

L7 - Designer plants I

From improving plant performance
to novel plant-produced products

L8 - Designer plants II

Synthetic biology of chloroplasts

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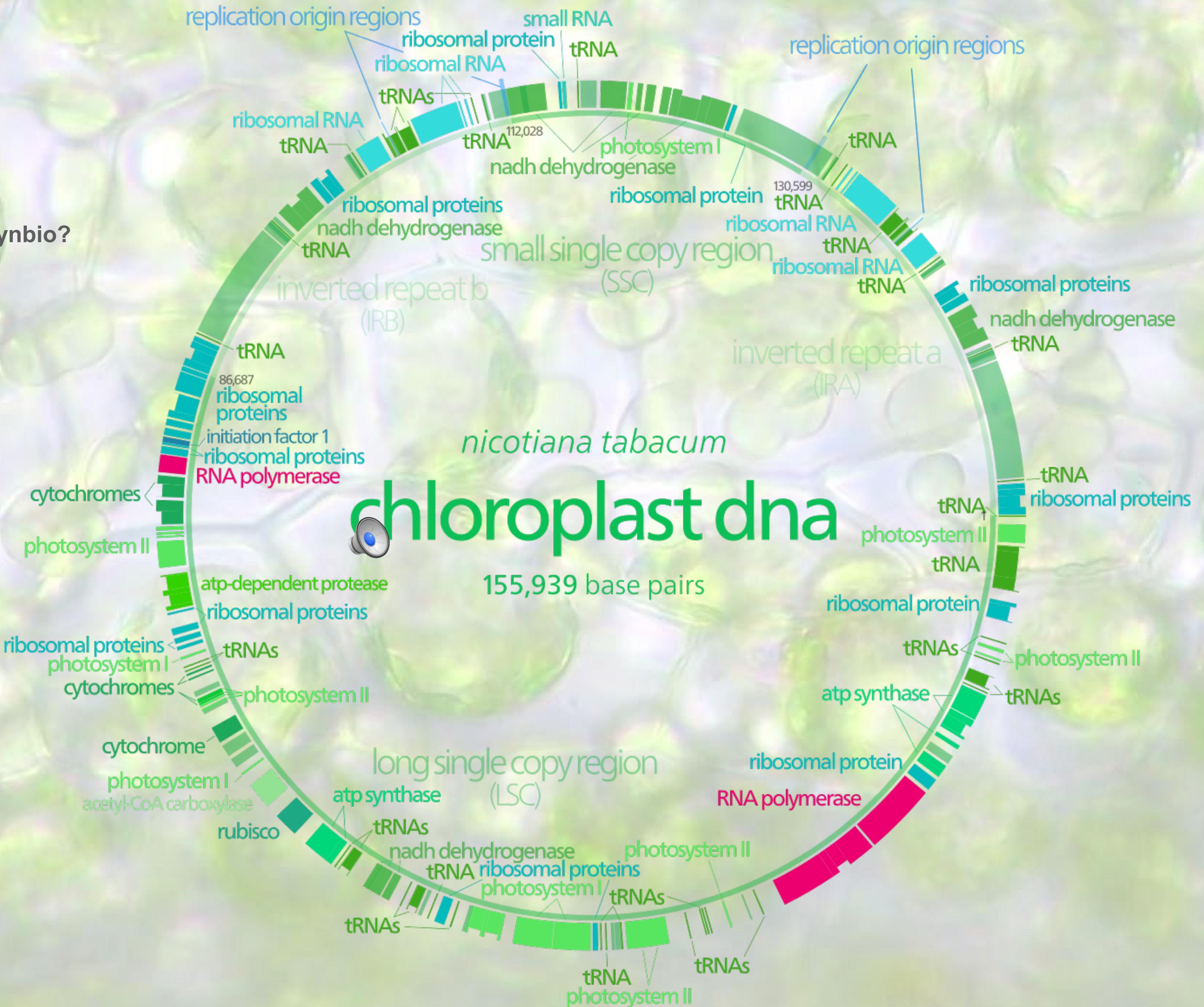
First part: Background, context — why are we interested in engineering these systems?

Second part: How do we engineer these systems? What is currently being done?

Third part: Where are we heading? What future can we look forward to?

Why are chloroplast genomes so well-suited to Synbio?

- ## 1. They're tiny



A structural phylogenetic map for chloroplast photosynthesis

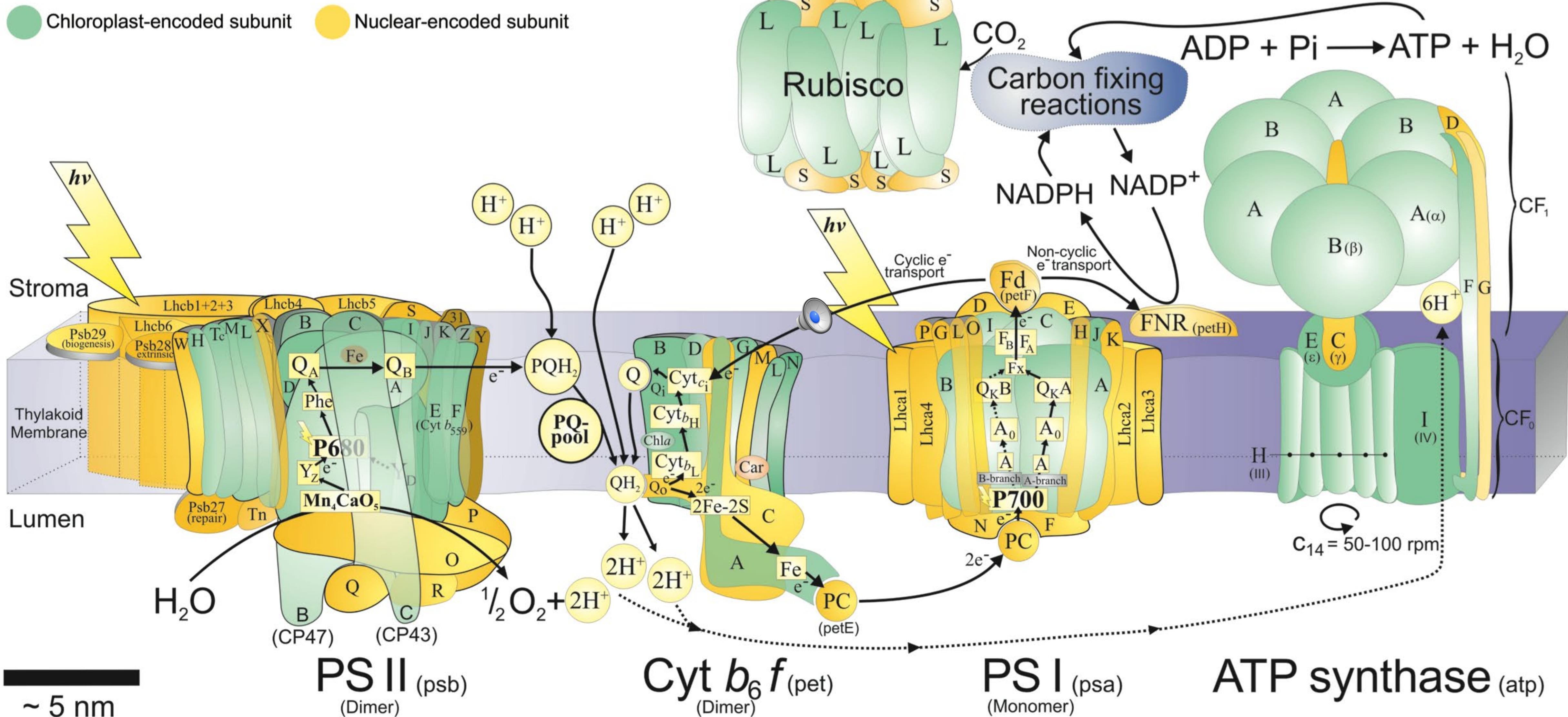
John F. Allen, Wilson B. M. de Paula, Sujith Puthiyaveetil, Jon Nield

School of Biological and Chemical Sciences, Queen Mary University of London

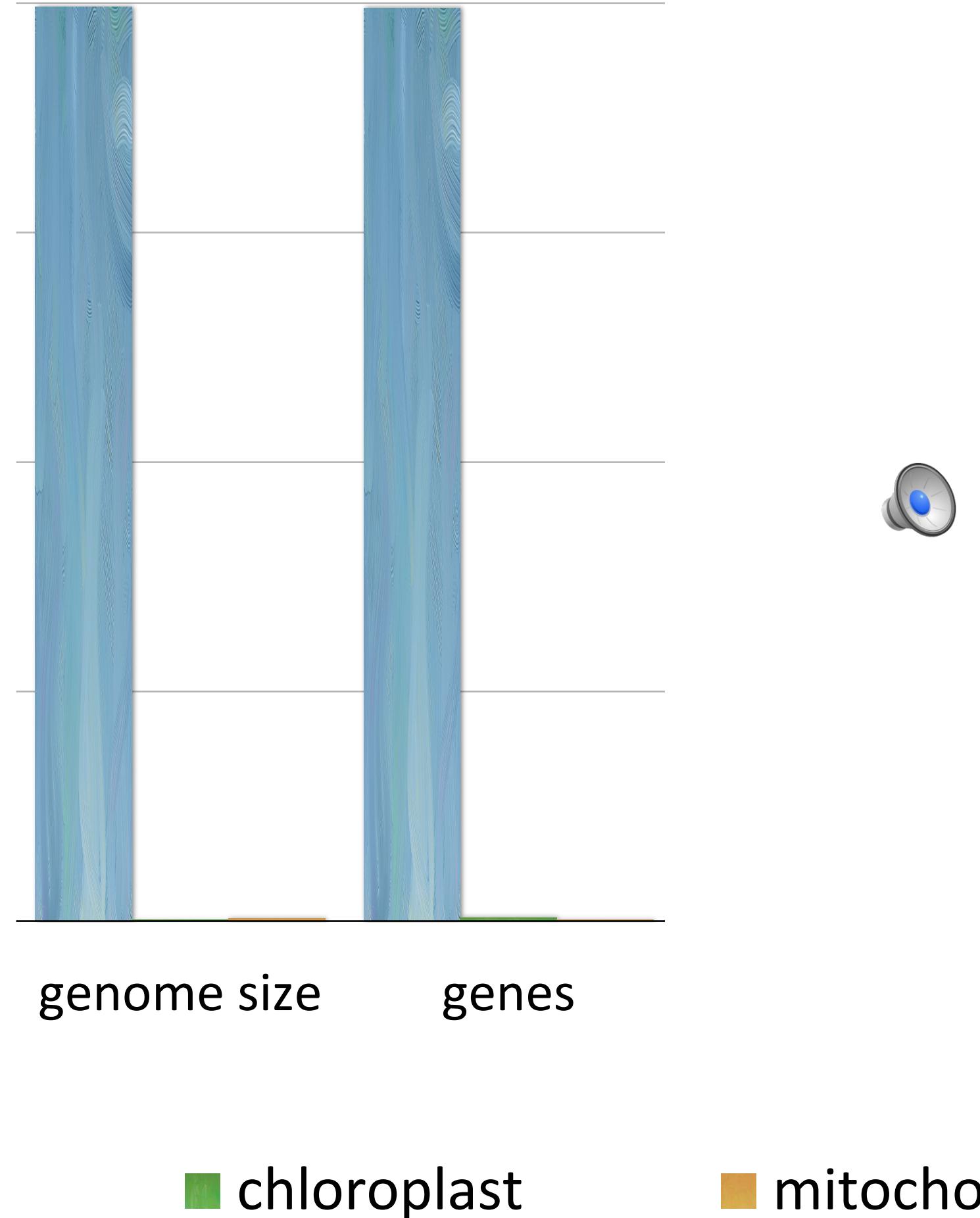
Why are chloroplast genomes so well-suited to Synbio?

2. They encode really important proteins

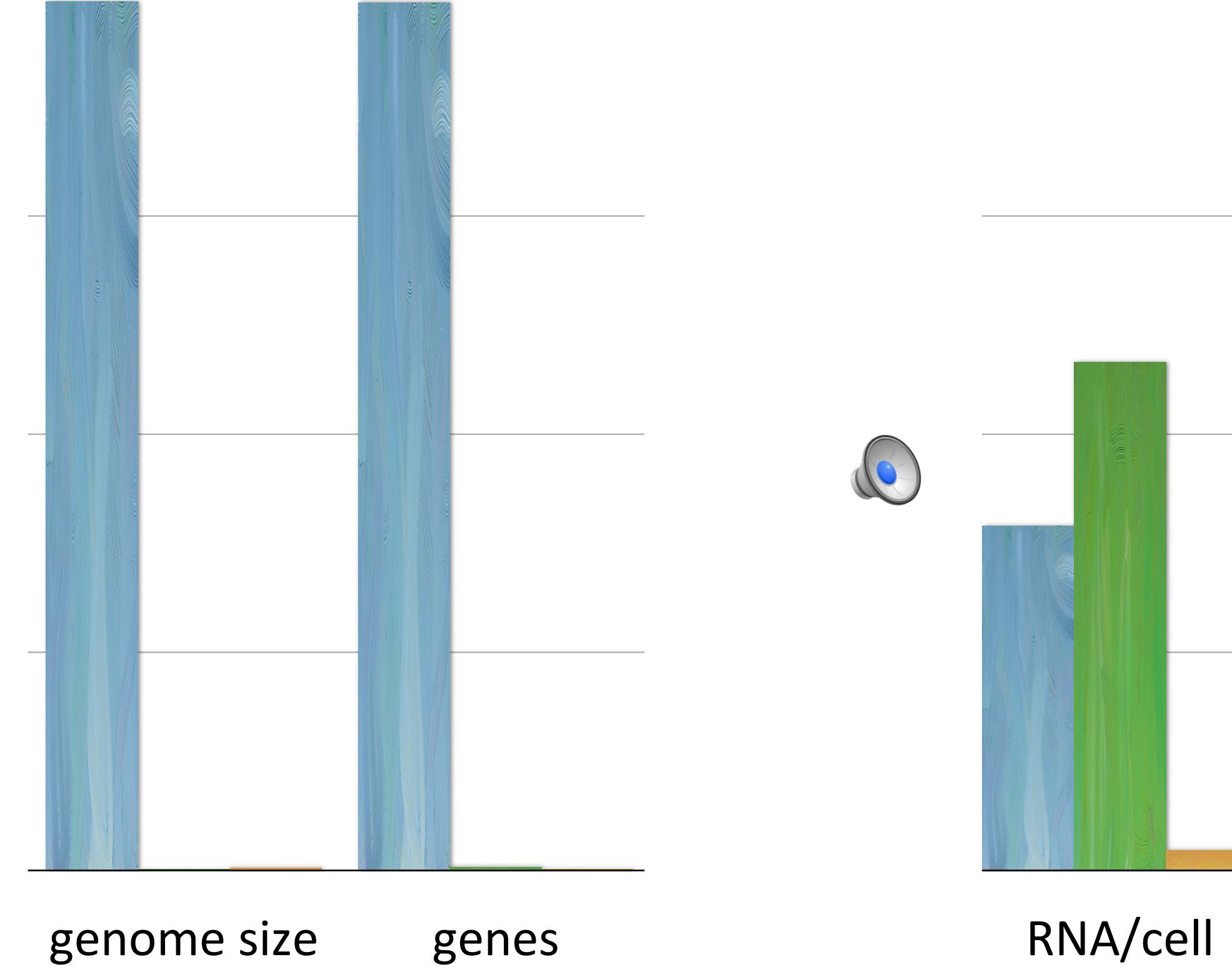
Embryophyte (eukaryote) *Arabidopsis thaliana*



Tiny genomes, huge impact



Tiny genomes, huge impact



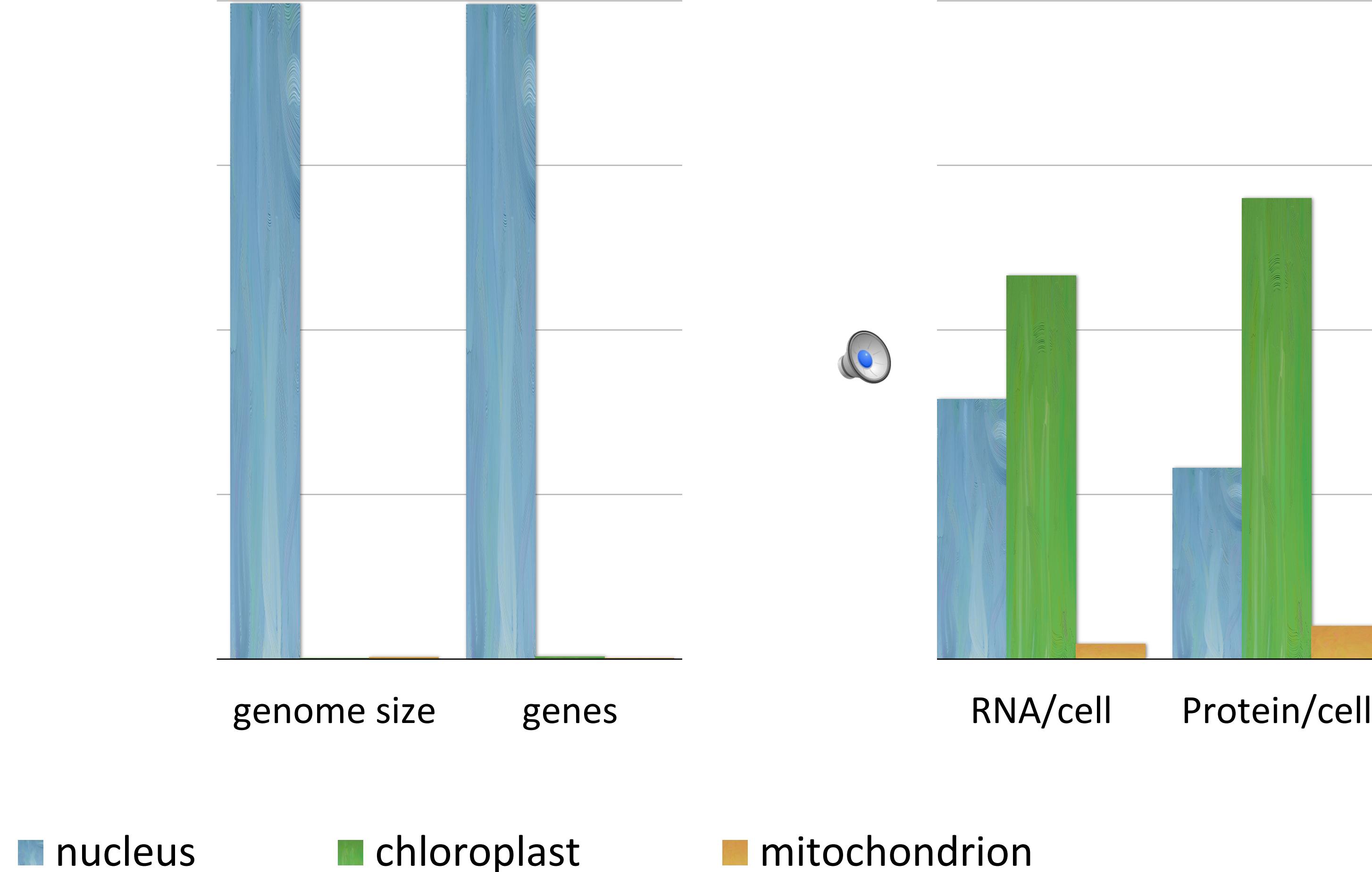
■ nucleus

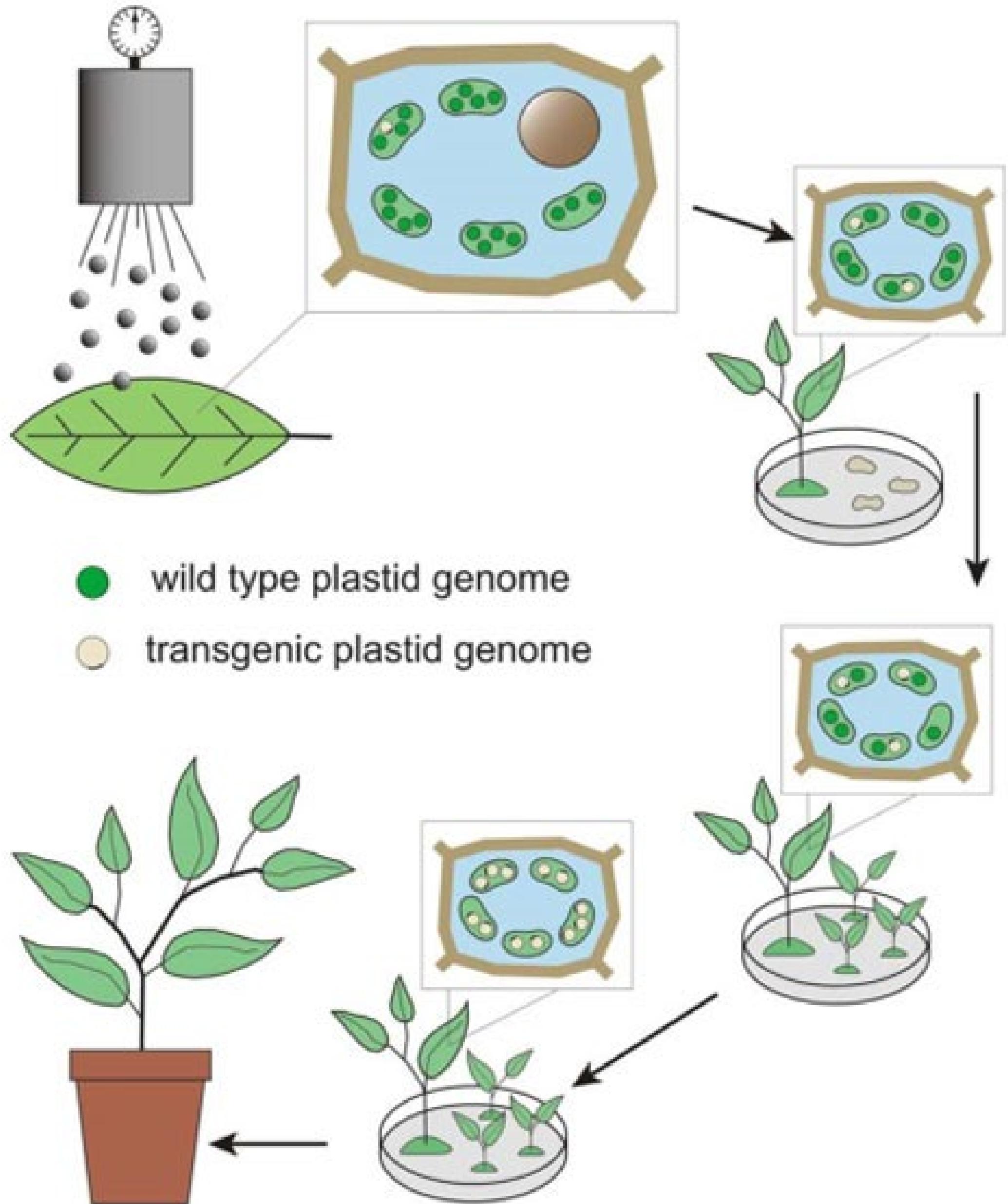
■ chloroplast

■ mitochondrion

Tiny genomes, huge impact

Why are chloroplast genomes so well-suited to Synbio?
3. They have prodigious production potential





Why are chloroplast genomes so well-suited to Synbio?

