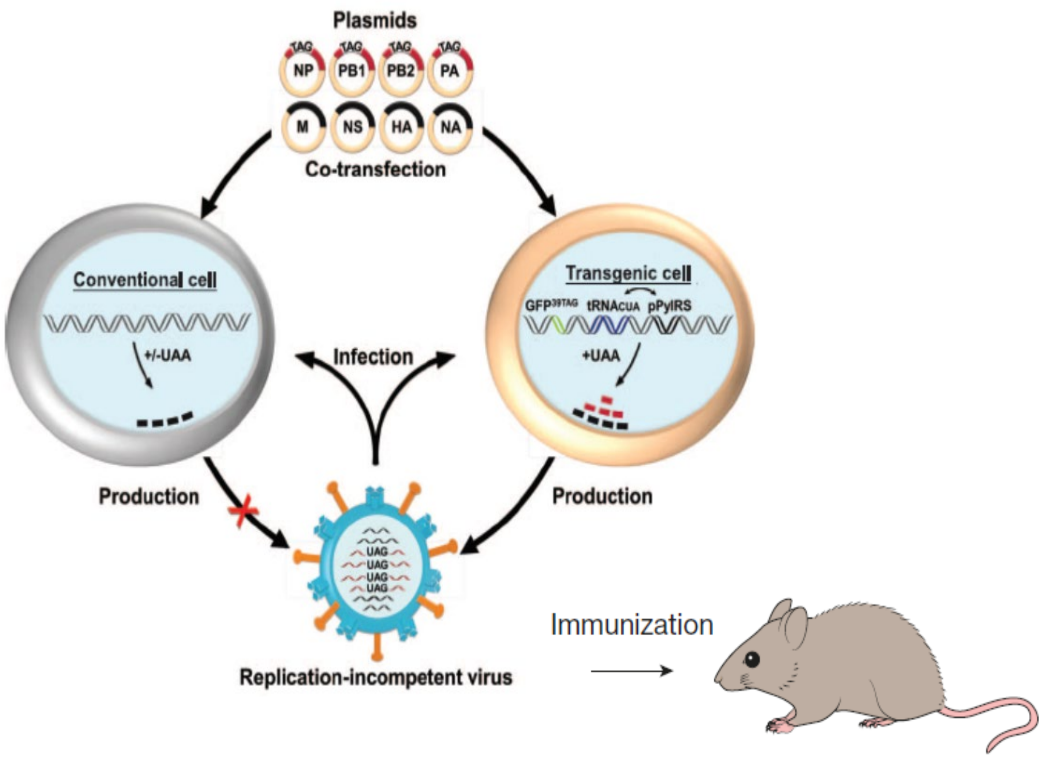

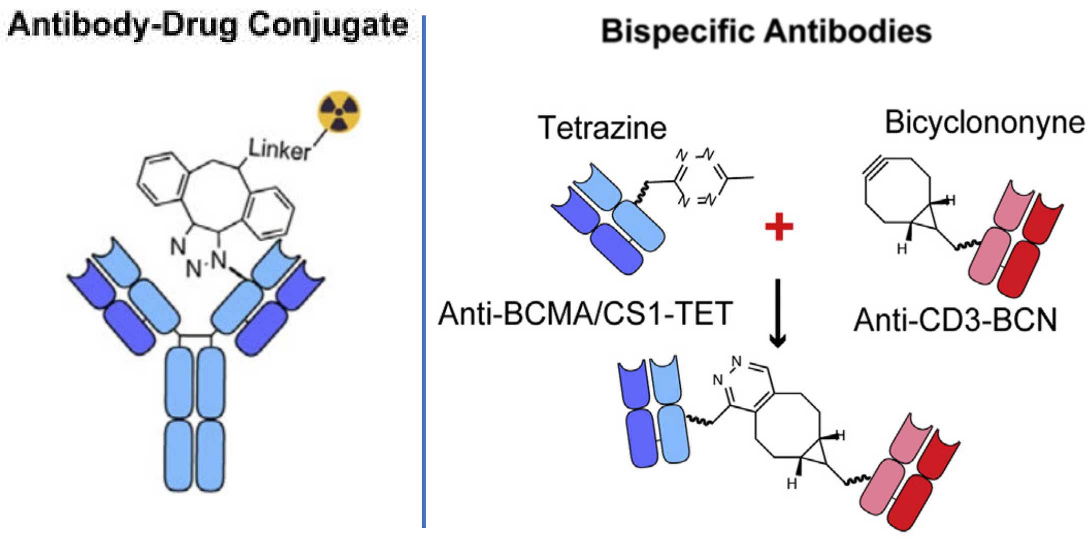

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SCIENCE

JACS — JOURNAL OF THE AMERICAN CHEMICAL SOCIETY — Communication — pubs.acs.org/JACS

An Anti-B Cell Maturation Antigen Bispecific Antibody for Multiple Myeloma

Nitya S. Ramadoss,† Andrew D. Schulman,† Sei-hyun Choi,‡ David T. Rodgers,† Stephanie A. Kazane,† Chan Hyuk Kim,† Brian R. Lawson,§ and Travis S. Young*,†

Bioconjugate Chemistry — Article — pubs.acs.org/bc

Synthesis of Site-Specific Radiolabeled Antibodies for Radioimmunotherapy via Genetic Code Expansion

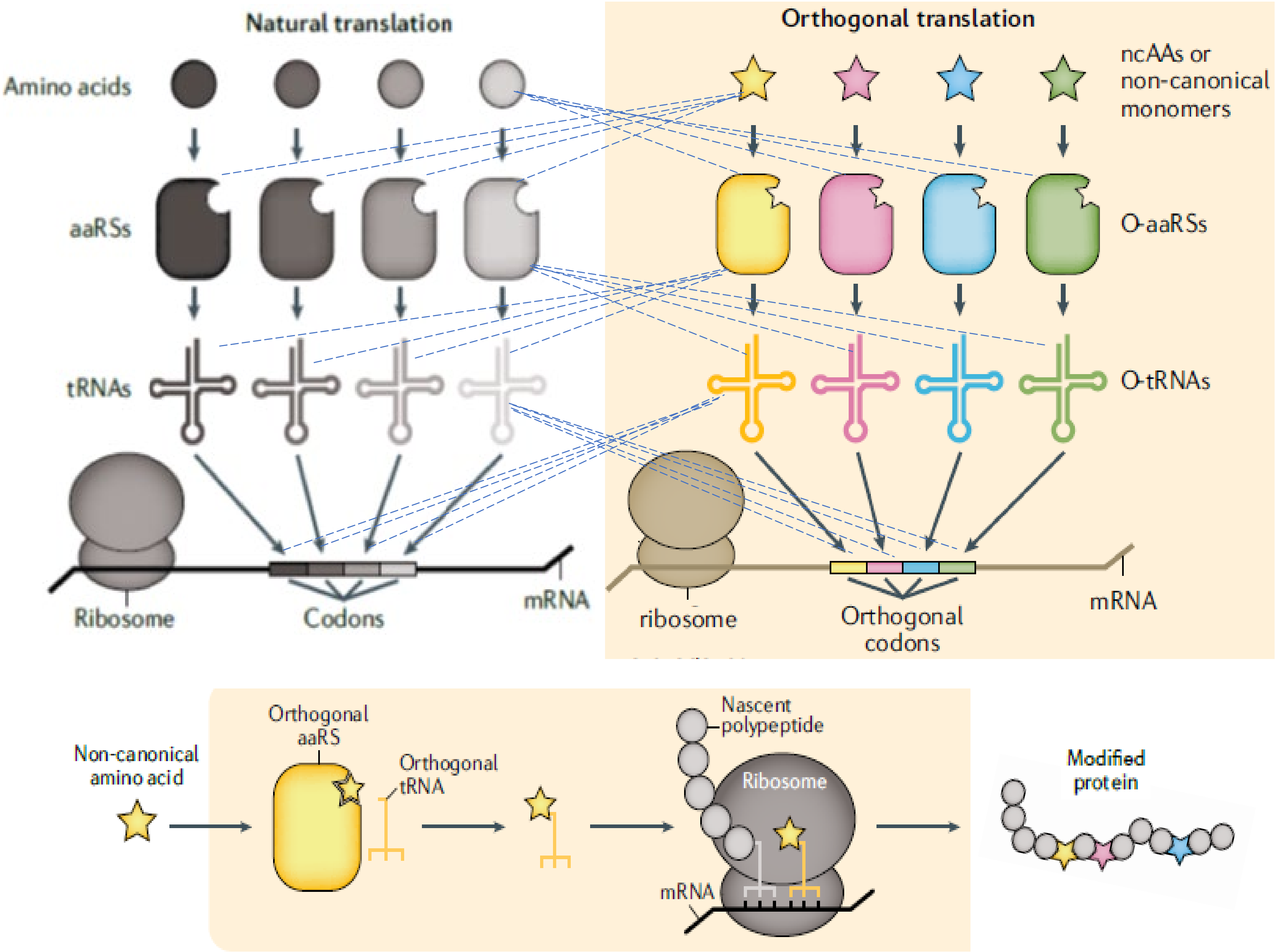
Yiming Wu,†,∥ Hua Zhu,‡,∥ Bo Zhang,§ Fei Liu,‡ Jingxian Chen,† Yufei Wang,† Yan Wang,† Ziwei Zhang,† Ling Wu,† Longlong Si,† Huan Xu,† Tianzhuo Yao,† Sulong Xiao,† Qing Xia,† Lihe Zhang,† Zhi Yang,*,‡ and Demin Zhou*,†

Chin 2017

Huang and Liu 2018

HOW TO REPROGRAM THE GENTIC CODE

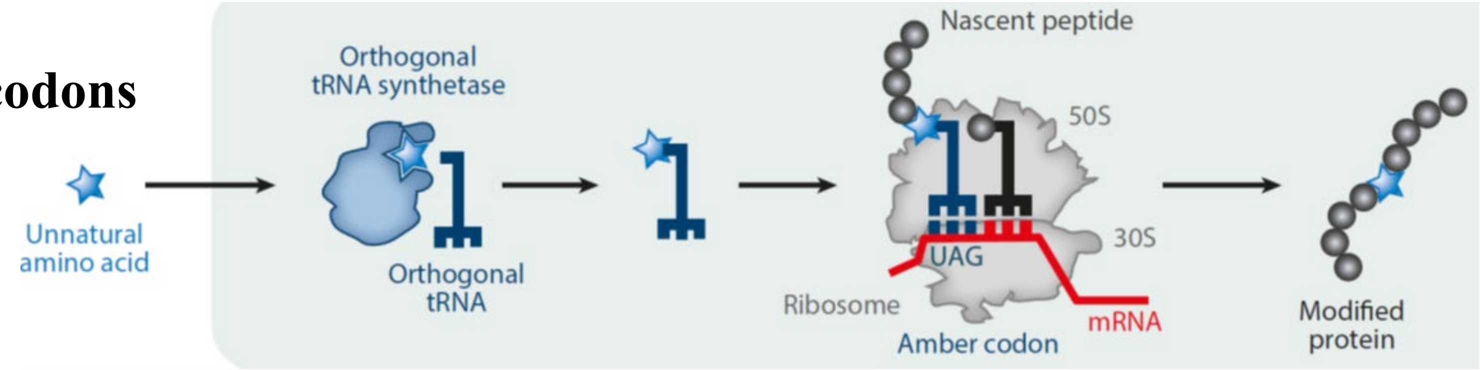
Selection and evolution of orthogonal components



de la Torre and Chin 2020

How to allocate codons for orthogonal translation ?

1. Recoding of stop codons



Chin 2014

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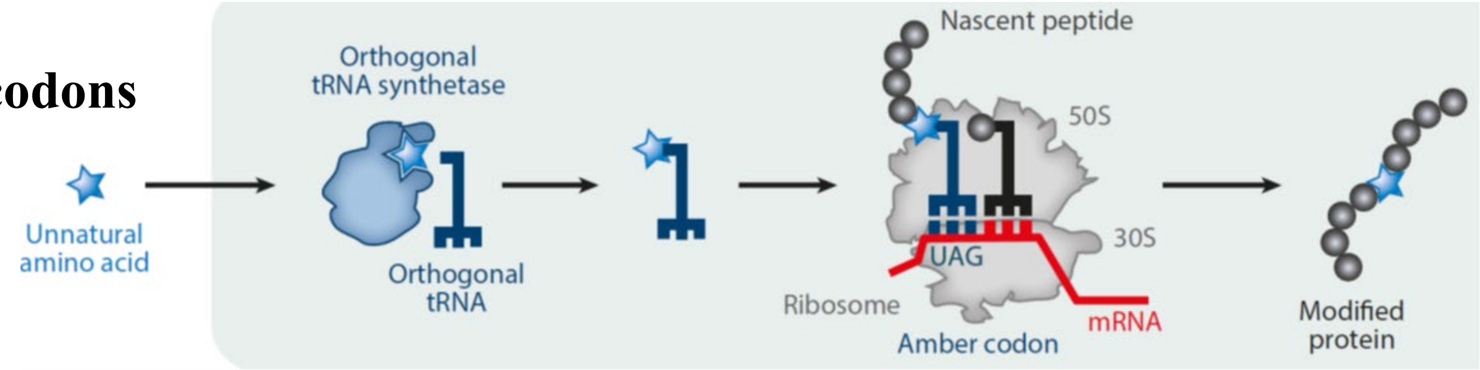

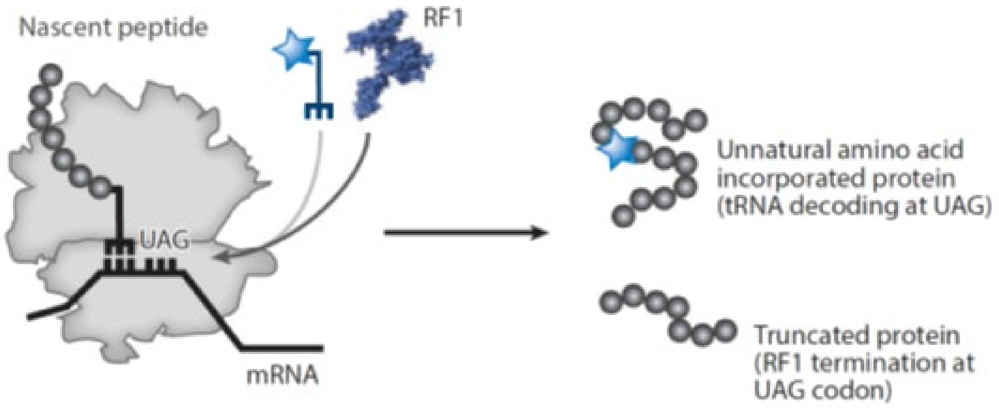

Not high efficiency !!

Chin 2014

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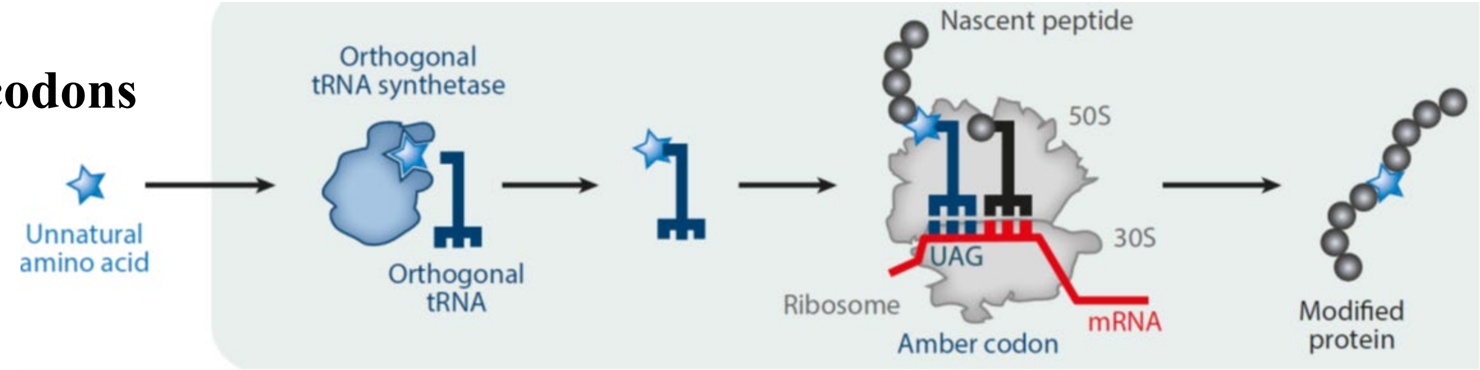

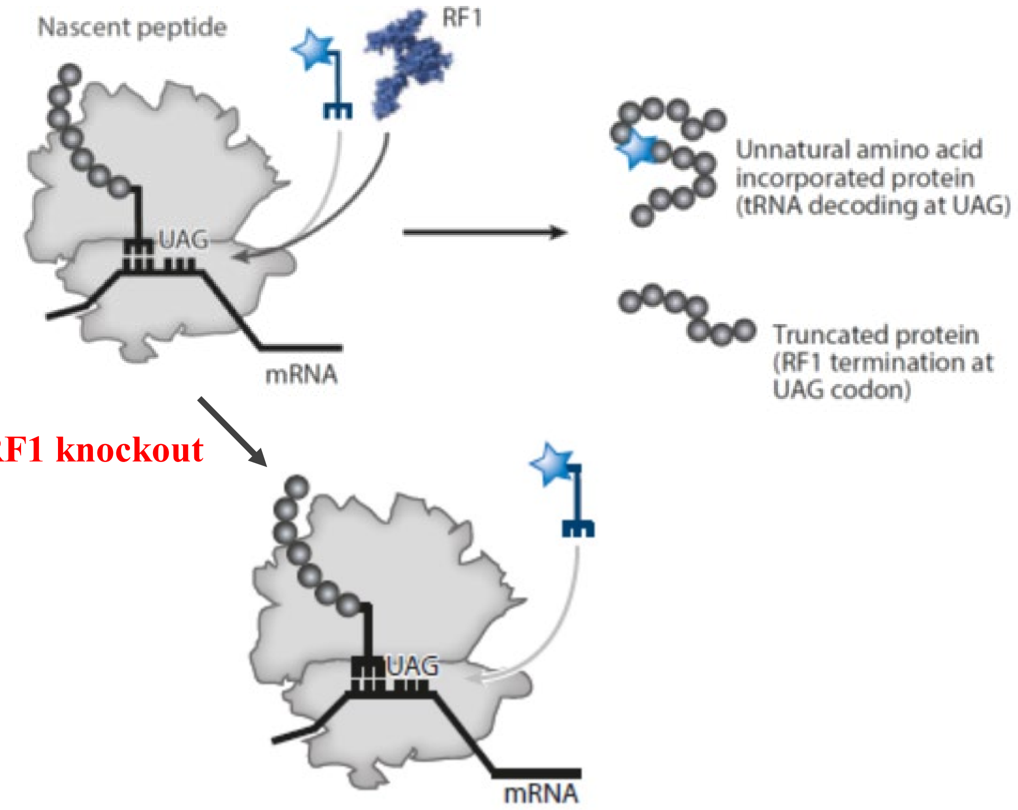

RF1 knockout

Not high efficiency !!

