

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?

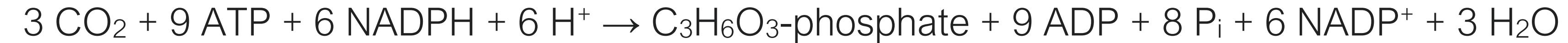
Second part: How do we engineer these systems? What is currently being done?

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

Why are we interested in engineering plants?

Photosynthetic organisms convert around **100–115 billion tons** of **carbon** into **biomass** per year

Field et al., Science 10 Jul 1998 Vol. 281, Issue 5374, pp. 237-240



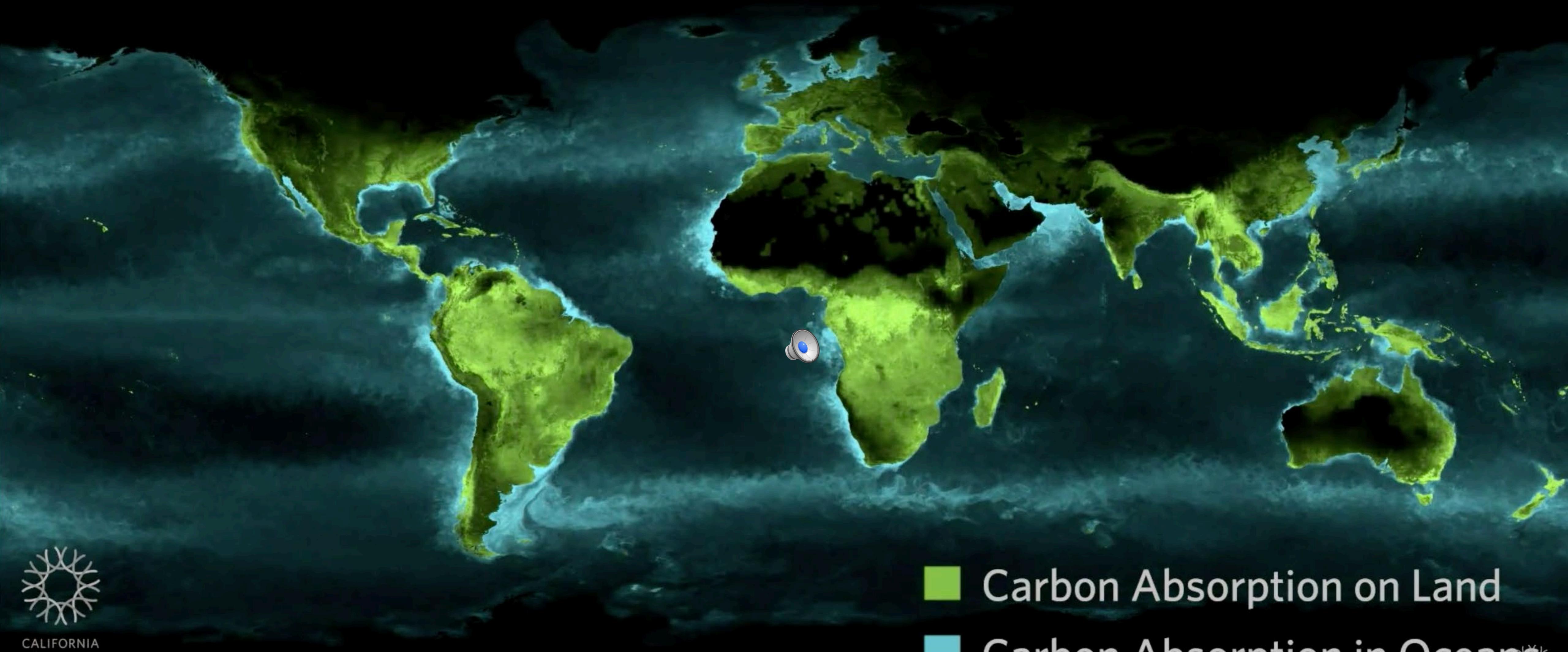
the average rate of energy capture by photosynthesis globally is approximately **100 terawatts**

Barber, Chem. Soc. Rev., 2009, 38, 185–196

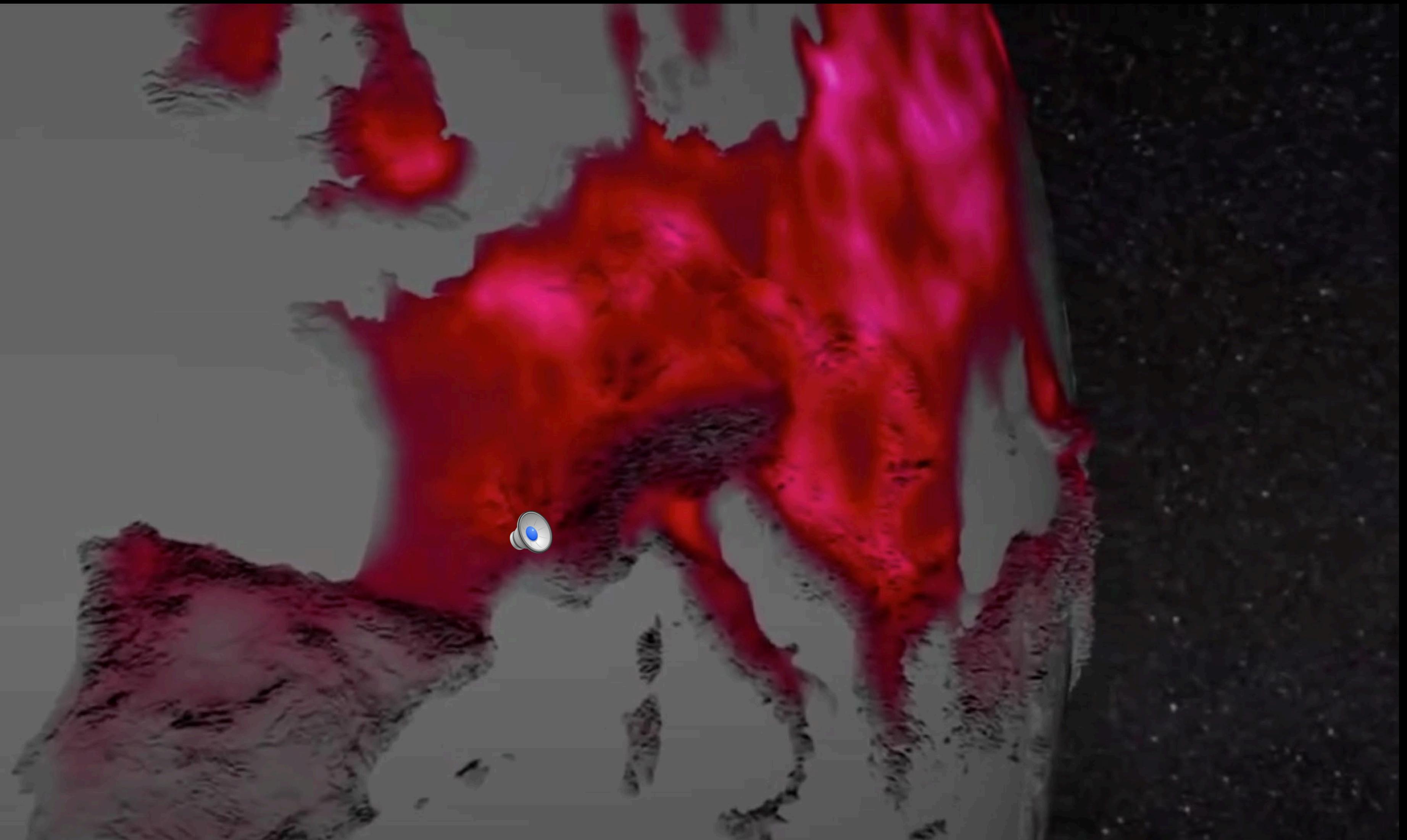
seven times the power consumption of human civilization

but the efficiency of photosynthesis is maximally **~4%** and usually below 1% in nature

Barber, Chem. Soc. Rev., 2009, 38, 185–196



CALIFORNIA
ACADEMY OF
SCIENCES

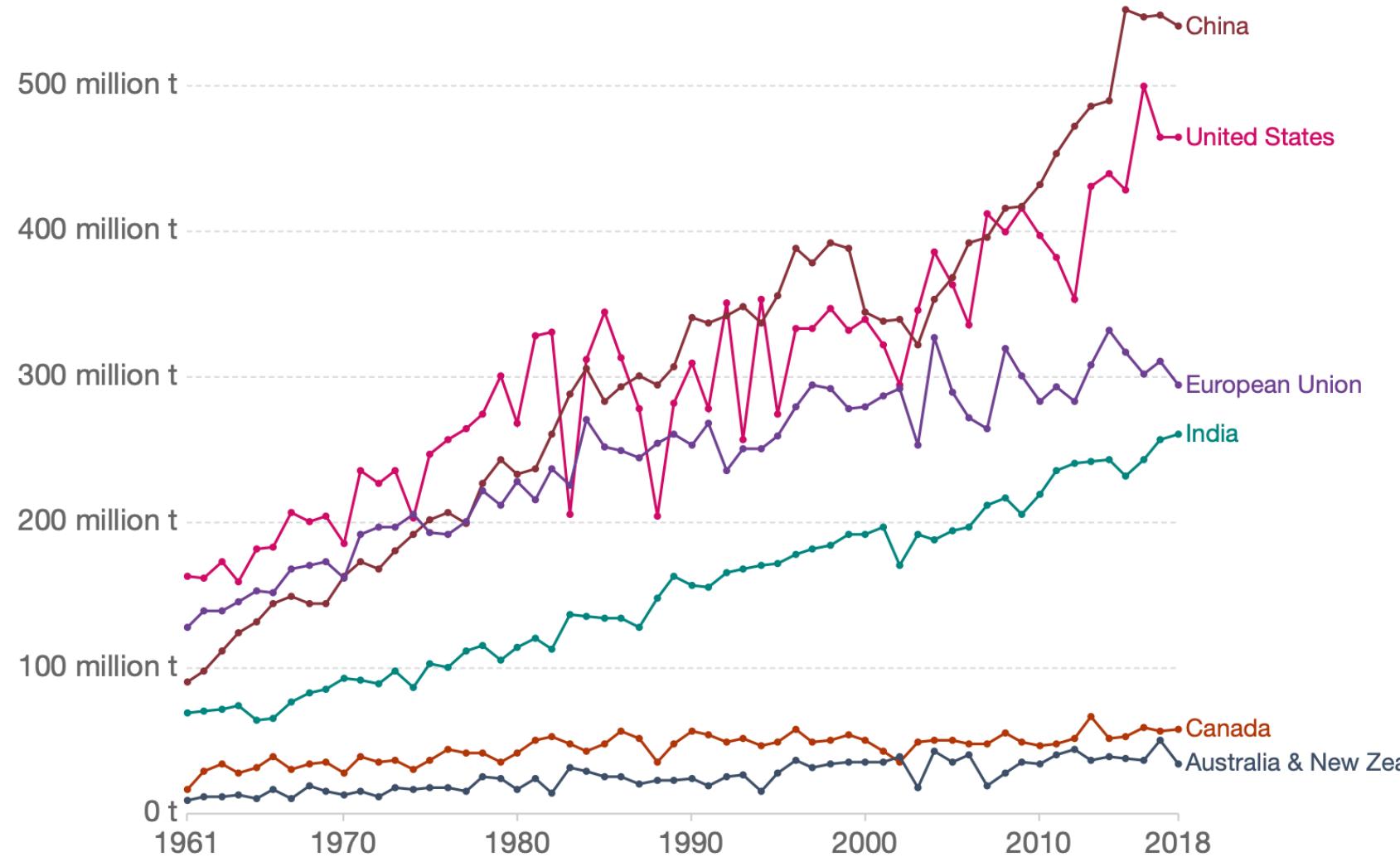


By observing changes in intensity over time, scientists can distinguish stressed, dead or dormant plants from healthy and growing vegetation.

Cereal production, 1961 to 2018

Cereal production is measured in tonnes, and represents the total of all cereal crops including maize, wheat, rice, barley, rye, millet and others.