- They can be genetically transformed

Specificities of chloroplast transformation

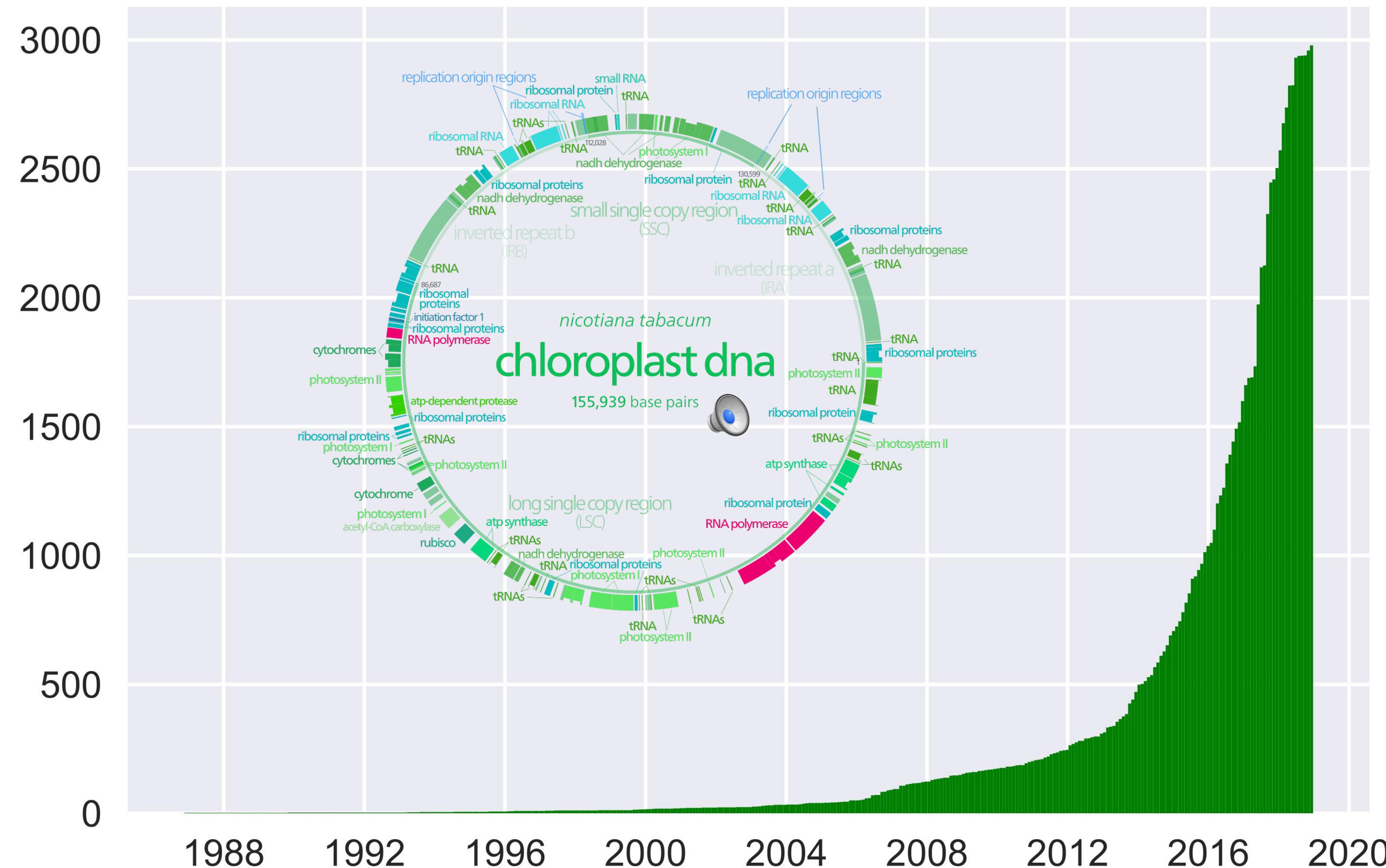
- biolistic transformation
- chloroplast-specific selectable markers
- active homologous recombination
- heteroplasmny



Why are chloroplast genomes so well-suited to Synbio?

5. They are extremely well understood

The accelerating pace of chloroplast genome sequencing

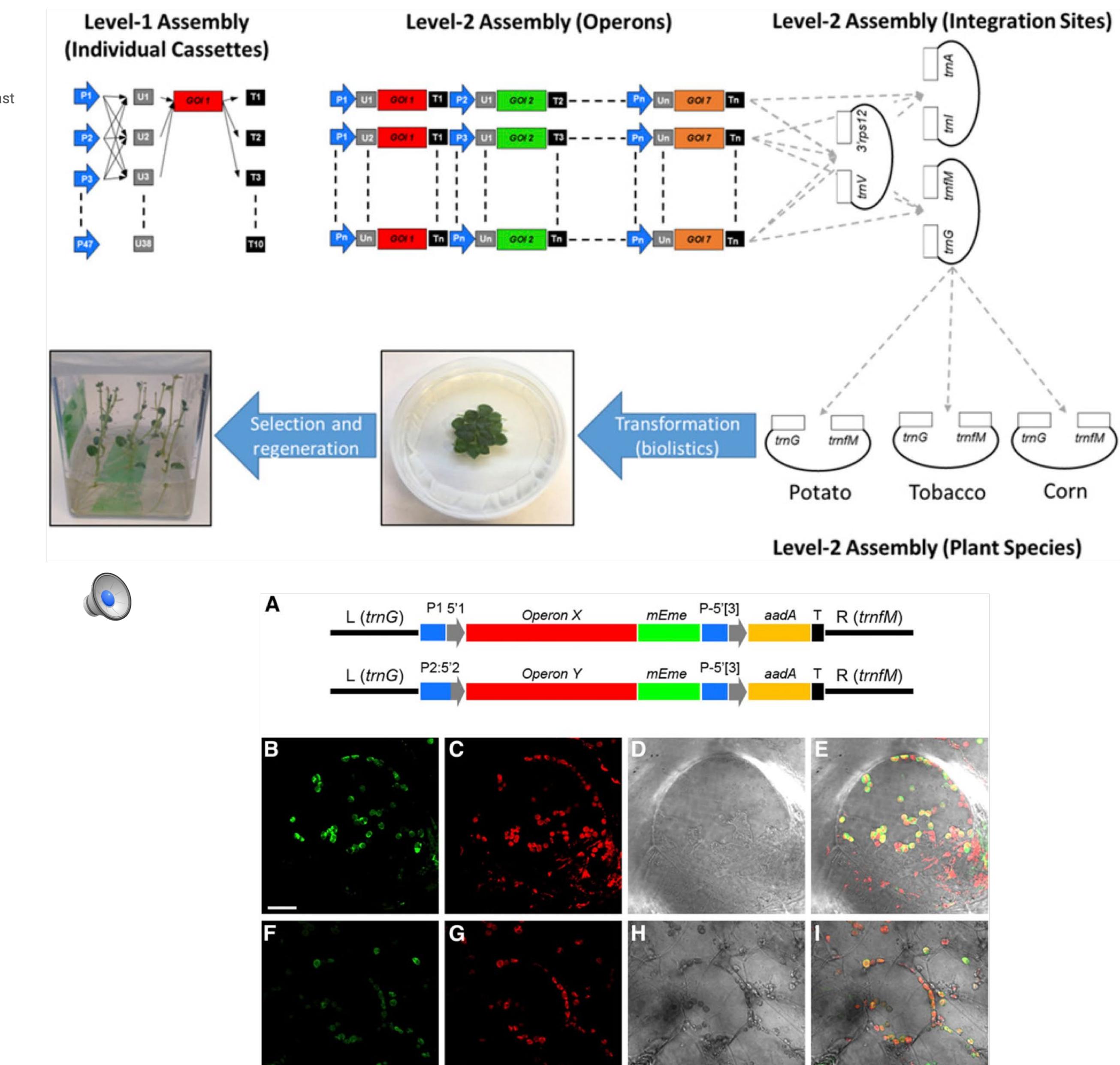
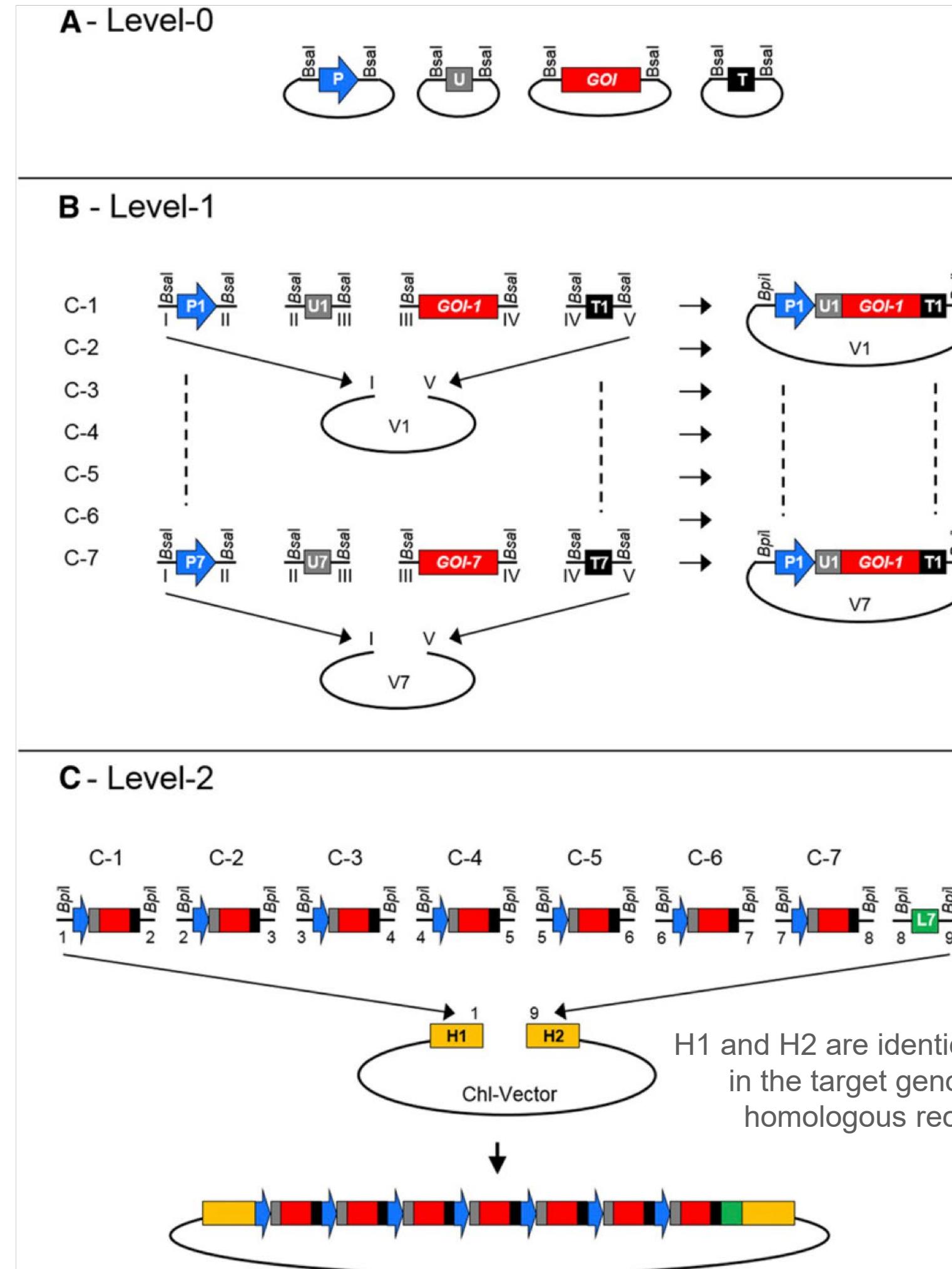


MoChlo: Modular Cloning Chloroplast Toolbox

(Kit #1000000156)

Depositing Lab: Scott Lenaghan

The MoChlo Toolbox consists of a library of standardized chloroplast-specific genetic modules for Golden-Gate cloning of synthetic operons into chloroplast transformation vectors. This kit includes a library of 115 level-0 modules (47 promoters, 36 5'UTRs, 9 promoter-5'UTR fusions, 10 3'UTRs and 13 genes of interest) along with 7 level-2 destination vectors specific for either tobacco (*Nicotiana tabacum*) or potato (*Solanum tuberosum*).



MoChlo: A versatile modular cloning toolbox for chloroplast biotechnology

Alessandro Occhialini, Agnieszka Piatek, Alexander C Pfotenhauer, Taylor P Frazier, C. Neal Stewart, Scott Lenaghan

Recent Advances and Current Challenges in Synthetic Biology of the Plastid Genetic System and Metabolism^[OPEN]

Christian R. Boehm and Ralph Bock^{1,2}

Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam-Golm, Germany

Published March 2019. DOI: <https://doi.org/10.1104/pp.18.00767>

	Bacteria	Yeast	Plant (nucleus)	Plant (plastid)
Biological features				
Genomic simplicity	**	*	*	***
Physiological simplicity	***	**	*	**
Homologous recombination	**	***	*	***
Polycistronic gene expression	***	*	*	***
Gene expression strength	**	**	*	***
Absence of transgene silencing	***	**	*	***
Biological transgene containment	*	*	*	**
Photoautotrophic growth	**	*	***	***
Available tools and techniques				
Ease of genetic manipulation	***	***	**	*
Speed of genetic manipulation	***	***	**	*
Genetic parts	***	**	*	*
Genetic part characterization	***	**	*	*
Genetic part assembly	***	***	**	**
Genetic part insulation	***	**	**	*
Gene expression control	***	**	**	*
Genetic & metabolic network models	***	**	*	*
Demonstrated applications				
Synthetic metabolic pathways	***	***	**	***
Synthetic genetic circuits	***	**	*	*
Synthetic subcompartments	**	**	*	*
Synthetic genomes	**	*	*	**

OUTSTANDING QUESTIONS

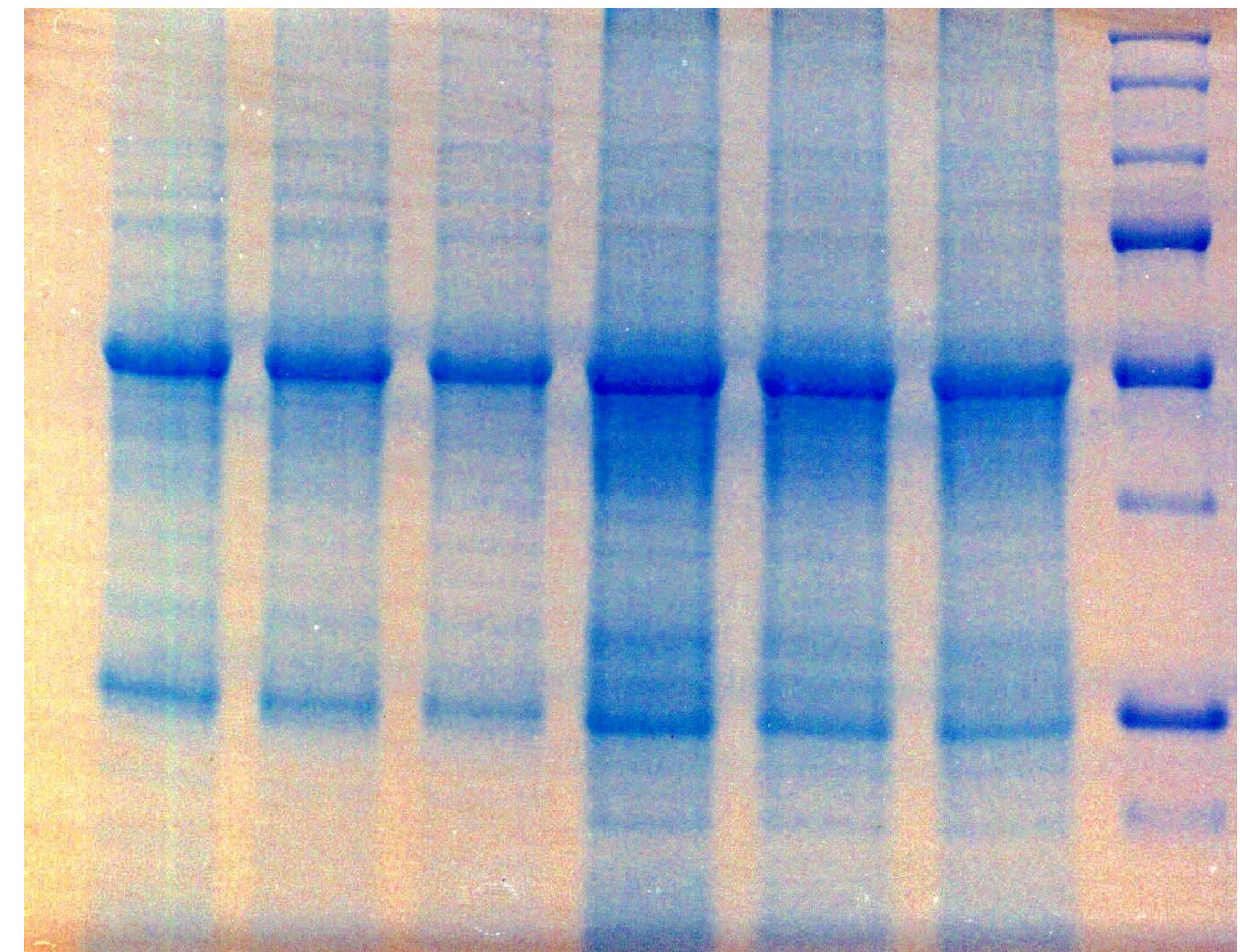
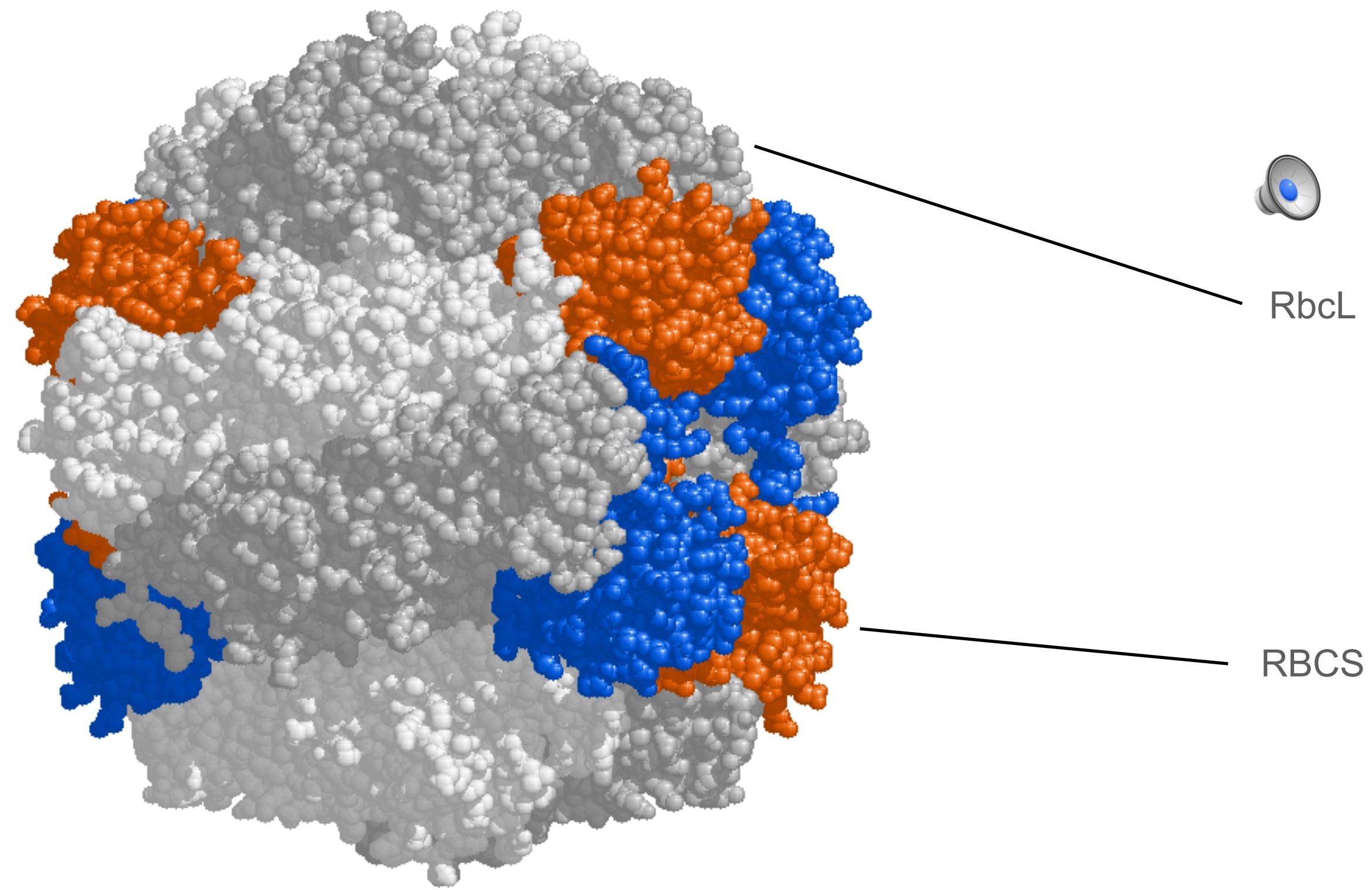
- How do we best characterize plastid genetic parts or devices in a consistent and efficient manner?
- How can individual plastid genetic parts or devices be functionally insulated from one another?
- Can we engineer RNA-binding proteins to tightly control plastid gene expression?
- How can we obtain quantitative parameters for modeling plastid gene expression?
- Can synthetic feedback loops and subcompartments enhance future plastid-based metabolic engineering efforts?
- Can synthetic genomes be introduced into plastids and booted up without undergoing recombination with the resident genome?