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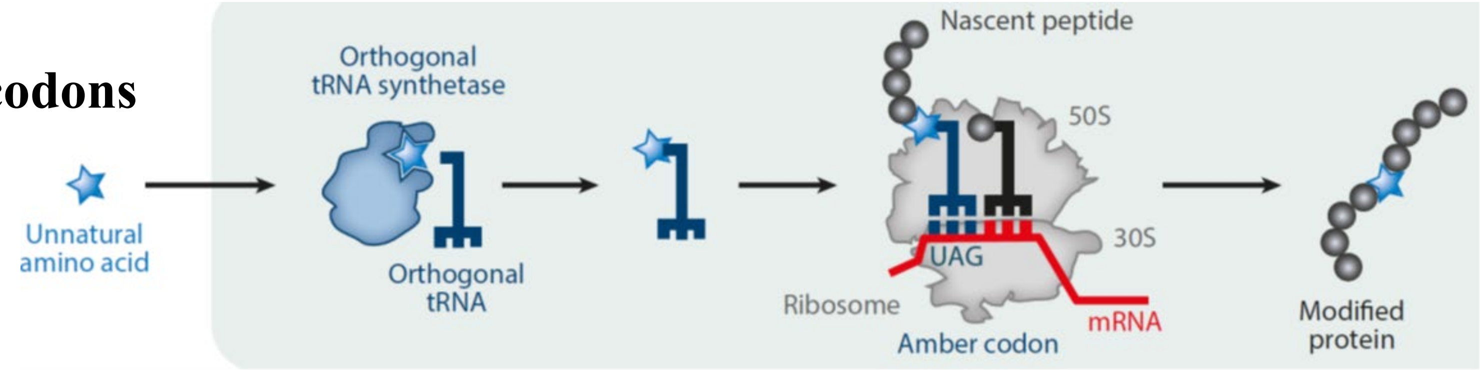

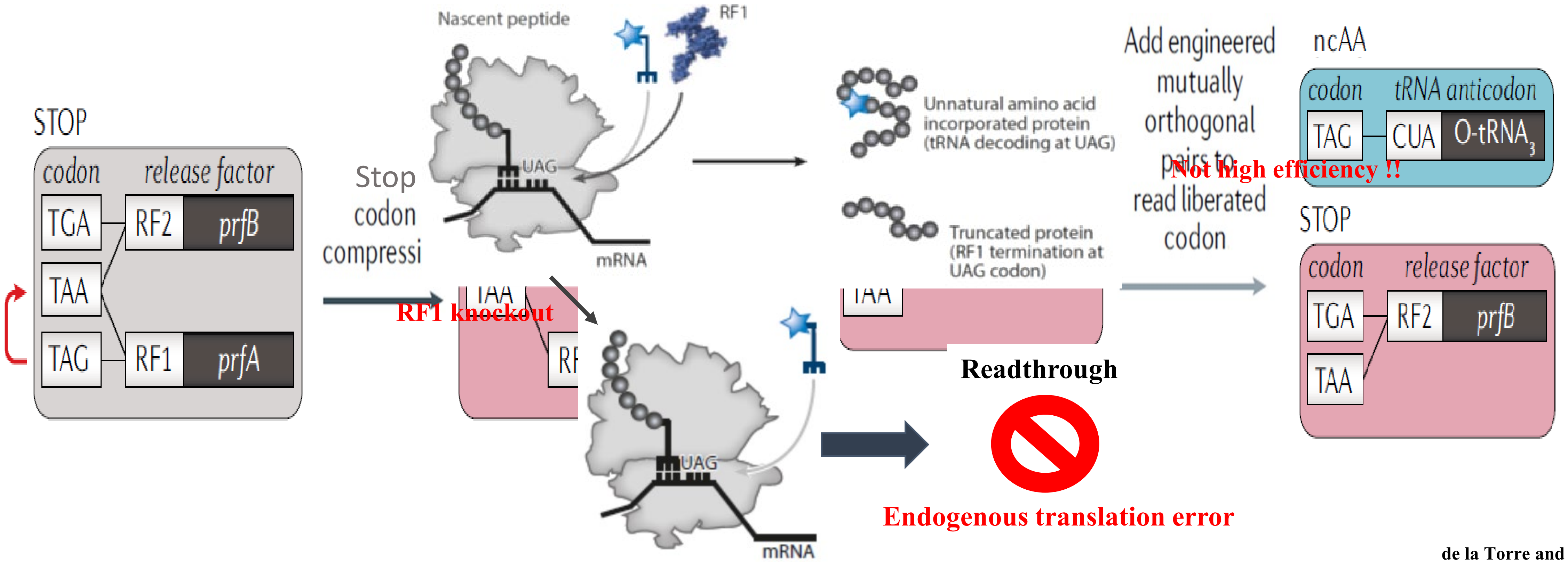

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de la Torre and Chin 2020

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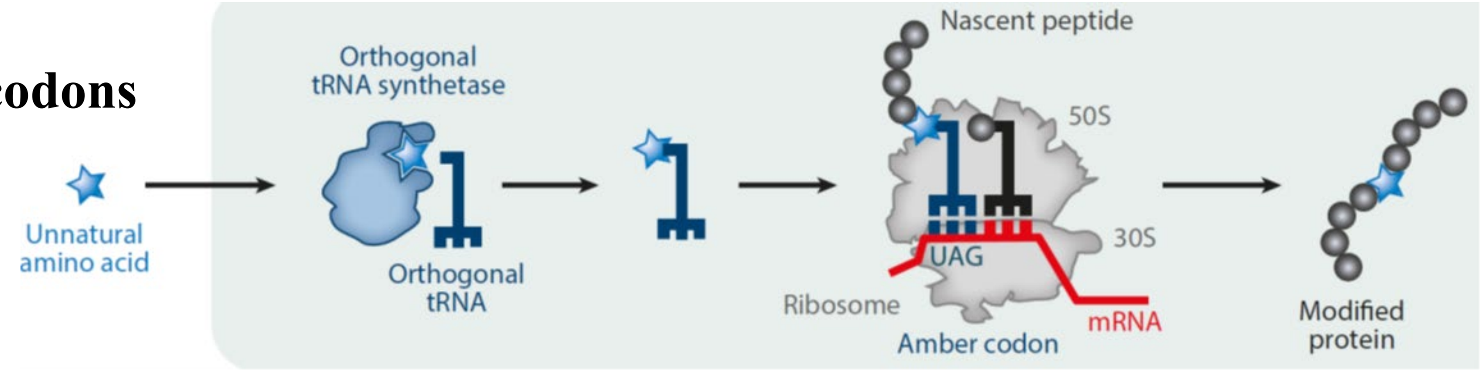
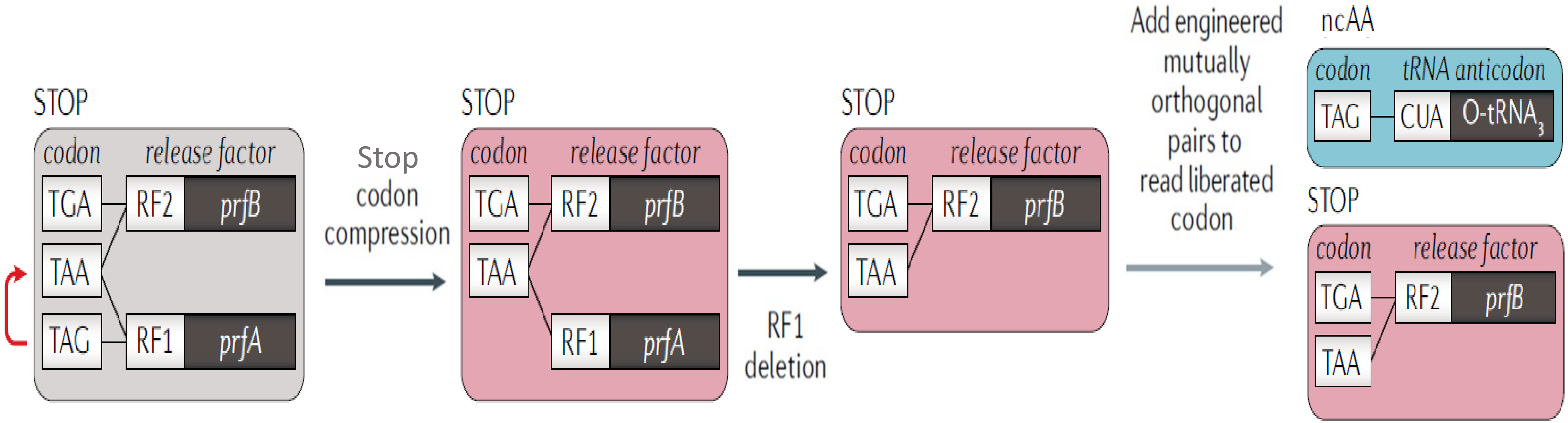


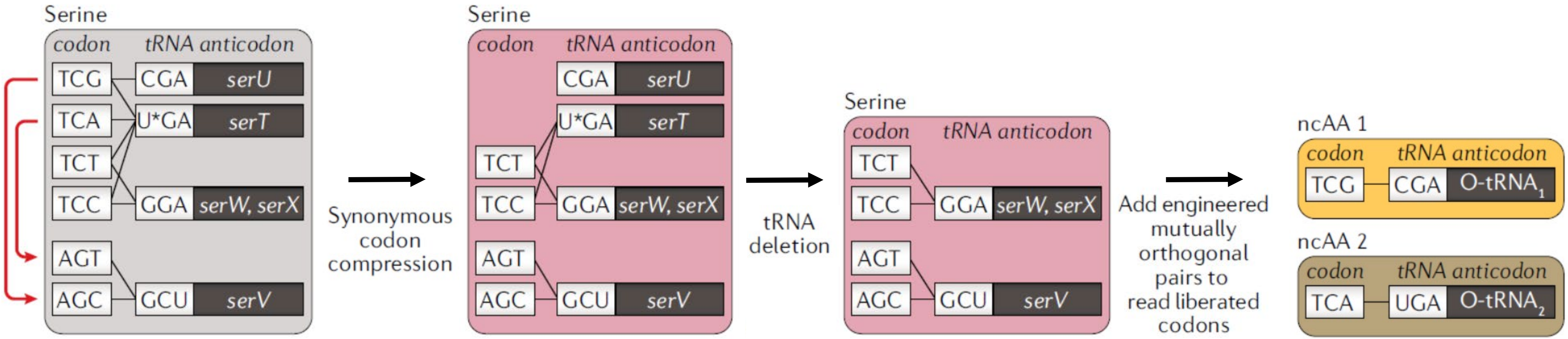


Genome complexity → not straightforward to transfer this approach to eukaryotes

de la Torre and Chin 2020

How to allocate codons for orthogonal translation ?

2. Recoding of synonymous codons (sense codon compression)



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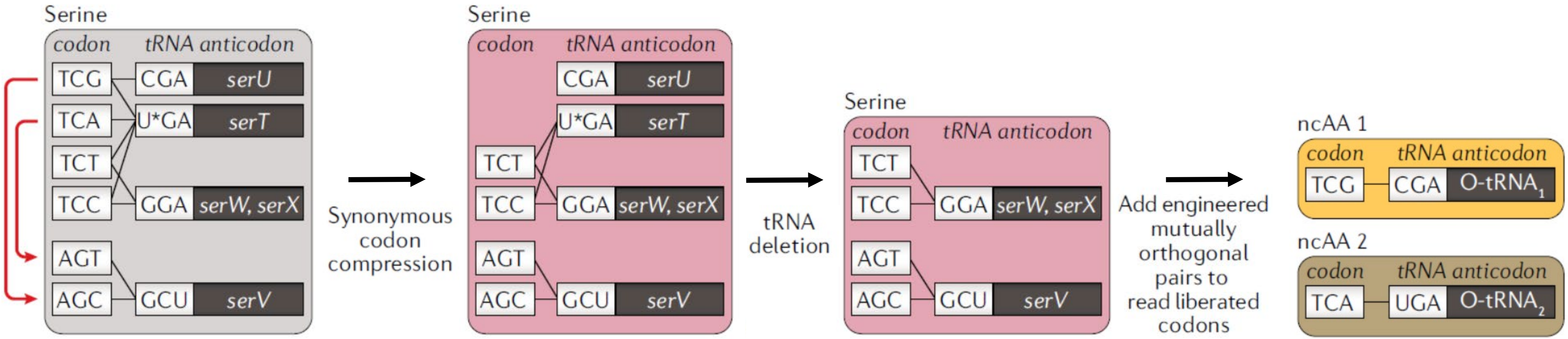

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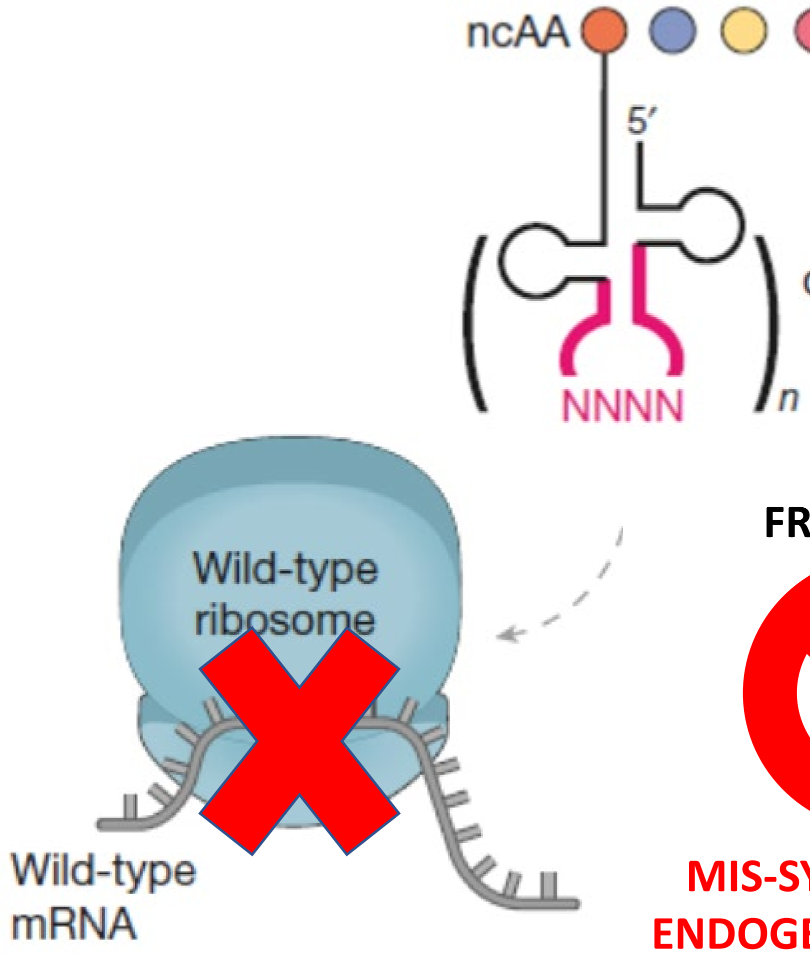
How to allocate codons for orthogonal translation ?