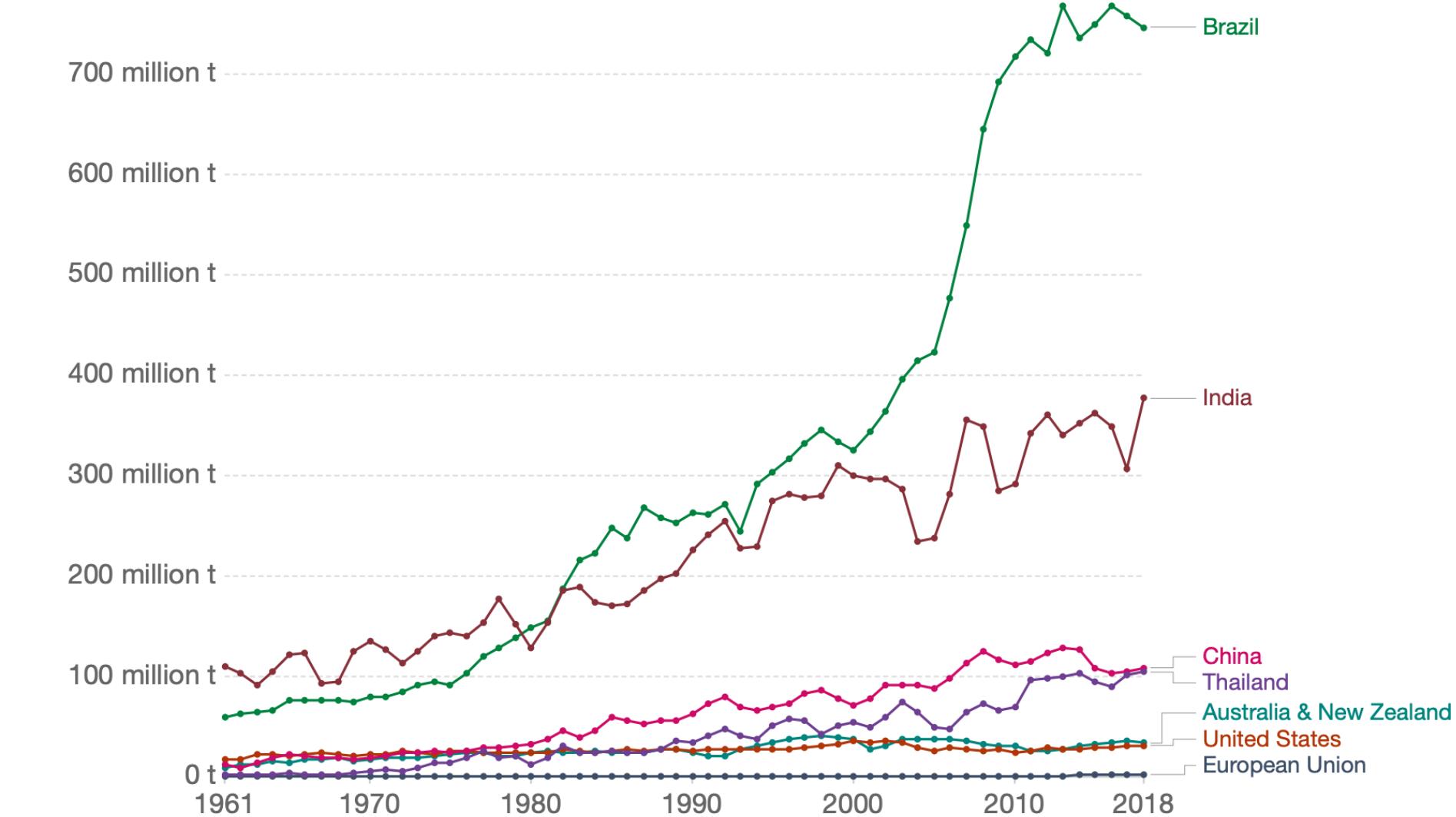
Our World
in Data



Sugar cane production, 1961 to 2018

Sugar cane production is measured in tonnes.

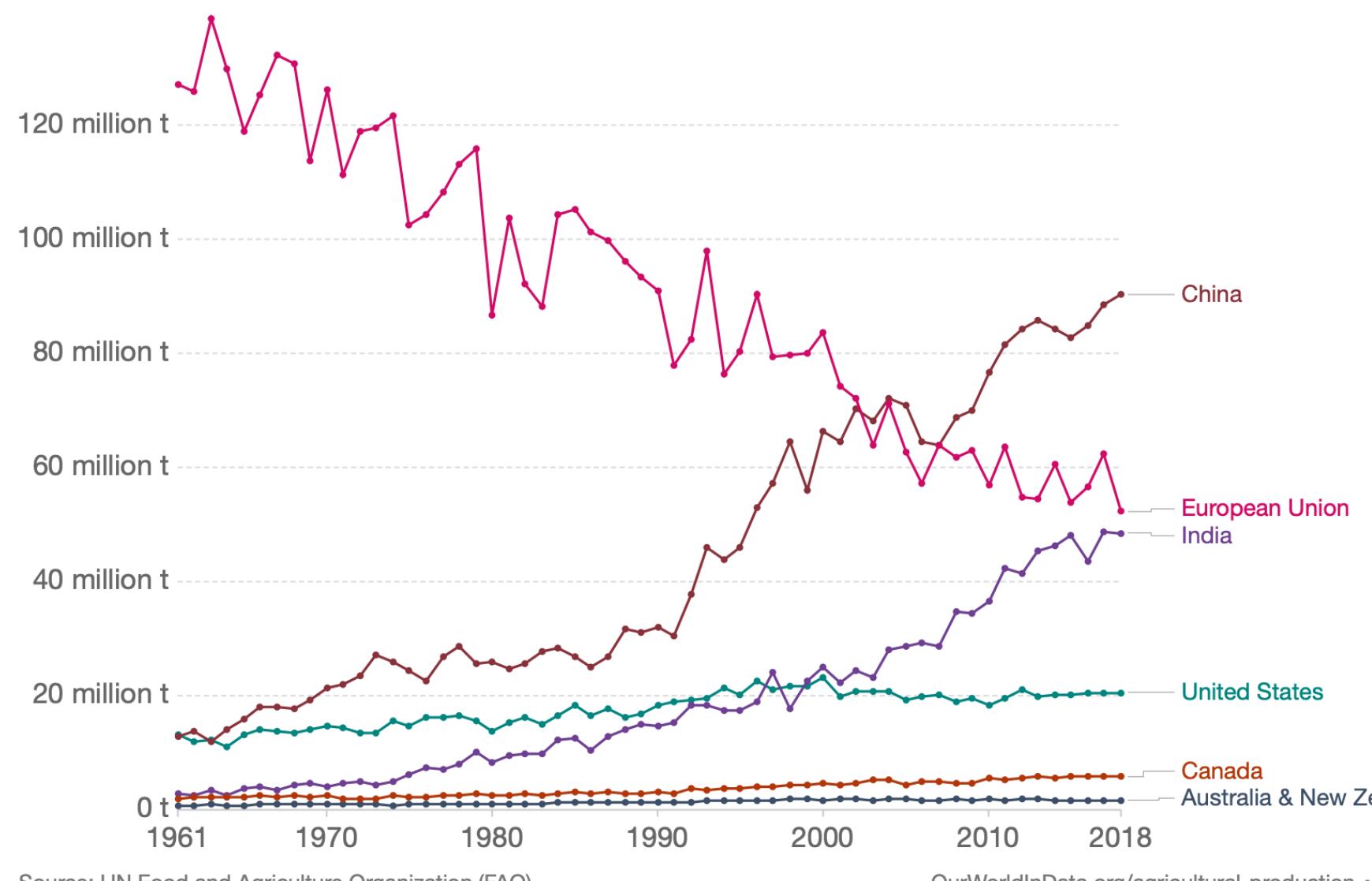
Our World
in Data



Potato production, 1961 to 2018

Potato production is measured in tonnes.

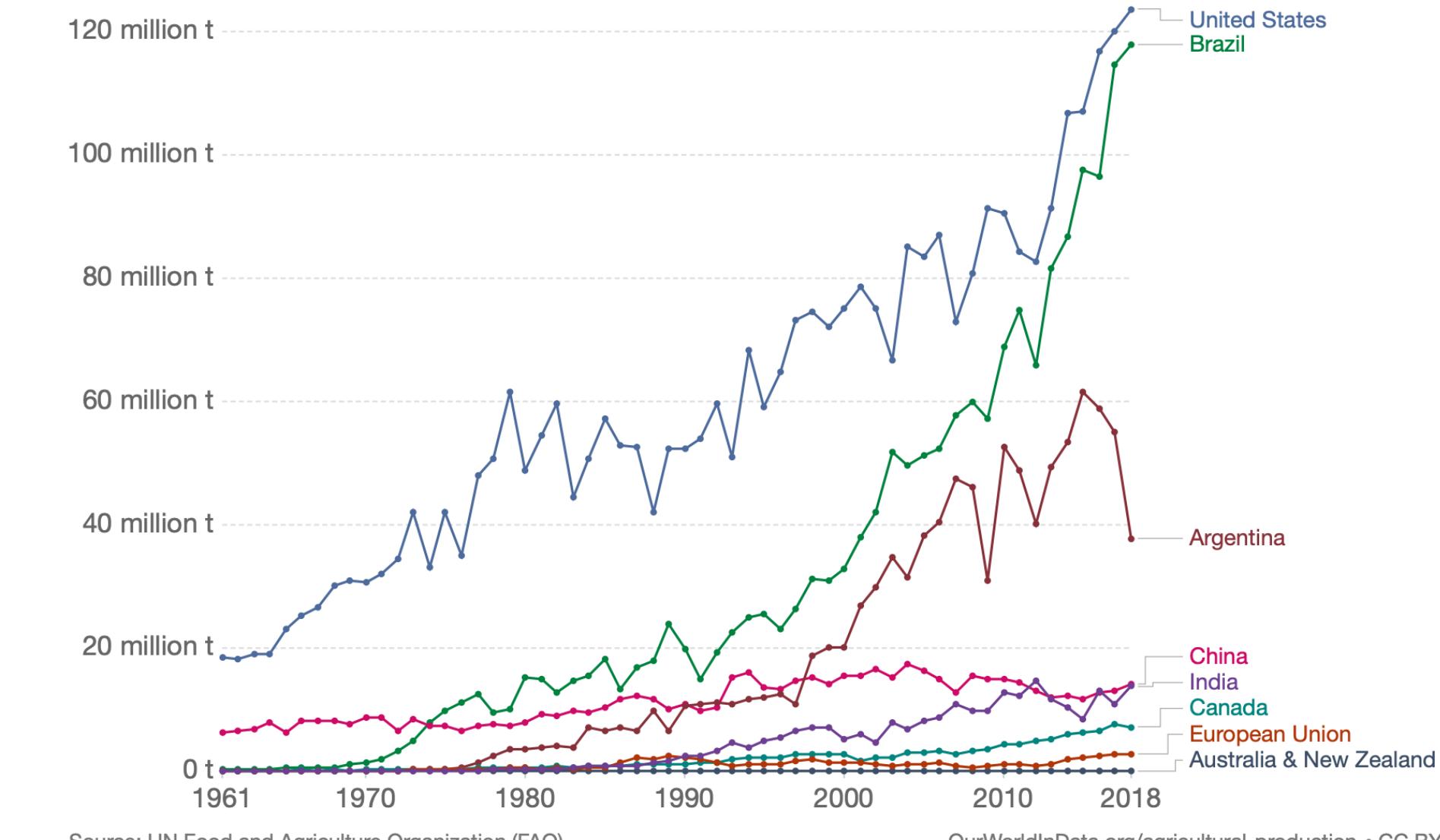
Our World
in Data



Soybean production, 1961 to 2018

Soybean production is measured in tonnes.