Chloroplasts as a protein production system

Rubisco (D-ribulose 1,5-bisphosphate carboxylase/oxygenase) is responsible for the vast majority of global carbon fixation and has been claimed to be the most abundant protein on Earth. We provide an updated and rigorous estimate for the total mass of Rubisco on Earth, concluding it is $\approx 0.7 \text{ Gt}$

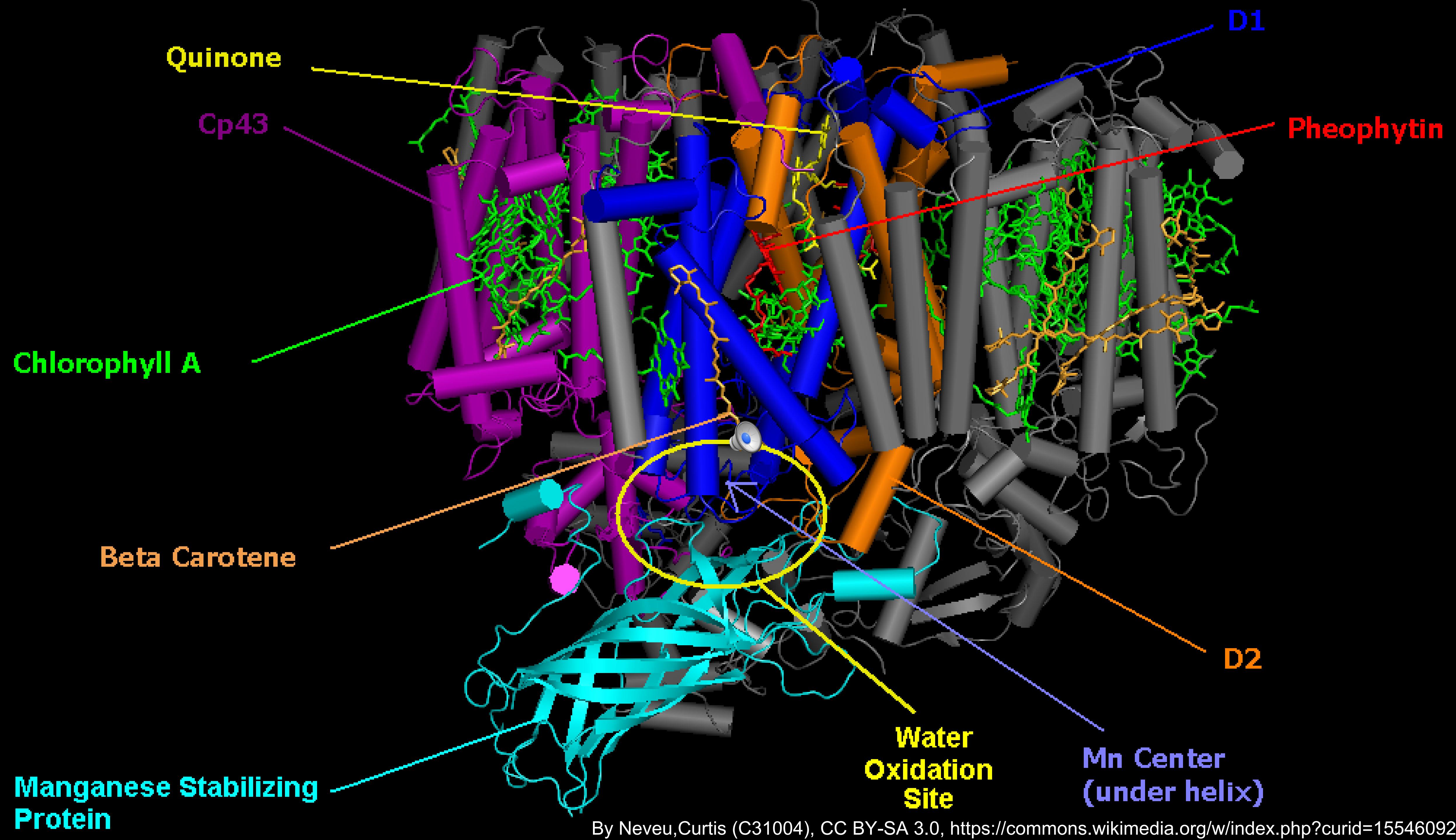
Yinon M. Bar-On, Ron Milo; PNAS March 5, 2019 116 (10) 4738-4743

>90% of Rubisco is found in the leaves of terrestrial plants, accounting for **30–50%** of the soluble protein in plant leaf



RNA-seq coverage of the *Arabidopsis* chloroplast genome





Plastids: The Green Frontiers for Vaccine Production

Mohammad T. Waheed^{1*}, Hammad Ismail¹, Johanna Gottschamel², Bushra Mirza¹ and Andreas G. Lössl^{3†}

¹ Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, ² NIBIO - Norwegian Institute of Bioeconomy Research, Ås, Norway, ³ Department of Applied Plant Sciences and Plant Biotechnology, University of Natural Resources and Applied Life Sciences, Tulln an der Donau, Austria

November 2015 | Volume 6 | Article 1005



FIGURE 1 | Diagrammatic presentation of constituents of expression cassette along with their respective expression levels for vaccine antigens.

Combinations of similar insertion sites, promoters, regulatory elements and terminators are shown in one color. SM, selection marker; RE, regulatory elements; UTR, untranslated region; psbA, psbA gene; TpsbA, Terminator of psbA gene; rrn16, rrn 16 gene; T7g10, leader sequence of gene 10 of the lambda phage T7; rbcL, rbcL gene; TrbcL, Terminator of rbcL gene; TrrnB, *Escherichia coli* rrnB terminator; TSP, total soluble protein; TLP, total leaf protein.

What is an edible vaccine?

An edible vaccine must trigger an immune response in the patient. Edible vaccines are based on food products that have been genetically transformed by adding specific genes into the genome to produce antigenic proteins. Plant-based edible vaccines can be cereals like wheat and barley, fruits like bananas or vegetables like lettuce and potatoes. Edible vaccines not only trigger systemic immunity, but also mucosal immunity when the antigenic material hits the digestive tract after ingestion. Mucosal immunity is very important as it is often the first line of defence against infecting pathogens.

Table 1 Developmental status of edible vaccines in clinical trials

Pathogen	Antigen	Host	Use	Clinical trial status
Enterotoxigenic E. coli	LT- B	Potato	Diarrhoea	Early phase 1
Enterotoxigenic E. coli	LT- B	Maize	Diarrhoea	Early phase 1
Norwalk Virus	CP	Potato	Diarrhoea	Early phase 1
Rabies Virus	GP/ NP	Spinach	Rabies	Early phase 1
HBV	HBsAg	Lettuce	Hepatitis B	Early phase 1
HBV	HBsAg	Potato	Hepatitis B	Phase 1
<i>Vibrio cholerae</i>	CTB	Rice	Cholera	Phase 1
HBV	HBV	<i>Saccharomyces cerevisiae</i>	Chronic HBV	Phase 2
HCV	HCV	<i>Saccharomyces Cerevisiae</i>	Chronic HCV	Phase 2

Chloroplasts as an RNA production system

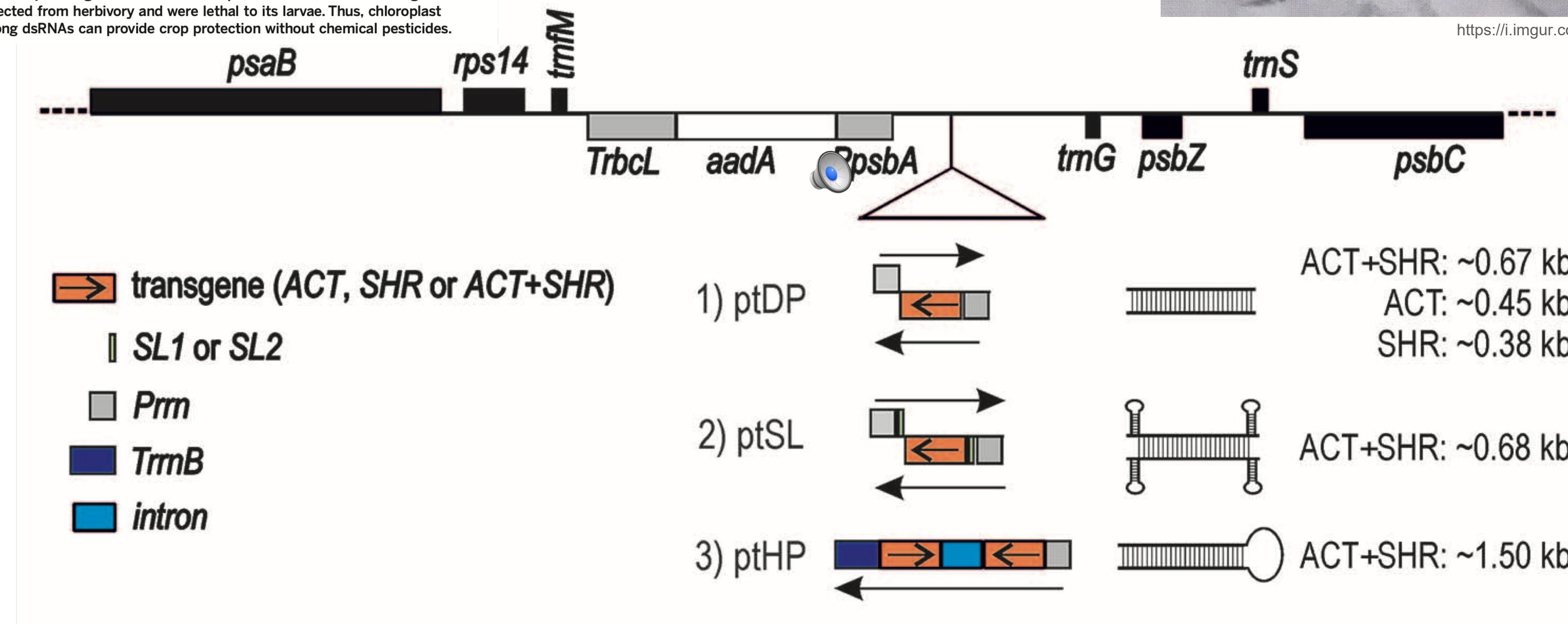
Full crop protection from an insect pest by expression of long double-stranded RNAs in plastids

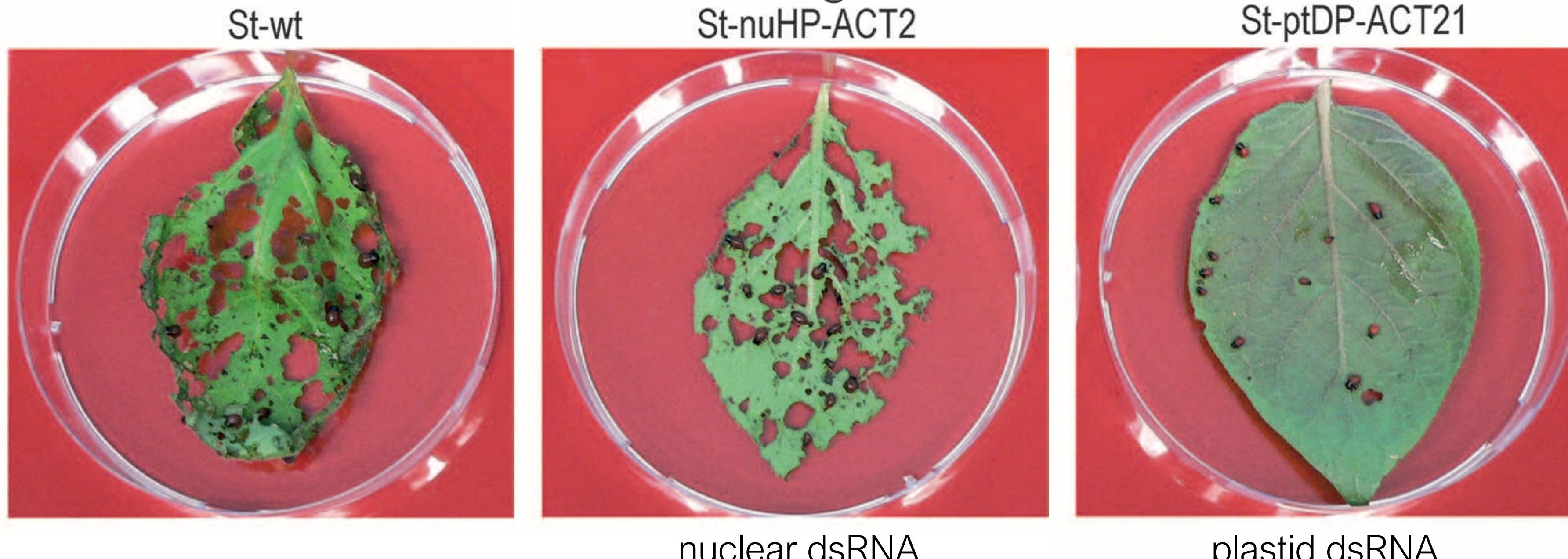
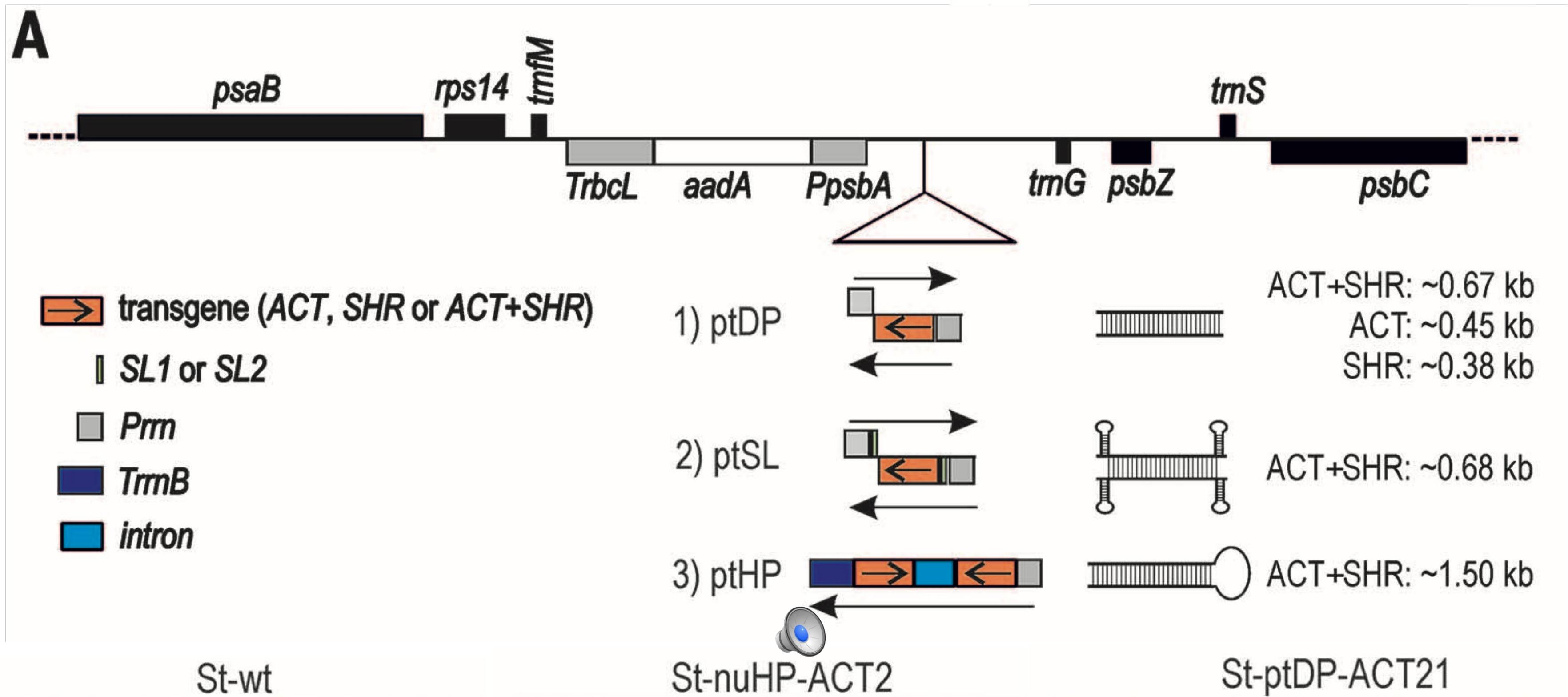
Jiang Zhang,¹ Sher Afzal Khan,² Claudia Hasse,¹ Stephanie Ruf,¹
David G. Heckel,² Ralph Bock^{1*}

Double-stranded RNAs (dsRNAs) targeted against essential genes can trigger a lethal RNA interference (RNAi) response in insect pests. The application of this concept in plant protection is hampered by the presence of an endogenous plant RNAi pathway that processes dsRNAs into short interfering RNAs. We found that long dsRNAs can be stably produced in chloroplasts, a cellular compartment that appears to lack an RNAi machinery. When expressed from the chloroplast genome, dsRNAs accumulated to as much as 0.4% of the total cellular RNA. Transplastomic potato plants producing dsRNAs targeted against the β -actin gene of the Colorado potato beetle, a notorious agricultural pest, were protected from herbivory and were lethal to its larvae. Thus, chloroplast expression of long dsRNAs can provide crop protection without chemical pesticides.



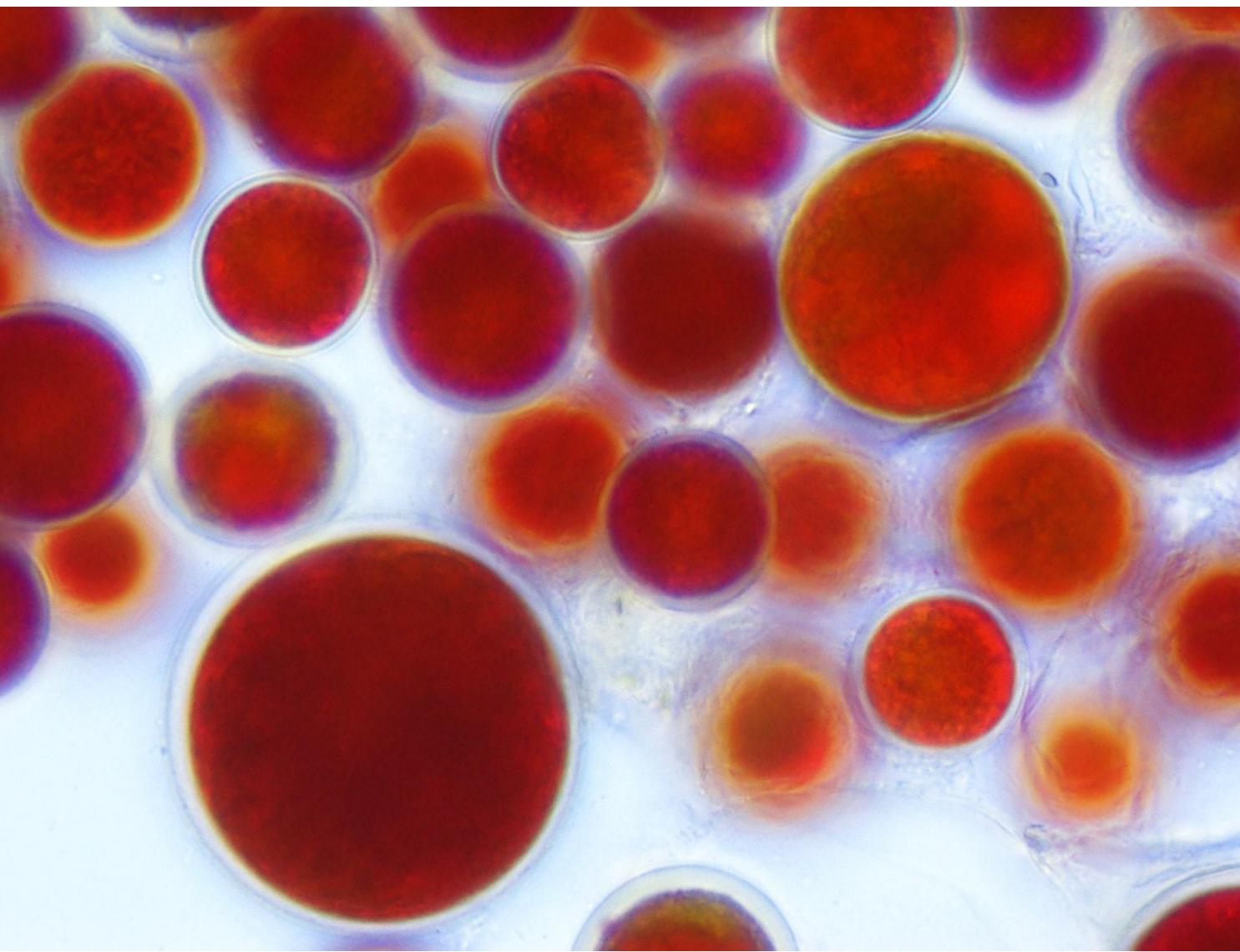
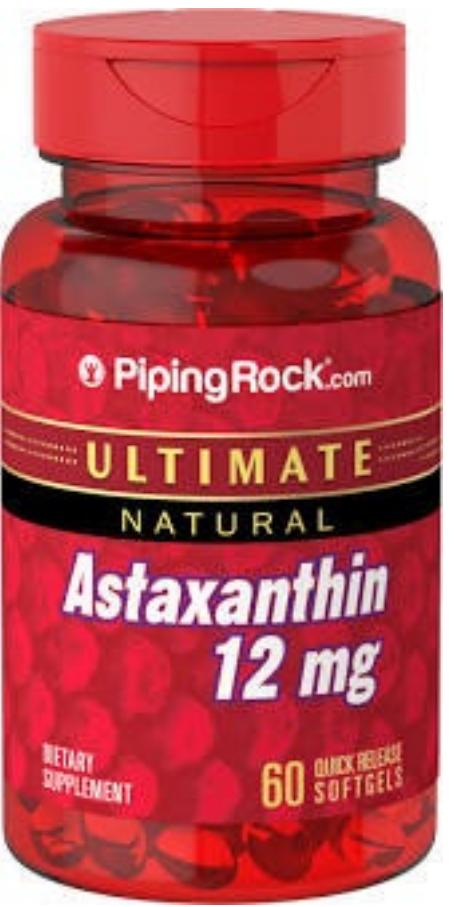
<https://i.imgur.com/QSZhPHa.mp4>





Astaxanthin is a blood-red pigment produced naturally in microalgae. Animals who feed on the algae, such as salmon, flamingos, and crustaceans (i.e. shrimp, krill, crab, lobster, and crayfish), subsequently reflect the red-orange astaxanthin pigmentation to various degrees.