3. Decoding of quadruplet codons



NOT EFFICIENT DECODING

FRAMESHIFT

MIS-SYNTHESIS OF THE ENDOGENOUS PROTEOME

Chin 2017

Minimization of background misincorporation

Decoding a specific codon only for the RNA of the protein of interest and not in the entire genome

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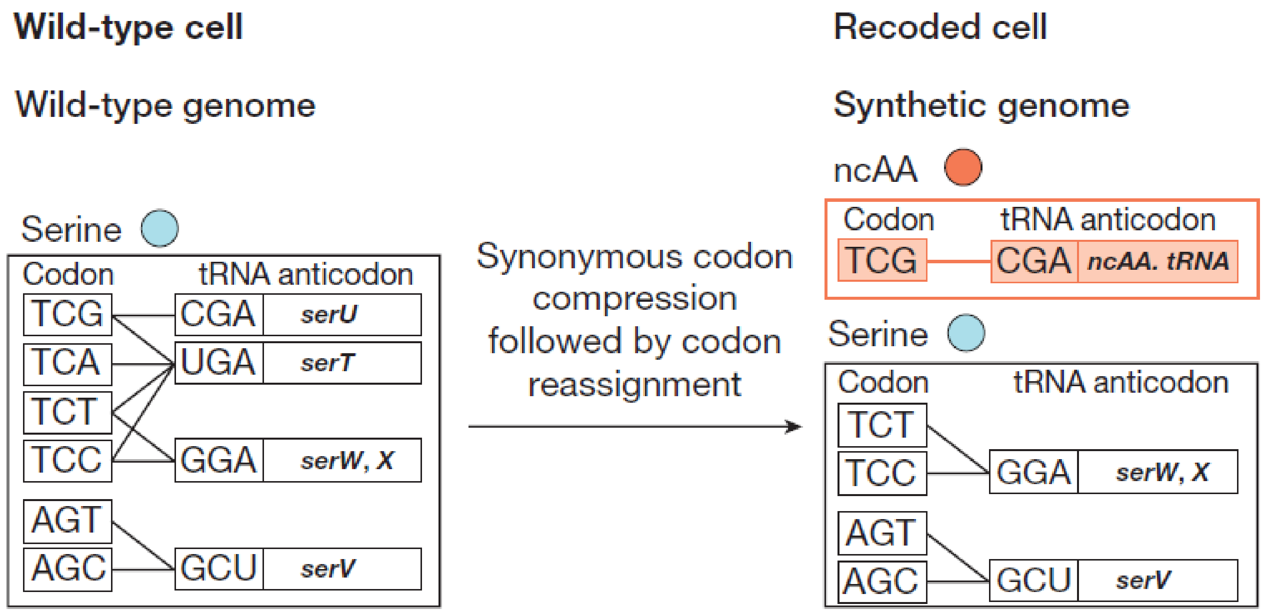

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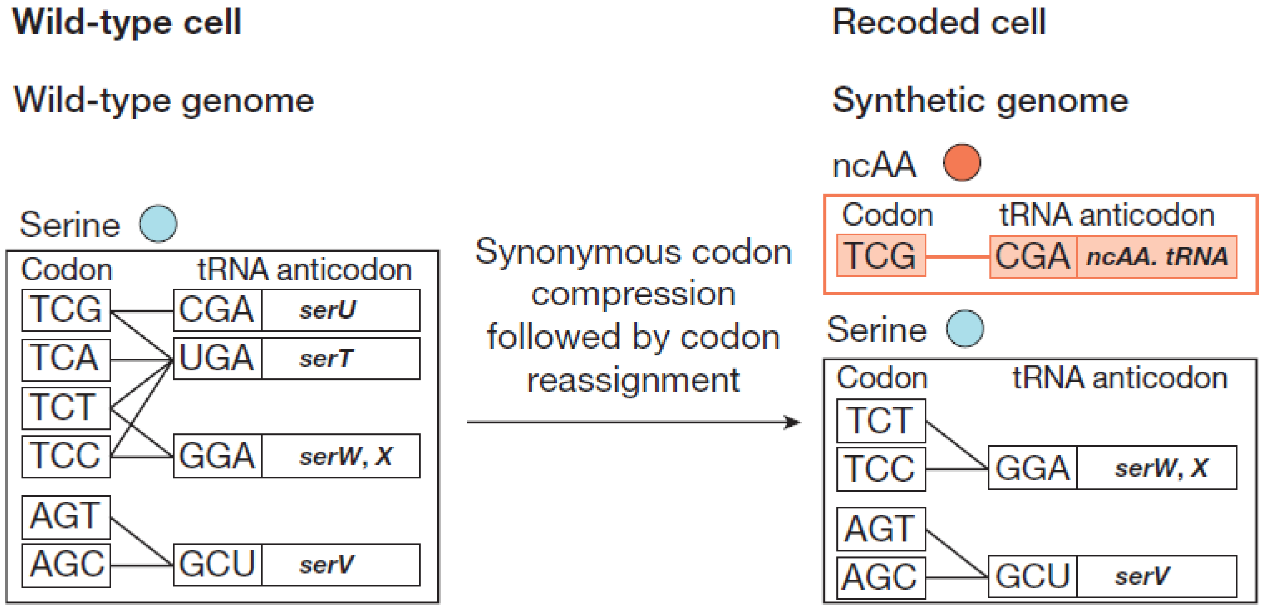

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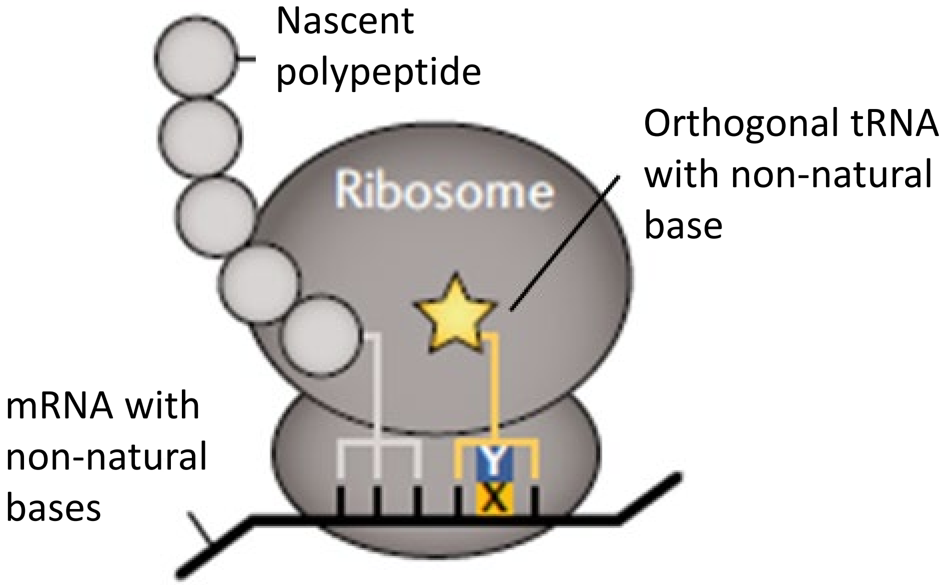

2. Decoding of non-natural base pairs

Chin 2017

de la Torre and Chin 2020

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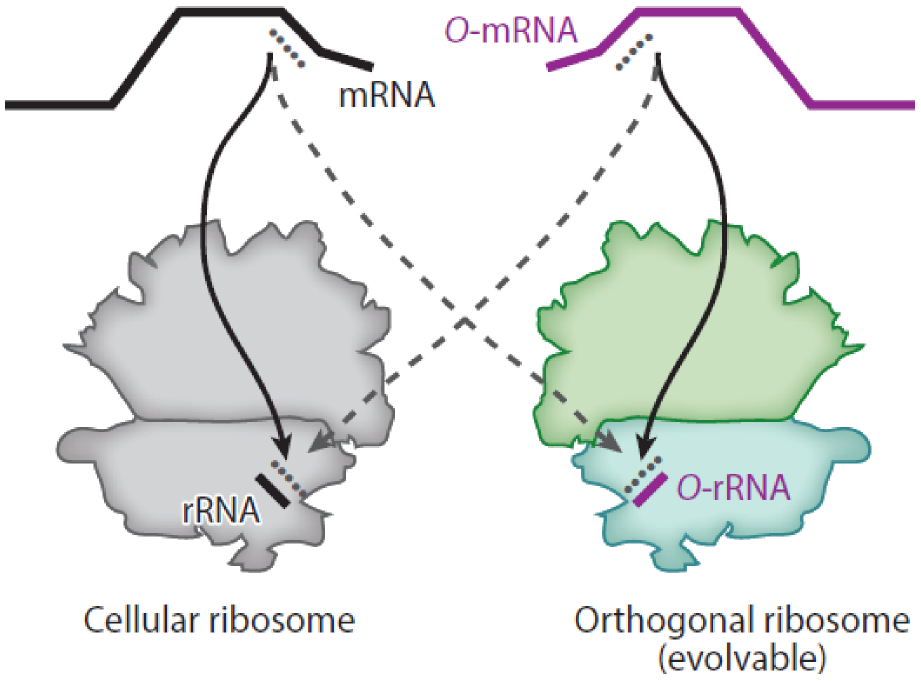

3. Decoding of orthogonal RNAs with orthogonal ribosomes

Chin 2014

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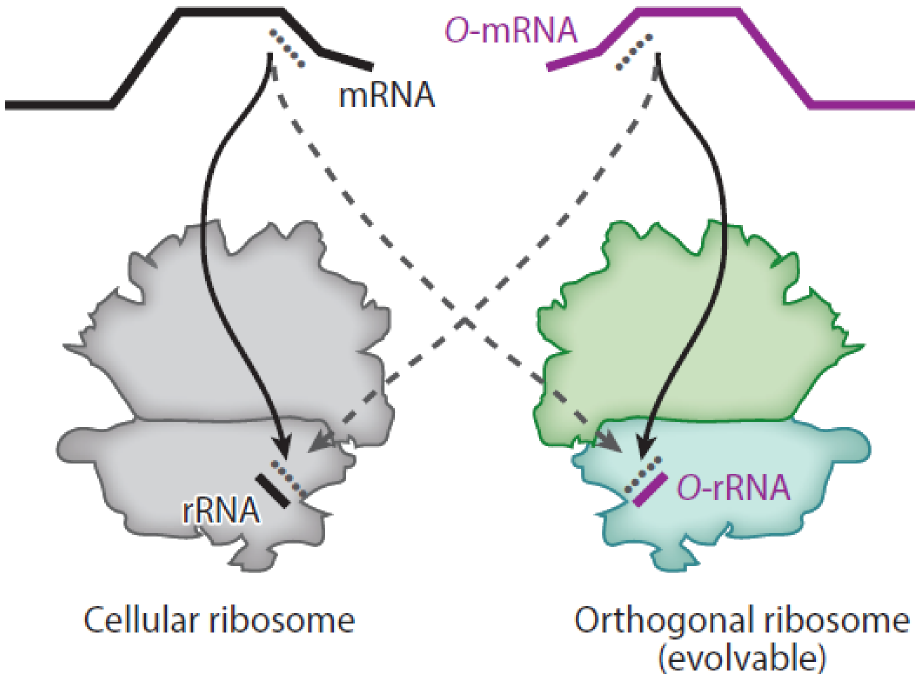

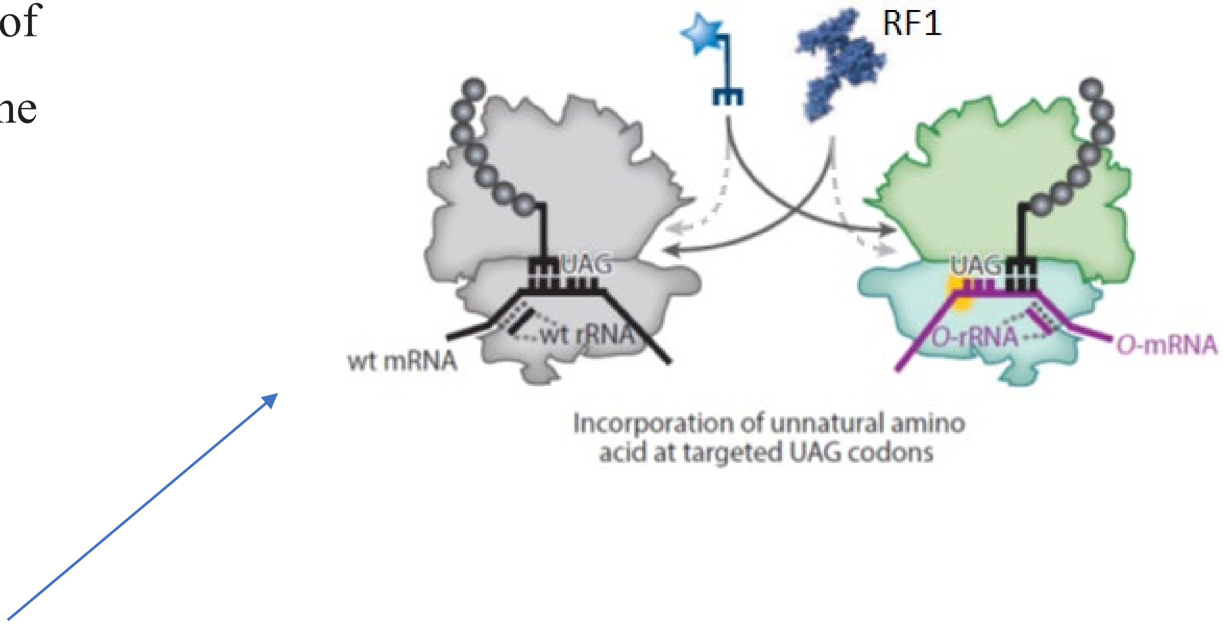

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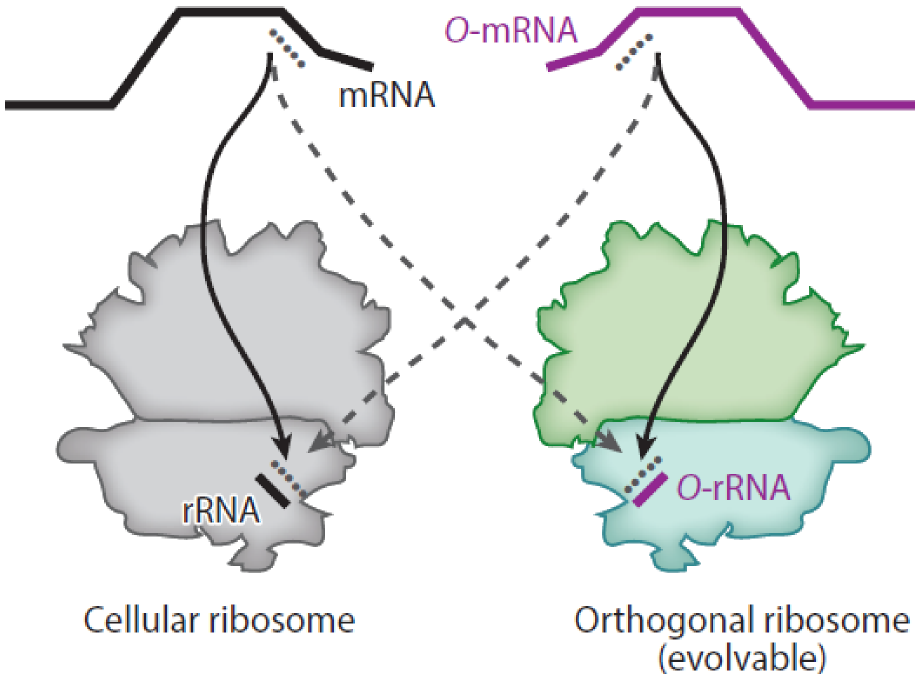

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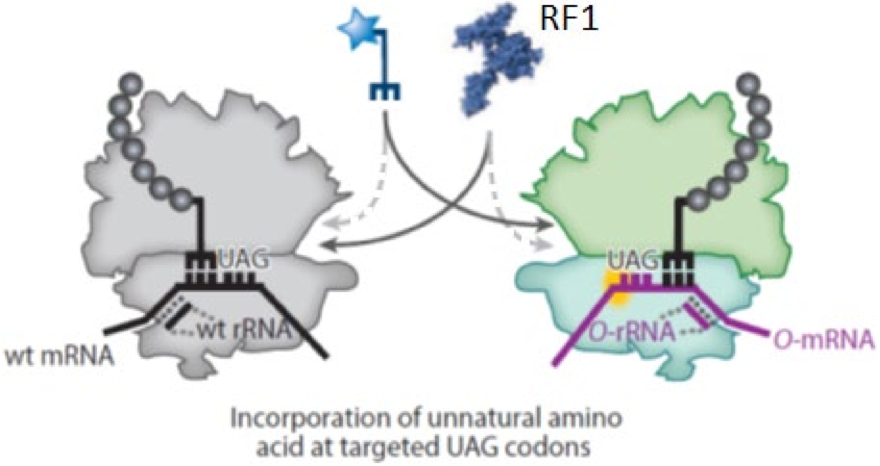

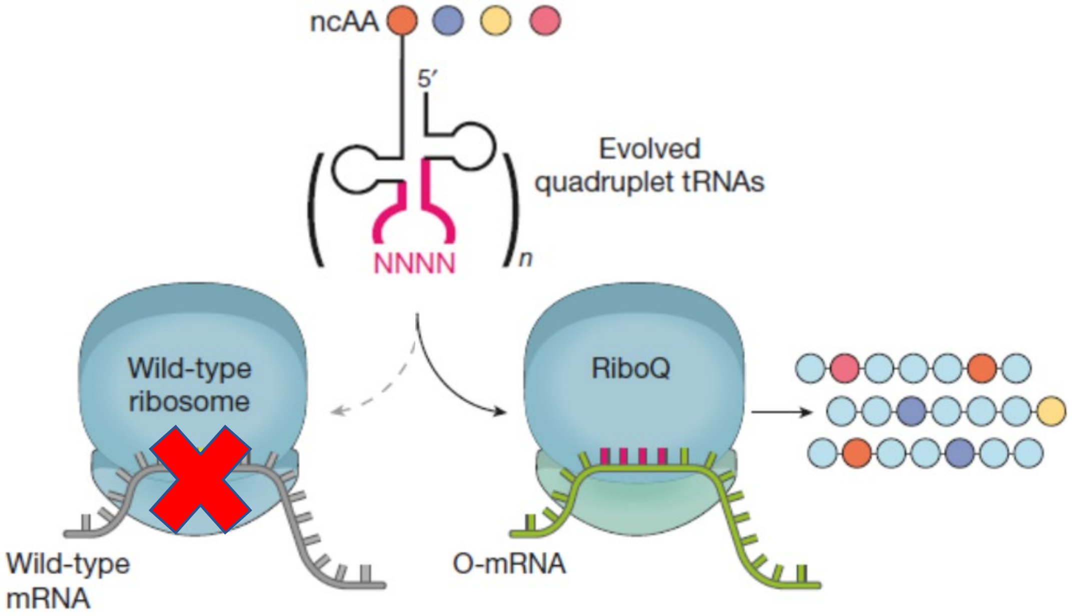

Chin 2017

LETTER

Controlling orthogonal ribosome subunit interactions enables evolution of new function

Wolfgang H. Schmied[1,4], Zakir Tnimov[1,4], Chayasith Uttamapinant[1,2,4], Christopher D. Rae[1], Stephen D. Fried[1,3] & Jason W. Chin[1]*

444 | NATURE | VOL 564 | 20/27 DECEMBER 2018

RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes

Christopher D. Reinkemeier[1,2,3]*, Gemma Estrada Girona[3]*, Edward A. Lemke[1,2,3]†