Our World
in Data





Agriculture has relatively low input costs

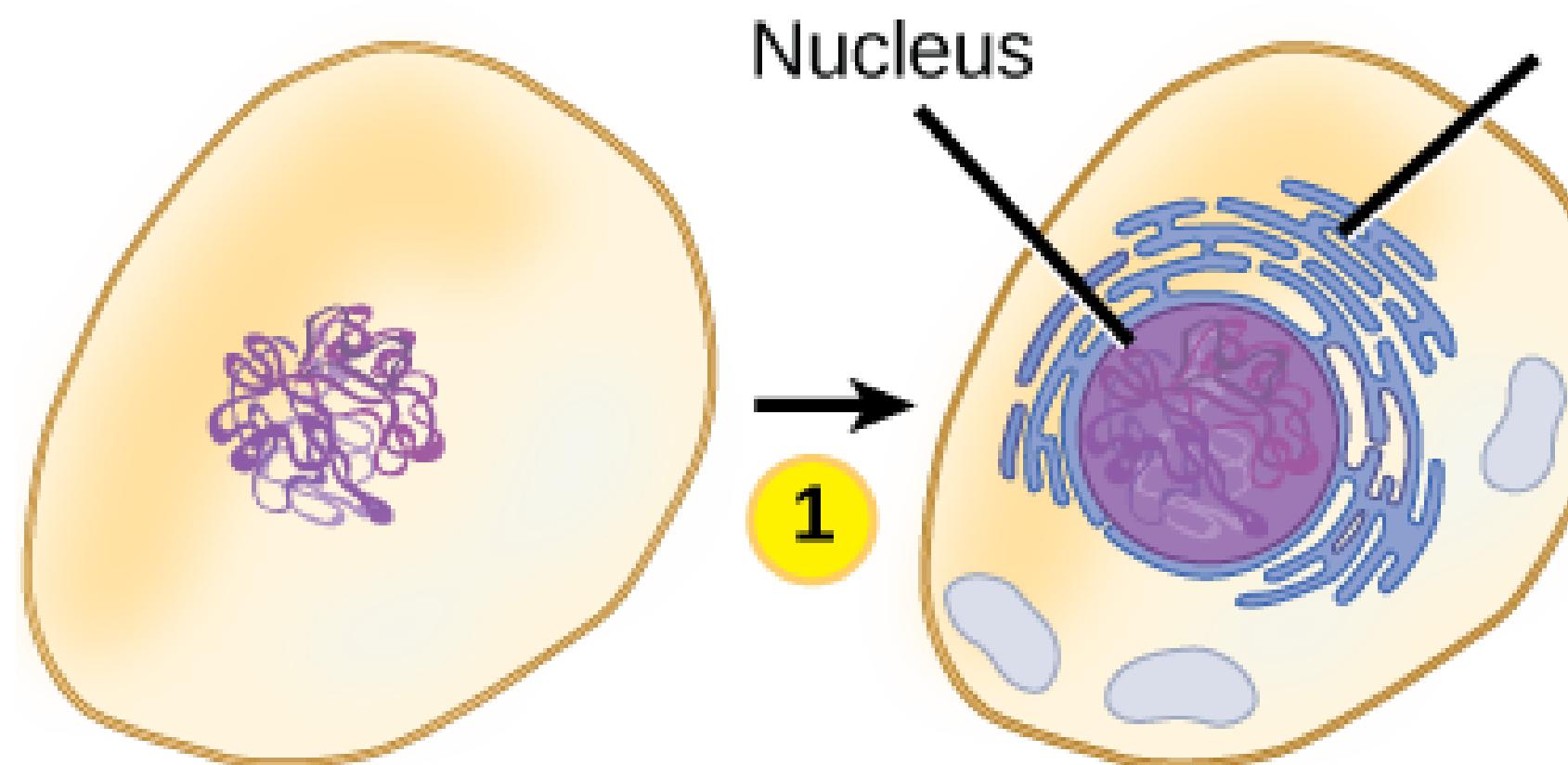




<https://www.hypergiant.com/green/>

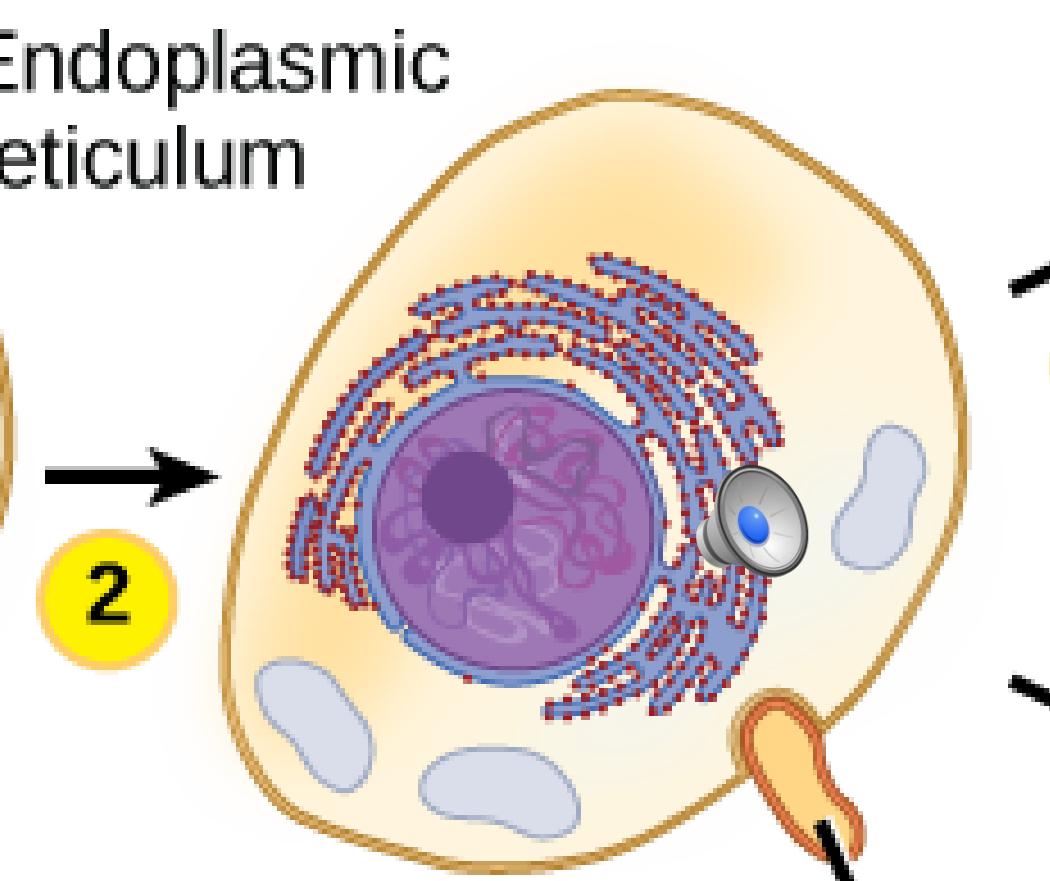
The ENDOSYMBIOTIC THEORY

1 Infoldings in the plasma membrane of an ancestral prokaryote gave rise to endomembrane components, including a nucleus and endoplasmic reticulum.

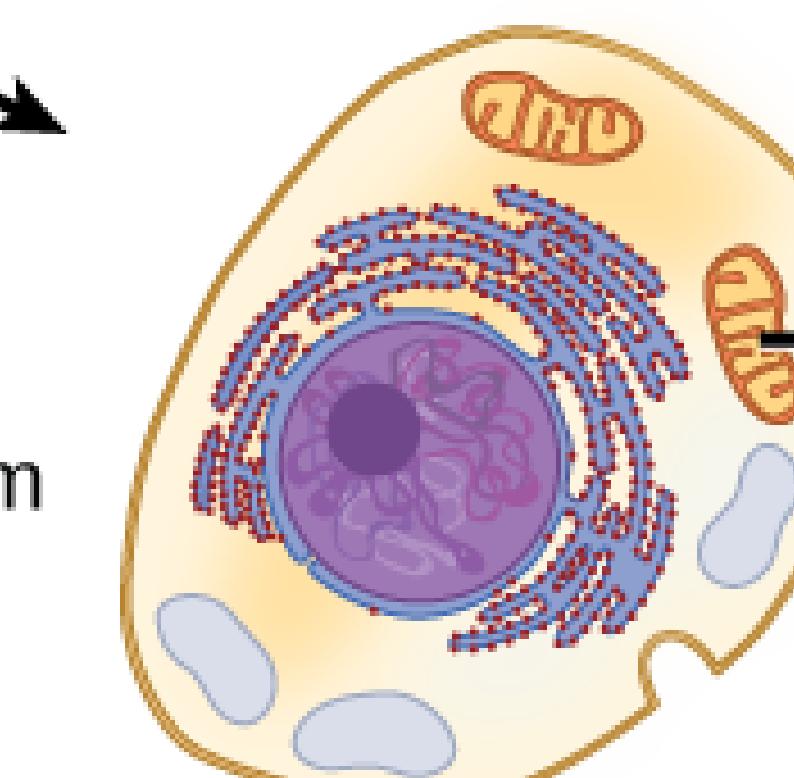


Proto-eukaryote

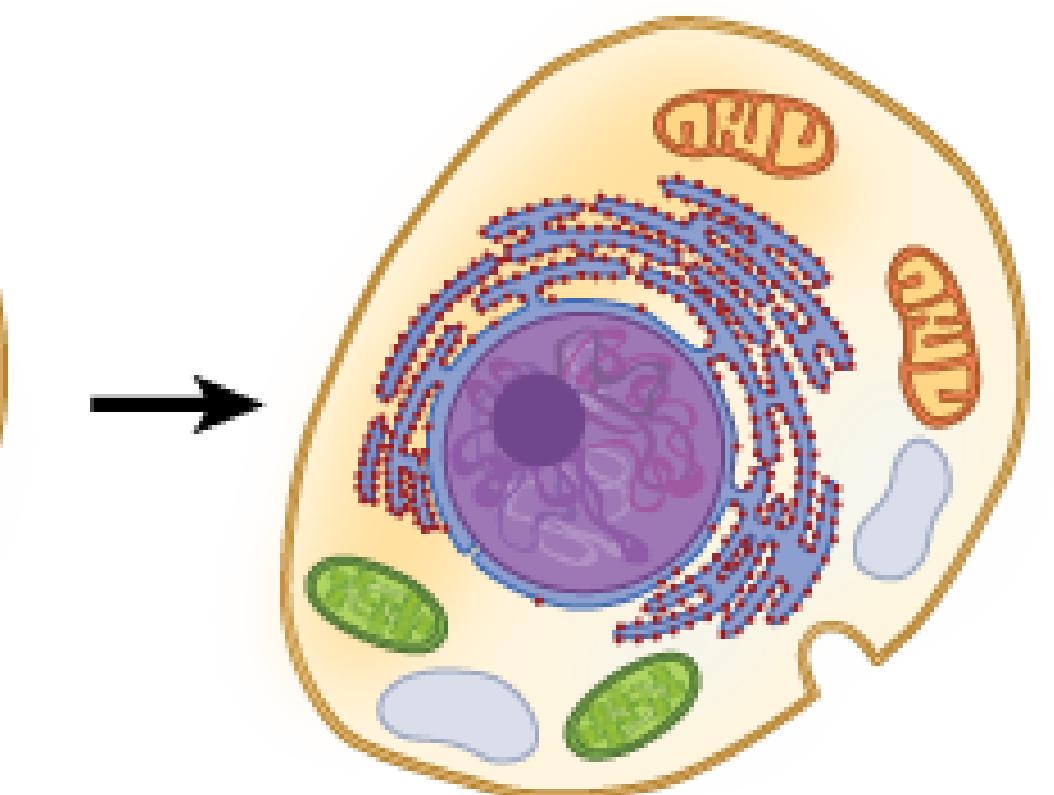
3 In a second endosymbiotic event, the early eukaryote consumed photosynthetic bacteria that evolved into chloroplasts.



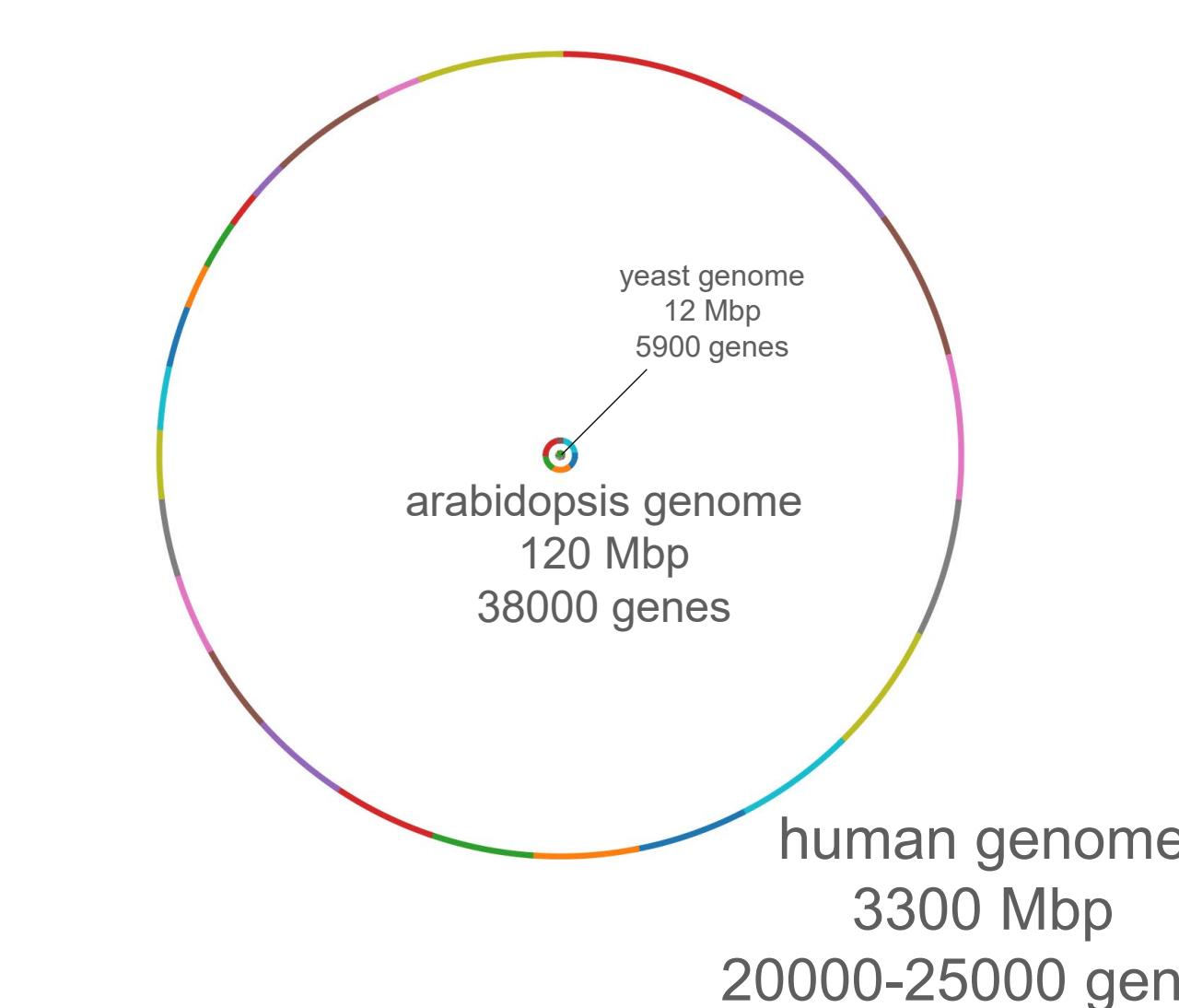
2 In a first endosymbiotic event, the ancestral eukaryote consumed aerobic bacteria that evolved into mitochondria.