Commercial-scale extraction of astaxanthin from marine microorganisms is very costly, and the market price of the pure compound is around US\$12,000 per kilogram



THE MOST POTENT ANTIOXIDANT NATURE HAS TO OFFER

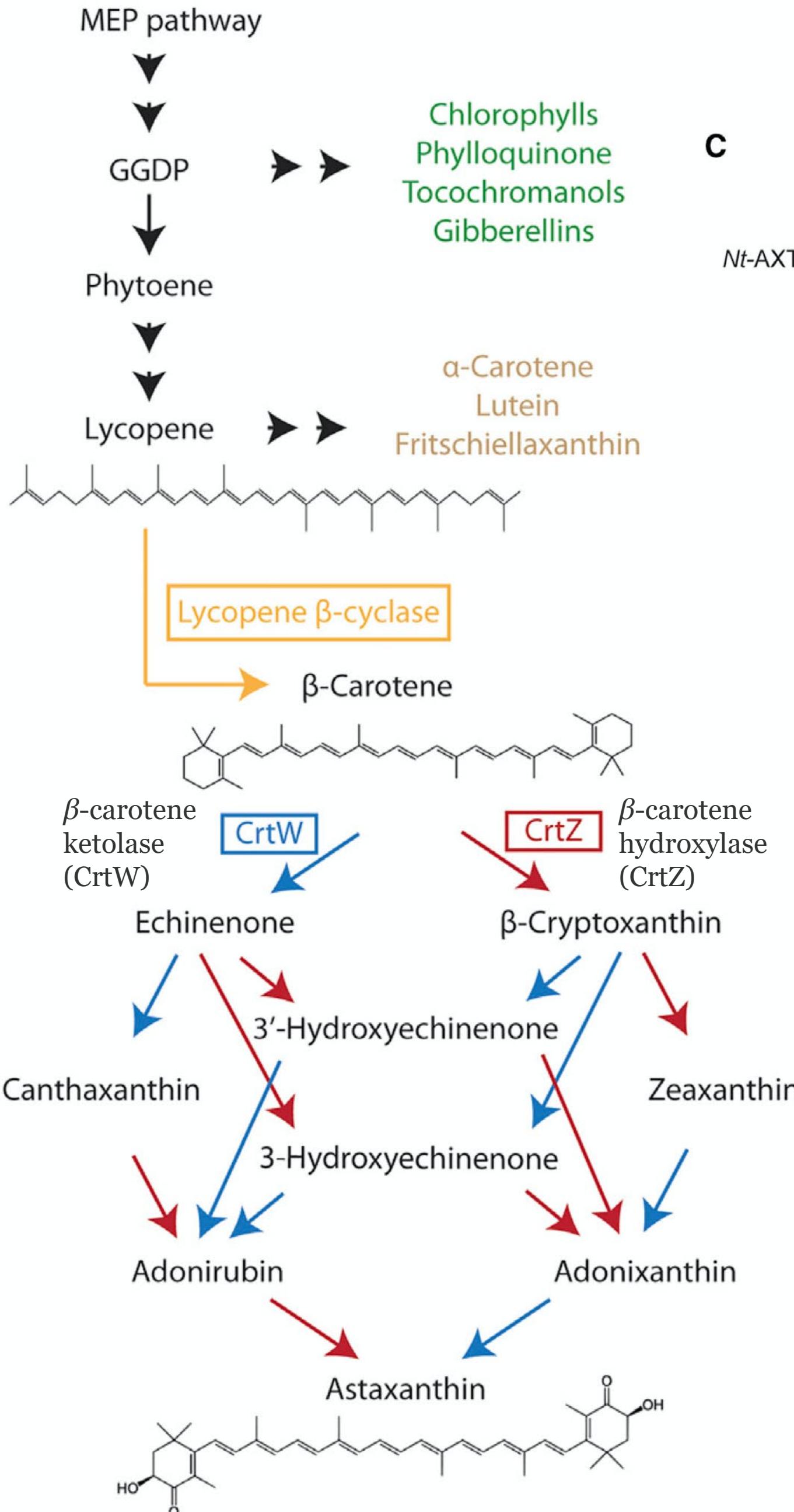
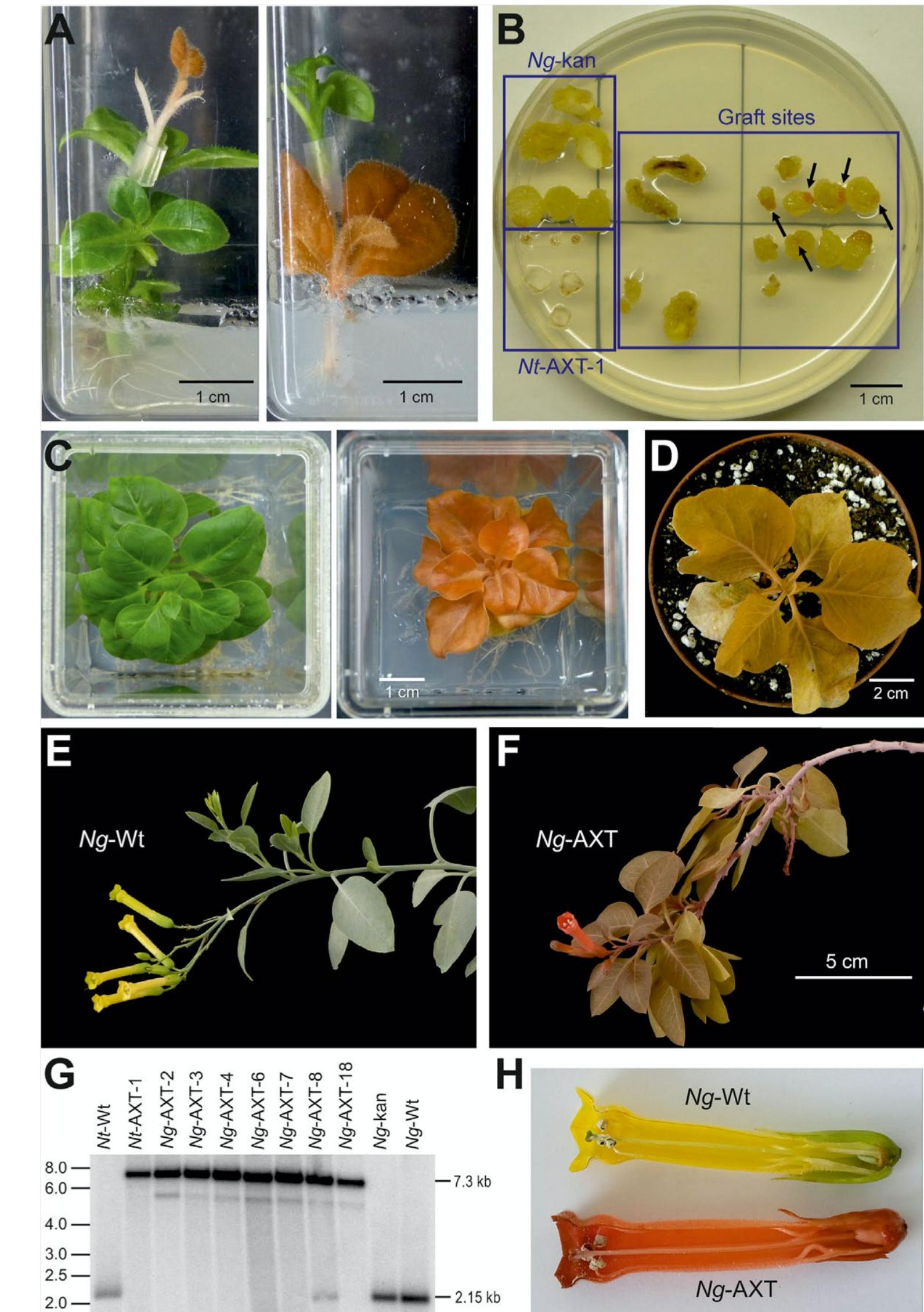
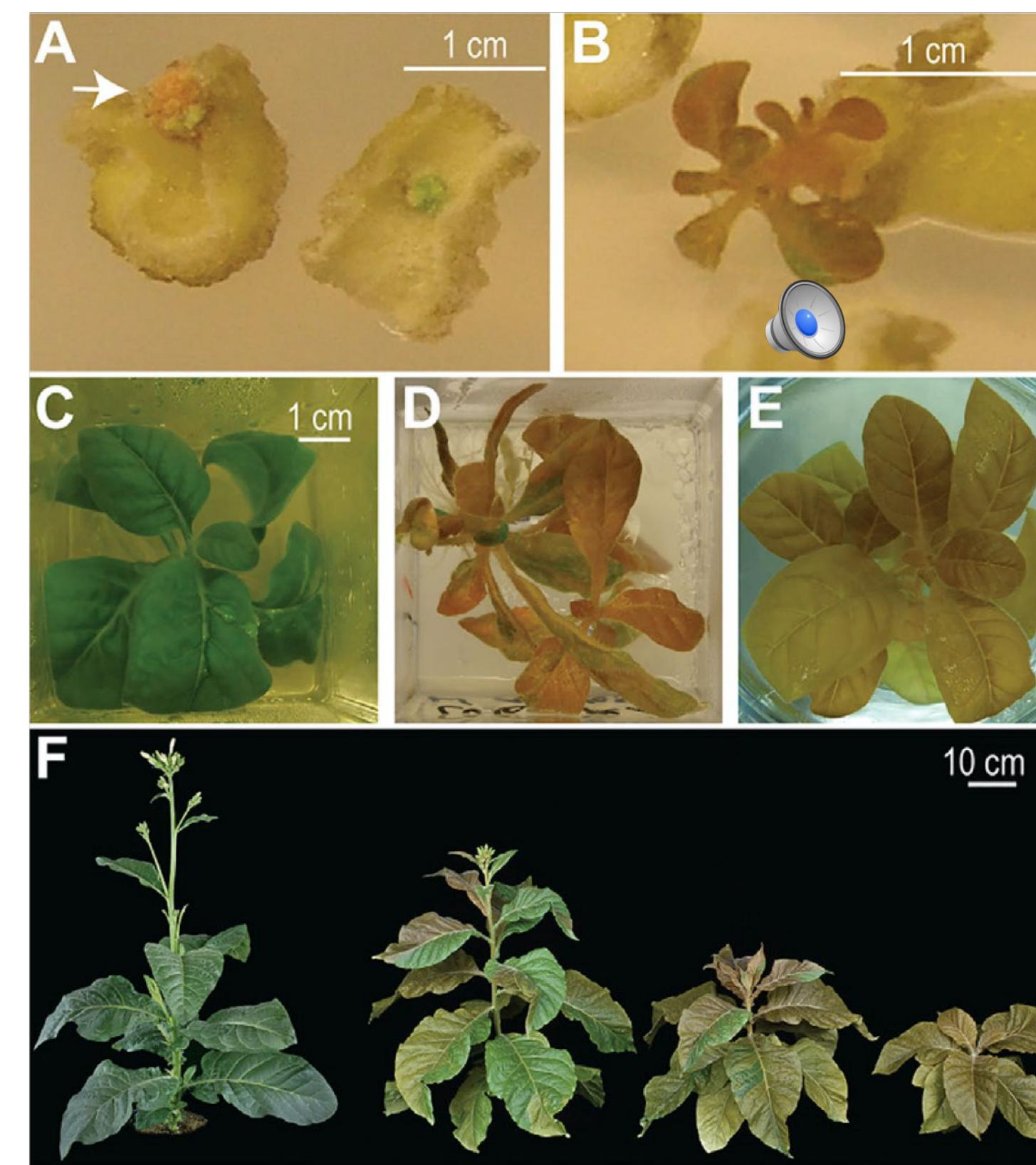
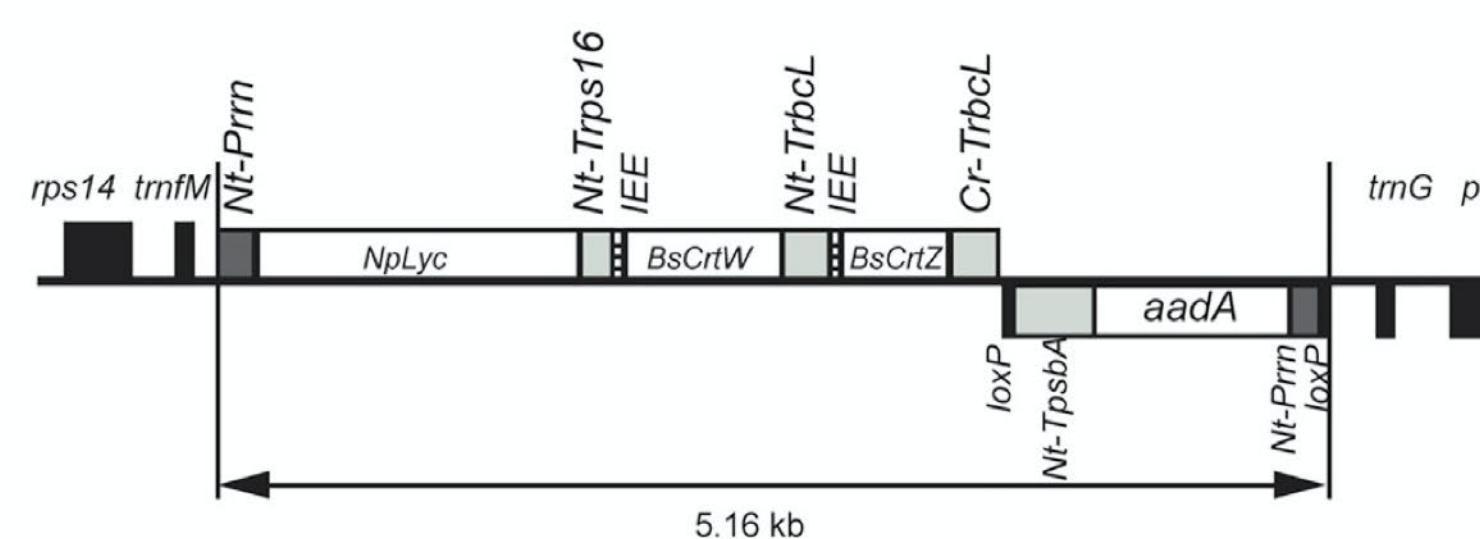
ASTAXANTHIN

- ✓ 6,000 TIMES STRONGER THAN VITAMIN C
- ✓ 800 TIMES STRONGER THAN GREEN TEA CATECHINS
- ✓ 550 TIMES STRONGER THAN VITAMIN E (A-TOCOPHEROL)
- ✓ 75 TIMES STRONGER THAN ALPHA LIPOIC ACID
- ✓ 40 TIMES STRONGER THAN BETA-CAROTENE
- ✓ 17 TIMES MORE POTENT THAN GRAPE SEED EXTRACTS
- ✓ SUPPRESSES DNA DAMAGE

STUDIES SHOW:

- ✓ ALLEVIATES SORE JOINTS AND MUSCLES
- ✓ ANTI-INFLAMMATORY
- ✓ ANTI-AGING (REVERSES EXTERNAL AGING, WRINKLES, AND SUN DAMAGE)
- ✓ BOOST IMMUNE SYSTEM
- ✓ HELPS BLOOD PRESSURE
- ✓ HELPS CARDIOVASCULAR SYSTEM
- ✓ PREVENTS CATARACTS, MACULAR DEGENERATION, AND GLAUCOMA
- ✓ REDUCES LACTIC ACID



**C**

Lu et al., 2017, Current Biology 27, 3034–3041
 October 9, 2017 © 2017 Elsevier Ltd.
<http://dx.doi.org/10.1016/j.cub.2017.08.044>

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,¹ John I. Glass,¹ Carole Lartigue,¹ Vladimir N. Noskov,¹ Ray-Yuan Chuang,¹ Mikkel A. Algire,¹ Gwynedd A. Benders,² Michael G. Montague,¹ Li Ma,¹ Monzia M. Moodie,¹ Chuck Merryman,¹ Sanjay Vashee,¹ Radha Krishnakumar,¹ Nacyra Assad-Garcia,¹ Cynthia Andrews-Pfannkoch,¹ Evgeniya A. Denisova,¹ Lei Young,¹ Zhi-Qing Qi,¹ Thomas H. Segall-Shapiro,¹ Christopher H. Calvey,¹ Prashanth P. Parmar,¹ Clyde A. Hutchison III,² Hamilton O. Smith,² J. Craig Venter^{1,2,*}

We report the design, synthesis, and assembly of the 1.08-mega-base pair *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *M. capricolum* recipient cell to create new *M. mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including “watermark” sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