Science **363**, 1415 (2019) 29 March 2019

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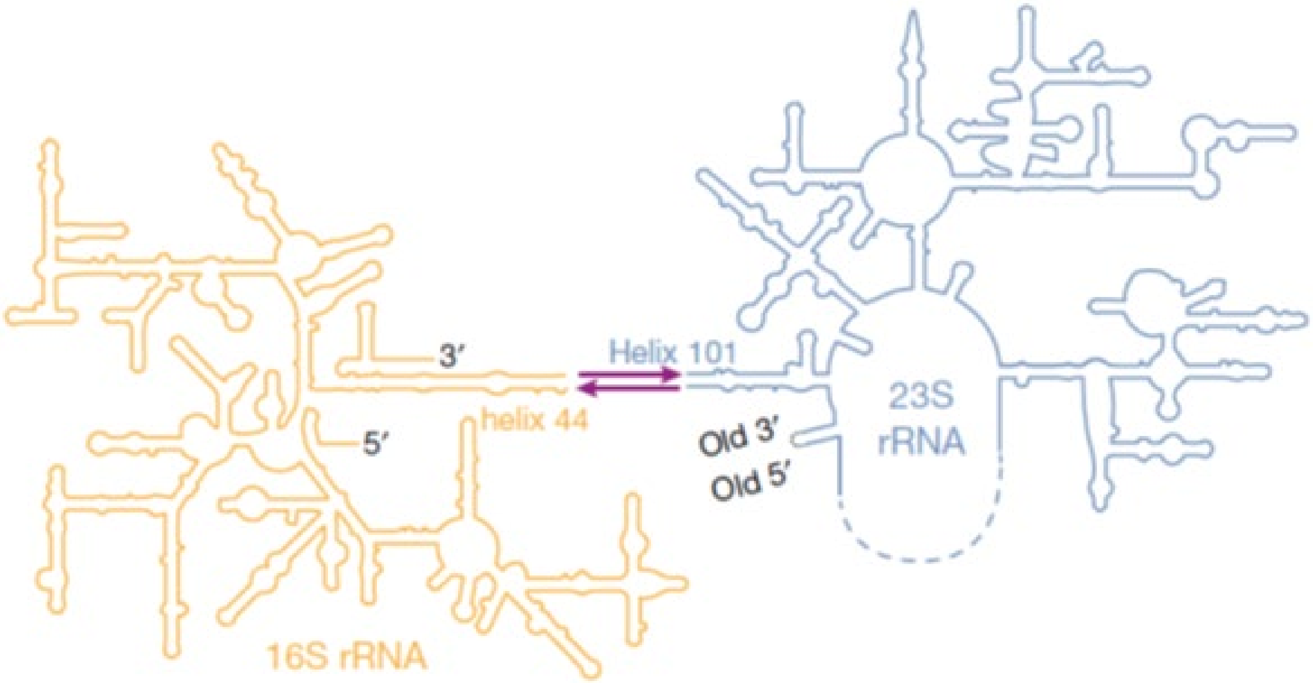
444 | NATURE | VOL 564 | 20/27 DECEMBER 2018

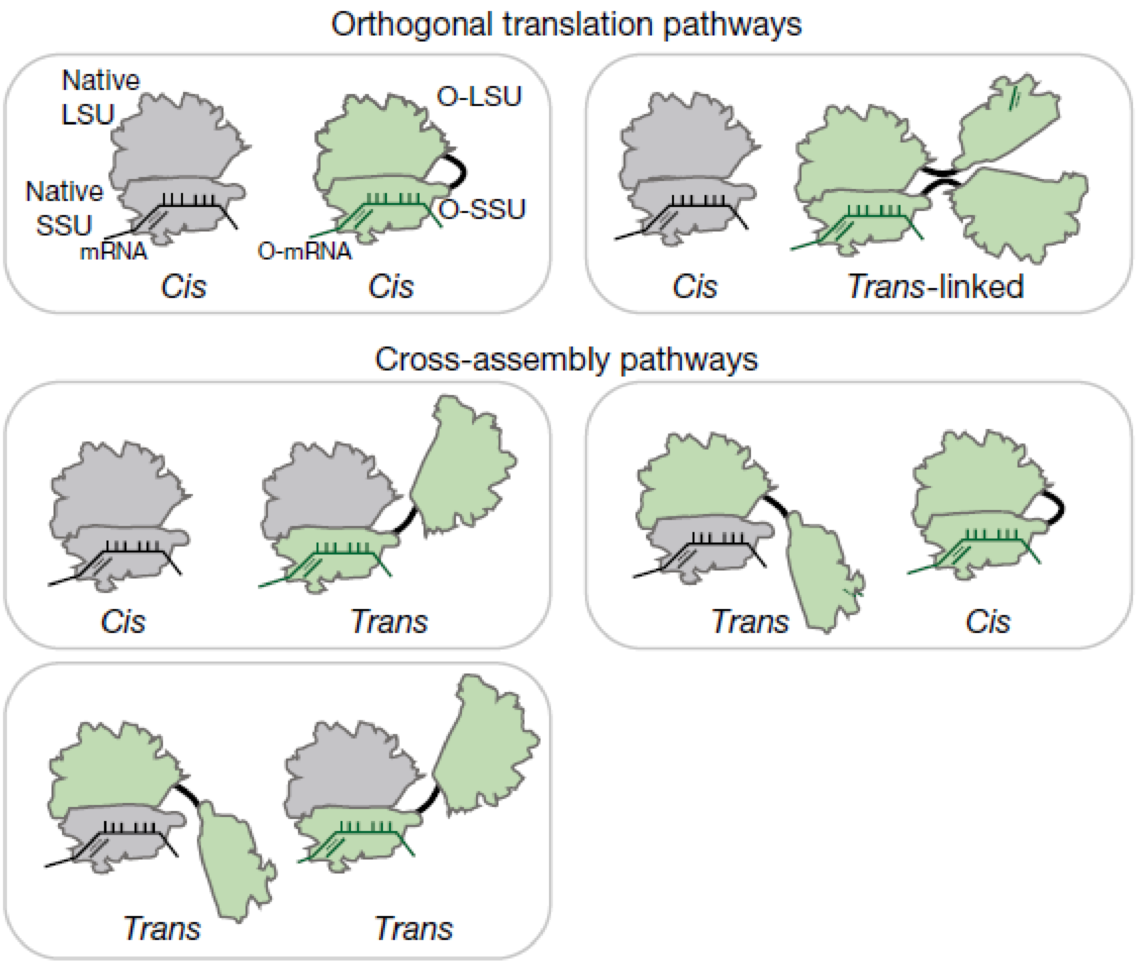
1) **Development of an engineered orthogonal "stapled" ribosome**

2) **Expansion of the chemical properties of ribosome-mediated polimerization**

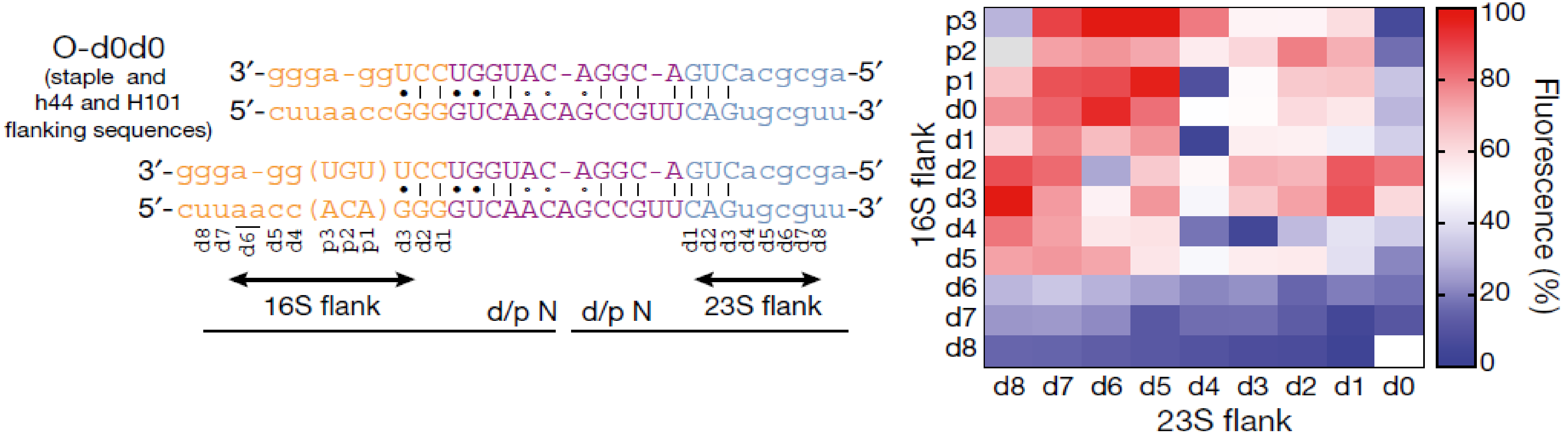
Engineered covalently linked ribosomal subunits

O-stapled ribosomes





Evolved covalently linked ribosomal subunits



O-sfGFP150TAG reporter

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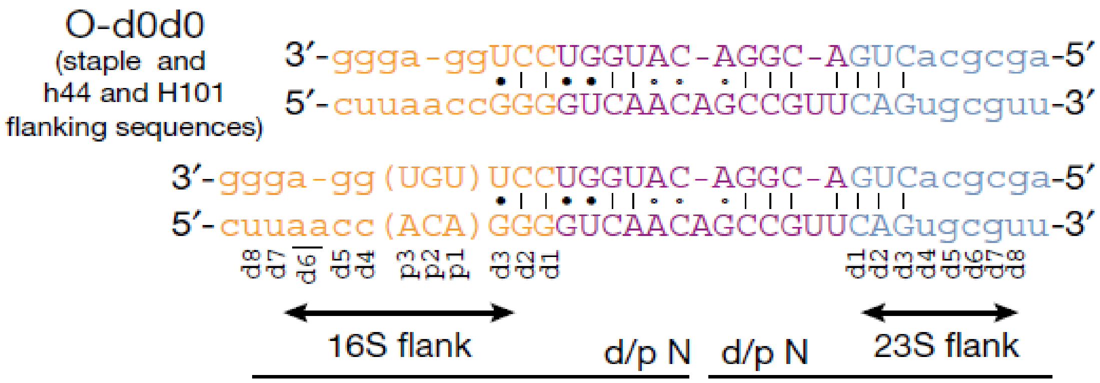

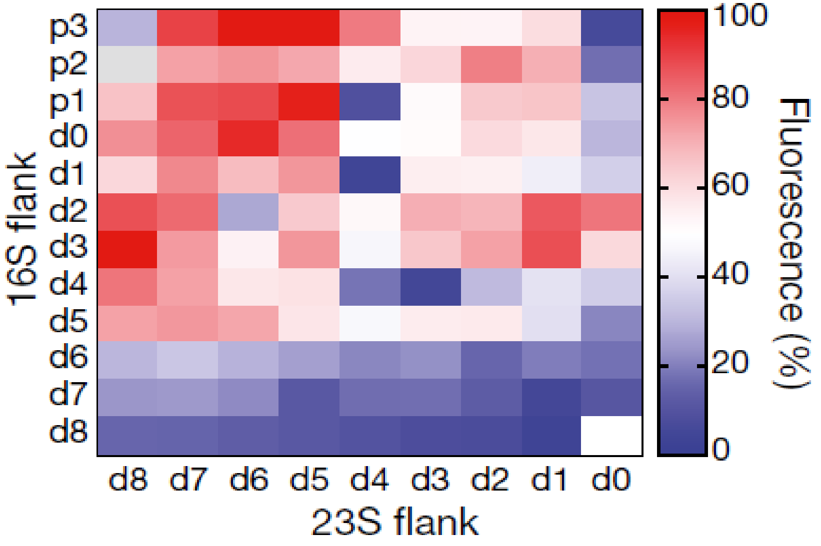

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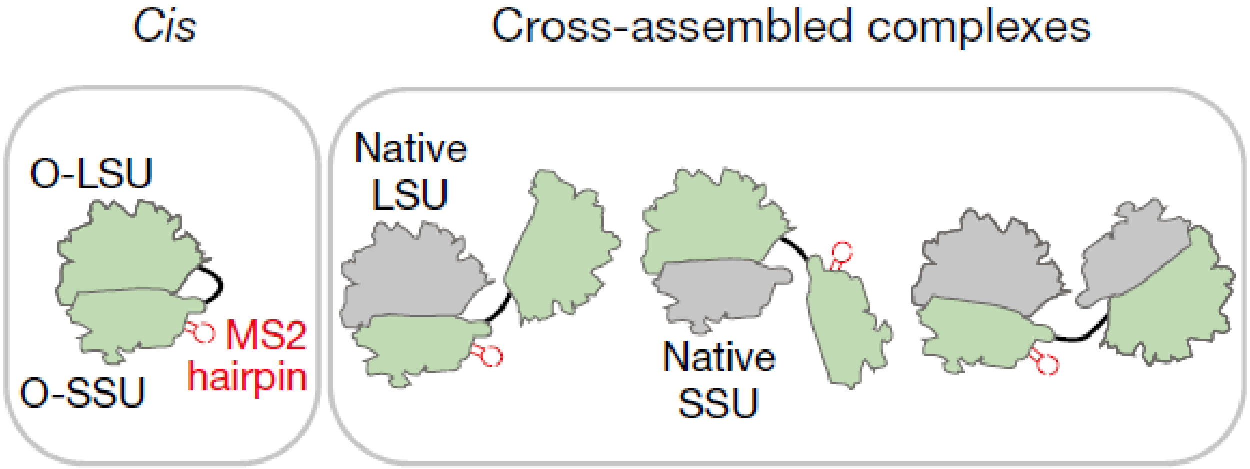
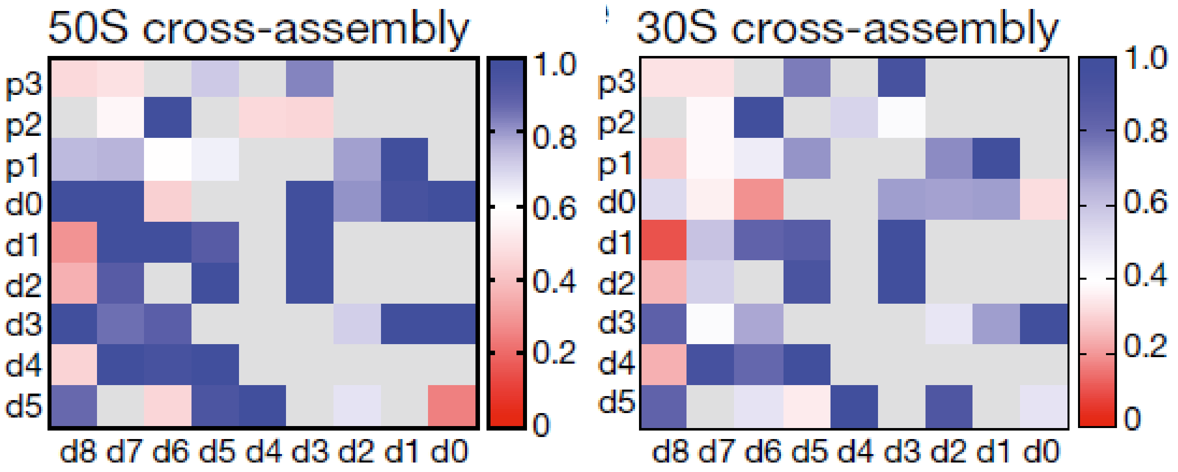
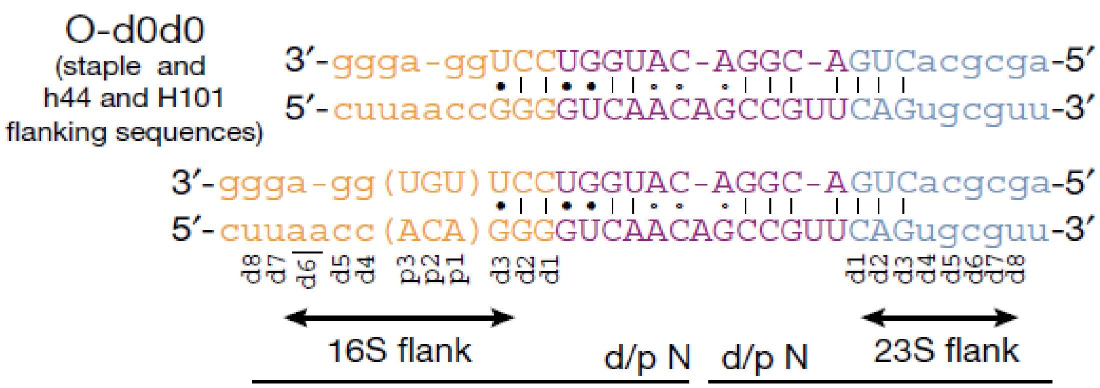
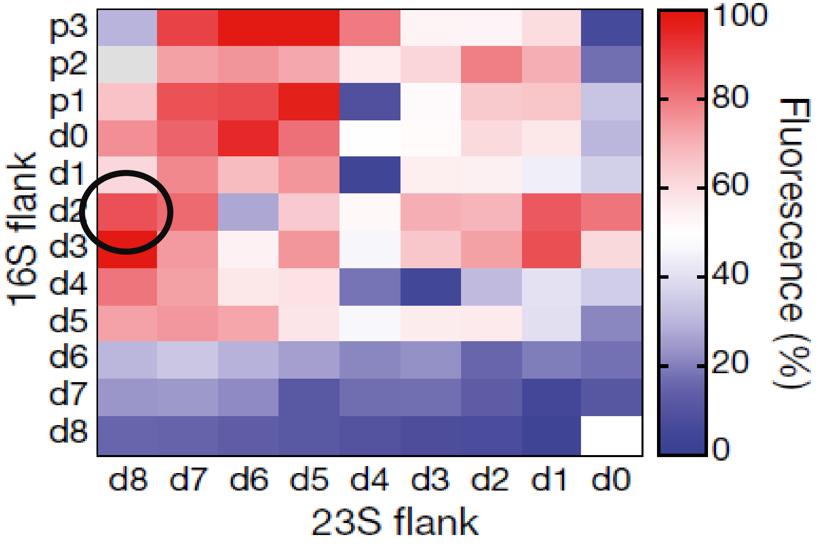