Modern heterotrophic eukaryote



Modern photosynthetic eukaryote



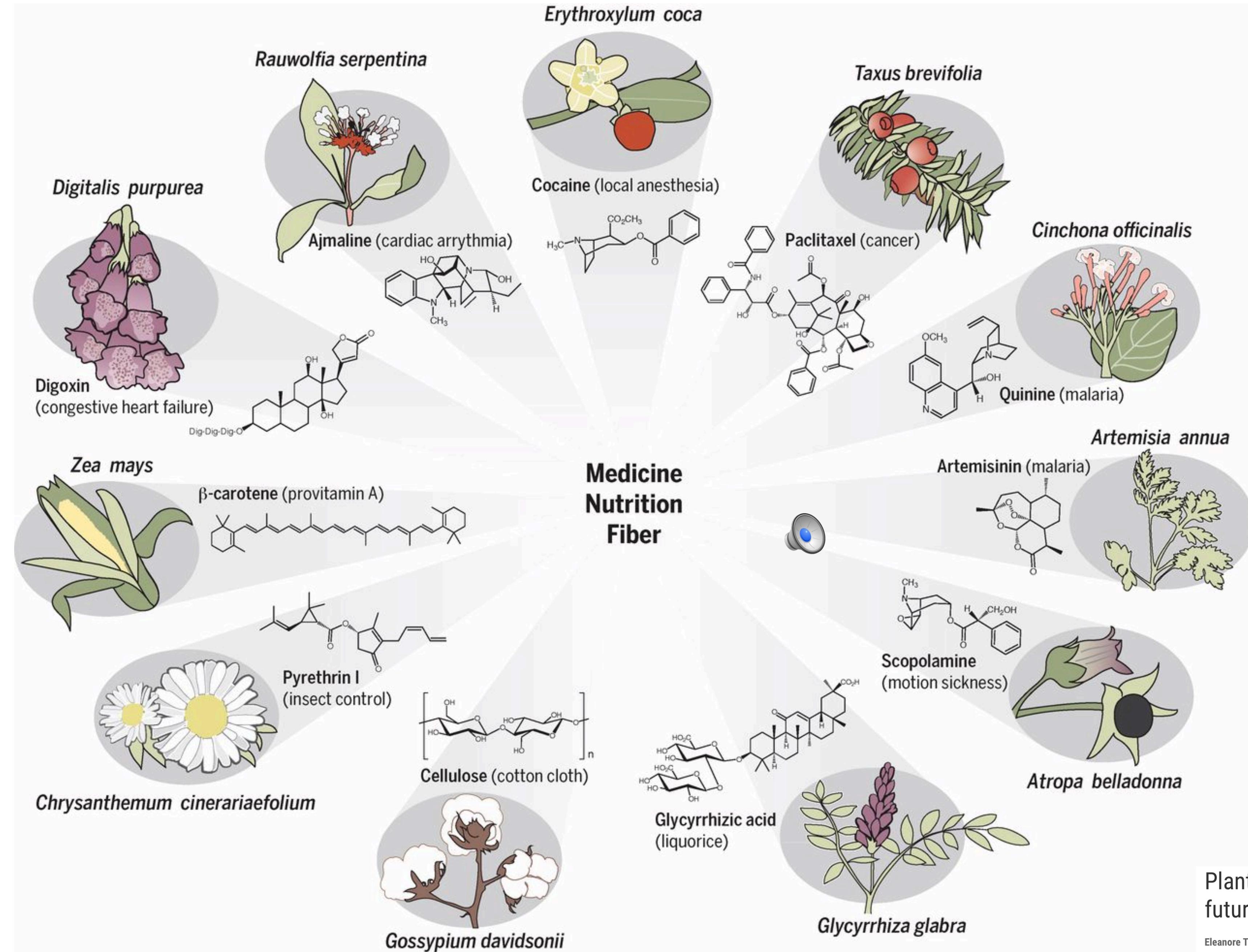
yeast genome
12 Mbp
5900 genes

arabidopsis genome
120 Mbp
38000 genes

human genome
3300 Mbp
20000-25000 genes



wheat genome
15000 Mbp
108000 genes

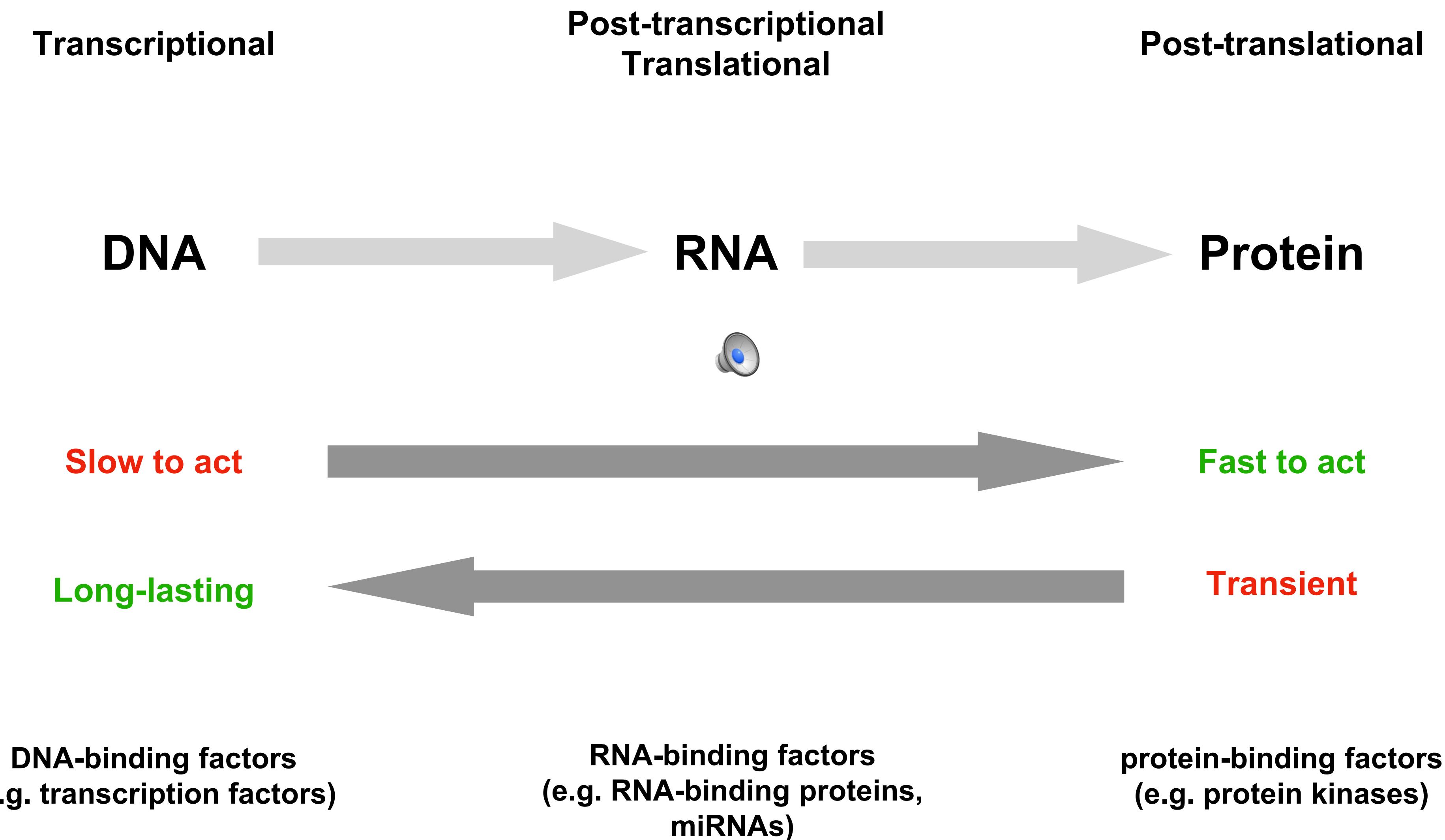


Plant metabolism, the diverse chemistry set of the future

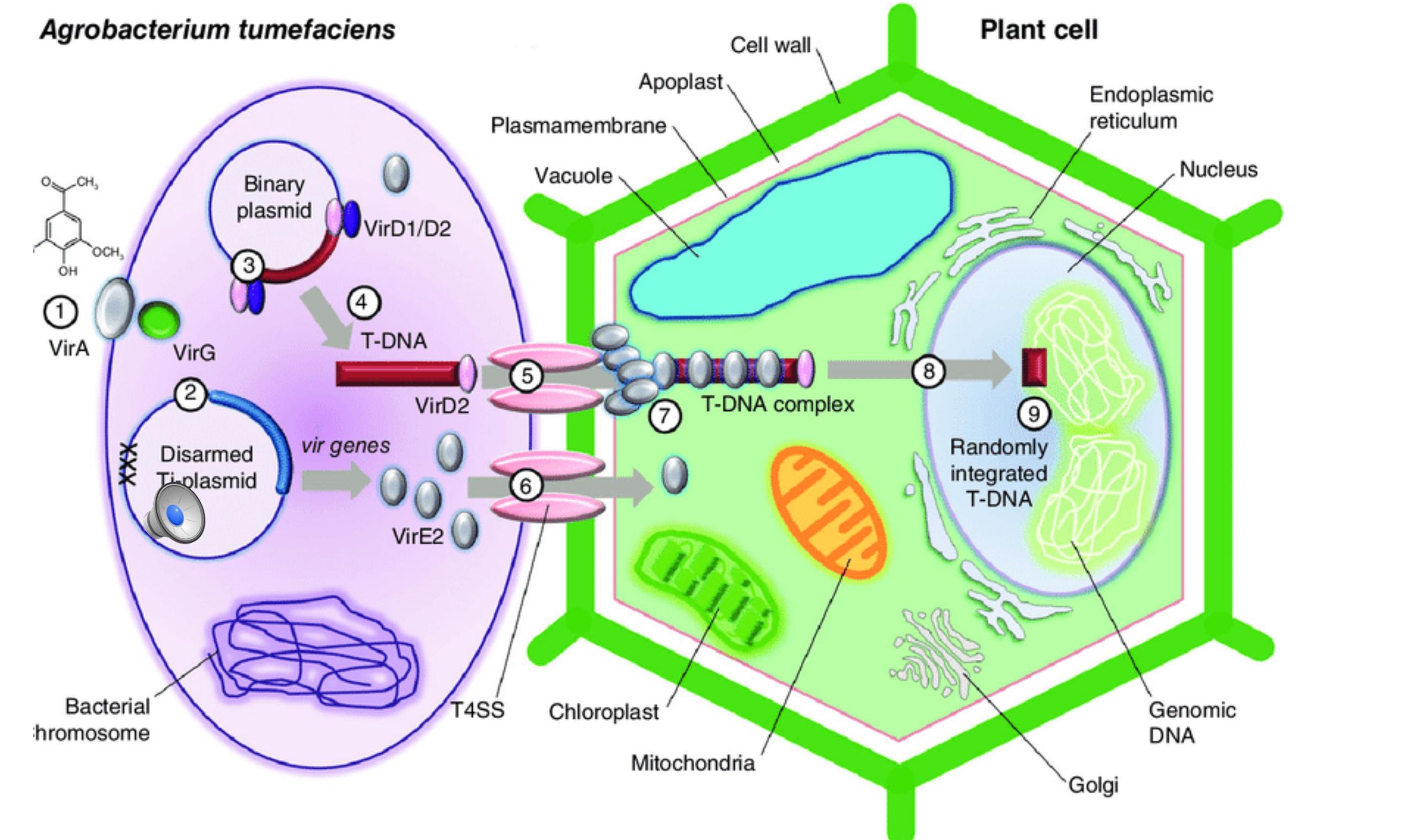
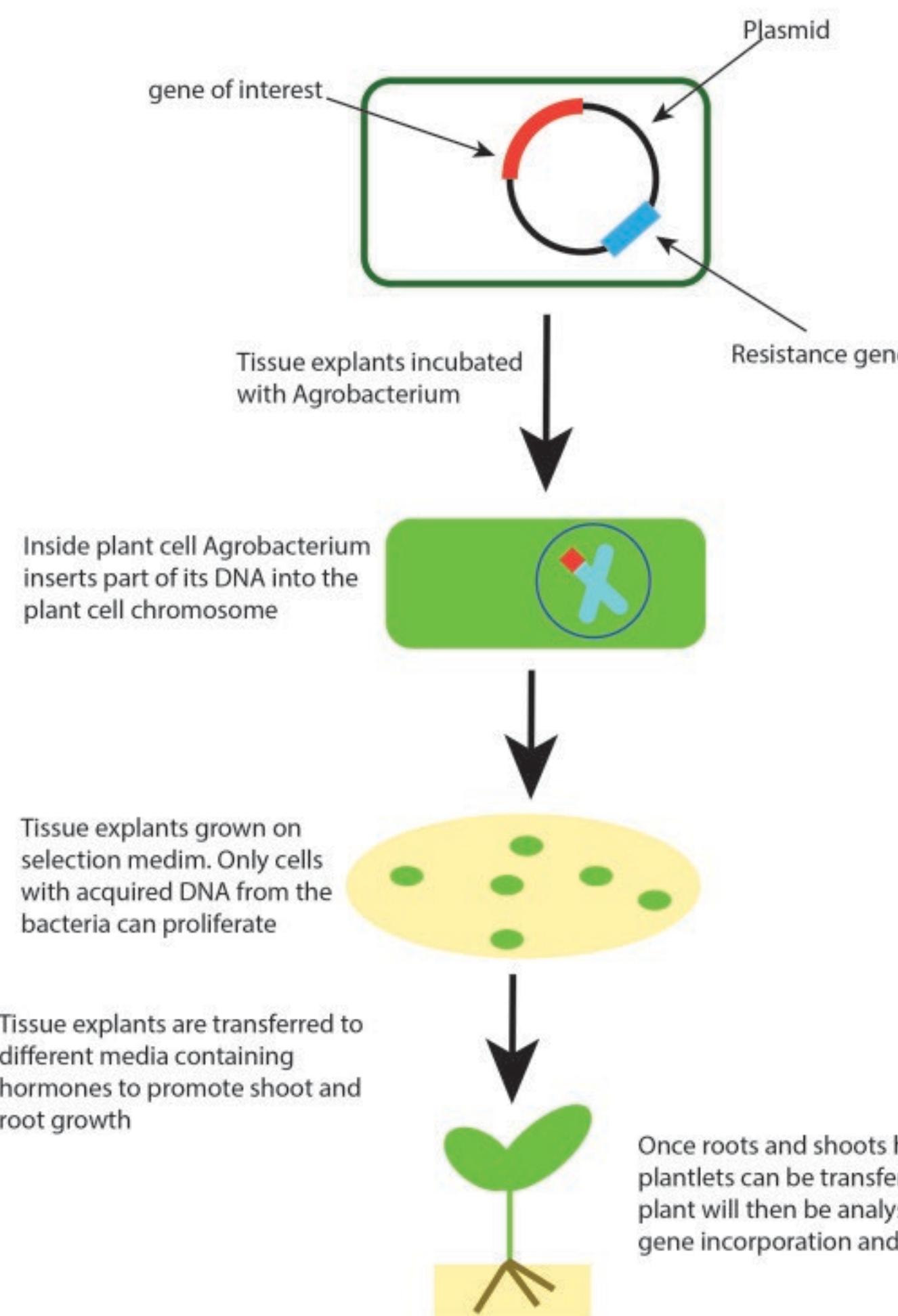
Eleanore T. Wurtzel^{1,2,*}, Toni M. Kutchan^{3,*}