2 JULY 2010 VOL 329 SCIENCE www.sciencemag.org

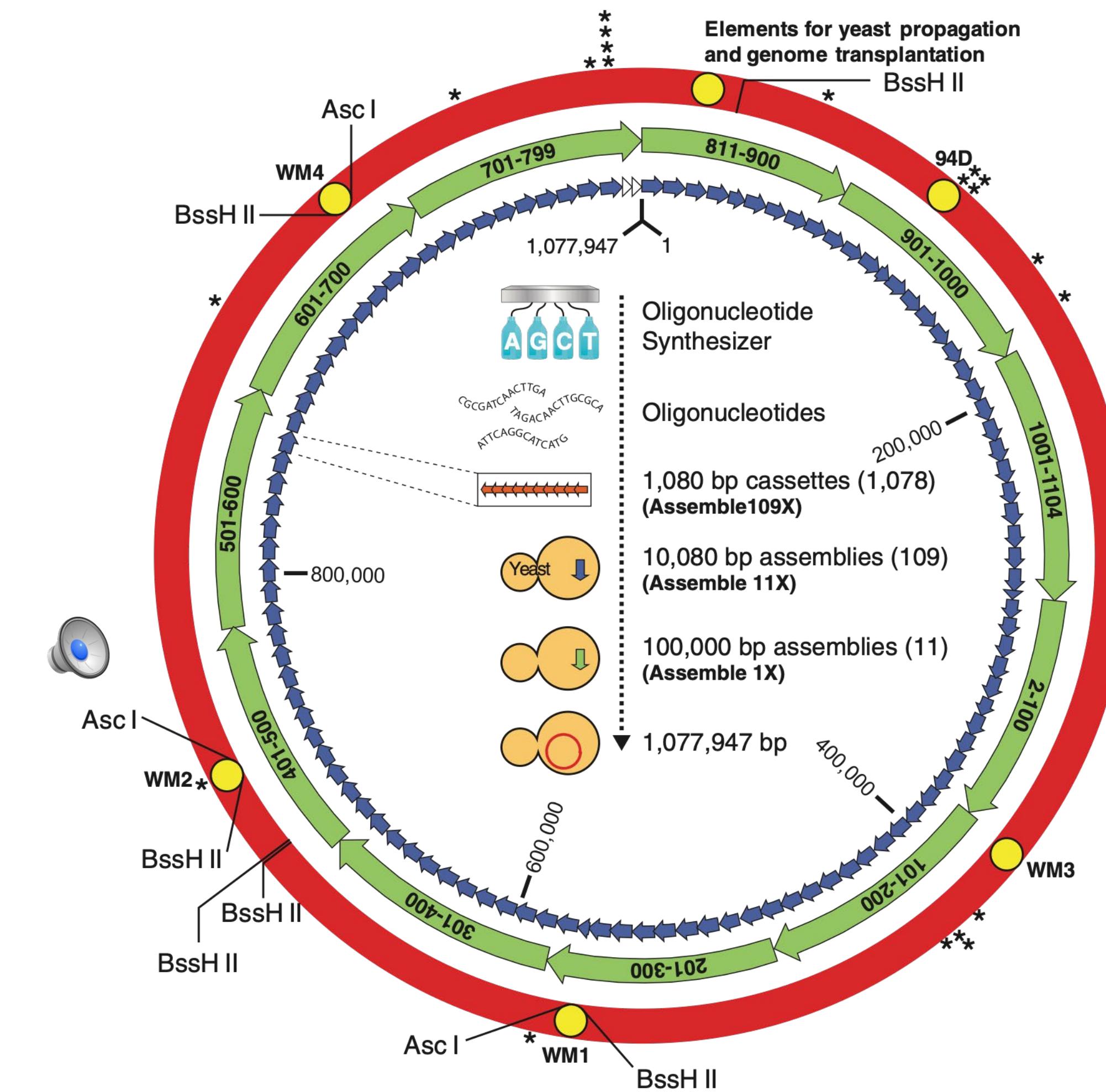
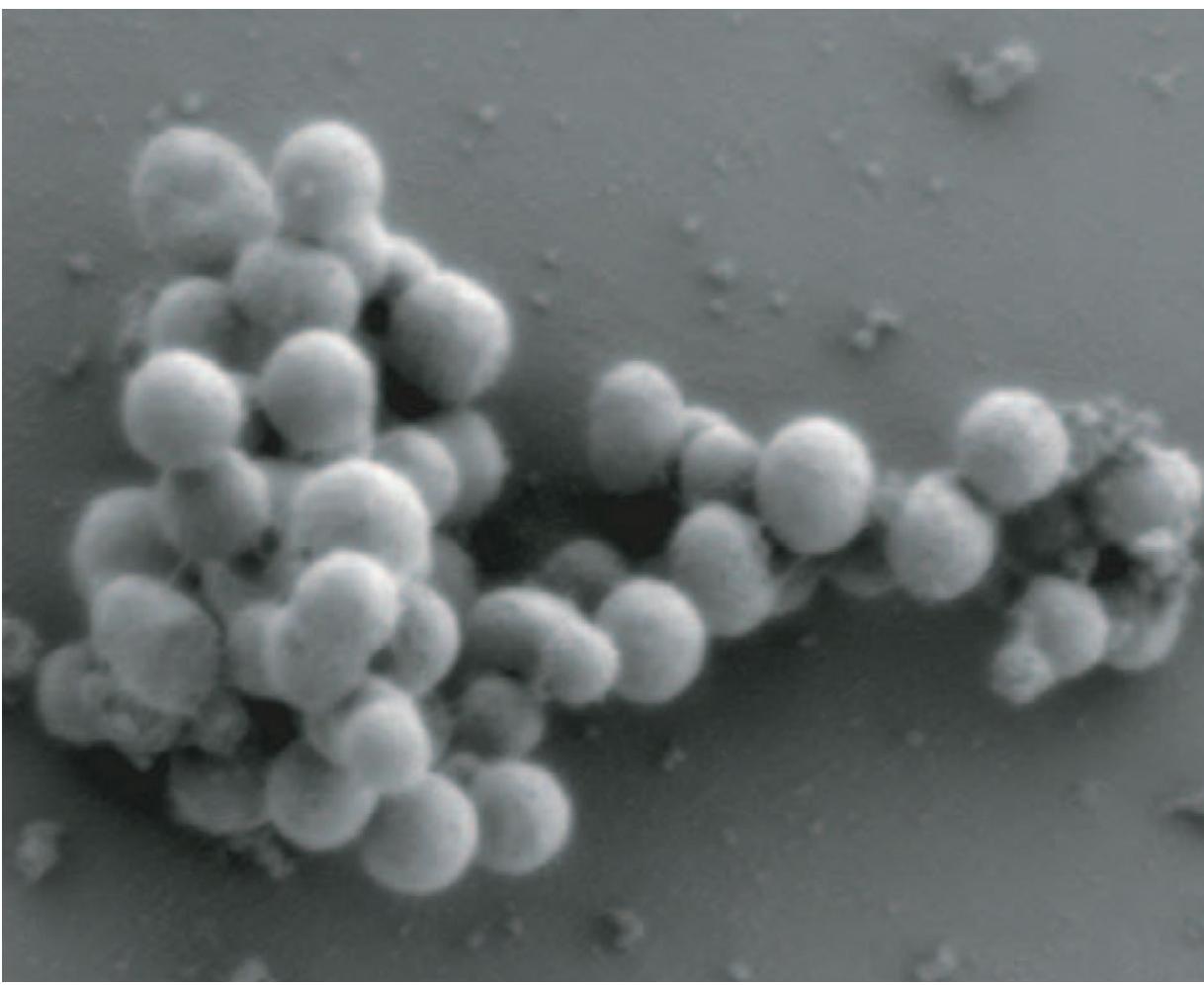
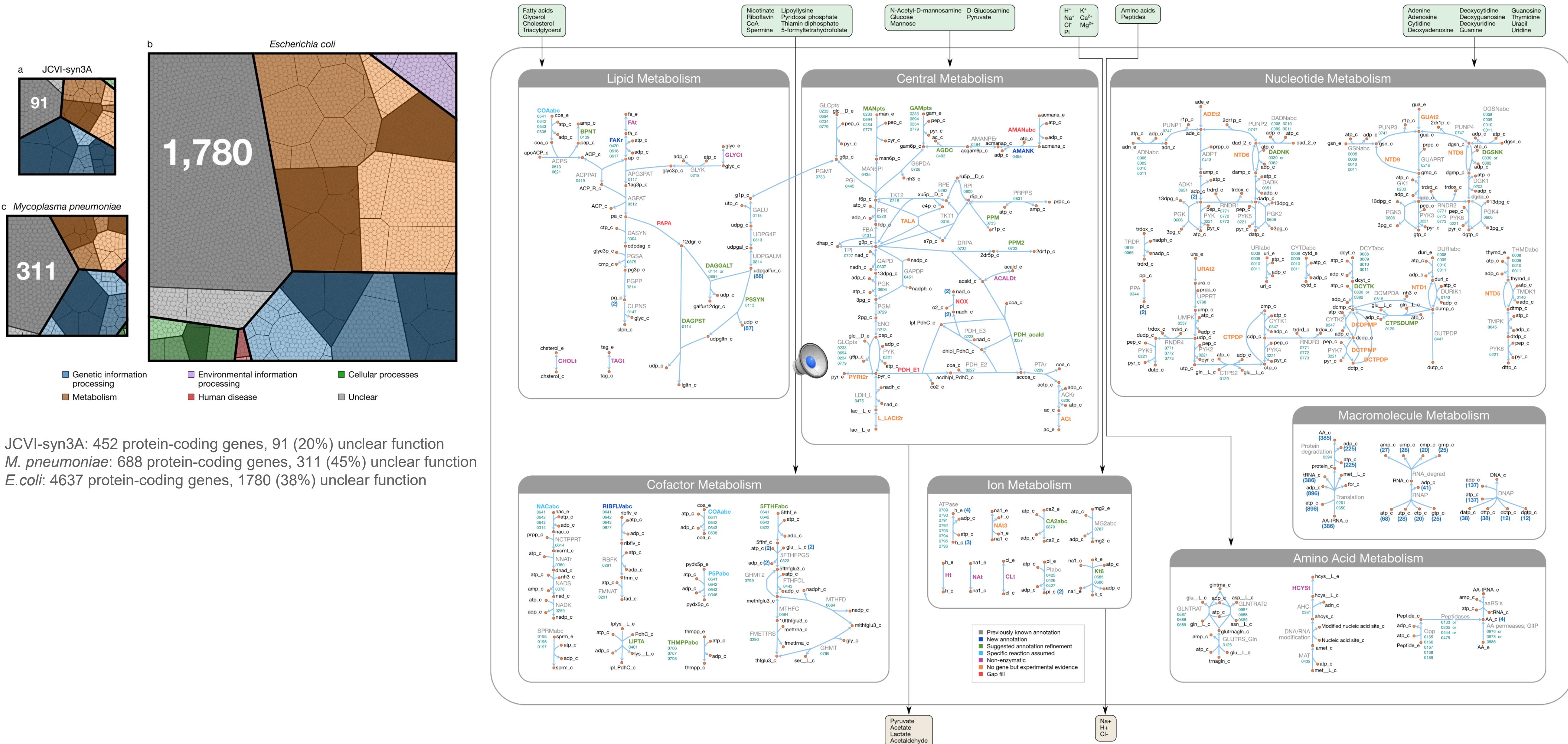
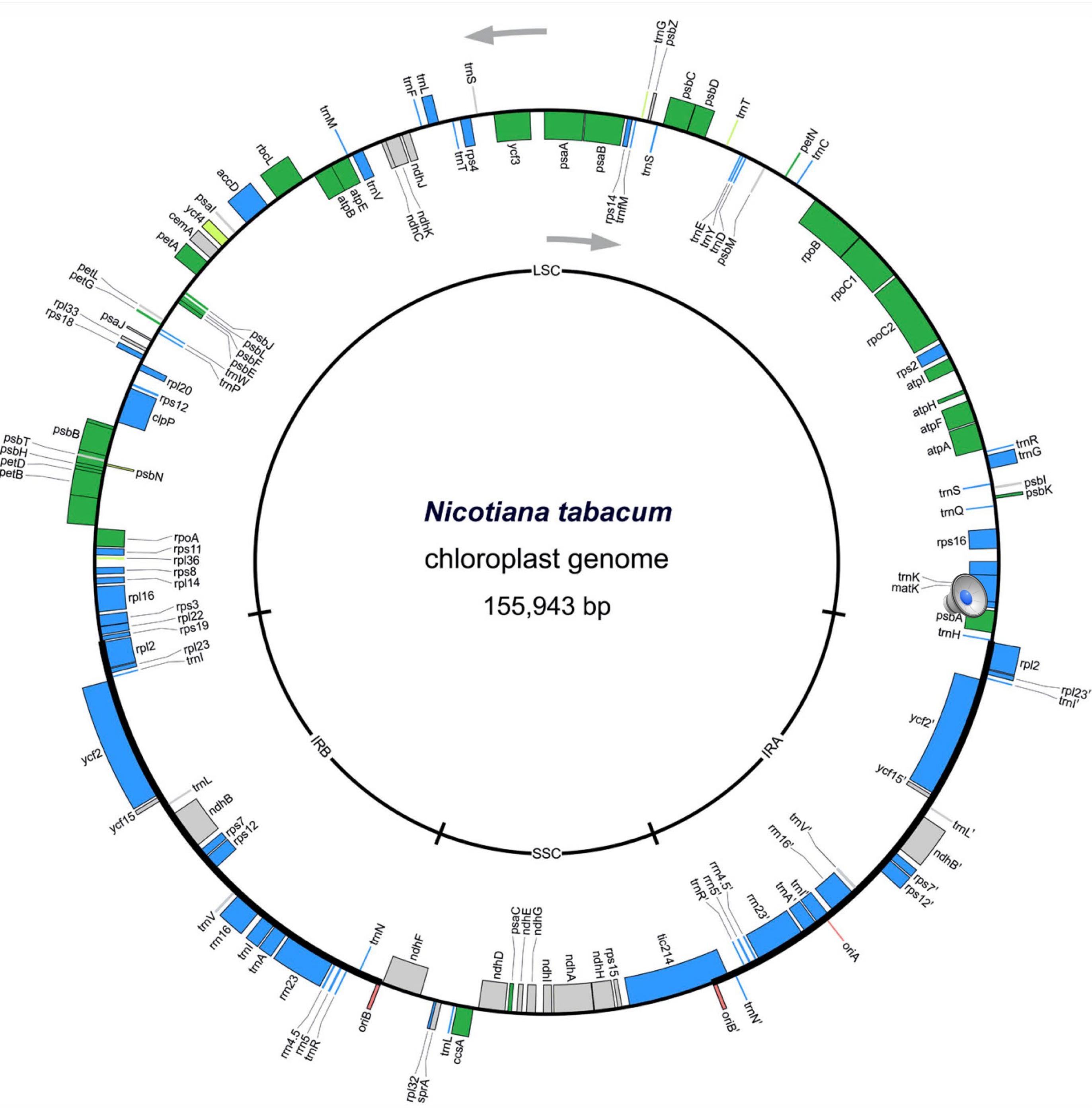


Fig. 1. The assembly of a synthetic *M. mycoides* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1078 overlapping DNA cassettes in three steps. In the first step, 1080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce 109 ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce 11 ~100-kb assemblies (green arrows). In the final stage of assembly, these 11 fragments were recombined into the complete genome (red circle).



Essential metabolism for a minimal cell





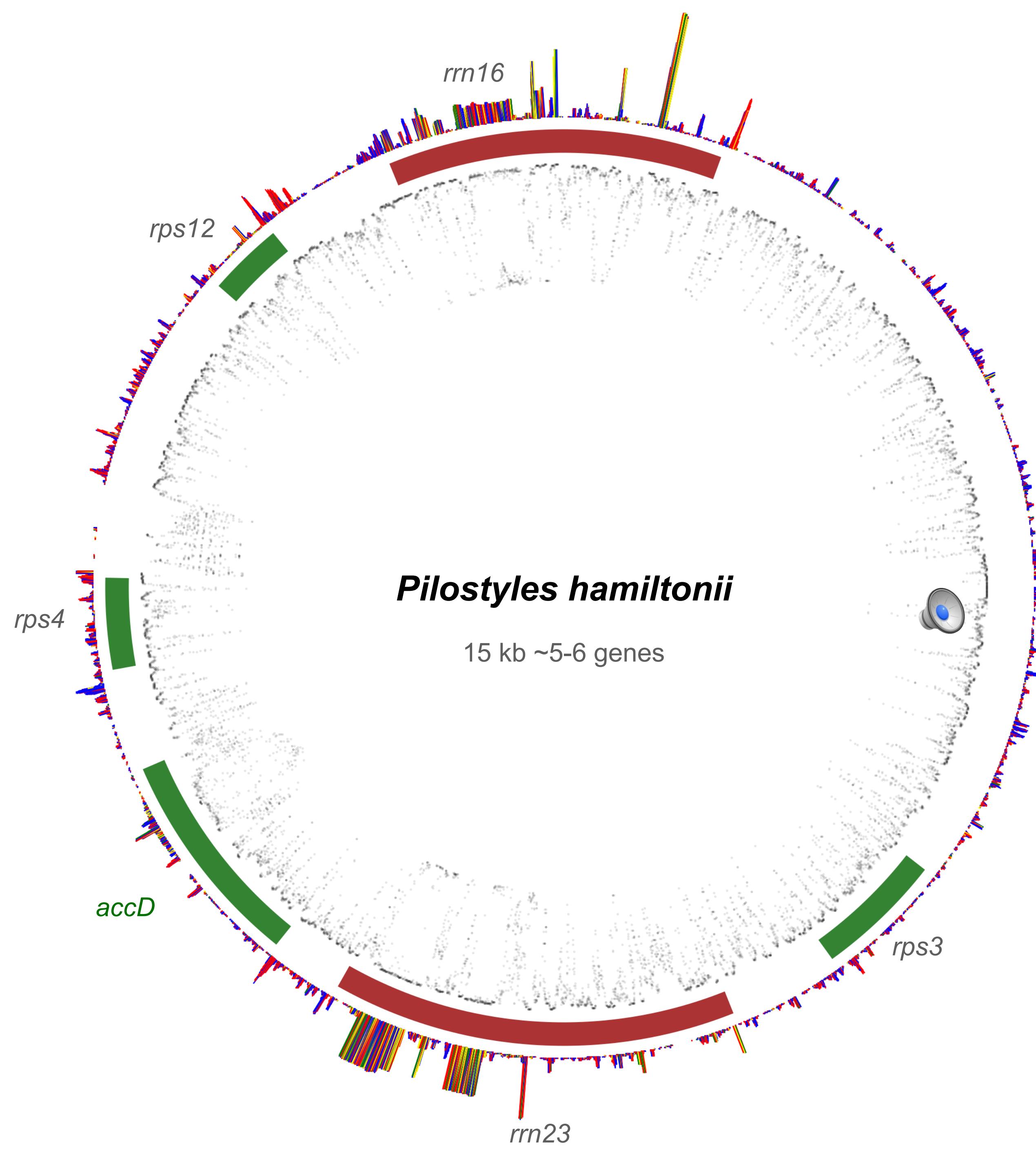
required for phototrophic growth

required for phototrophic and heterotrophic growth



Pilostyles hamiltonii

Kevin Thiele



The Plastomes of Two Species in the Endoparasite Genus *Pilostyles* (Apodanthaceae) Each Retain Just Five or Six Possibly Functional Genes
[Genome Biol Evol](#). 2016 Jan; 8(1): 189–201

Pilostyles retains a plastid genome to make one single protein: subunit D of acetyl-CoA carboxylase

(Re)design of the chloroplast genome - towards a synthetic organelle.

Lead Research Organisation: [University College London](#)

Department Name: Structural Molecular Biology

Funded Value:

£462,698

Funded Period:

Oct 18 - Sep 21

Funder:

BBSRC

Project Status:

Active

Project Category:

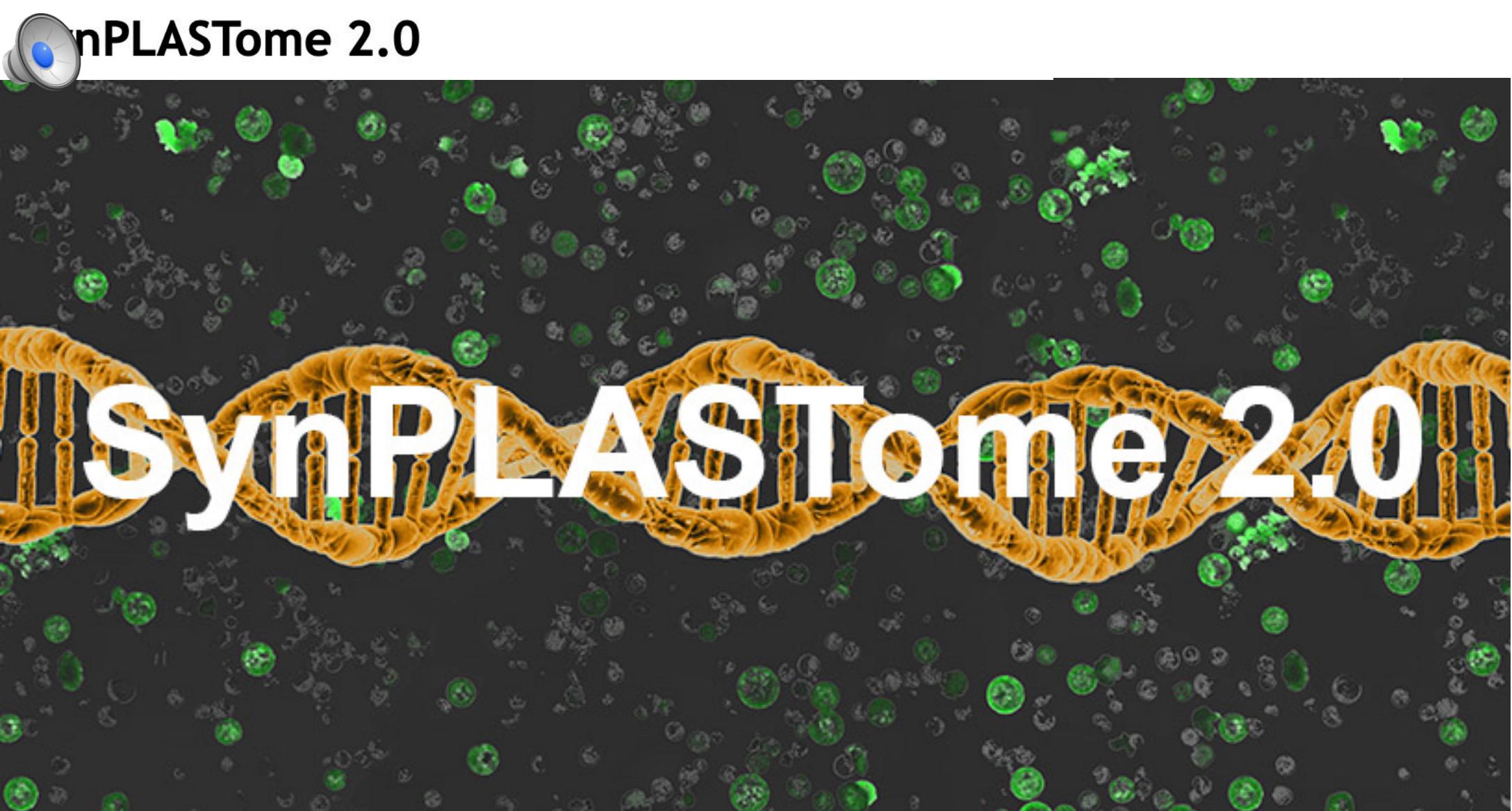
Research Grant

Project Reference:

BB/R016534/1

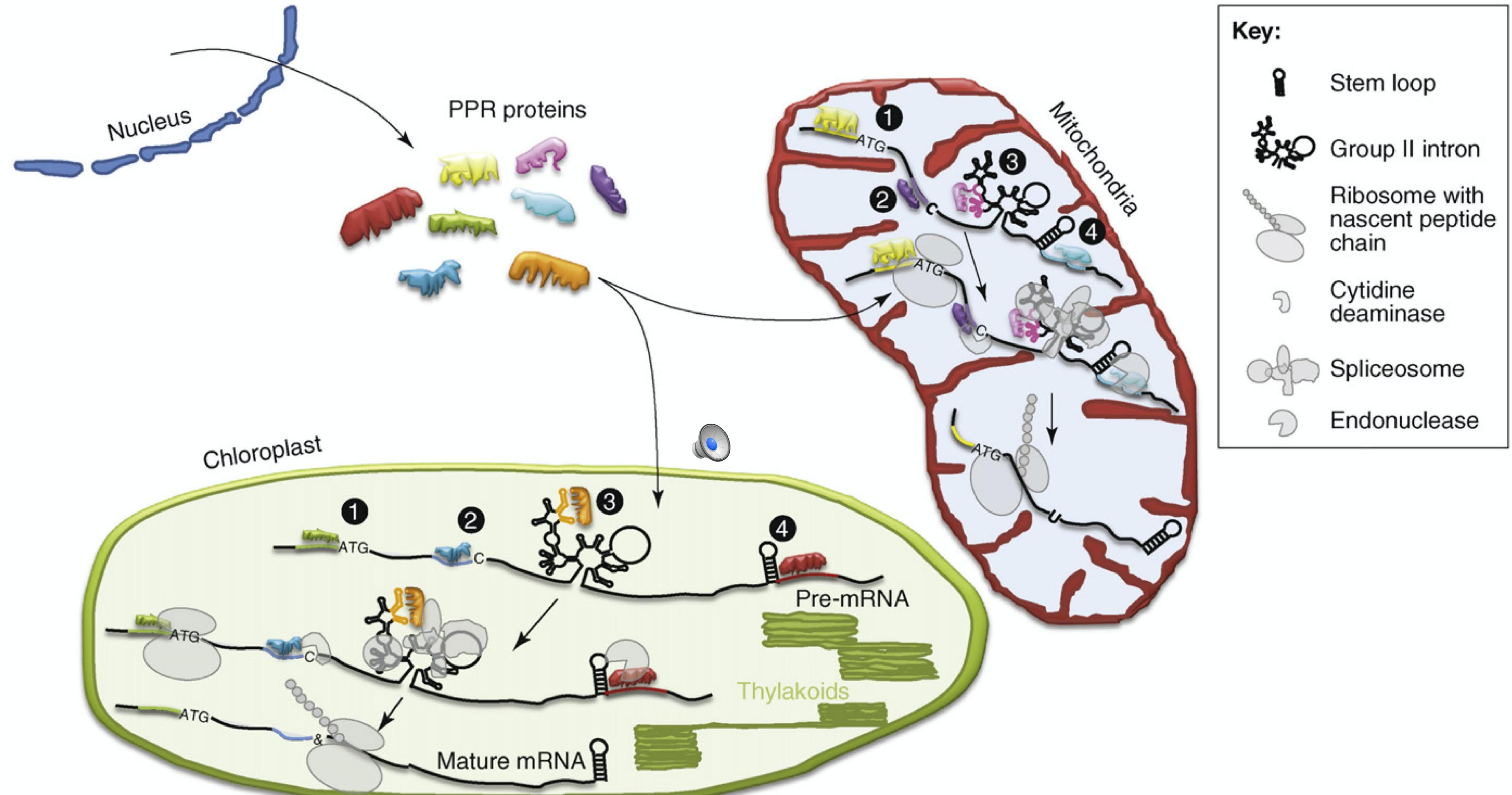
Principal Investigator:

[Saul Purton](#)



**WE'RE
HIRING!**

Gene expression in organelles is regulated not by transcription, but by RNA processing and translation initiation

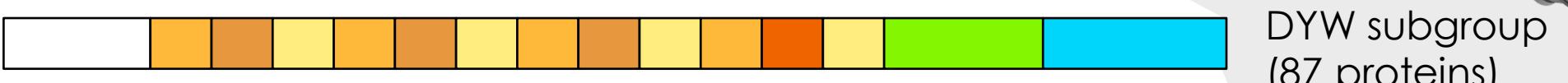
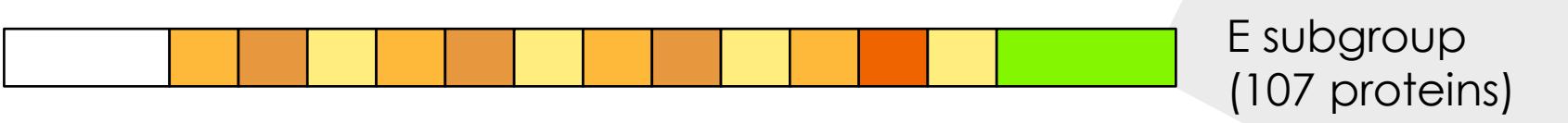
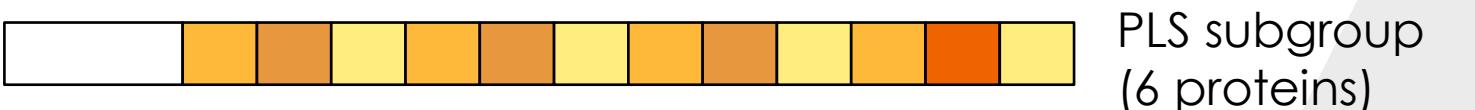


The PPR family (pentatricopeptide repeat)

P subfamily



PLS subfamily



Motifs P [orange] L1 [orange] L2 [orange] S [yellow] E [green] DYW [blue]

plant organelles contain hundreds of PPR RNA-binding proteins,
each of which binds a different RNA sequence