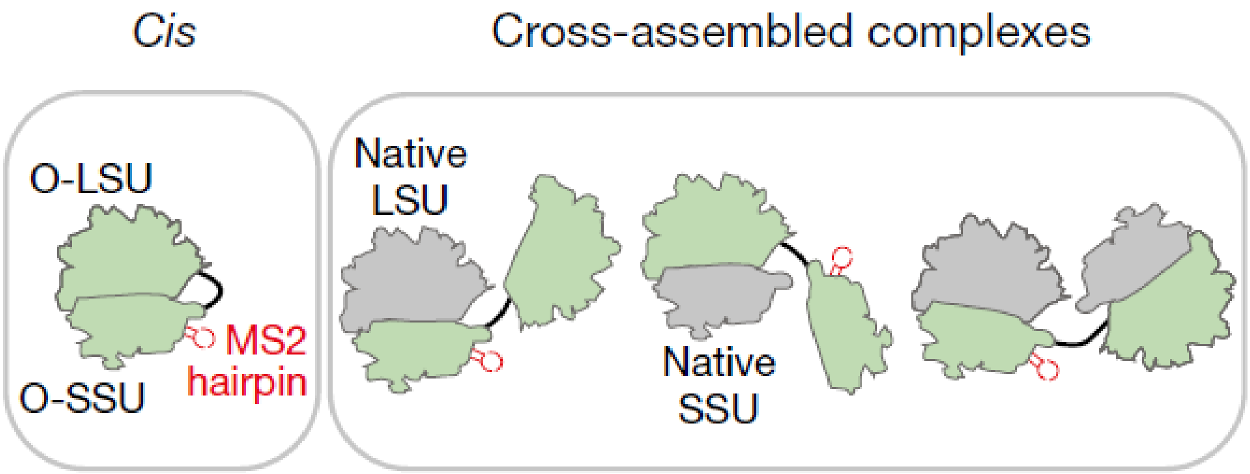


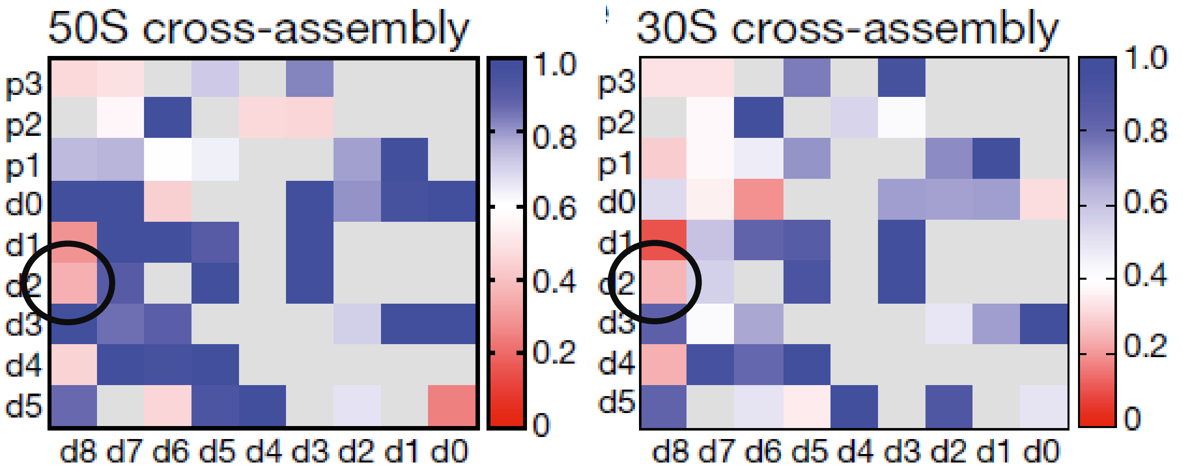
Evolved covalently linked ribosomal subunits



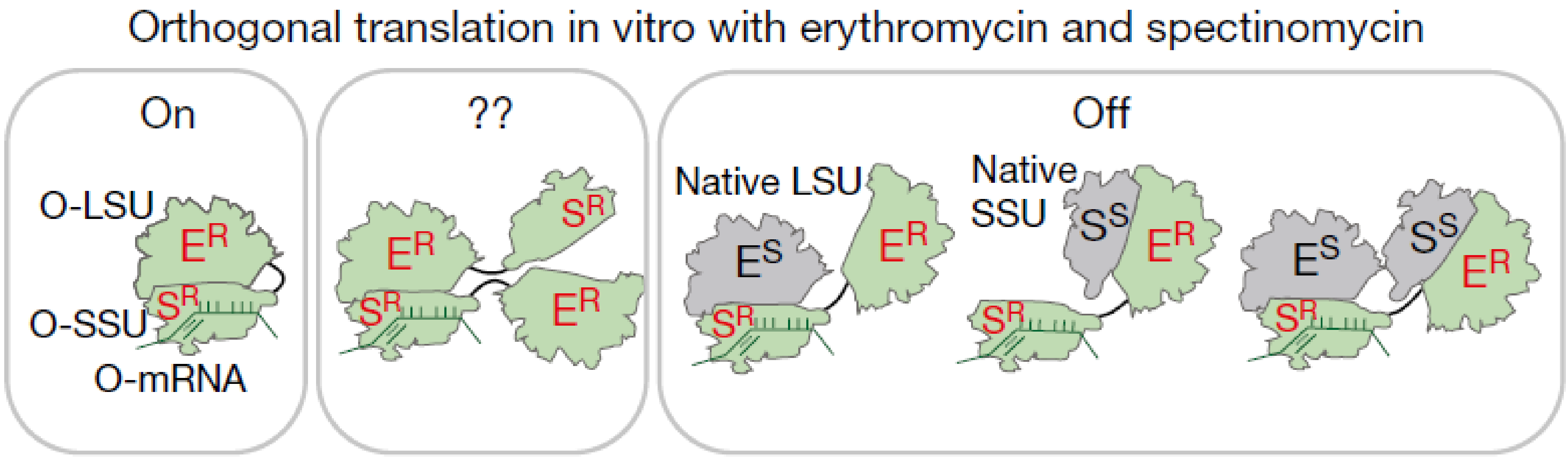


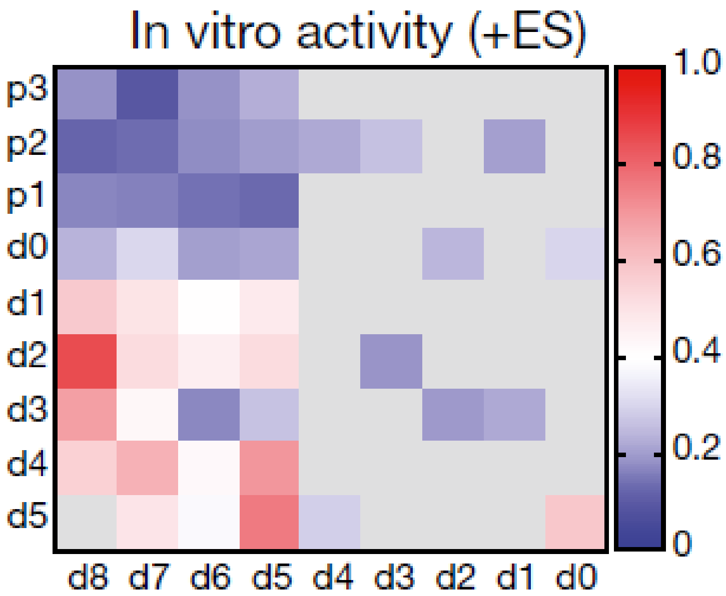
O-sfGFP150TAG reporter





O-stapled ribosomes function without cross-assembling with endogenous subunits





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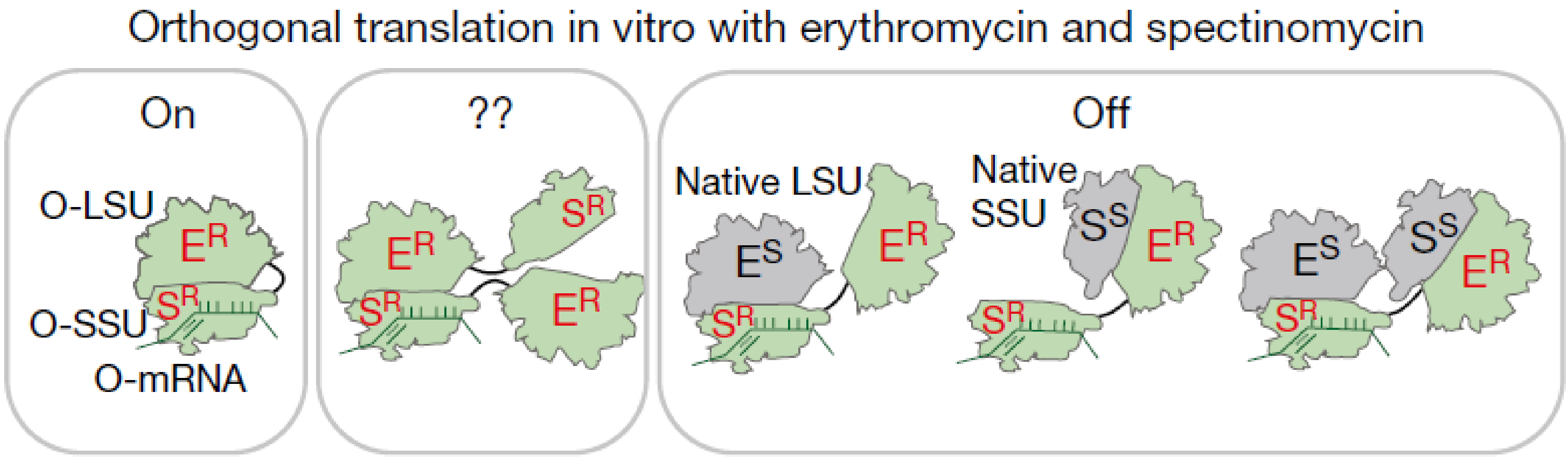
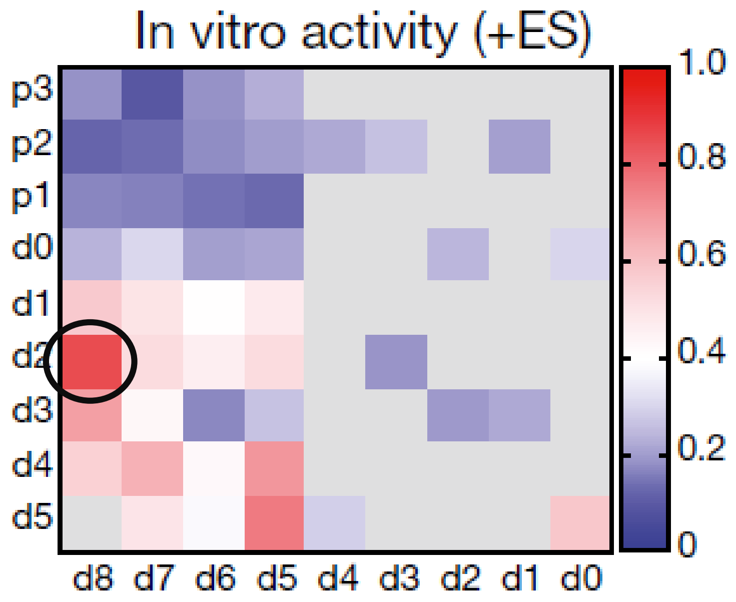
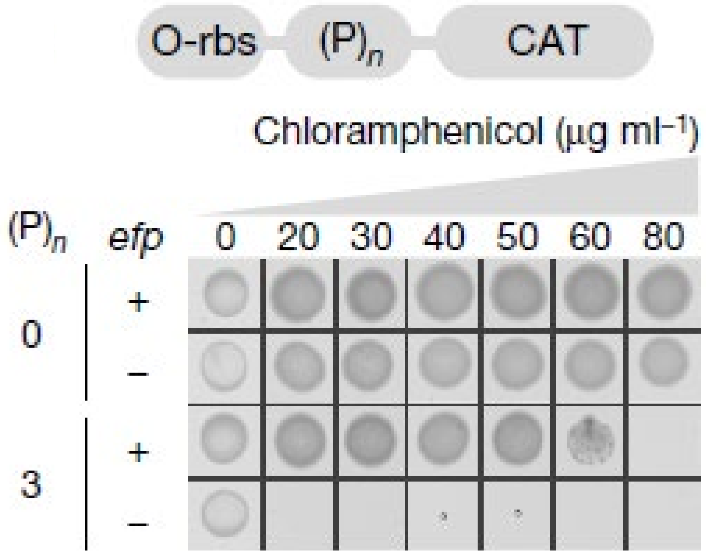
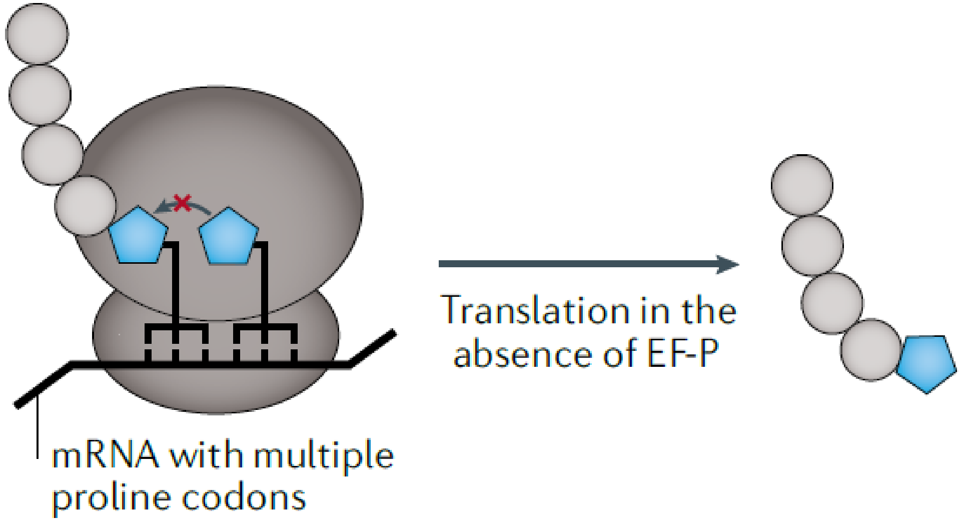




O-stapled ribosomes with new polymerizing function





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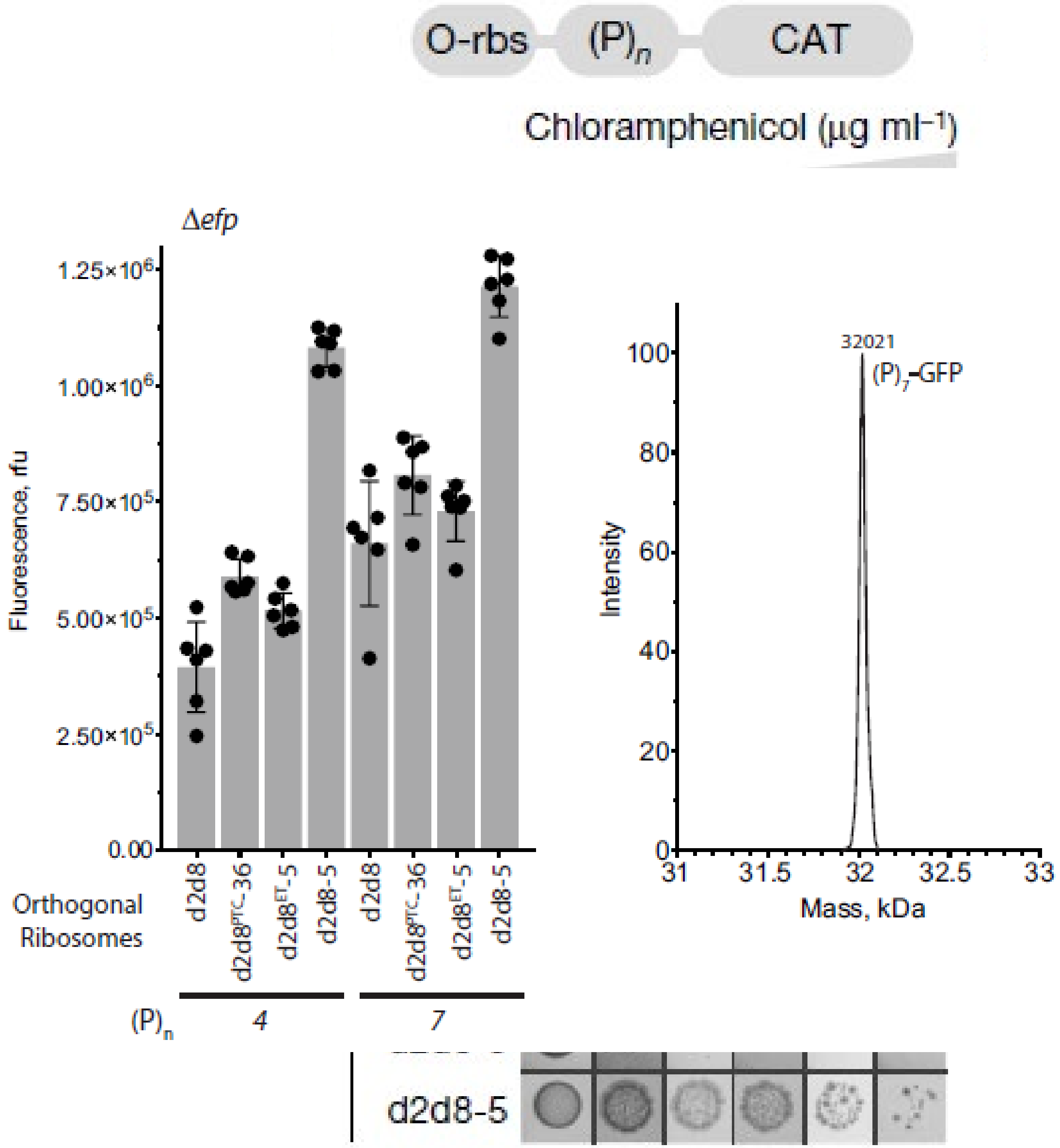