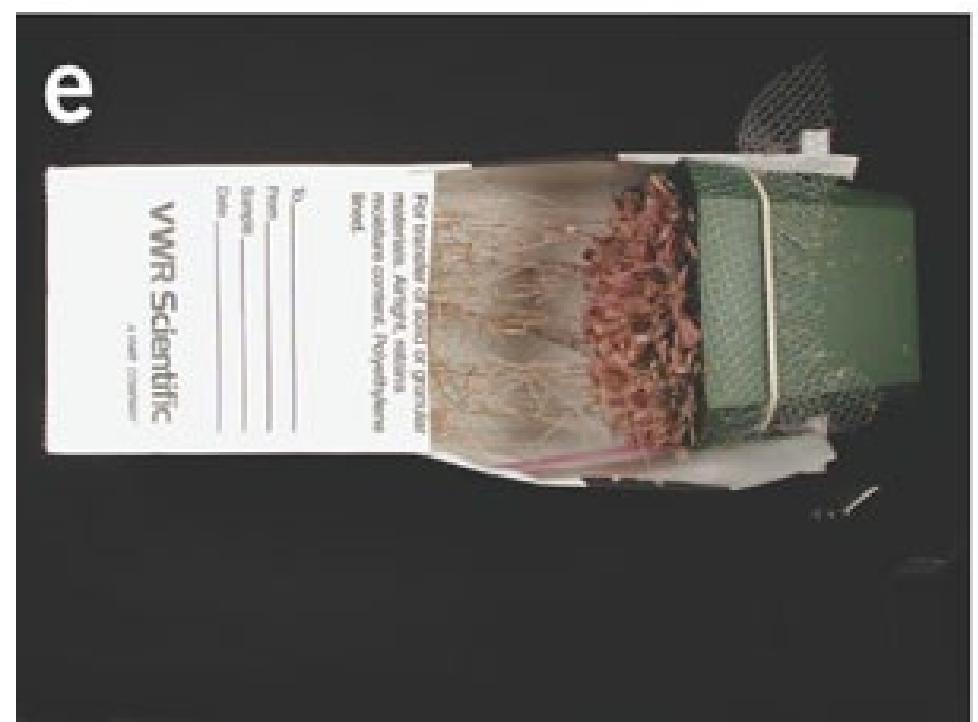
* See all authors and affiliations

Levels at which you can regulate gene expression



Agrobacterium tumefaciens — nature's genetic engineer





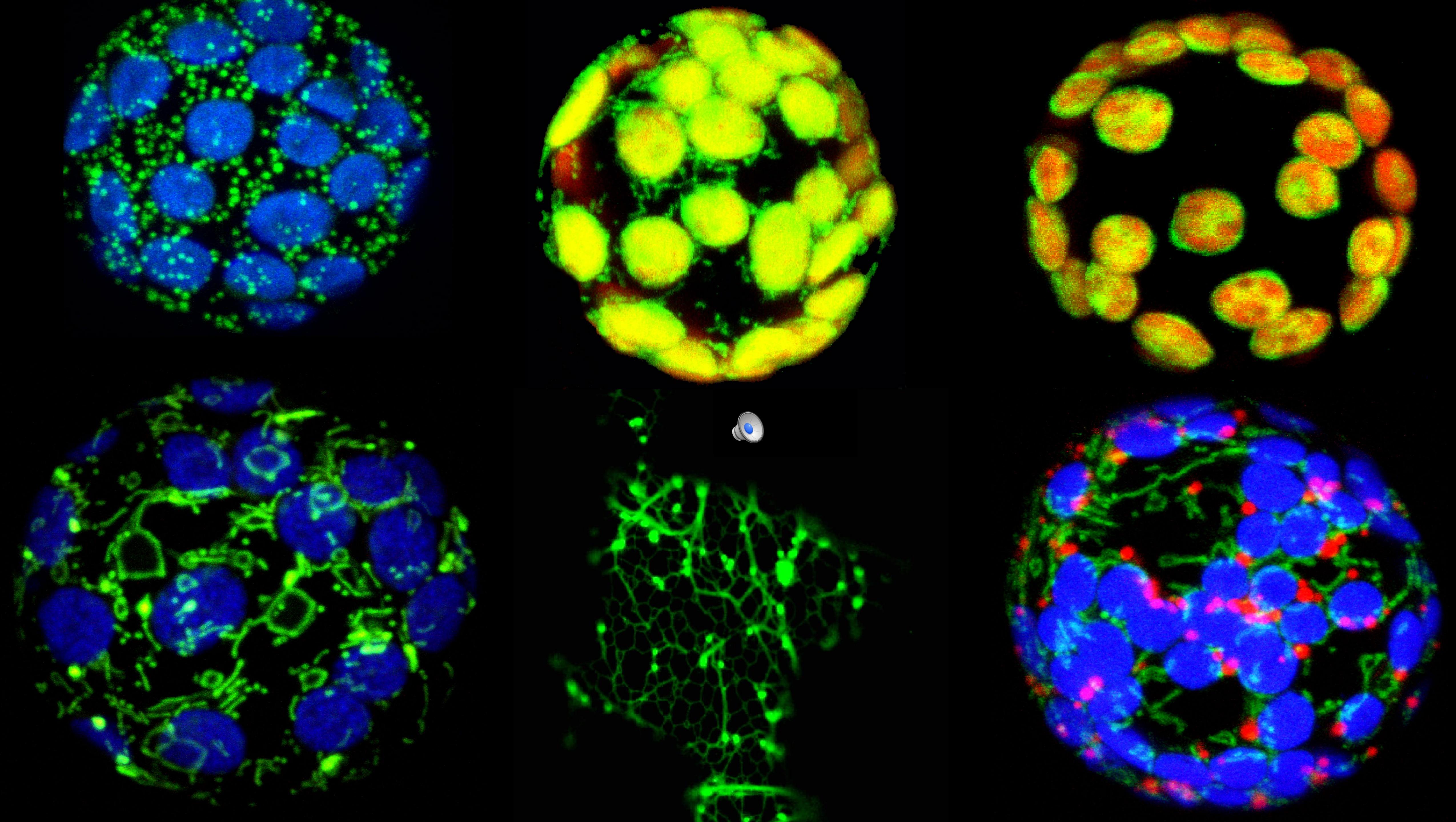
**Agrobacterium-mediated transformation of
Arabidopsis thaliana using the floral dip method**
[Nature Protocols volume 1, pages 641–646](https://doi.org/10.1038/nprot.2006.100)
(2006)



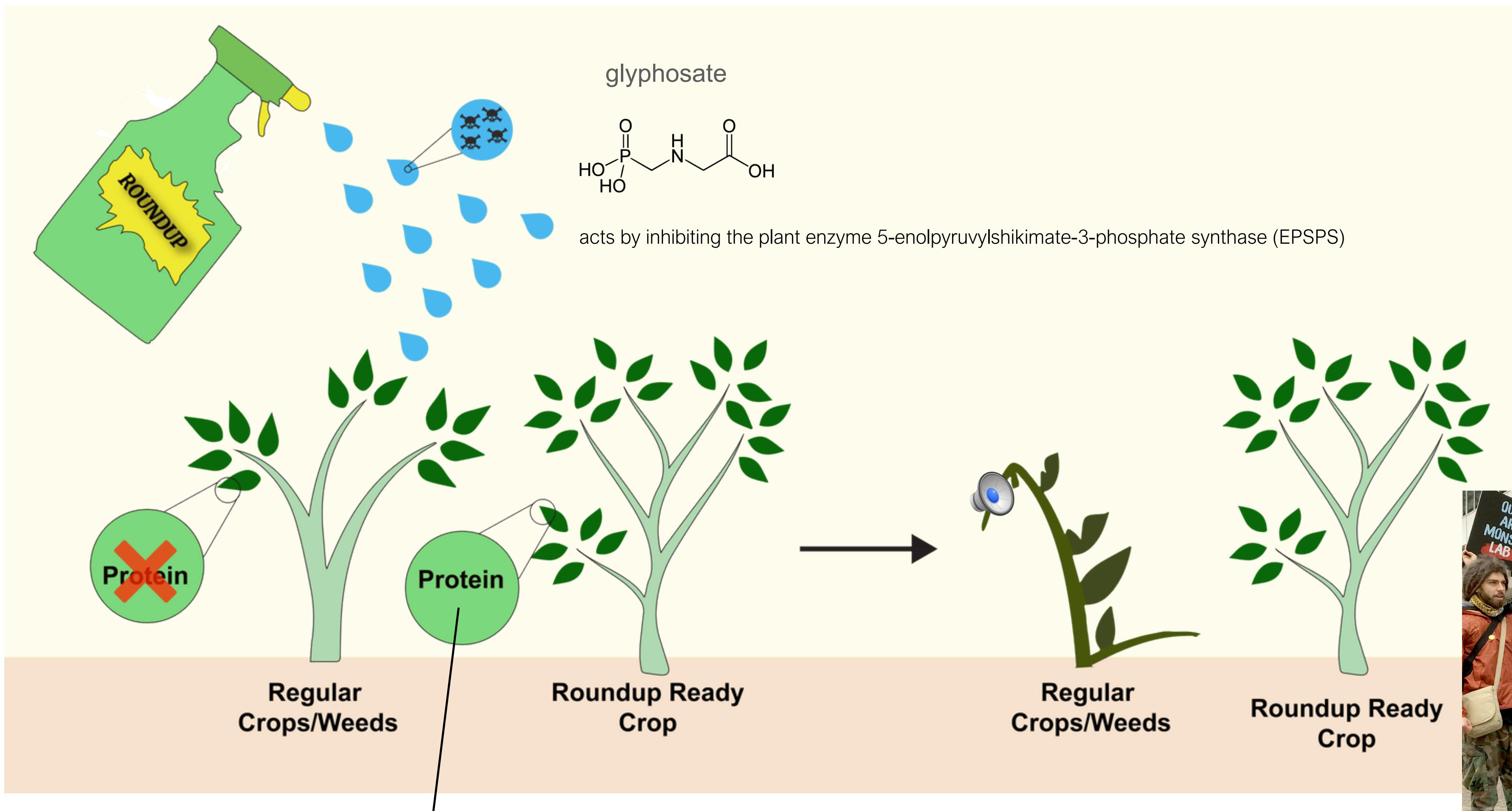
<https://www.youtube.com/watch?v=wDt7s9euS4A>