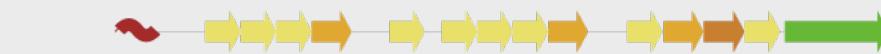
AtPPR_1g64310: OTP71



AtPPR_5g19020: MEF18



AtPPR_1g62260: MEF9



AtPPR_3g18970: MEF20



AtPPR_3g05240: MEF19



AtPPR_2g45350: CRR4



AtPPR_2g20540: MEF21



AtPPR_1g05750: CLB19



AtPPR_3g13880: OTP72



AtPPR_5g55740: CRR21



AtPPR_5g59200: OTP80



AtPPR_4g14850: MEF11



AtPPR_3g12770: MEF22



AtPPR_2g29760: OTP81



AtPPR_2g02980: OTP85



AtPPR_3g63370: OTP86



AtPPR_5g13270: RARE1



AtPPR_5g48910: LPA66



AtPPR_3g22690: YS1



AtPPR_1g15510: ECB2



AtPPR_1g11290: CRR22



AtPPR_1g59720: CRR28



AtPPR_1g08070: OTP82



AtPPR_3g57430: OTP84



AtPPR_5g52630: MEF1

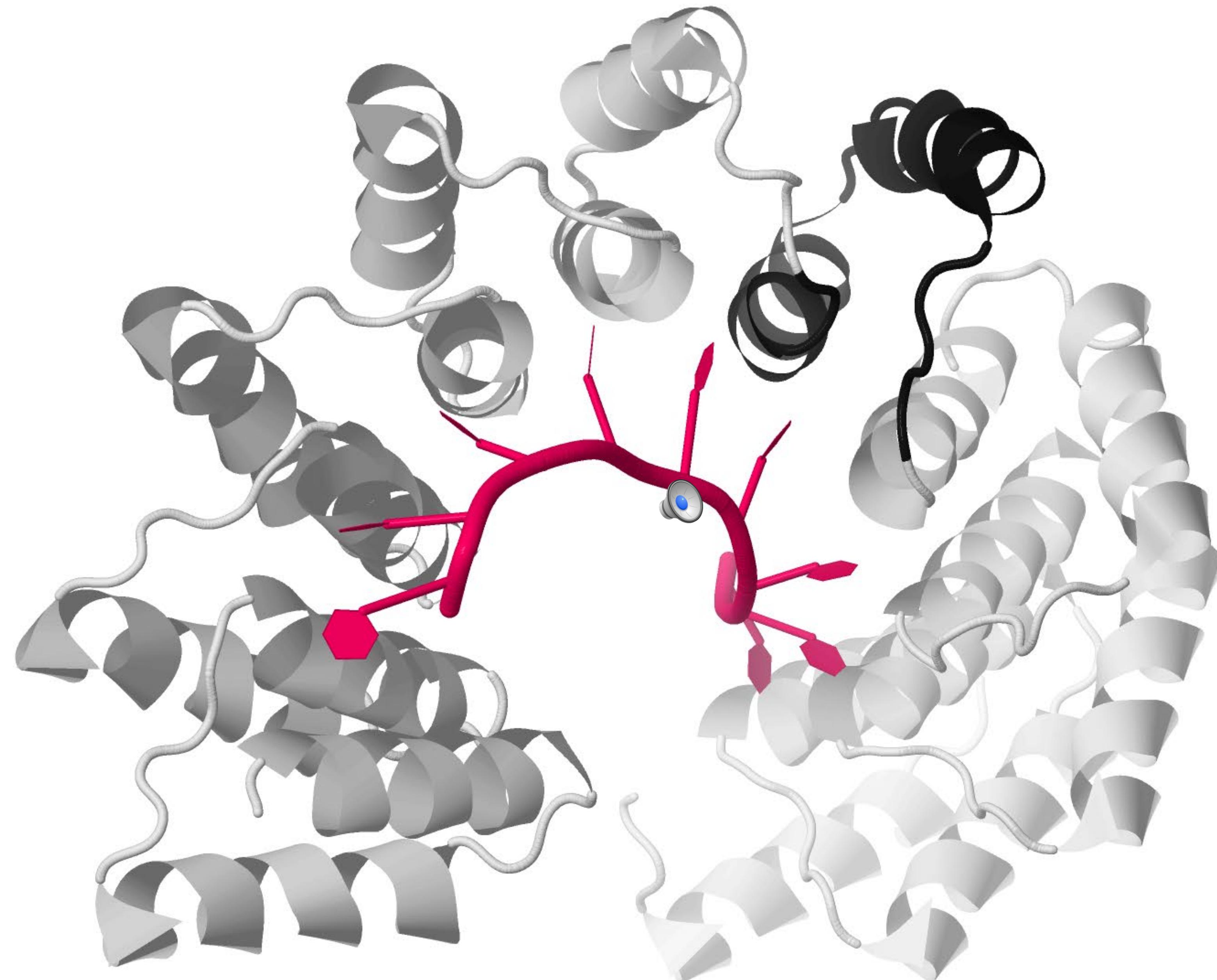


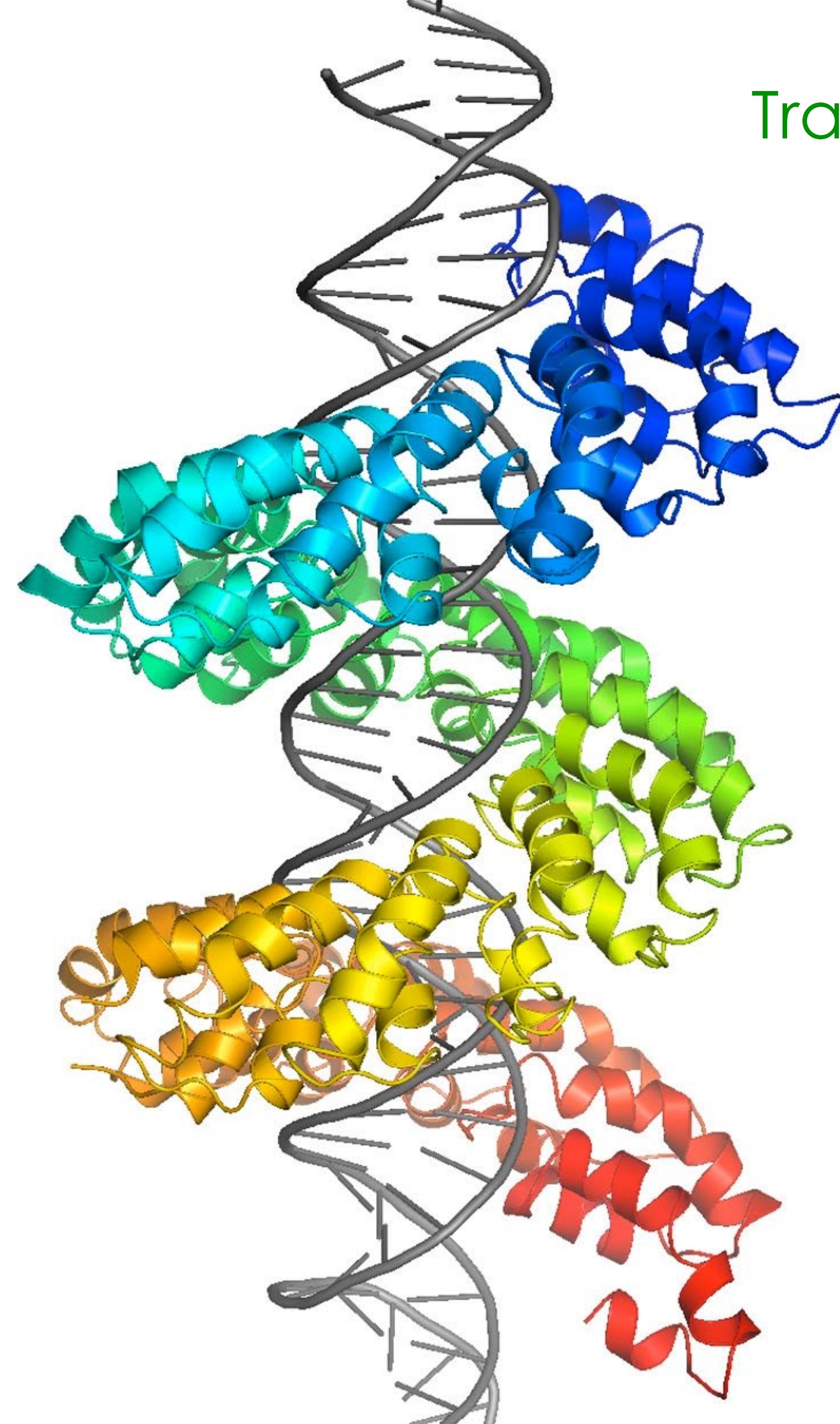
OsPPR_12g17080: OGR1



Targeting Sequence Mitochondria P L S E DYW
Plastid

Each PPR motif recognises one RNA nucleotide in the sequence





Transcription activator-like (TAL) effectors



nature.com ▶ journal home ▶ archive ▶ issue ▶ editorial ▶ full text

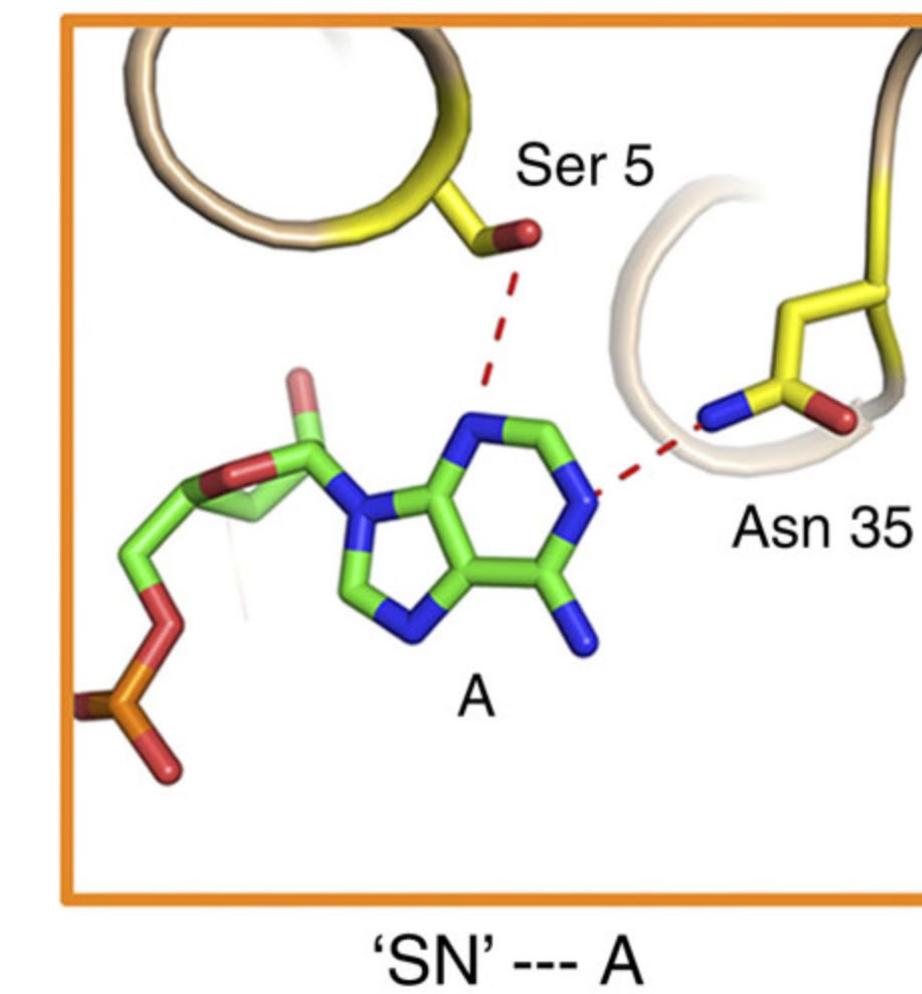
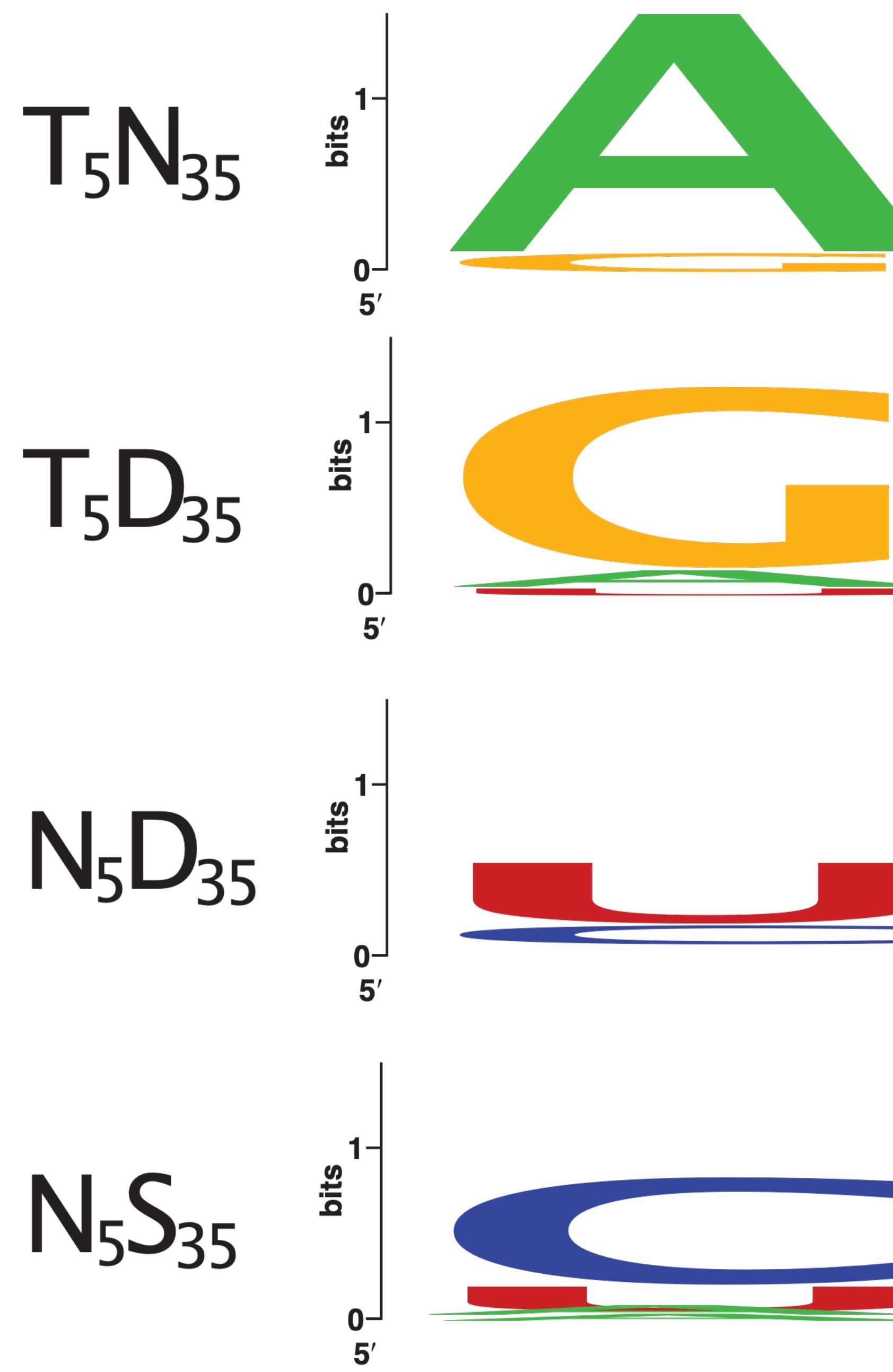
NATURE METHODS | EDITORIAL

Method of the Year 2011

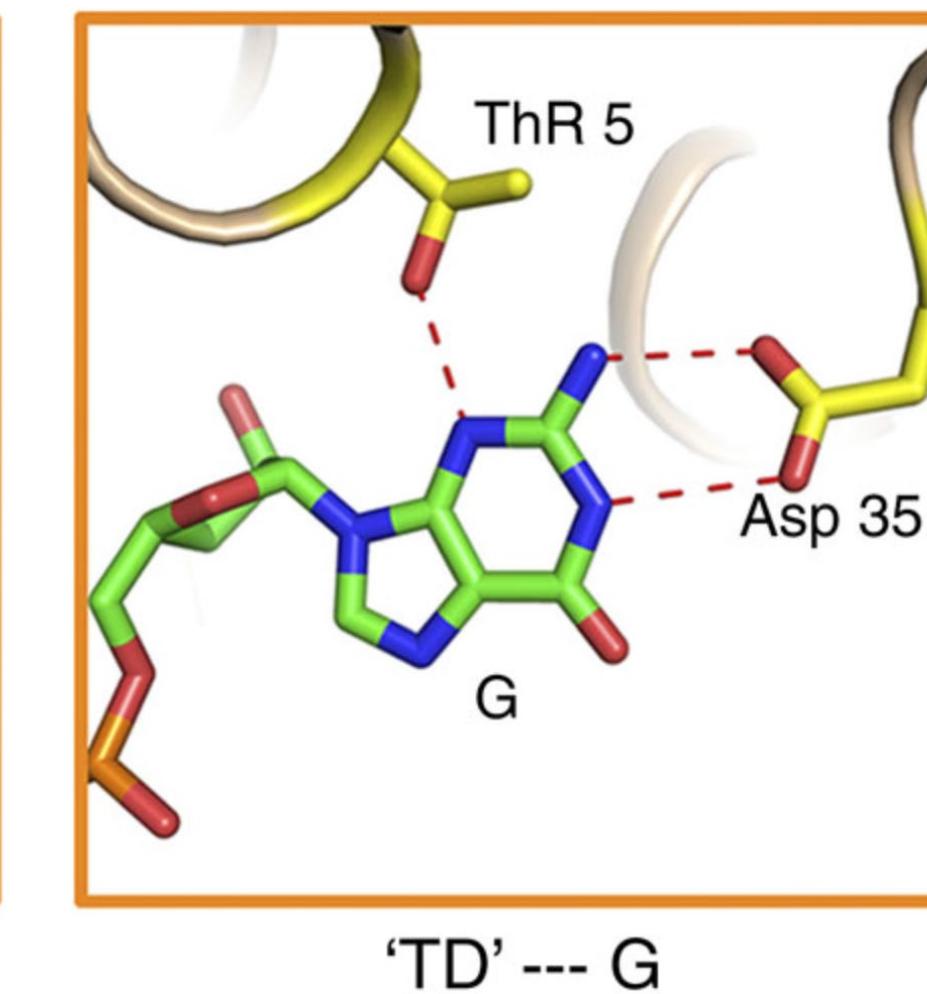
Nature Methods 9, 1 (2012) | doi:10.1038/nmeth.1852

Published online 28 December 2011

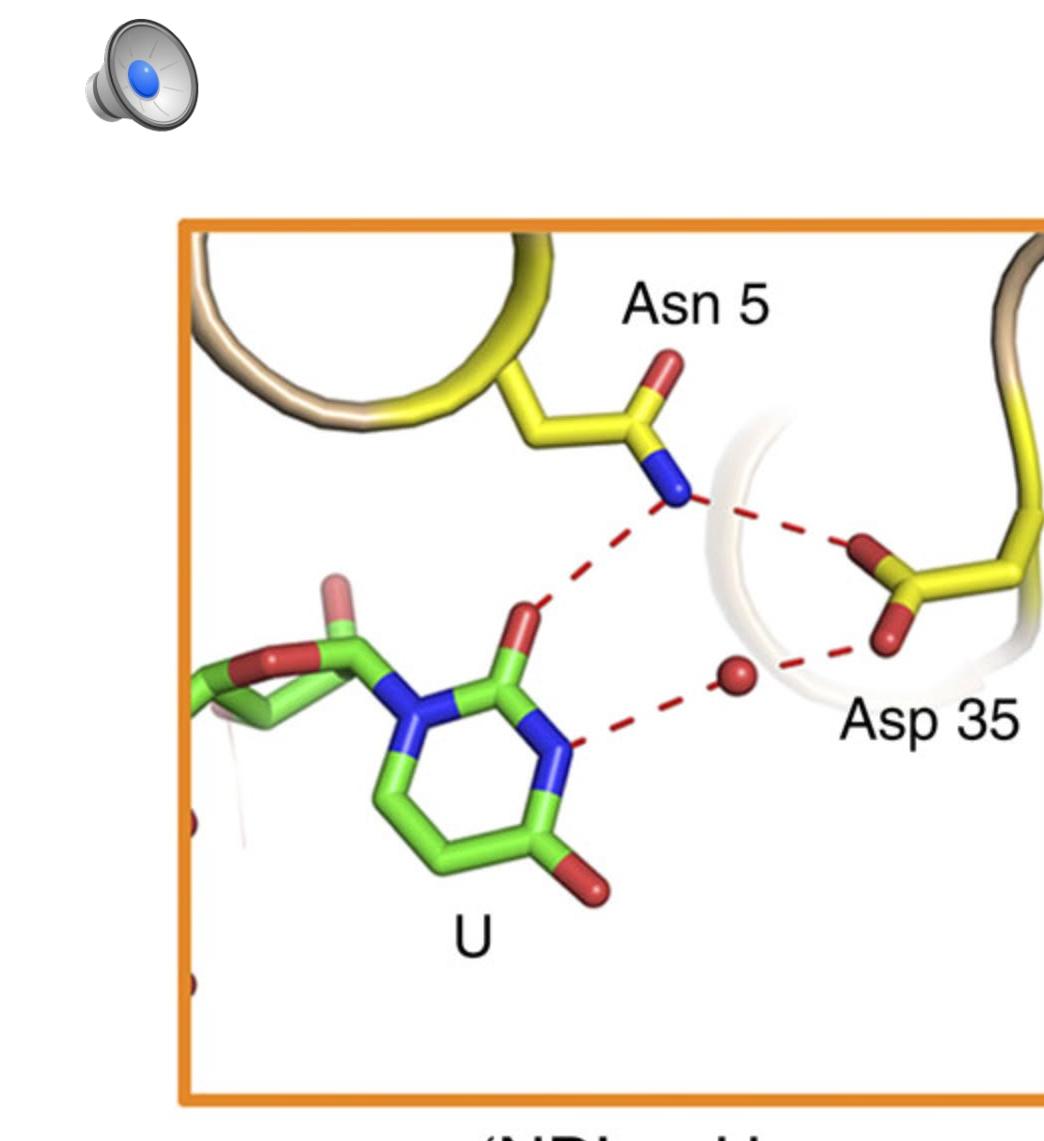
The ability to introduce targeted, tailored changes into the genomes of several species will make it feasible to ask more precise biological questions.