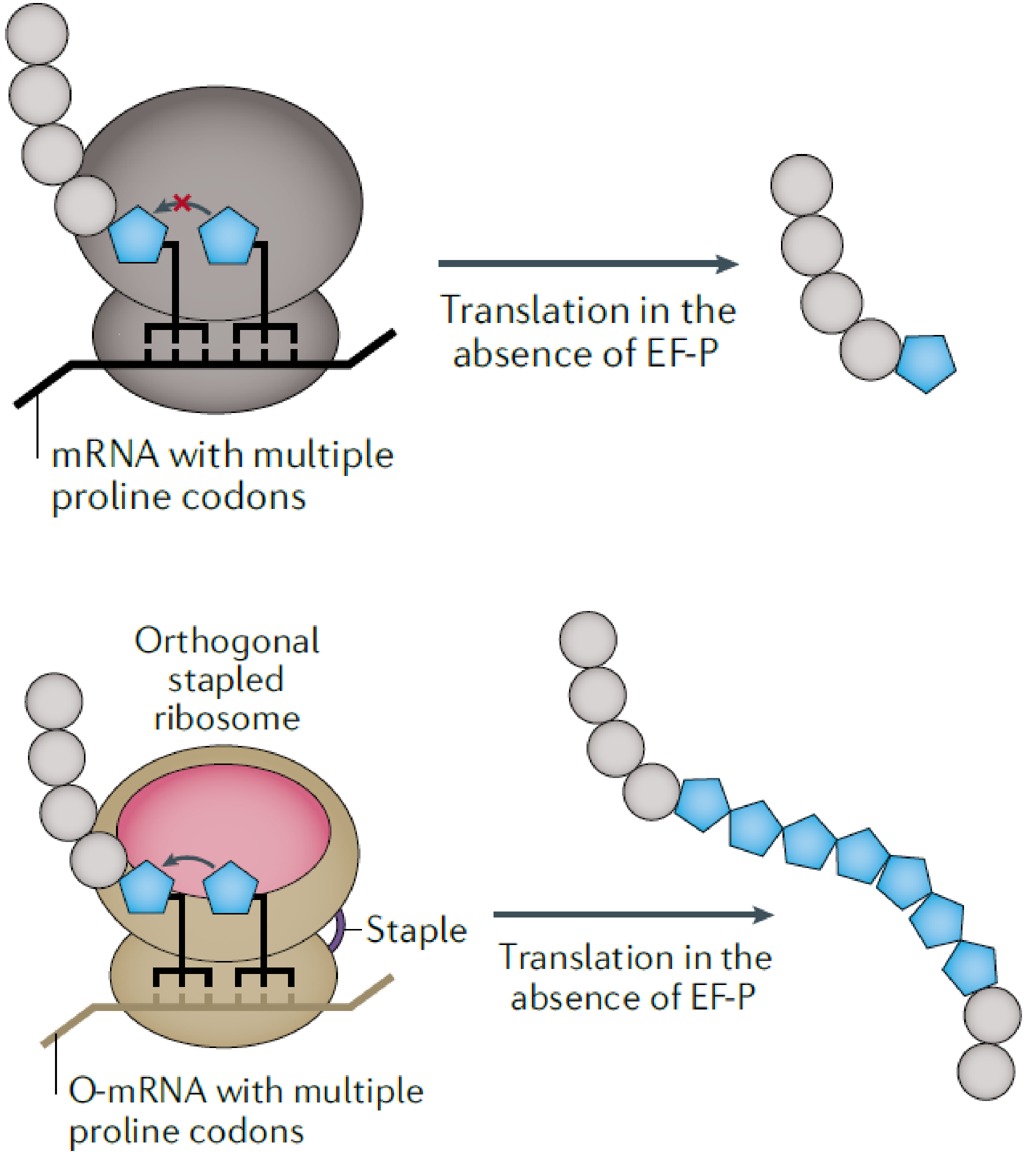

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Summary

Creation of an orthogonal ribosome in which both subunits are directed to an orthogonal message, minimizing the cross-assembly with endogenous subunits.

Evolution of new large-subunit polymerizing function that has not been accessed in natural ribosomes.

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Creation of an orthogonal ribosome in which both subunits are directed to an orthogonal message, minimizing the cross-assembly with endogenous subunits.

Evolution of new large-subunit polymerizing function that has not been accessed in natural ribosomes.

Future perspectives

Expand strategies to aminoacylate tRNA with substrates for non-canonical polymerization *in vivo.*

Transform cells into factories for the encoded synthesis of biopolymers with non-natural backbone chemistries, which may include new materials and medicines.

RESEARCH ARTICLE

SYNTHETIC BIOLOGY

Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes

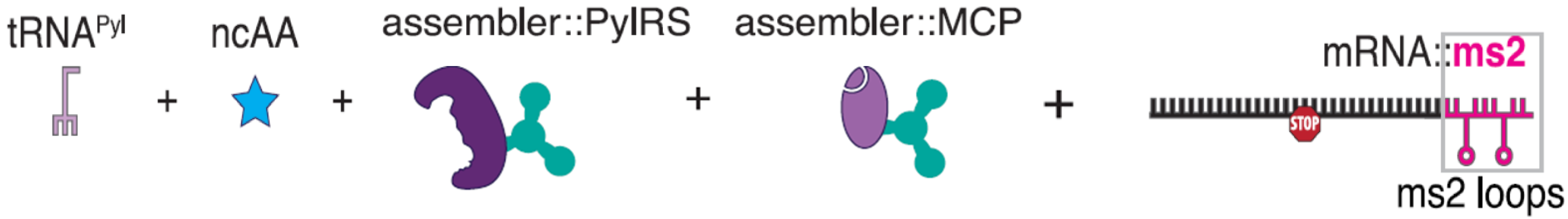
Christopher D. Reinkemeier[1,2,3*], Gemma Estrada Girona[3*], Edward A. Lemke[1,2,3†]

Science **363**, 1415 (2019) 29 March 2019

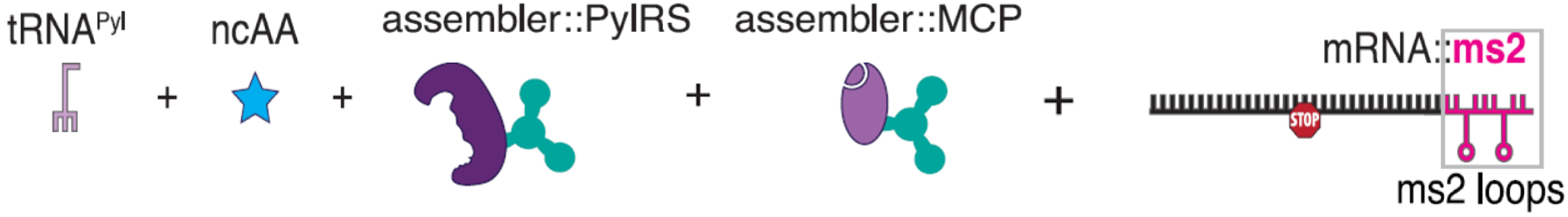
1) **Design of an artificial membraneless organelle exploiting phase and spatial separation**

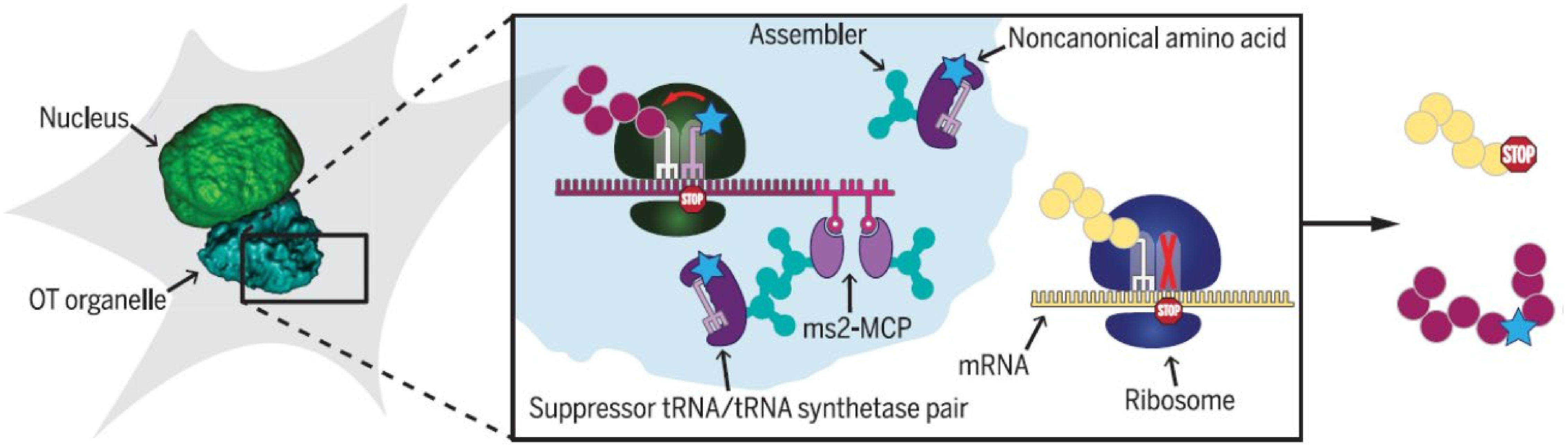
2) **Development of a fully orthogonal translation system in eukaryotes**

Create an orthogonal translation system by local enrichment of specific components

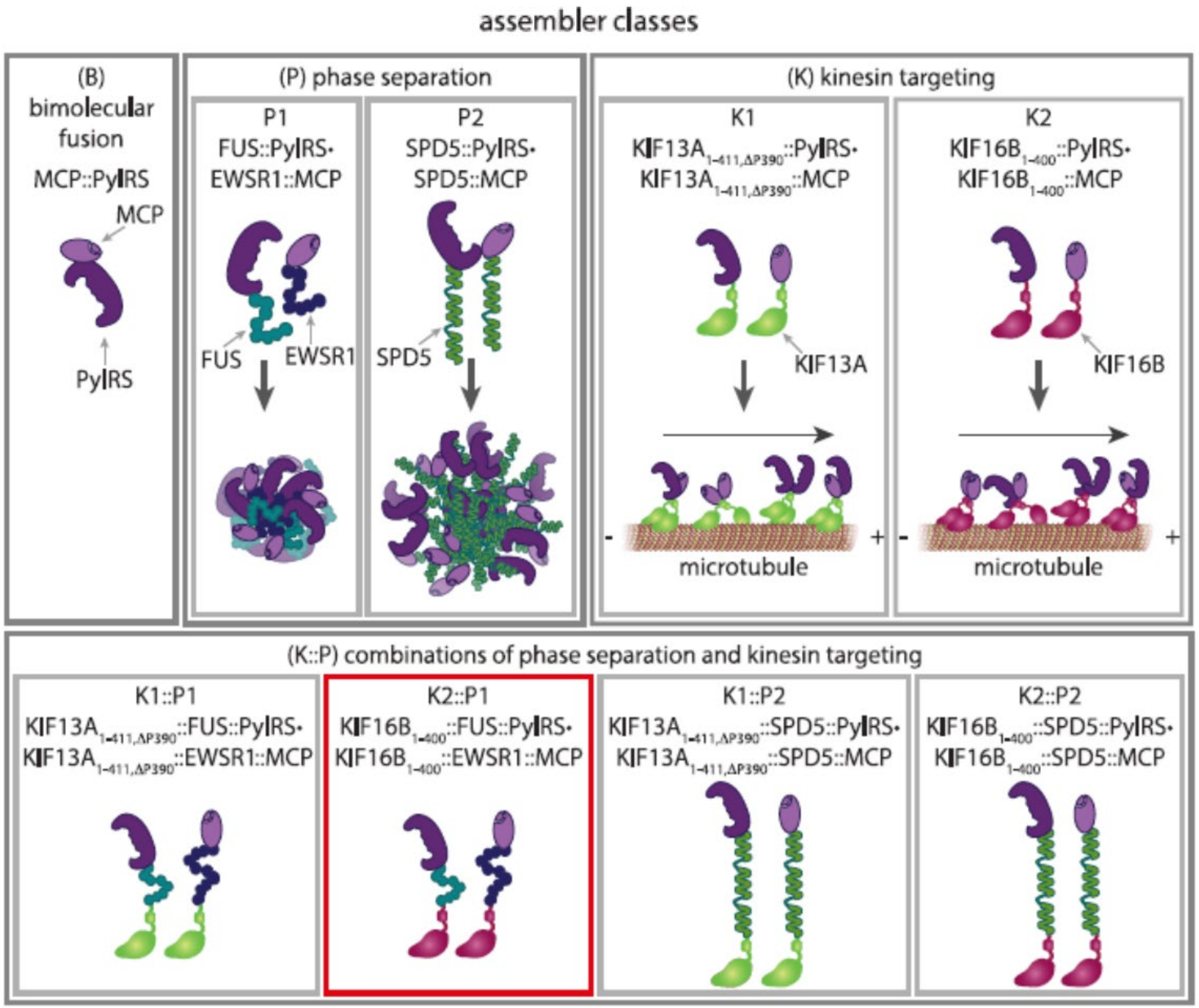


Create an orthogonal translation system by local enrichment of specific components

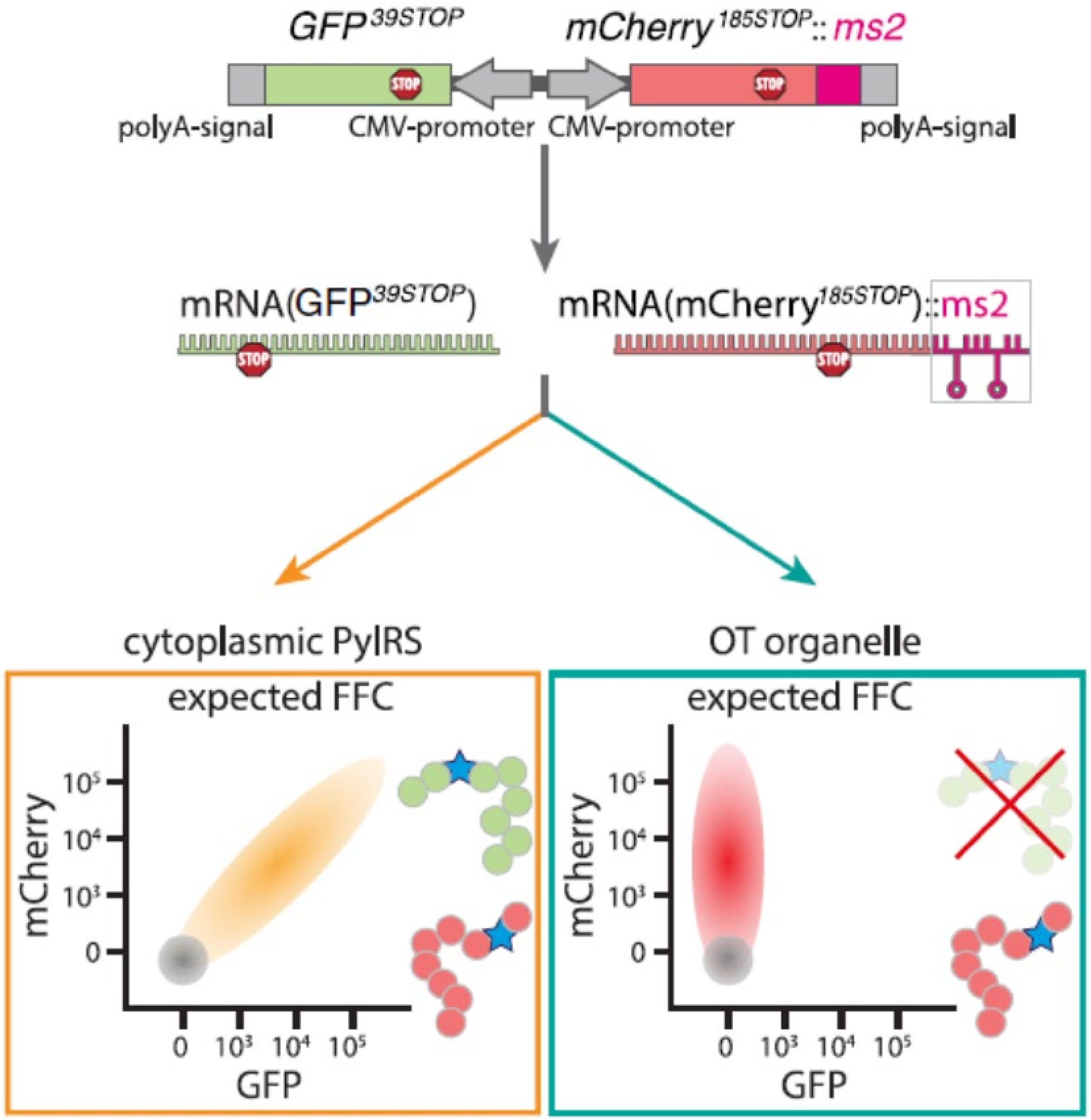




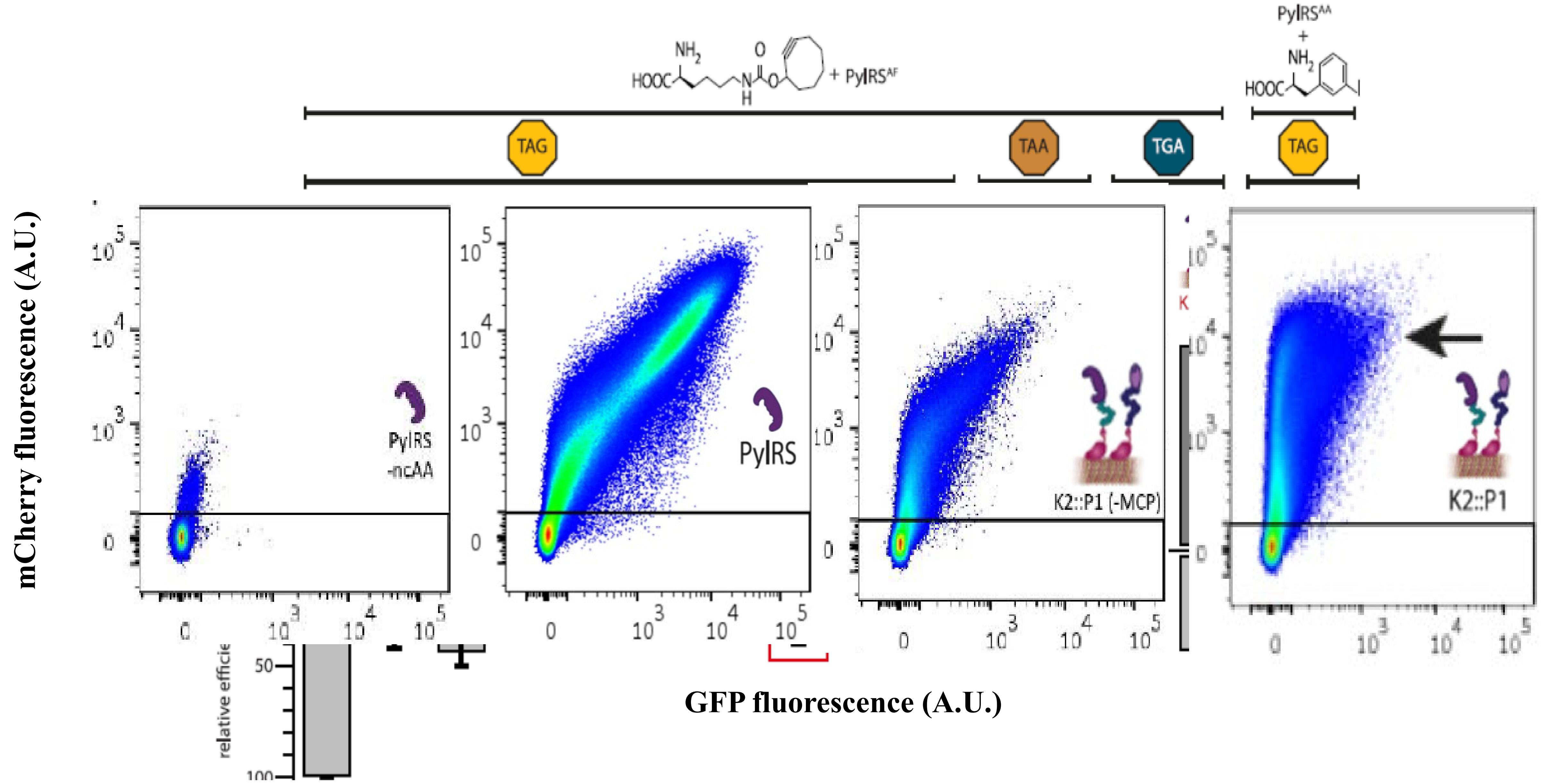
Assembler strategies for local enrichment by means of phase and/or spatial separation