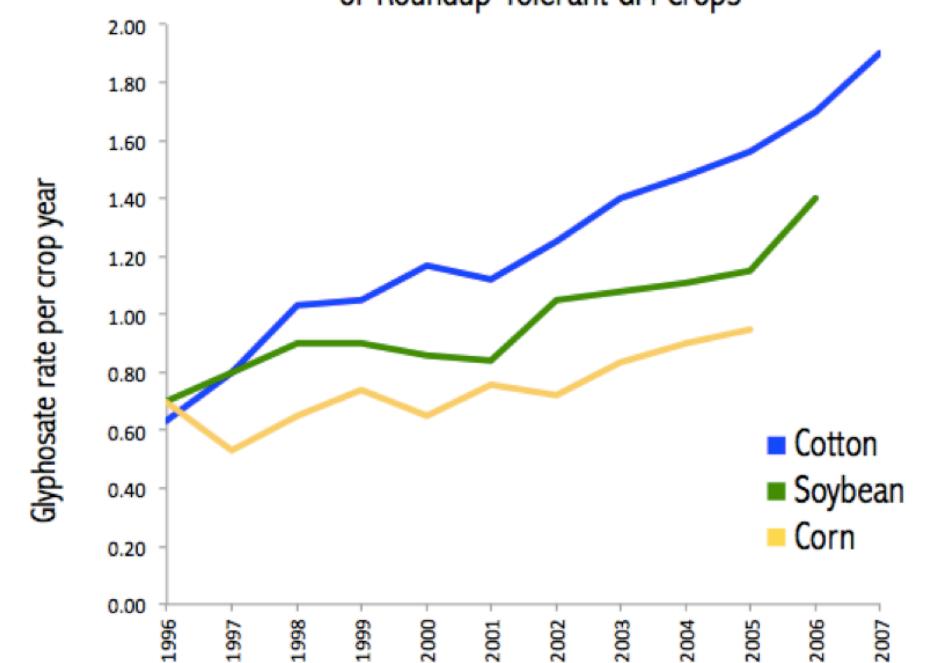
<https://www.youtube.com/watch?v=DsoS7isMg1g>



GM crops are highly controversial...



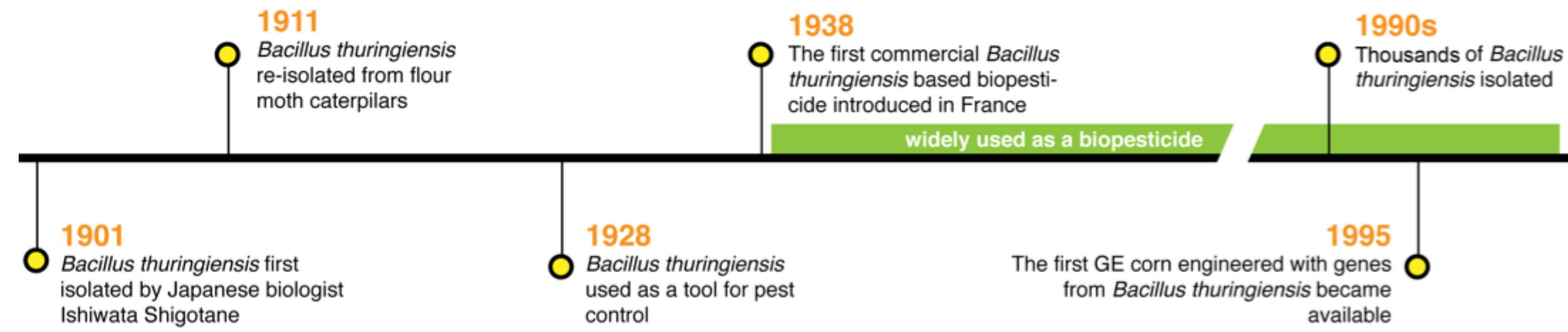
Herbicide use on corn, cotton, and soybean since the introduction of Roundup-Tolerant GM Crops



<http://sitn.hms.harvard.edu/flash/2015/gmos-and-pesticides/>

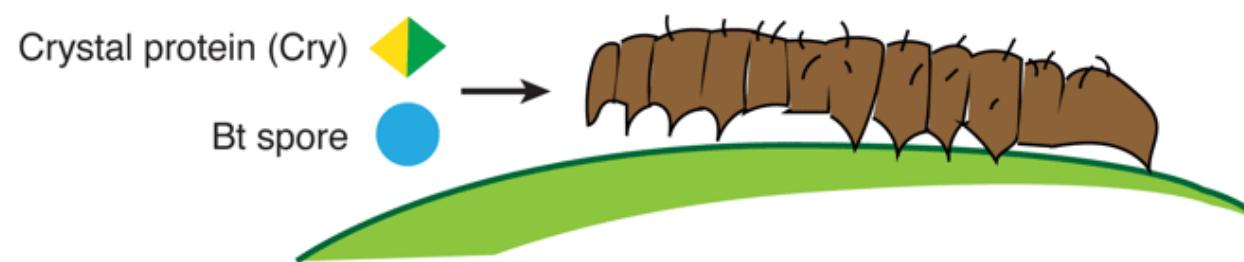


At the highest concentration in bread of 0.080 mg/kg, a person of average body weight (around 70 kg) could eat more than 370 loaves (261 kg) of bread every day over their lifetime without exceeding safe levels of glyphosate dietary exposure.

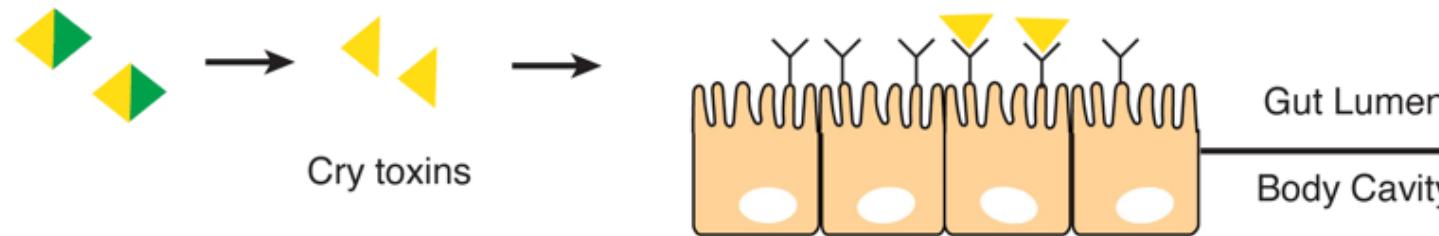


Bacillus thuringiensis (*Bt*) is a very common bacterium found in a variety of soil environments from desert to tundra.

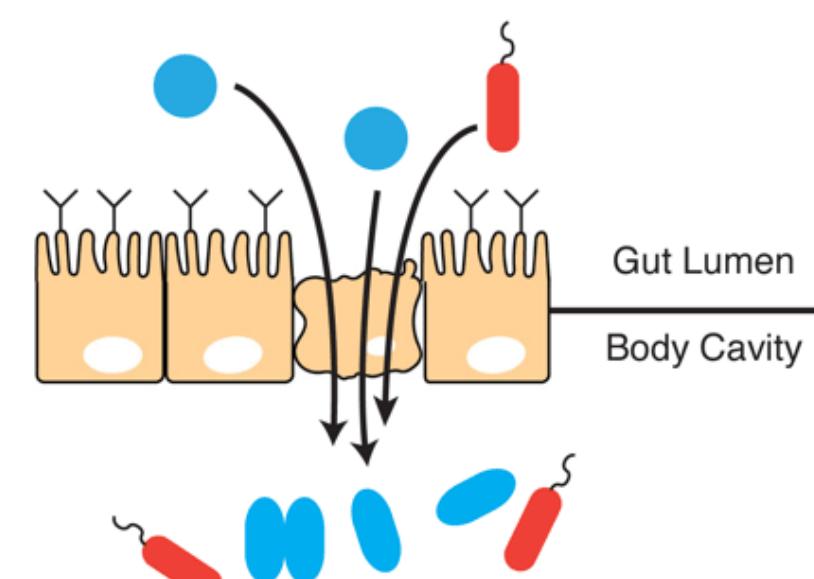
(A) Larvae ingest Bt spores and Cry proteins



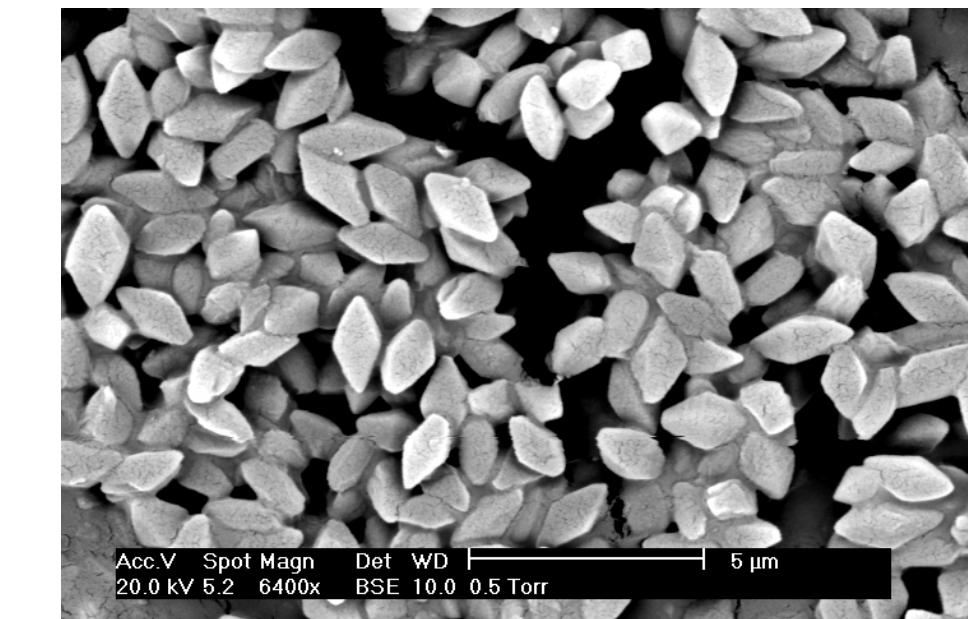
(B) In larval midgut, proteolytic digestion of proteins release Cry toxins, which bind to epithelial receptors