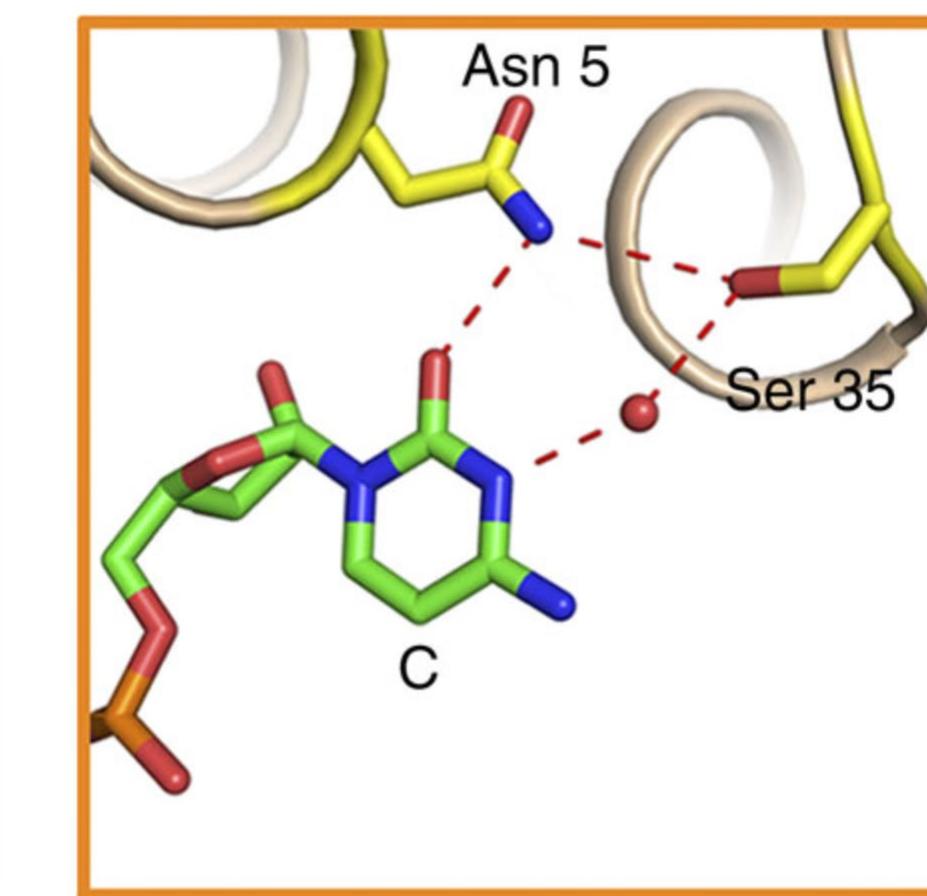
'SN' ---



'TD' --- C



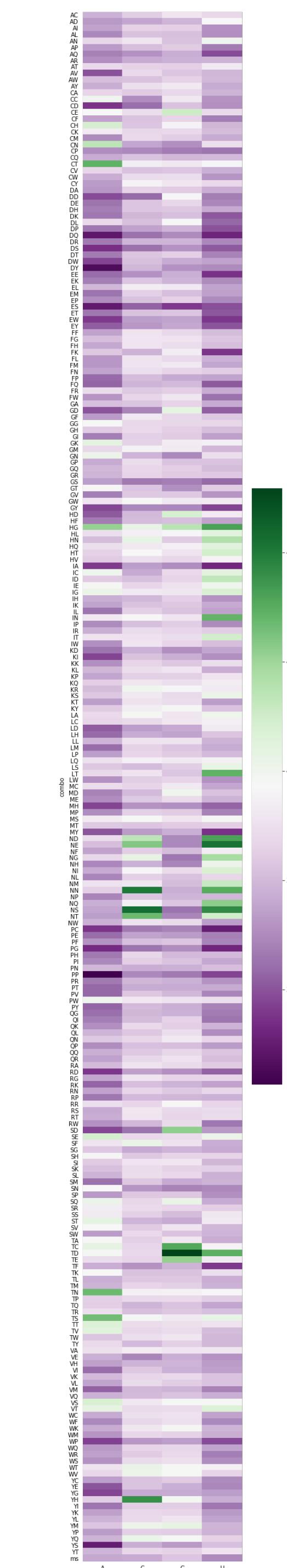
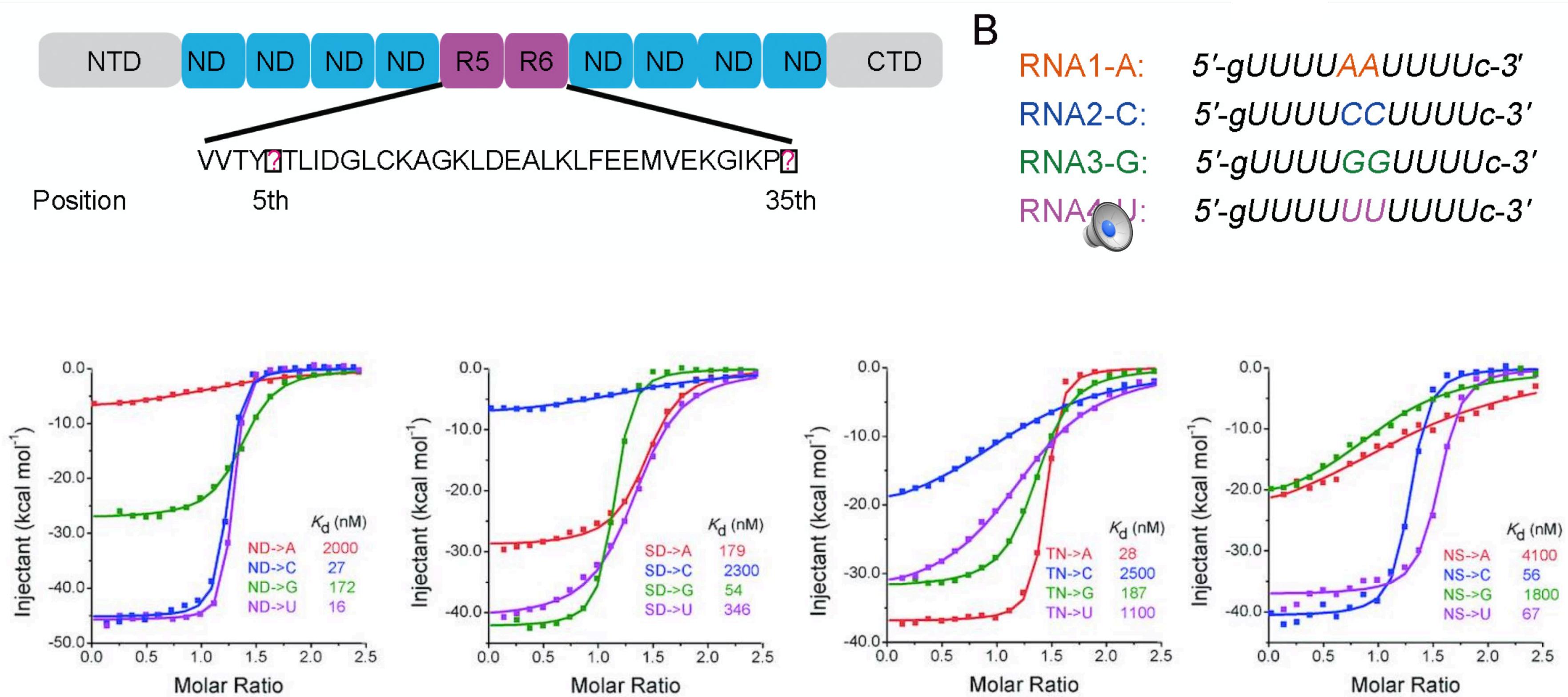
'ND' ---



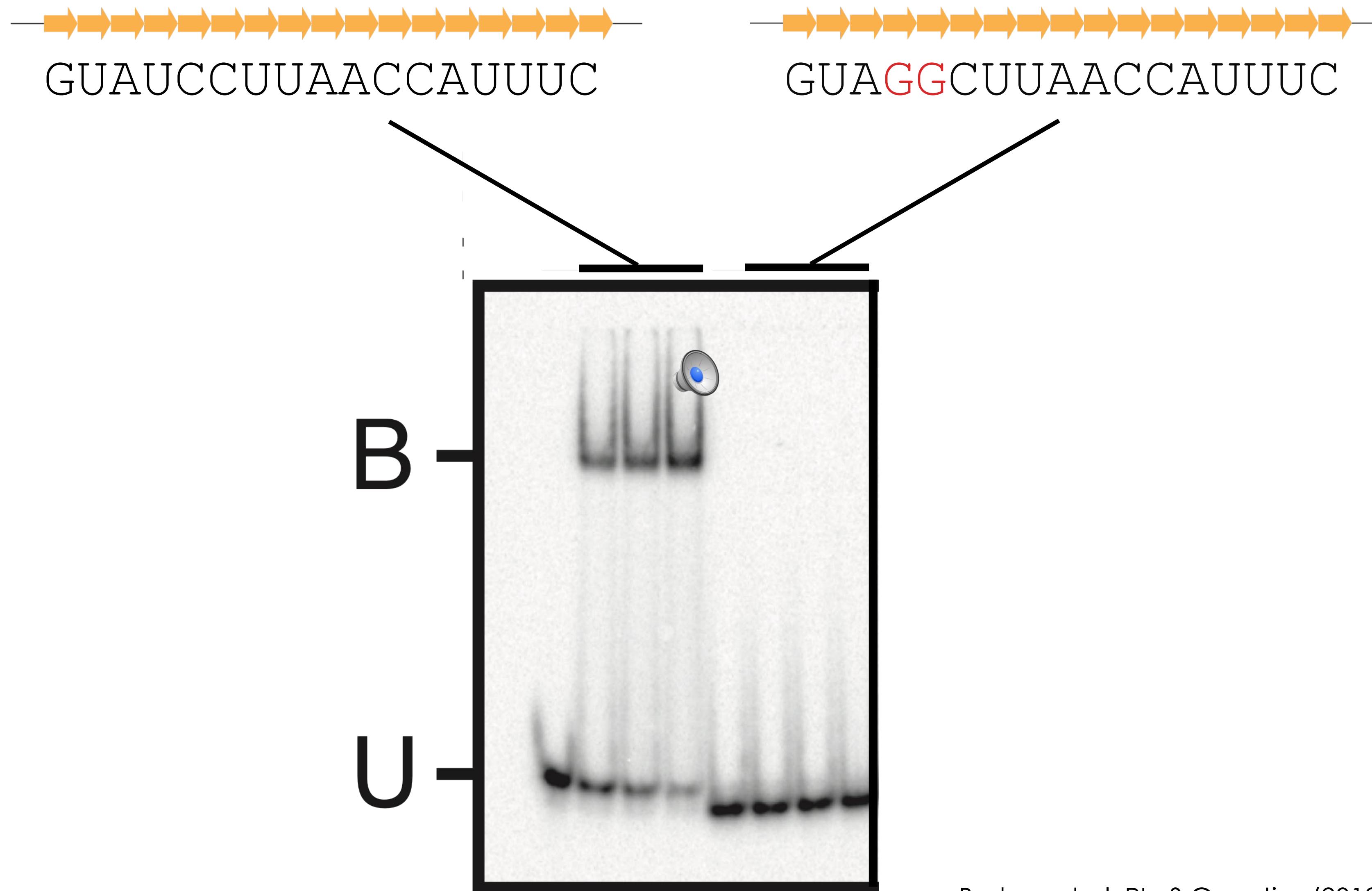
'NS' --- C

Delineation of pentatricopeptide repeat codes for target RNA prediction

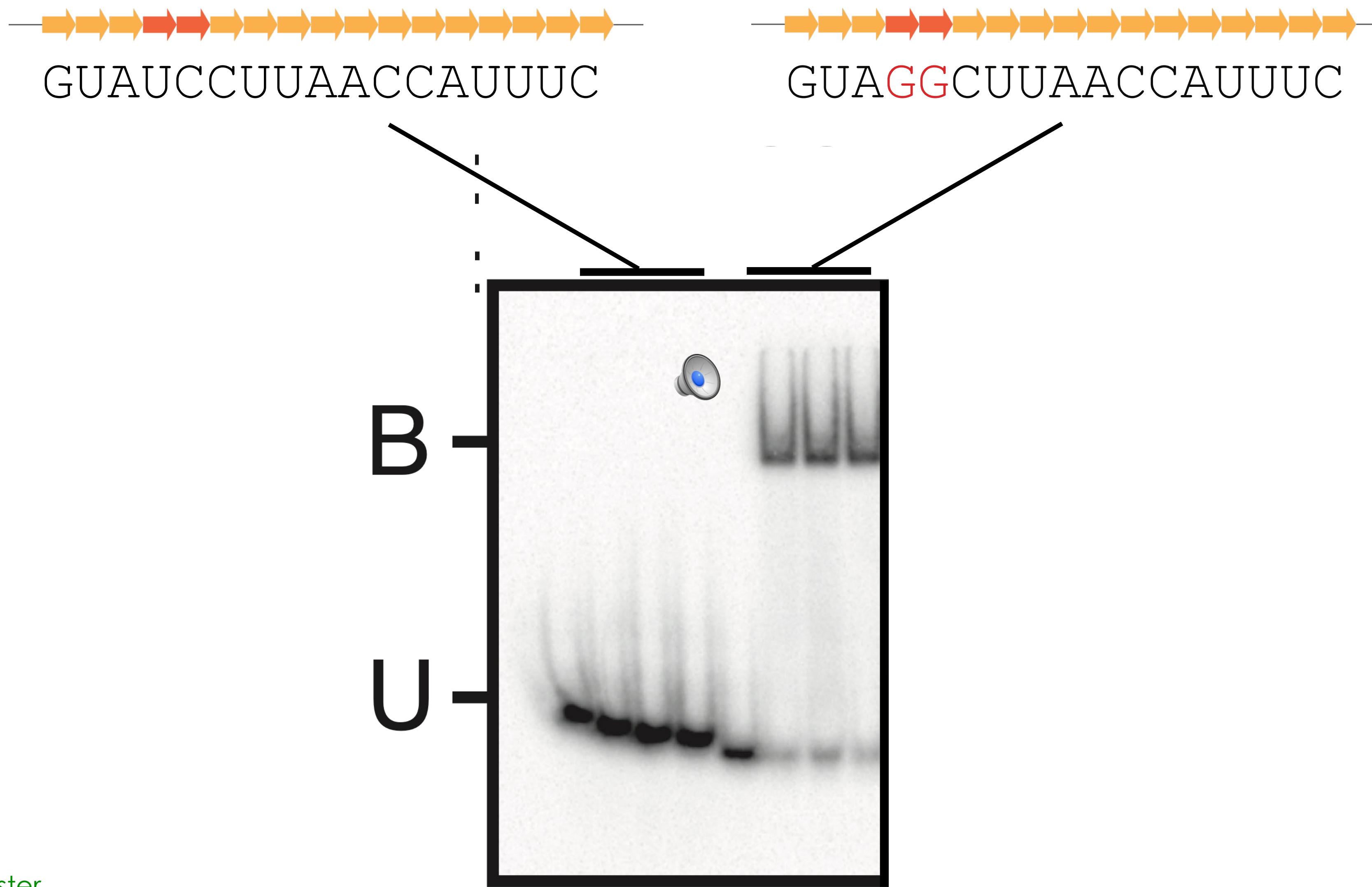
Junjie Yan^{1,†}, Yinying Yao^{1,†}, Sixing Hong¹, Yan Yang¹, Cuicui Shen¹, Qunxia Zhang¹, Delin Zhang¹, Tingting Zou² and Ping Yin^{1,*}



PPR10 fails to bind to a mutated target RNA



mutated PPR10 binds to mutated target RNA



see poster
Wed-316

Barkan et al. PLoS Genetics (2012)

Engineered RNA-binding protein for transgene activation in non-green plastids

Qiquo Yu, Alice Barkan & Pal Maliga

Nature Plants volume 5, pages 486–490 (2019)

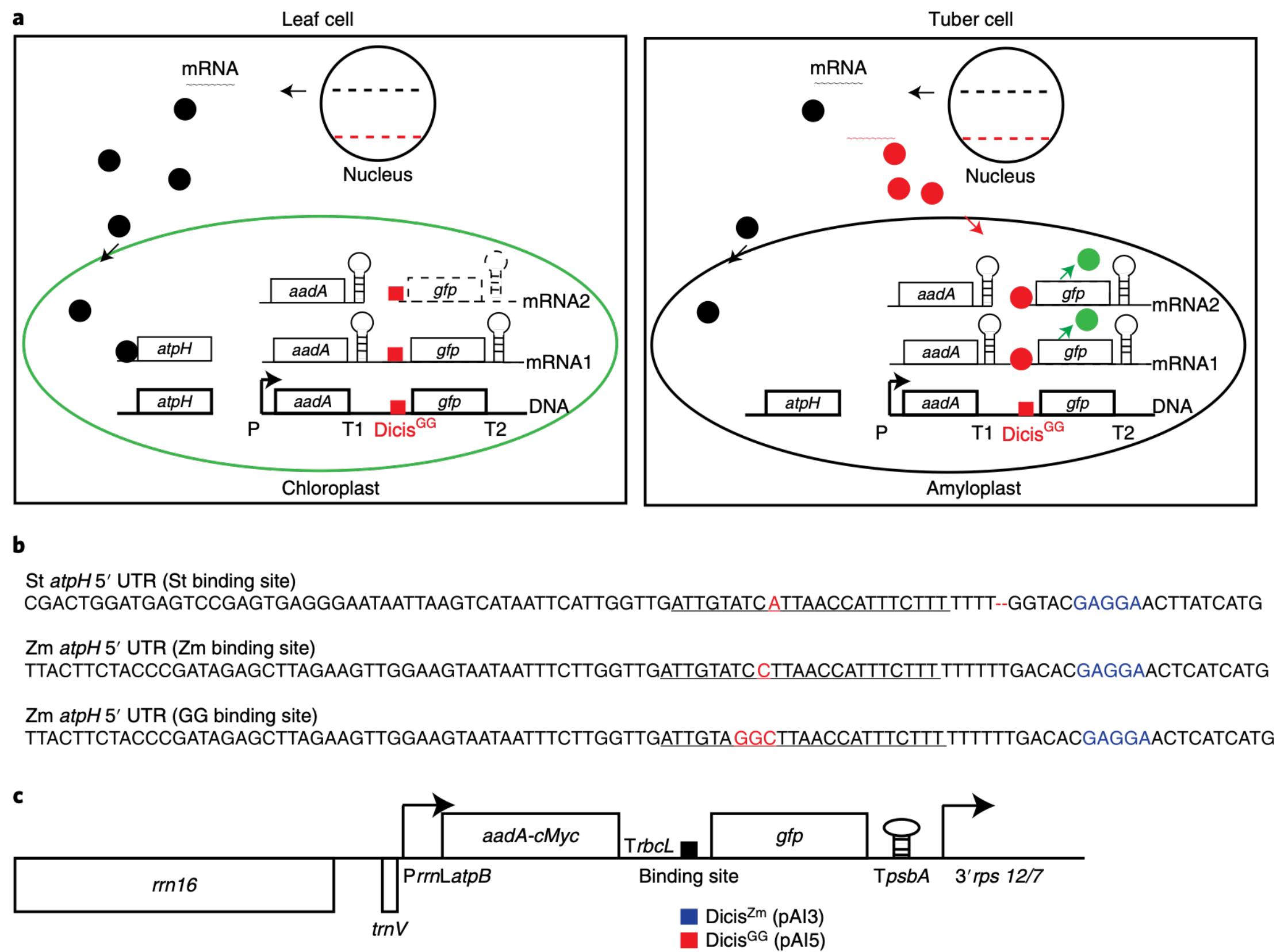


Fig. 1 | PPR10 binding site system for regulating gene expression in potato amyloplasts. a, Schematic of a leaf cell (left) showing the nuclear and chloroplast compartments, and a tuber cell (right) showing the nuclear and amyloplast compartments. The black dashed line indicates the endogenous potato PPR10; the red dashed line indicates the engineered maize PPR10^{GG} expressed from the patatin promoter. Black circles indicate the endogenous potato PPR10; green circles indicate GFP; red circles indicate the engineered maize PPR10^{GG}. P indicates *PrrnLatpB* the 16S rRNA operon promoter fused with the *atpB* gene leader and N-terminal amino acids; T1 indicates the plastid *rbcl* gene 3' UTR; T2 indicates plastid *psbA* gene 3' UTR. mRNA1 indicates the dicistronic transcript; mRNA2 is its two processed monocistronic *aadA* and *gfp* derivatives. Dashed lines indicate *gfp* mRNA, indicating rapid turnover of the processed form. **b,** An alignment of the sequence upstream of the *atpH* translation initiation codon (AUG) containing the St (WT potato; top), Zm (WT maize; middle) and GG (mutant maize; bottom) binding sites. The PPR10 binding sites are underlined; the ribosome binding site (Shine-Dalgarno sequence) is shown in blue. Nucleotides that differ among binding sites are highlighted in red. The dash in the sequence was inserted to fill a gap in the alignment. **c,** Schematic of the pAI3 and pAI5 dicistronic plastid transformation vectors that yielded transplastomeric Dicis^{Zm} and Dicis^{GG} plants. *PrrnLatpB* is the 16S rRNA operon promoter fused with the *atpB* gene leader and N-terminal amino acids; *TrbcL* is the 3' UTR of the plastid *rbcl* gene; *TpsbA* is the 3' UTR of the plastid *psbA* gene.