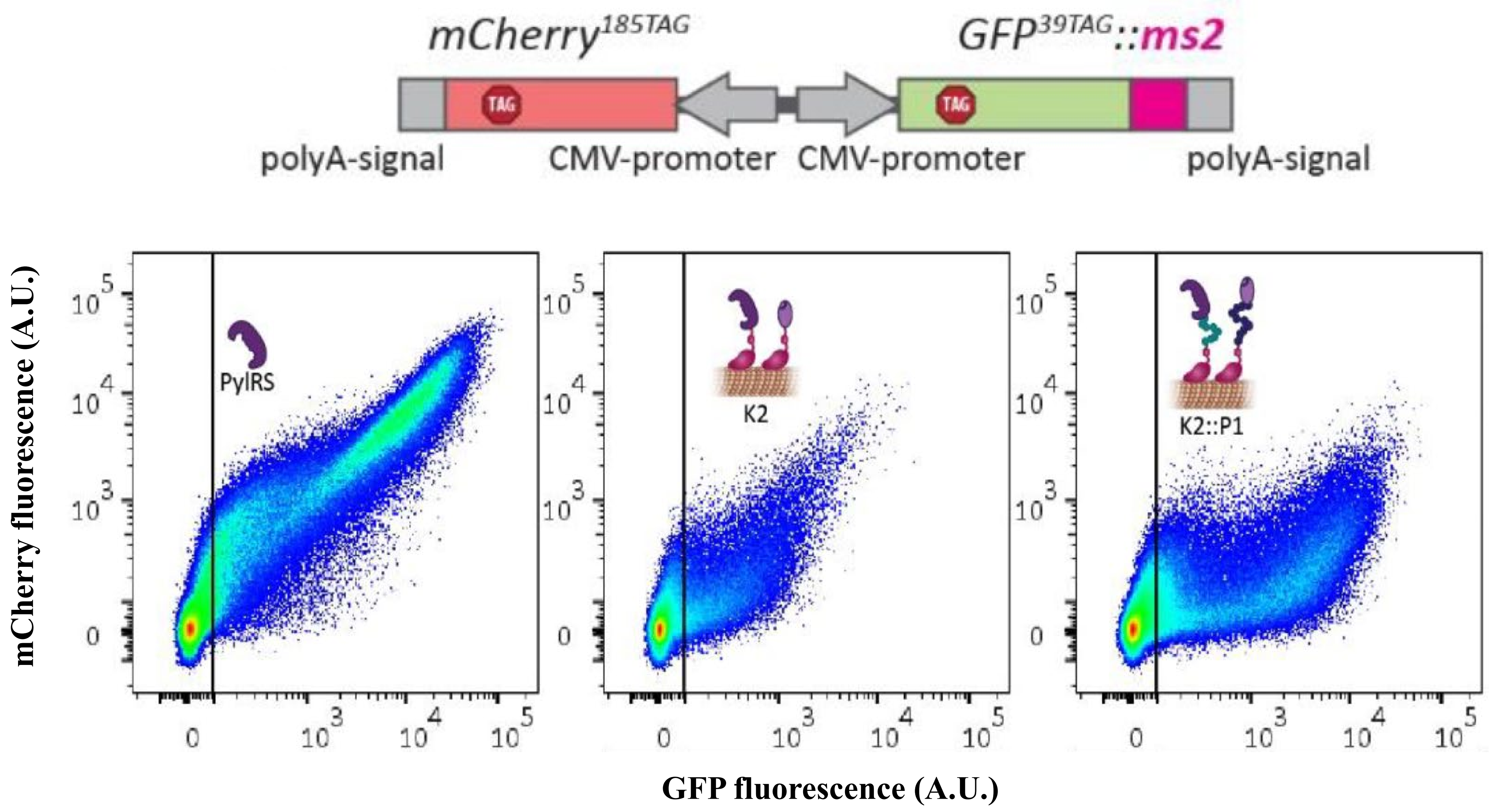
A dual-color reporter to test the selectivity and efficiency of the orthogonal translation



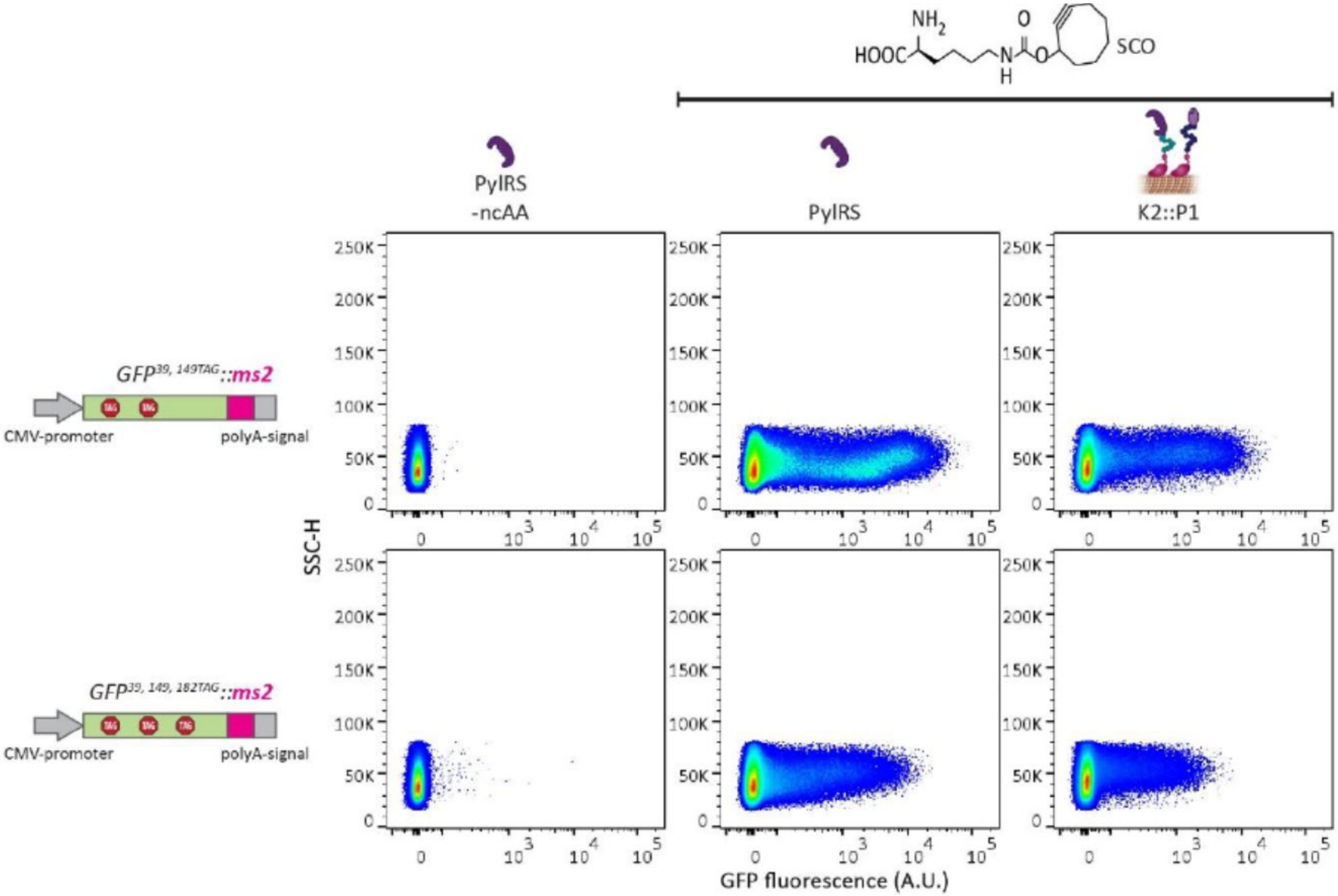
The combination of phase and spatial separation strategies shows the best performance



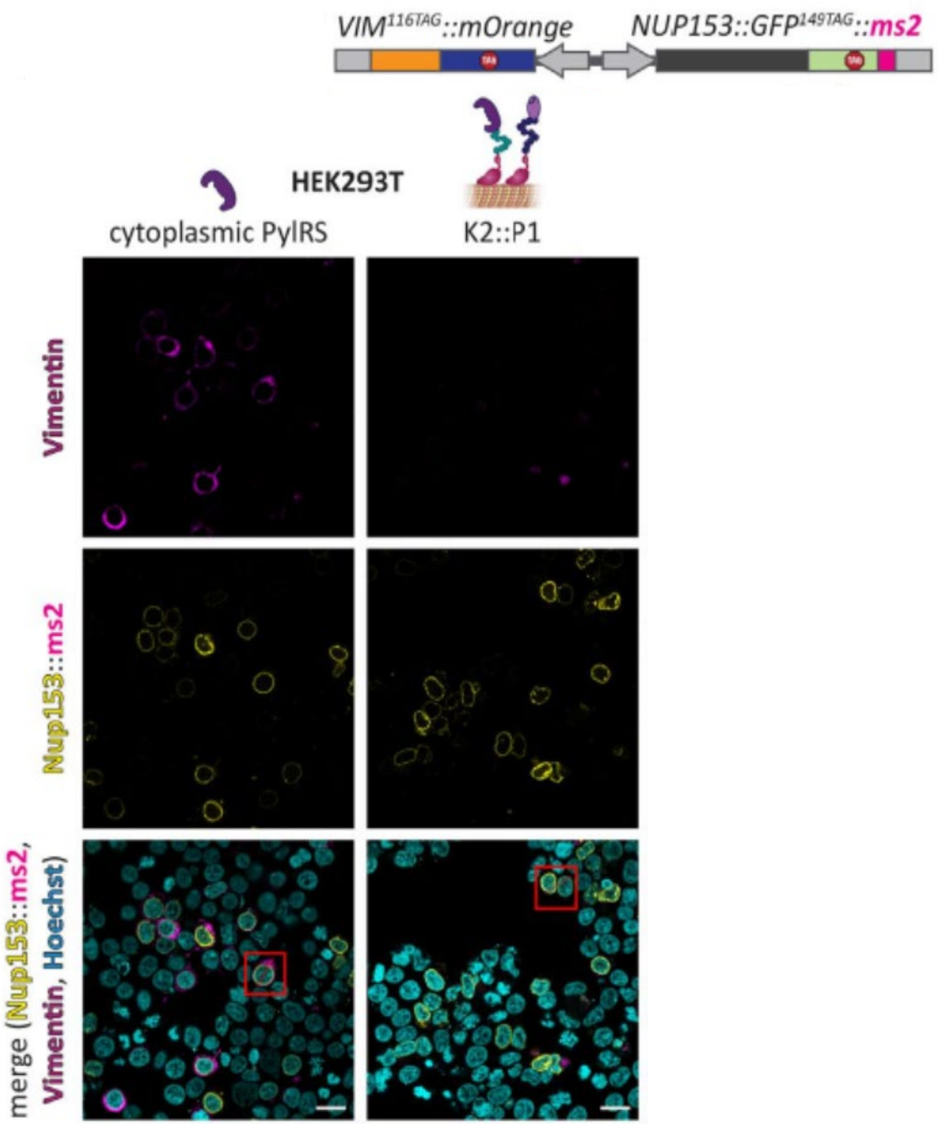
Inverted dual-color reporter to test the robustness of the approach



The system permits suppression of multiple Amber codons



The synthetic organelle enables orthogonal translation of proteins of various cellular compartments



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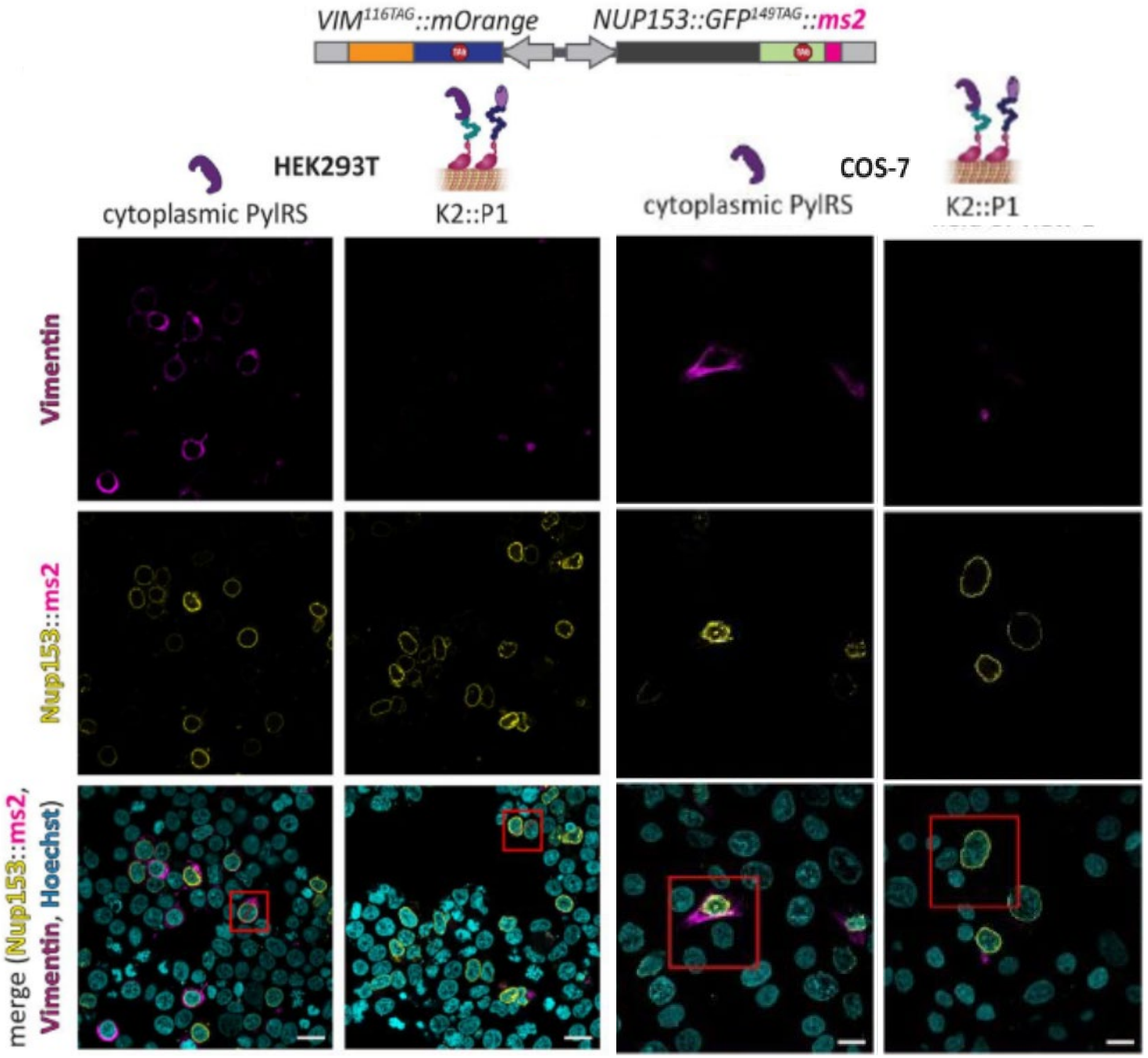