(C) Toxin binding causes cell lysis destroying barrier to body cavity



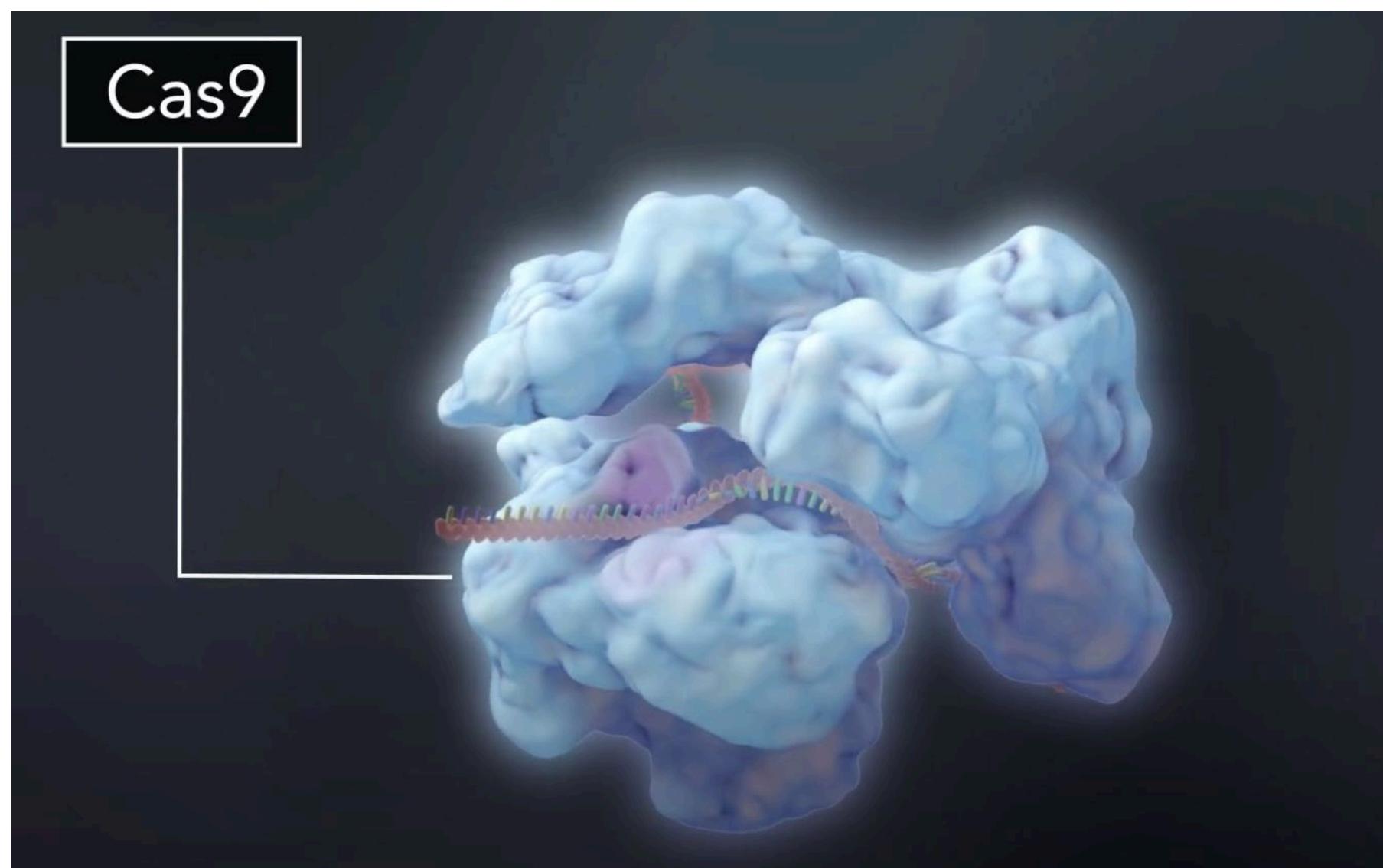
Bt toxin



GM cotton was first grown in Australia in 1996. Over 99.5% of cotton grown in Australia now is genetically modified.			
Licence number	Trade name	Modified trait/s	Issued
DIR 062/2005	LibertyLink® and LibertyLink® x Roundup Ready®	Glufosinate and glyphosate herbicide tolerance	08 Aug 2006
DIR 066/2006	Bollgard II®, Roundup Ready®, Roundup Ready Flex®, Bollgard II® x Roundup Ready® and Bollgard II® x Roundup Ready Flex®	Glyphosate herbicide tolerance; insect resistance	26 Oct 2006
DIR 091	Widestrike™	Insect resistance; glufosinate herbicide tolerance	25 Nov 2009
DIR 118	Pima cotton ⁽¹⁾ Roundup Ready Flex®	Glyphosate herbicide tolerance	16 Aug 2013
DIR 124	Bollgard® 3 and Bollgard® 3 x Roundup Ready Flex®	Glyphosate herbicide tolerance, insect resistance	19 Jun 2014
DIR 143	GlyTol® and GlyTol® x TwinLink Plus®	Insect resistance; glufosinate and glyphosate herbicide tolerance	08 Dec 2016
DIR 145	Xtendflex® and Bollgard® 3 x Xtendflex®	Insect resistance; glufosinate, dicamba and glyphosate herbicide tolerance	20 Dec 2016
DIR 157	COT102 (VIPCOT™ Cotton)	Insect resistance	14 Feb 2018

currently grown GM cotton in Australia (Office of the Gene Technology Regulator, <http://www.ogtr.gov.au>)

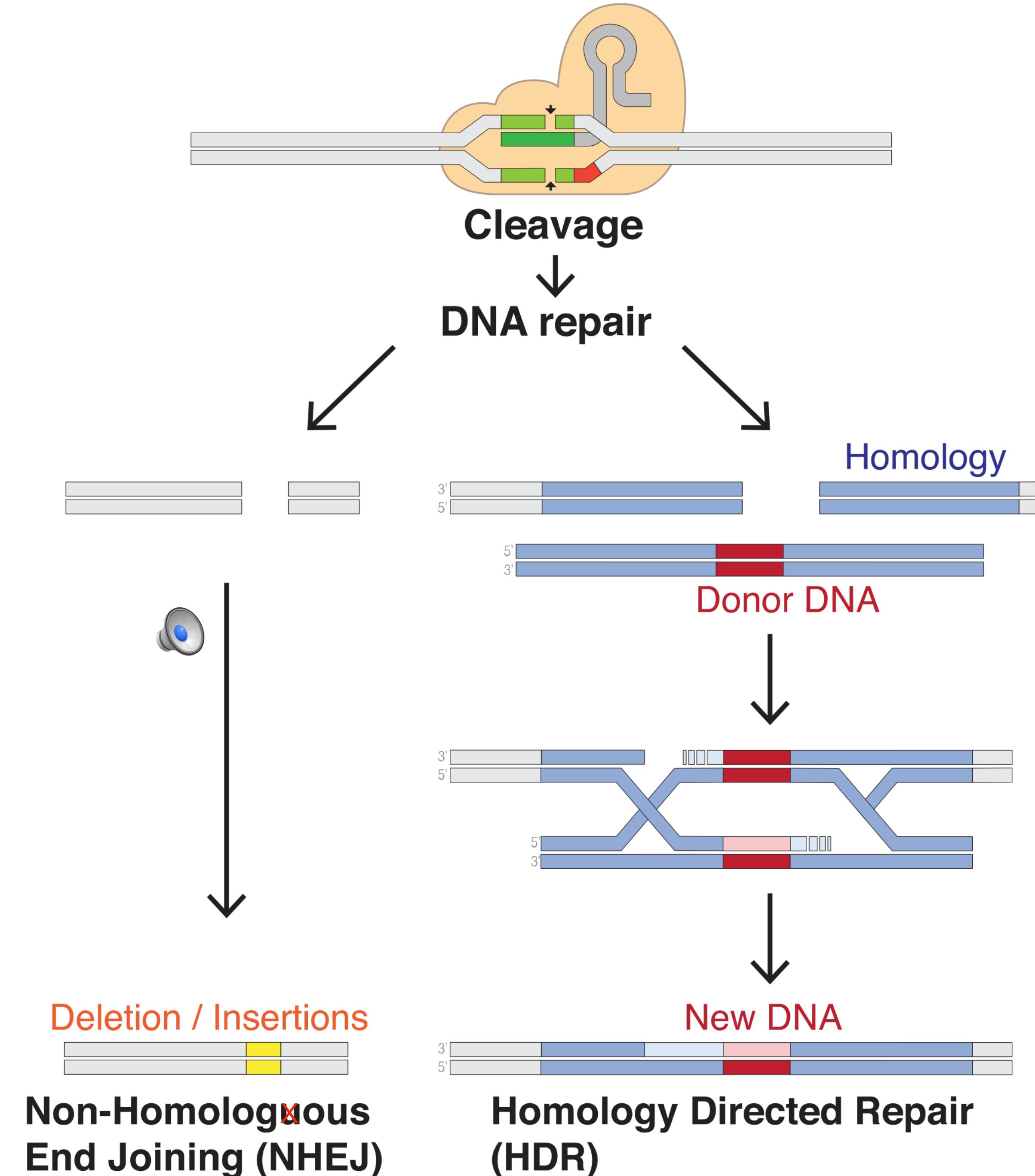
The CRISPR-Cas revolution



<https://www.youtube.com/watch?v=4YKFw2KZA5o>

in plants, only one out of 10,000 breaks is repaired by HDR
(given an ectopic DNA template)

Puchta, *Journal of Experimental Botany*, Volume 56, Issue 409, January 2005, Pages 1–14

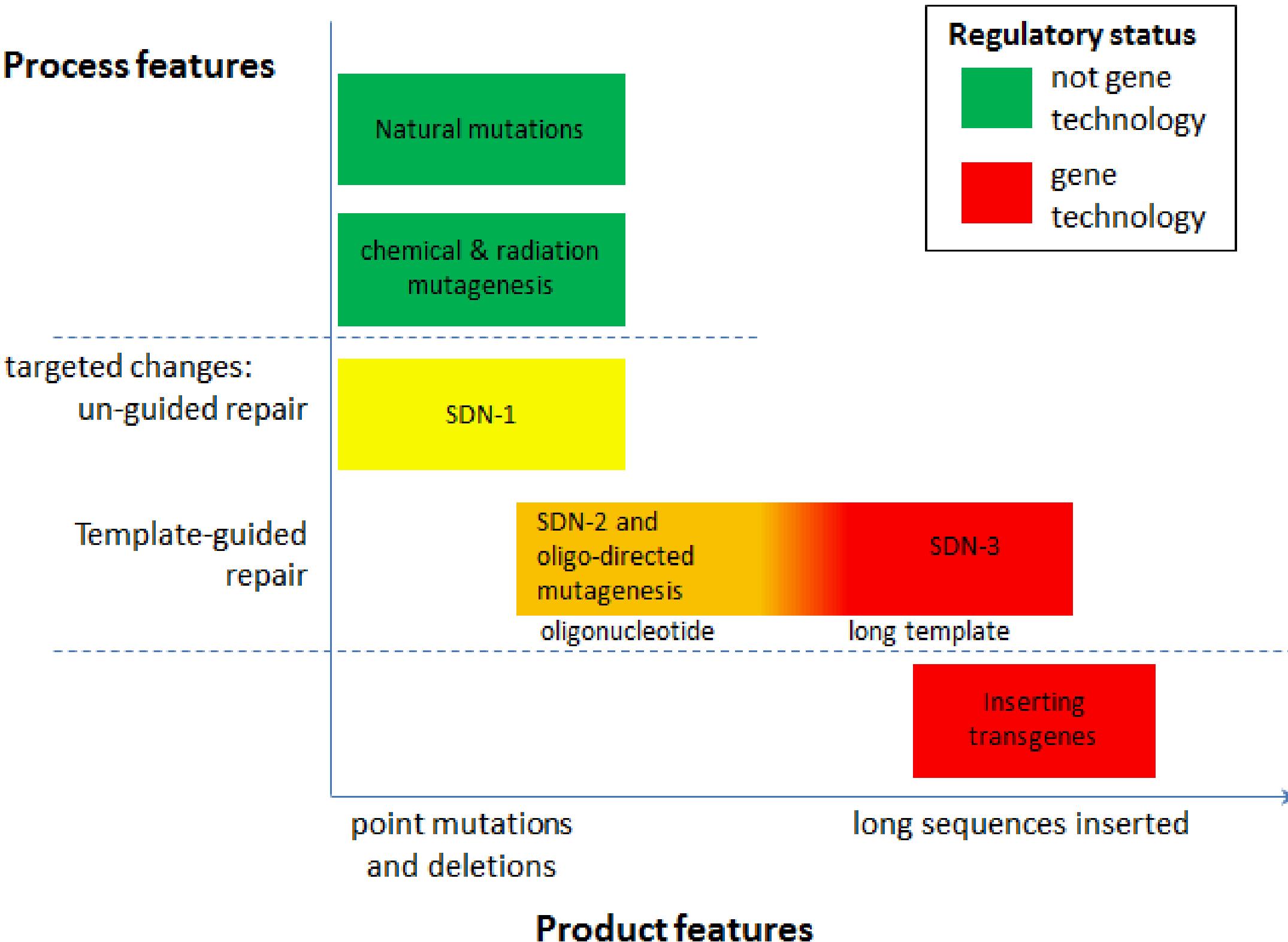


In Australia, GM technology is regulated by the *Gene Technology Act 2000*, administered by the Gene Technology Regulator