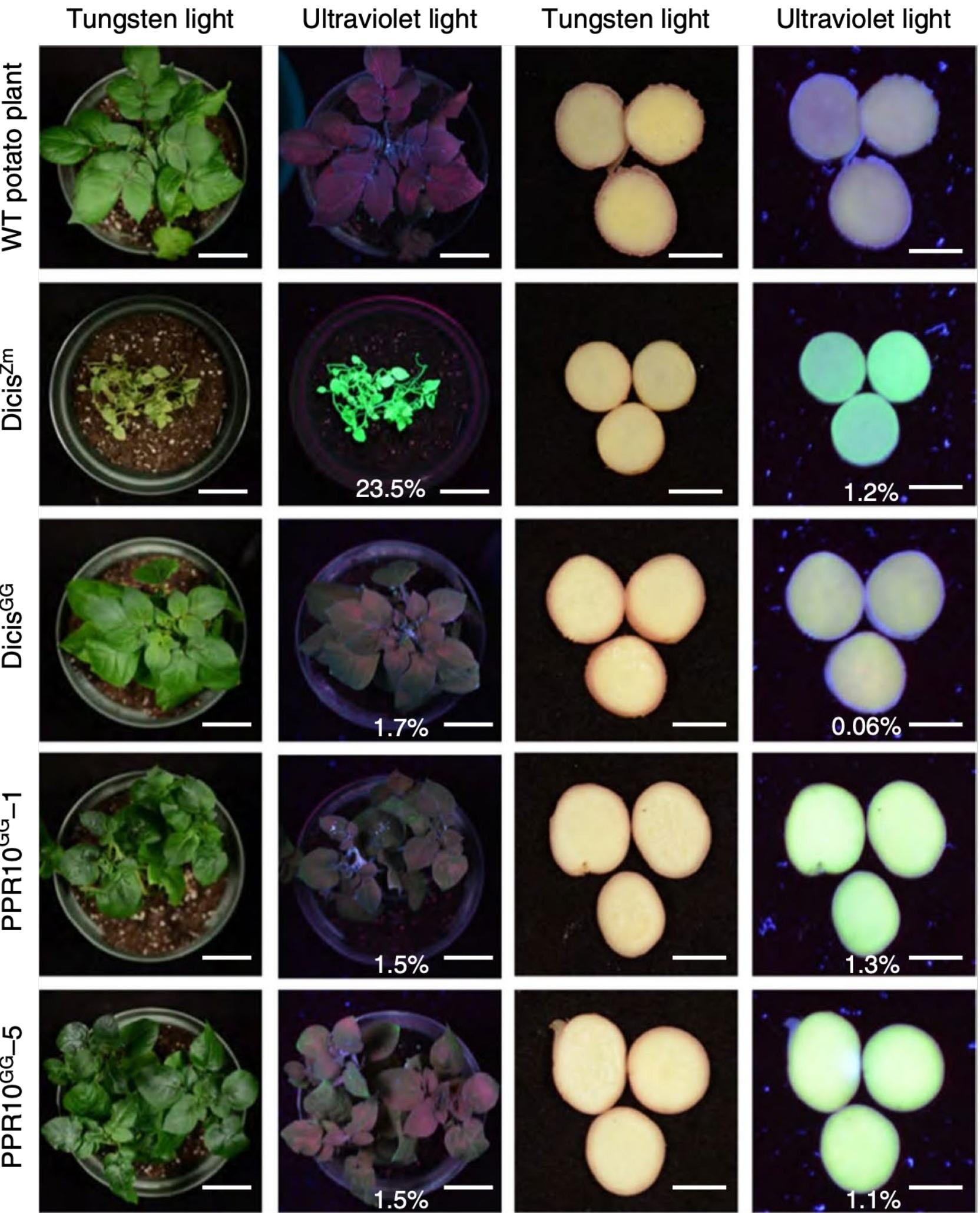
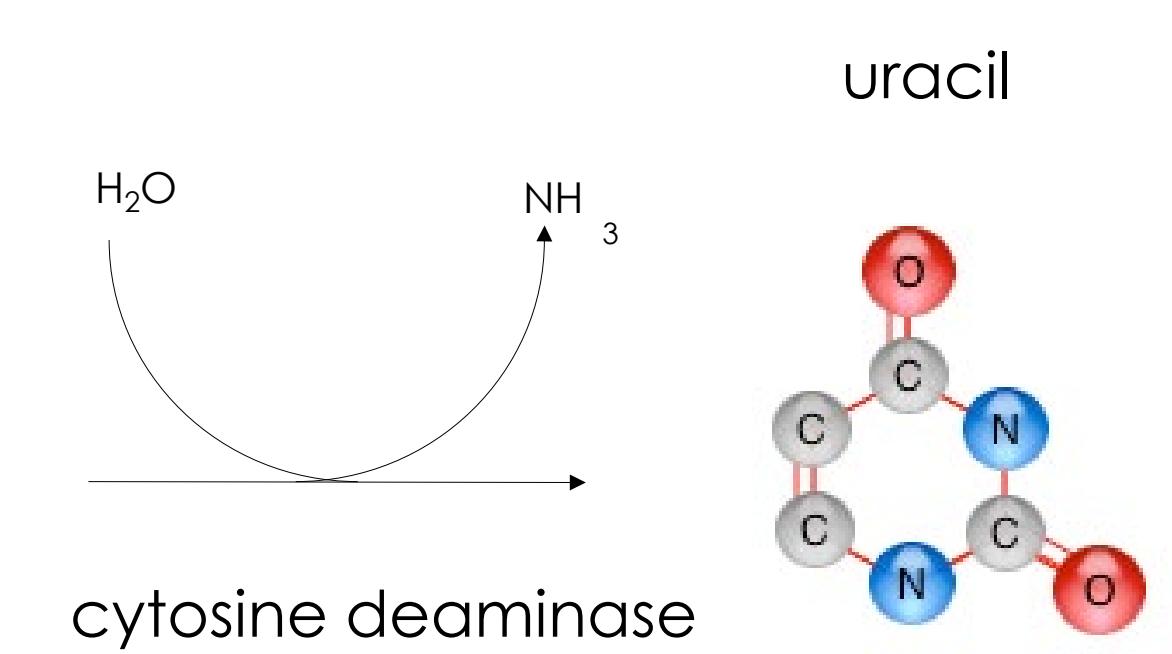
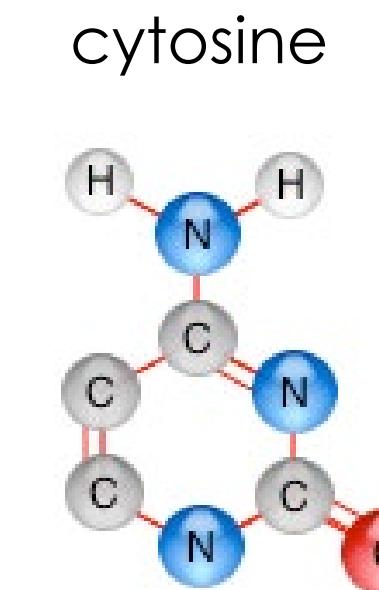
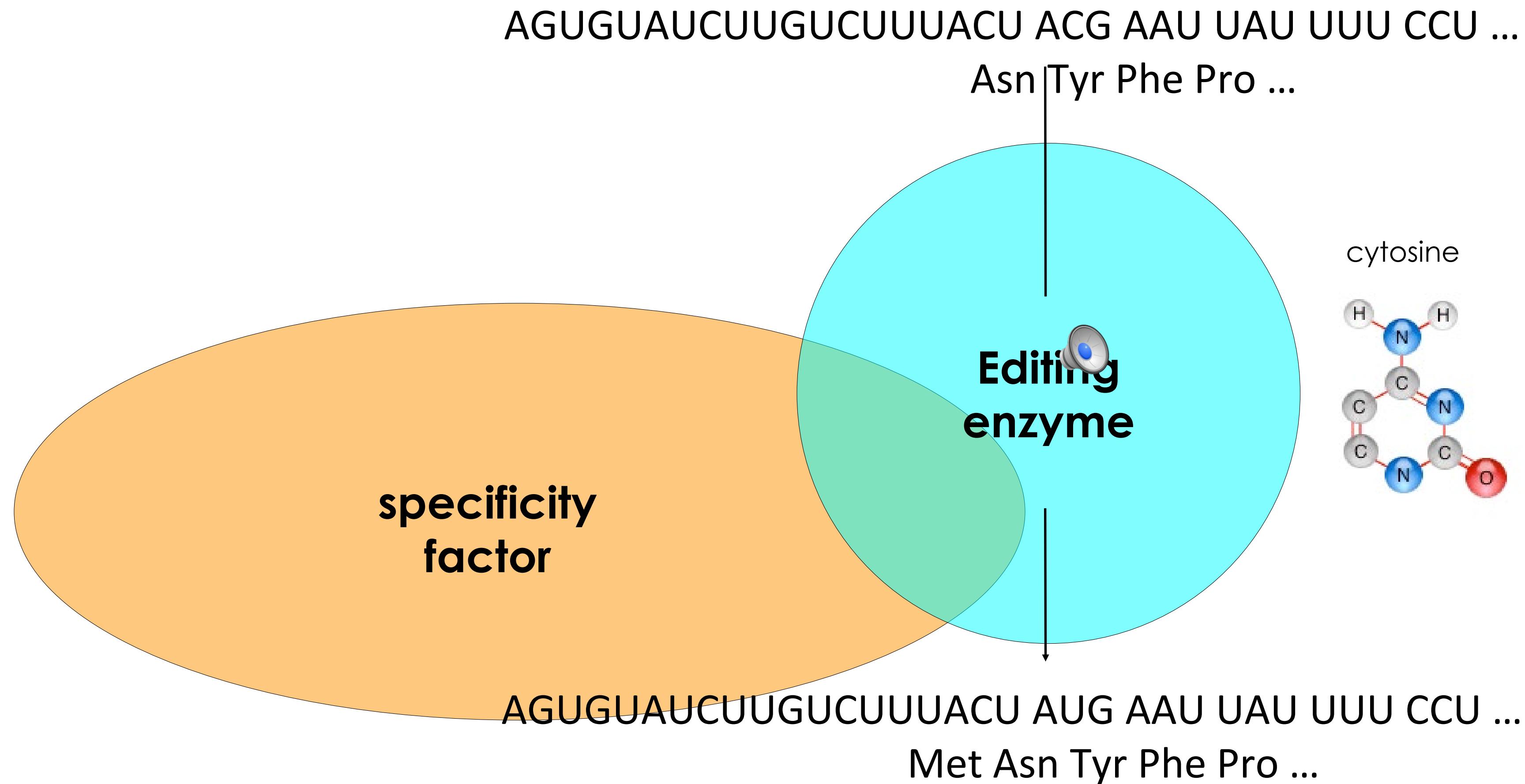
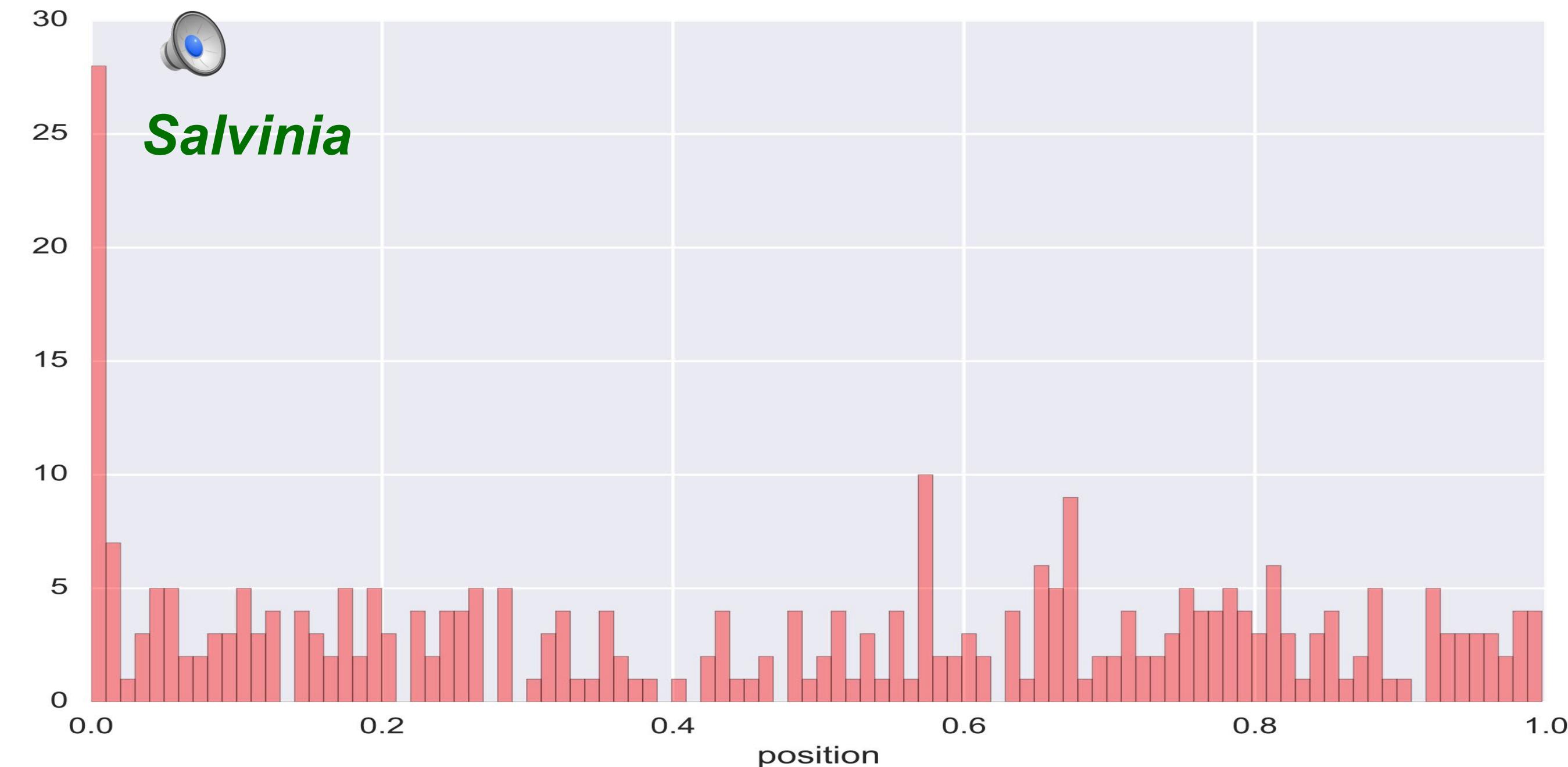
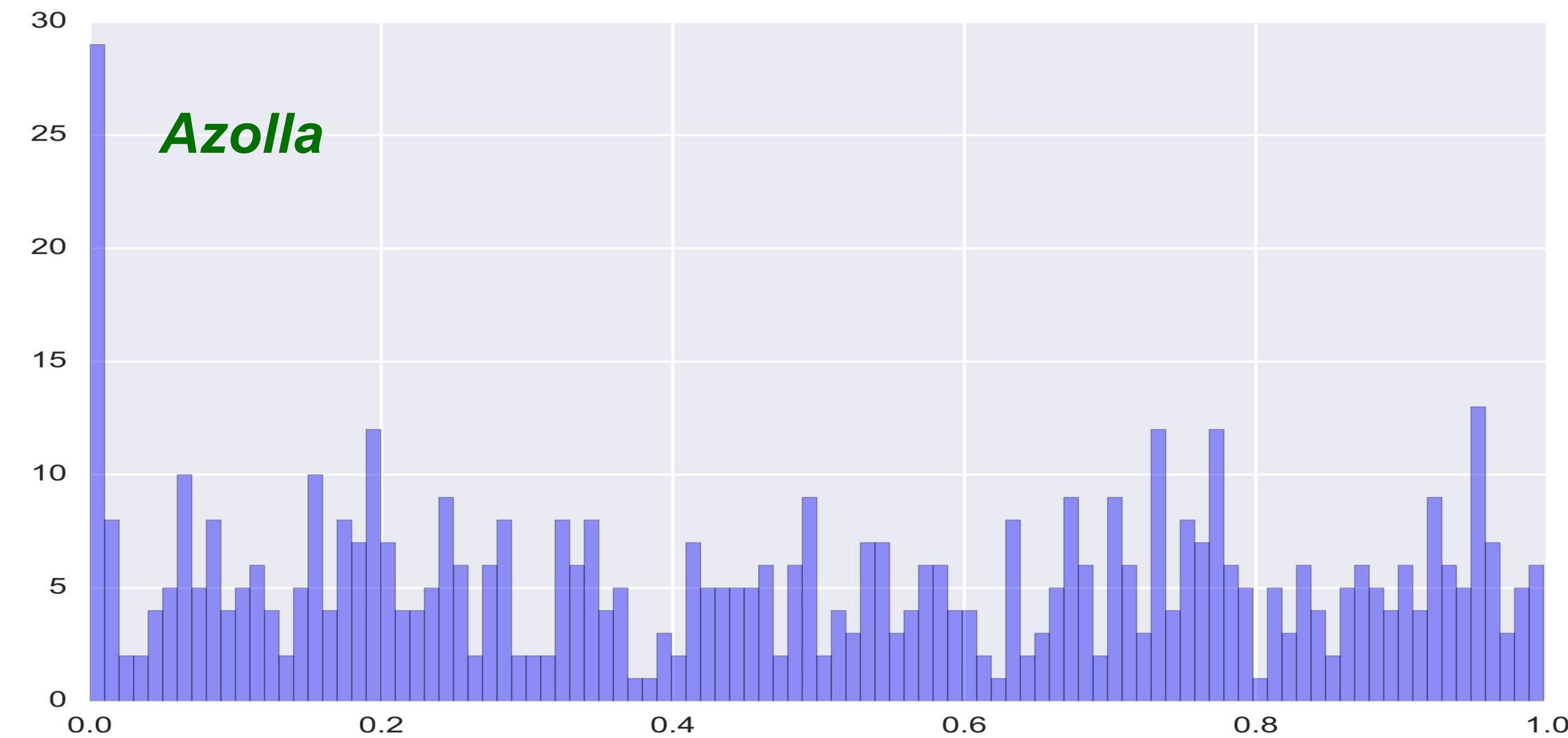
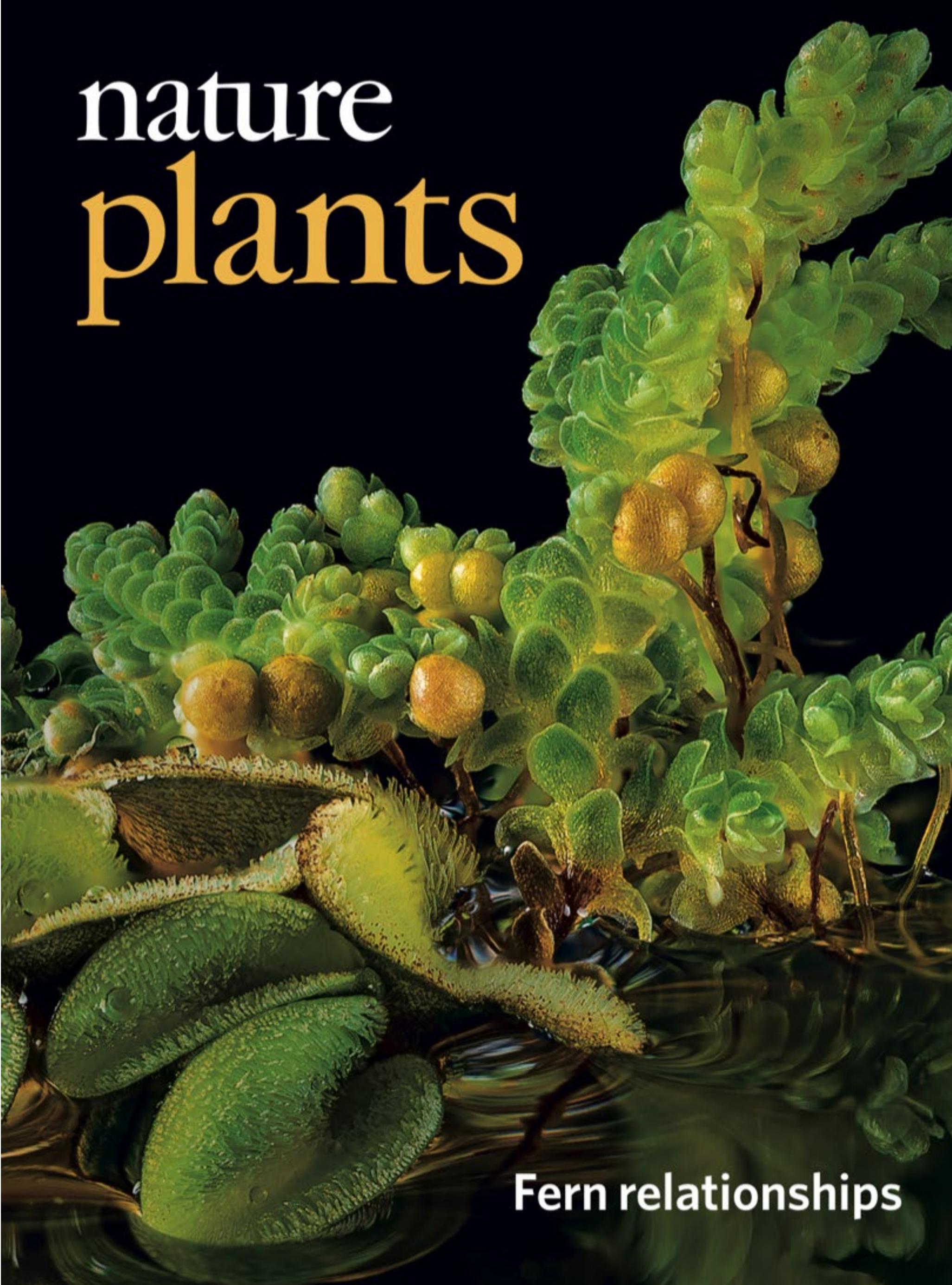


Fig. 2 | Leaf and tuber phenotypes of potato plants illuminated with tungsten or ultraviolet light. Tungsten or ultraviolet light (366 nm) illumination of potato plant leaves (first and second columns) and tubers (third and fourth columns), four months after planting in vitro-cultivated shoots in soil. The suffixes -1 and -5 indicate two independent nuclear transgenic lines. GFP abundance (% TSP) in leaves and tubers is given in the images. Plant scale bar, 5 cm; tuber scale bar, 1 cm. Testing of phenotypes was performed three times independently with similar results.

C-to-U RNA editing



nature plants



A synthetic SS protein can specifically edit its target site in *E. coli*

P L S P L S P L S P L S E E D Y W

N P S N C I N T P S N T G G V
D D N S I T D D D S R N S K HxE...CxxC

The figure displays a Sanger sequencing chromatogram with four colored traces: Red (Adenine A), Green (Thymine T), Blue (Cytosine C), and Yellow (Guanine G). The x-axis shows the sequence of the DNA strand being sequenced, with labels for each base: A, U, U, A, C, A, C, G, U, G, C, A, A, A, A, U, C, U, G, A. The traces show characteristic peaks for each base at their respective positions, allowing for the sequence to be read from left to right.

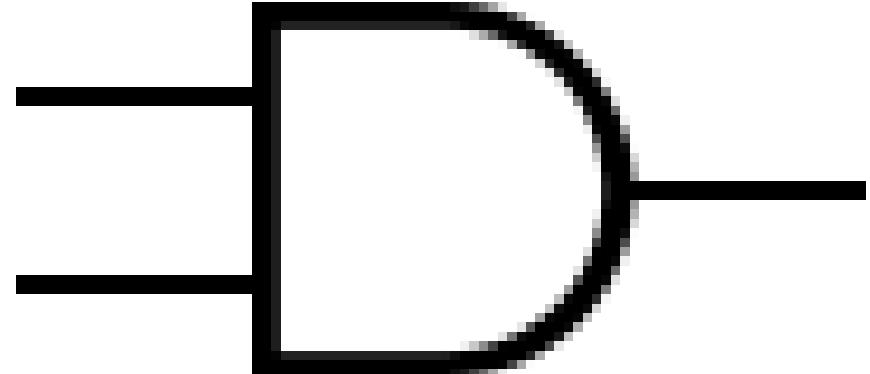
syn(PLS)₃DYW + MORF9

S S S S S S S S P L S E E D Y W

N D N D N S N S D D D S R N T G G V K HxE...CxxC

The figure displays a Sanger sequencing chromatogram with four sequence traces: Adenine (red), Thymine (green), Cytosine (blue), and Guanine (yellow). The x-axis represents the sequence of bases: A U U A C A C G U G C A A A A U C U G A. The y-axis represents fluorescence intensity. Each trace shows peaks corresponding to the sequence of bases, with the most intense peak at each position. The Adenine trace has peaks at positions 1, 5, 6, 8, 10, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 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syn(SS)₃DYW

Gate	Symbol	A	B	Input	Output
AND		0	0	0	0
		0	1	0	0
		1	0	0	0
		1	1	1	1
OR		0	0	0	0
		0	1	1	1
		1	0	1	1
		1	1	1	1
NAND		0	0	0	1
		0	1	1	1
		1	0	1	1
		1	1	0	0
NOR		0	0	0	1
		0	1	1	0
		1	0	0	0
		1	1	1	0
XOR		0	0	0	0
		0	1	1	1
		1	0	1	1
		1	1	0	0
		0	0	0	1
		0	1	1	0