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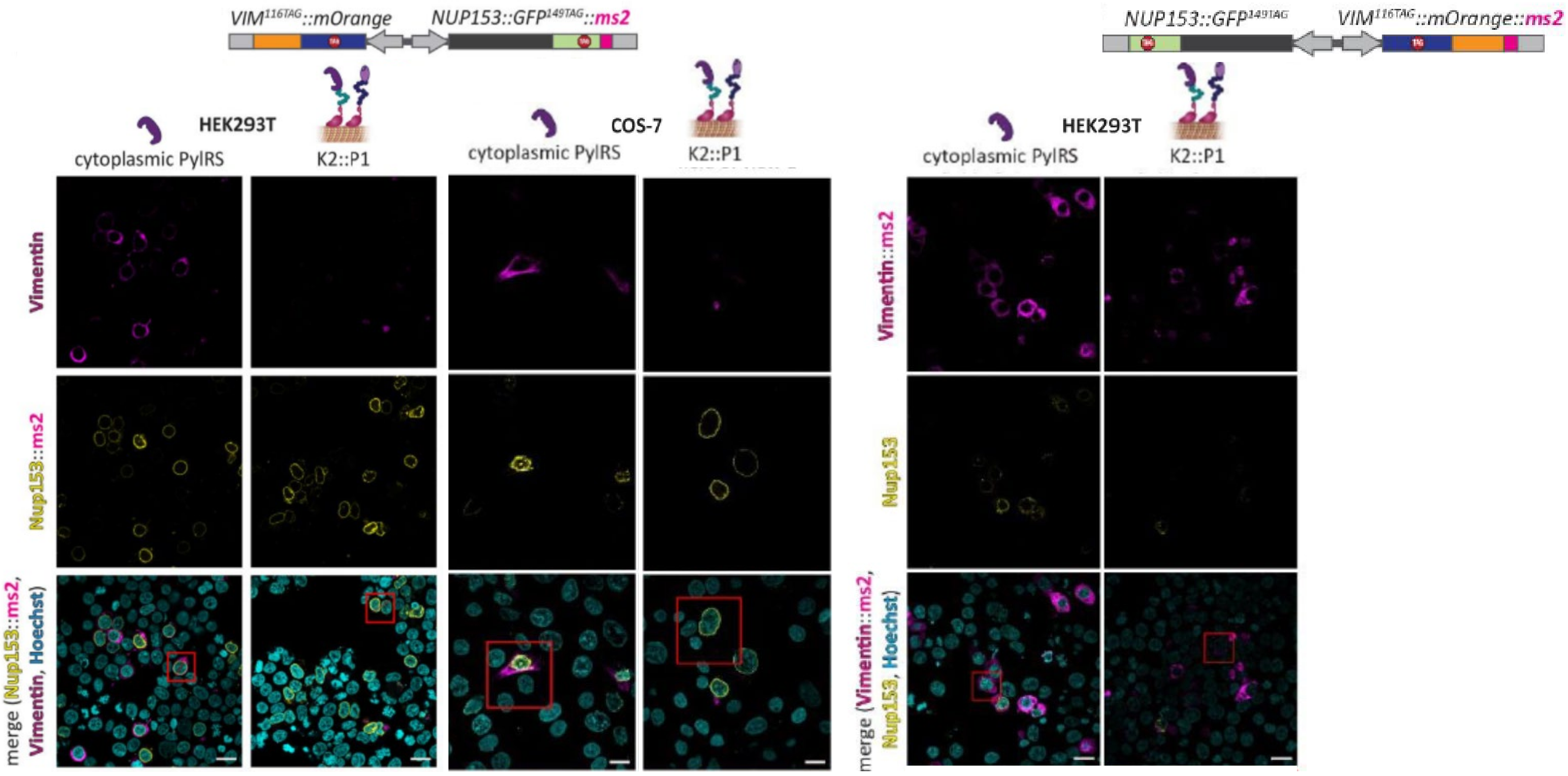

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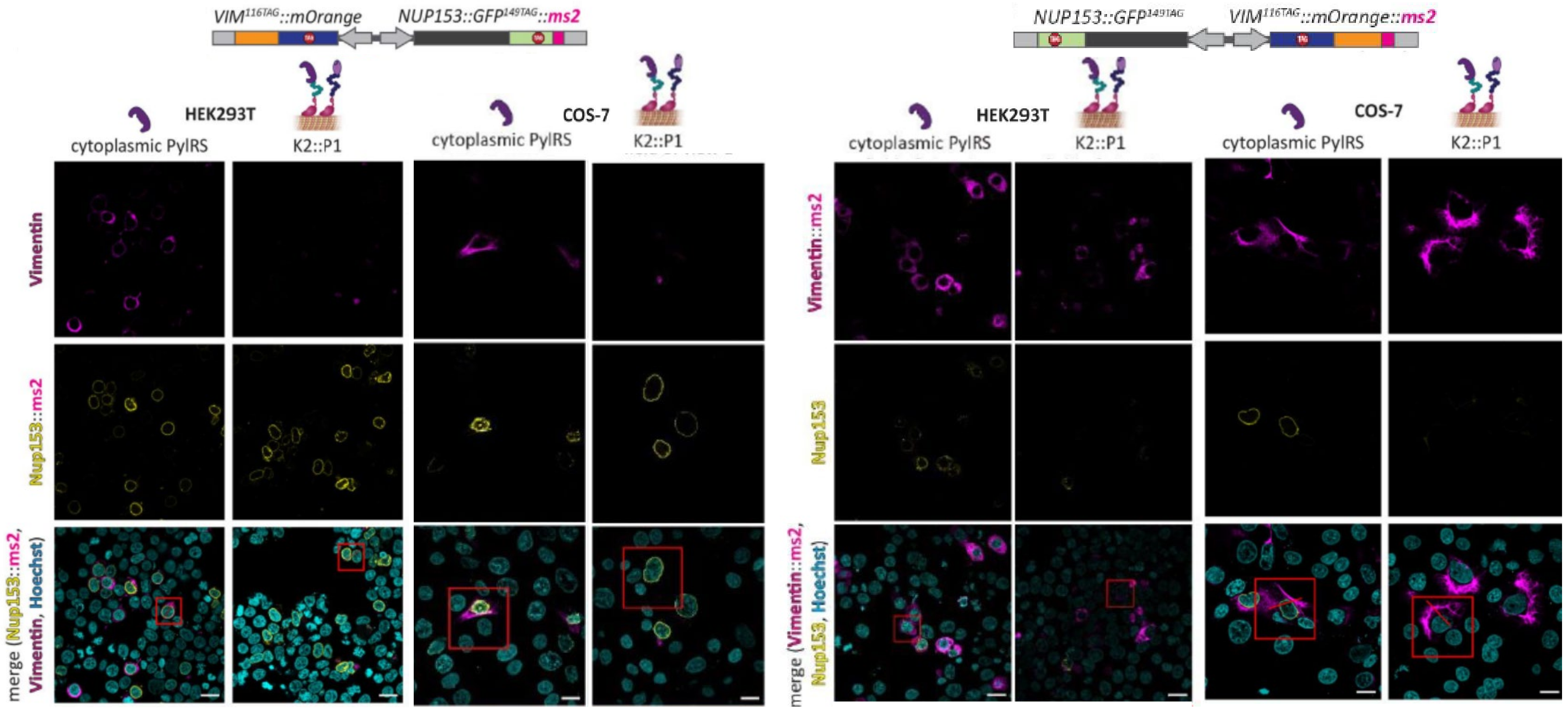

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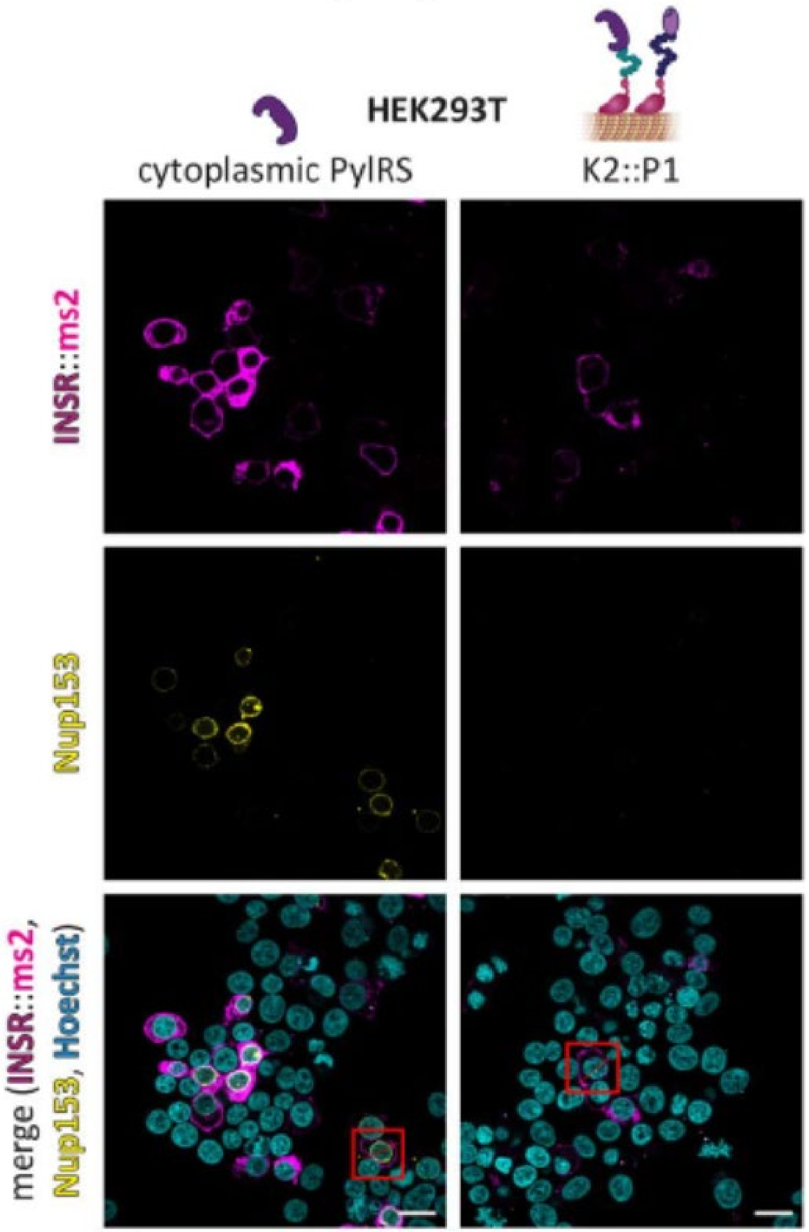
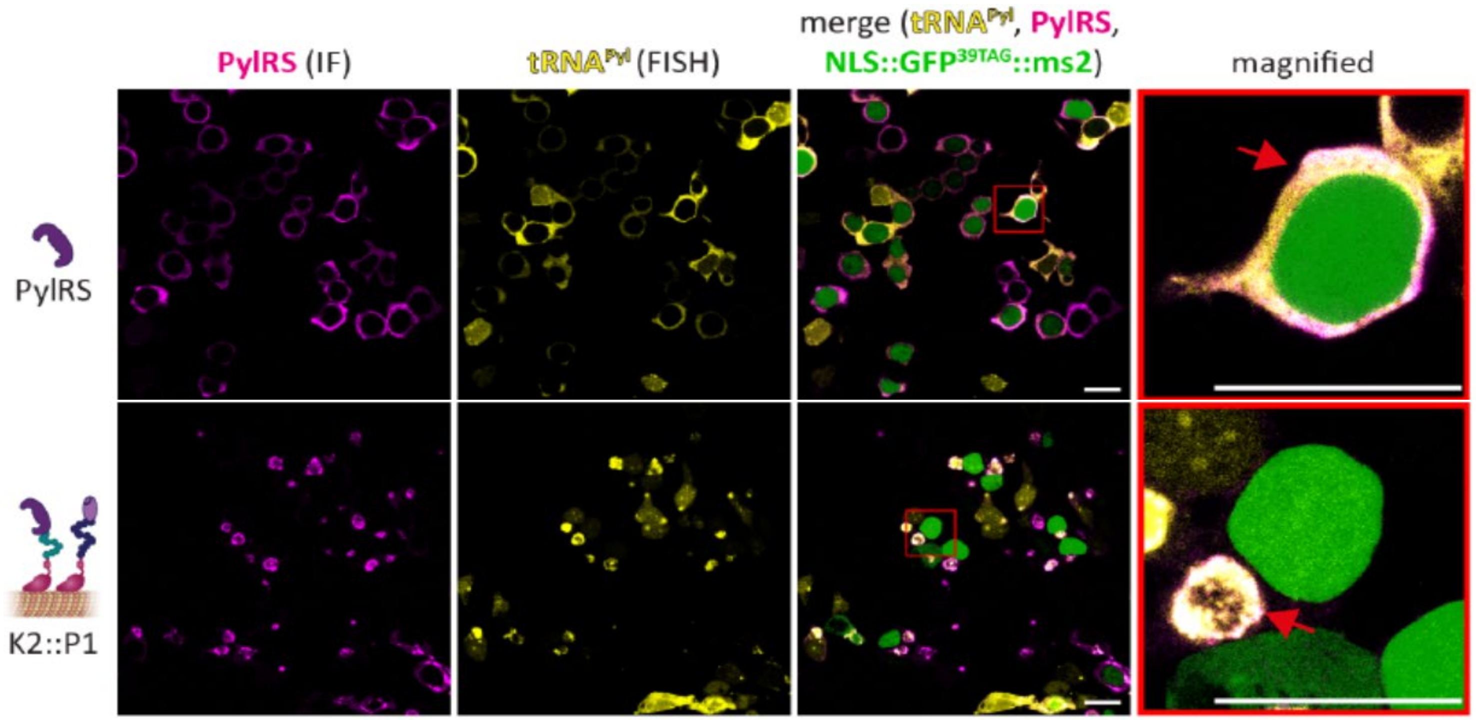
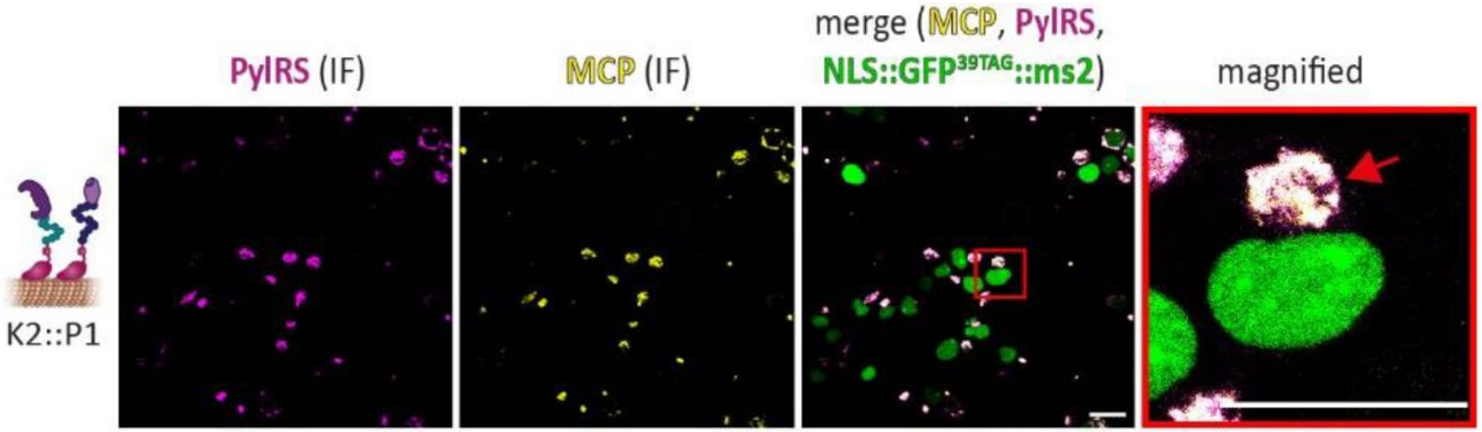


The synthetic organelle functions by recruiting ribosomes, tRNA, aaRS and mRNA





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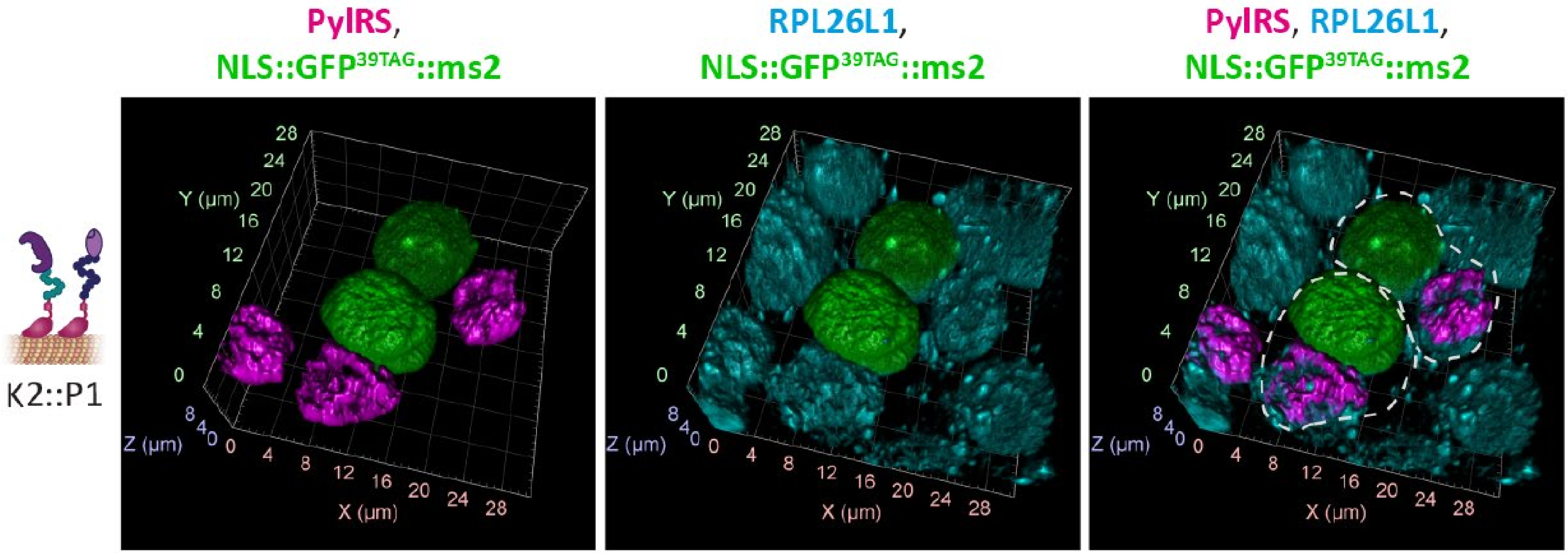

Summary

Combine phase and spatial separation inside cells allows the concentration of a custom designed task into a distinct specially designed membraneless organelle.

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This synthetic system requires only five extra components to enable orthogonal translation.

Future perspectives

This synthetic system represents an important step toward generating semisynthetic eukaryotic organisms with an "orthogonal central dogma".

The general concept of this strategy could be developed in a scalable platform for further organelle engineering and generation of new functions.

Concluding remarks and prospects

The ability to incorporate ncAAs with diverse structures and properties into proteins in living organisms provides unique opportunities.

Combining orthogonal modalities at multiple nodes of the central dogma is greatly improving circuits performance.

Numerous strategies have improved ncAA incorporation efficiency with orthogonal aaRS–tRNA pairs while limiting host fitness consequences.

To date, bacterial systems enable the greatest repertoire of orthogonal elements. Further development of these strategies are required also in eukaryotic organisms.

Thank you for the attention

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