Amendments commencing 8 October 2019

Organisms modified using SDN-1 are not GMOs

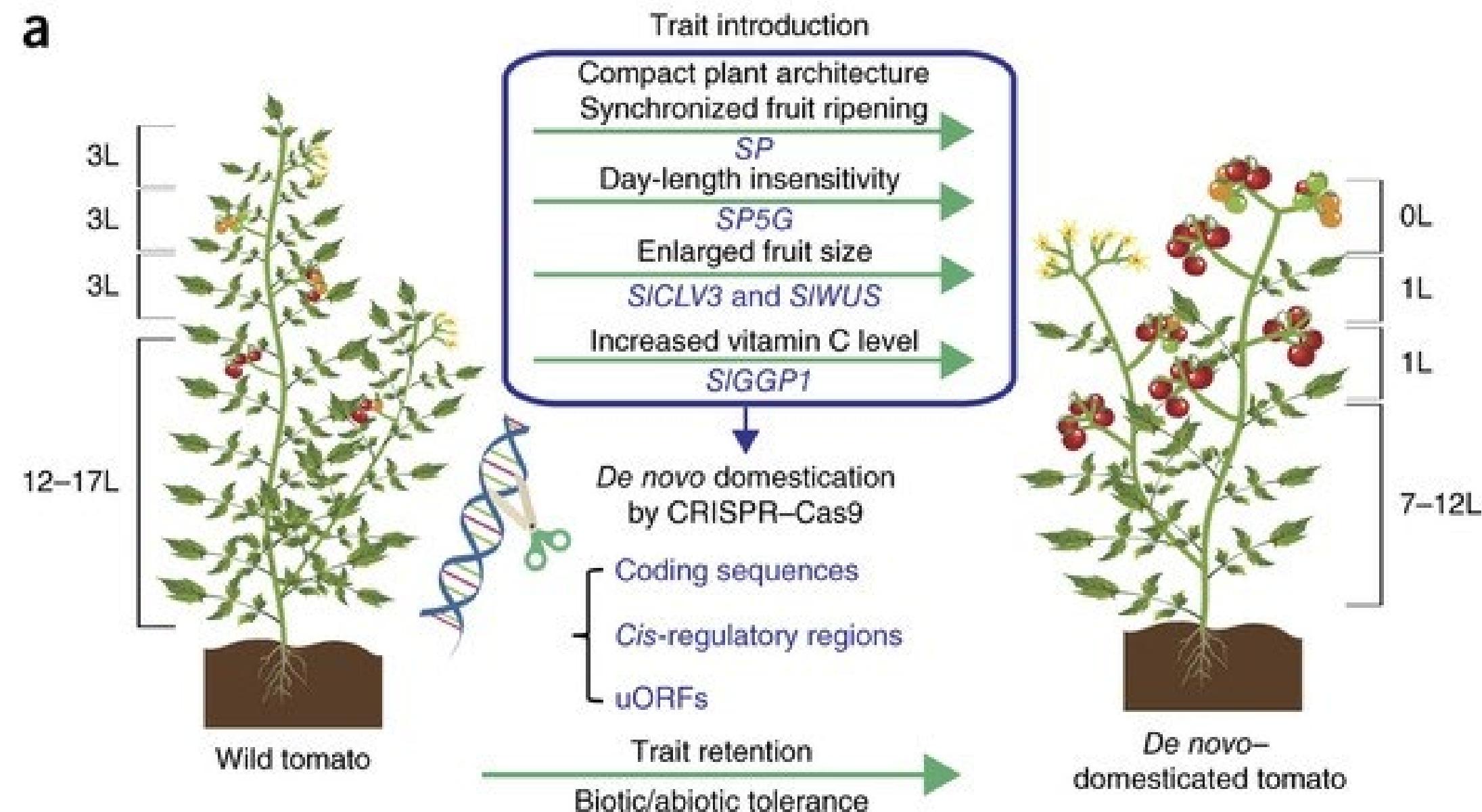
Schedule 1 lists organisms that are not GMOs for the purposes of the Gene Technology Act 2000 (the Act). The amendments add items to this list to exclude organisms modified through unguided repair of site-directed nuclease activity, also known as SDN-1 organisms, from regulation as GMOs. Unguided repair means that no nucleic acid template was added to cells to guide genome repair following SDN application. SDNs include, but are not limited to, CRISPR/Cas9, zinc finger nucleases, meganucleases and TALENs.



Method of site-directed nuclease application			
	SDN Protein (with or without sgRNA)	SDN expressed from a transgene that is only transiently present in the organism	SDN expressed from transgene integrated in the genome
Status of the initial organism modified by SDN-1	Not a GMO (Schedule 1 item 4)	GMO while transgene or its expressed products are present	GMO
		Not a GMO when transgene and expressed products have degraded (Schedule 1 items 4+10)	
Status of offspring inheriting the SDN-1 modification	Not a GMO (Schedule 1 item 9(a))	Not a GMO (Schedule 1 item 9(b))	GMO if SDN transgene also inherited Not a GMO if no SDN transgene inherited (Schedule 1 item 9(b))

'Domestication' of wild species to form farmable crops mostly involves loss-of-function mutations

a



Crop species	Gene target	Function	Mutation type	Genetic effect	Phenotypic outcome	Refs.
Maize	<i>Tb1</i>	TCP-family transcription factor	Retrotransposon insertion in regulatory region	Gain of function	Inhibition of side branching, altering source–sink relations and increasing yield	31, 32
	<i>lg1</i>	Squamosa-promoter binding protein	Retrotransposon insertion	Loss of function	Leaf is upright due to absent ligules and auricles	28, 33
	<i>tga1</i>	SBP-box transcription factor	SNP altering single amino acid	Gain of function	Changes encased to naked kernels	34
	<i>ZmCCT</i>	CCT domain-containing protein	Retrotransposon insertion in regulatory region	Loss of function	Reduction of photoperiod sensitivity	35, 36
Soybean	<i>DT1</i>	CETS family of regulatory genes	SNPs altering amino acids	Loss of function	Changes growth from indeterminate to determinate, producing a shorter, more compact plant	37, 38 ^a
	<i>GA20ox</i>	Gibberellin biosynthesis enzyme	Variation in promoter region	Loss of function	Seed weight	39
	<i>SHAT1-5</i>	NAC-family transcription factor	20-bp deletion disrupting a repressive element	Gain of function	Increased secondary wall biosynthesis promoting thickening of fiber cap cells, leading to reduced shattering	40

Domestication of wild tomato is accelerated by genome editing

Tingdong Li, Xinping Yang, Yuan Yu, Xiaomin Si, Xiawan Zhai, Huawei Zhang, Wenxia Dong, Caixia Gao & Cao Xu

Nature Biotechnology volume 36, pages 1160–1163 (2018)

De novo domestication of wild tomato using genome editing

Agustín Zsögön, Tomáš Čermák, Emmanuel Rezende Naves, Marcela Morato Notini, Kai H Edel, Stefan Weinl, Luciano Freschi, Daniel F Voytas, Jörg Kudla & Lázaro Eustáquio Pereira Peres

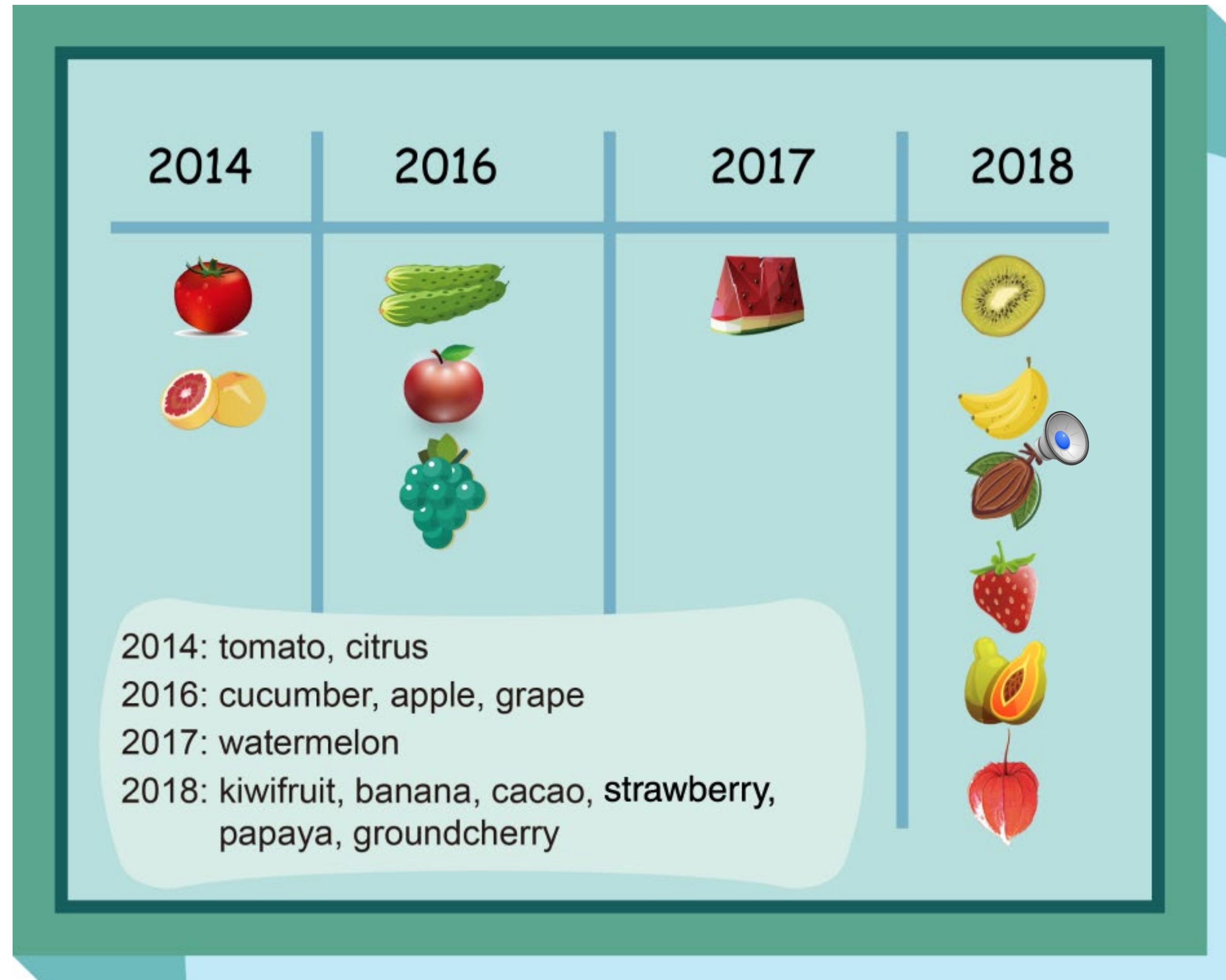
Nature Biotechnology volume 36, pages 1211–1216 (2018)

loss-of-function mutations can appear to be a gain-of-function if it involves loss-of-function of a repressor protein or a repressor element in a promoter

CRISPR technology is revolutionizing the improvement of tomato and other fruit crops

Tian Wang, [Hongyan Zhang](#) & [Hongliang Zhu](#)

Horticulture Research volume 6, Article number: 77 (2019)



Crop species	Target genes	Target traits	Refs.
Resistance to biotic stresses			
Tomato	<i>CP</i> and <i>Rep</i> of virus	Resistance against tomato yellow leaf curl virus	83
Tomato	<i>DCL2</i>	Susceptibility to potato virus X, tobacco mosaic virus, and tomato mosaic virus	84,85
Tomato	<i>DMR6</i>	Resistance against downy mildew	86
Tomato	<i>MLO1</i>	Resistance against powdery mildew	87
Tomato	<i>PMR4</i>	Resistance against powdery mildew	88
Tomato	<i>Solyco8g075770</i>	Susceptibility to <i>Fusarium</i> wilt disease	89
Tomato	<i>MAPK3</i>	Susceptibility to gray mold disease	90
Tomato	<i>JAZ2</i>	Resistance against bacterial speck disease	91
Banana	ORF region of virus	Resistance against banana streak virus	92
Cucumber	<i>eIF4E</i>	Resistance against cucumber vein yellowing virus, zucchini yellow mosaic virus, and papaya ring spot mosaic virus	93
Grape	<i>MLO7</i>	Resistance against powdery mildew	94
Grape	<i>WRKY52</i>	Resistance against gray mold disease	95
Cacao	<i>NPR3</i>	Resistance against <i>Phytophthora tropicalis</i>	96
Papaya	<i>alEPIC8</i>	Resistance against <i>Phytophthora palmivora</i>	97
Citrus	<i>LOB1 promoter</i>	Resistance against citrus canker	98,99
Apple	<i>DfPM1, 2, 4</i>	Resistance against fire blight disease	94
Resistance to abiotic stresses			
Tomato	<i>BZR1</i>	Decrease in heat stress tolerance	100
Tomato	<i>CBF1</i>	Decrease in chilling stress tolerance	101
Tomato	<i>MAPK3</i>	Decrease in drought stress tolerance	102
Watermelon	<i>ALS</i>	Resistance against herbicide	103
Fruit quality improvement			
Tomato	<i>CLV3, Ic</i>	Fruits with increasing locule numbers	104
Tomato	<i>PSY1</i>	Yellow-colored tomato	105
Tomato	<i>MYB12</i>	Pink-colored tomato	106
Tomato	<i>ANT2</i> (gene insertion)	Purple-colored tomato	107
Tomato	<i>PL</i>	Long-shelf life tomato	108
Tomato	<i>ALC</i>	Long-shelf life tomato	109
Tomato	<i>MPK20</i>	Repression of genes controlling sugar metabolism	110
Tomato	<i>ANT2</i> (gene insertion)	Increase in anthocyanin content	107
Tomato	<i>GAD2, GAD3</i>	Increase in GABA content	111
Tomato	<i>GABA-TP1, GABA-TP2, GABA-TP3, CAT9, SSADH</i>	Increase in GABA content	112
Tomato	<i>SGR1, LCY-E, Blc, LCY-B1, LCY-B2</i>	Increase in lycopene content	113
Tomato	<i>ALMT9</i>	Decrease in malate content	114
Fruit crop domestication			
Tomato	<i>AGL6</i>	Production of parthenocarpic fruit	115
Tomato	<i>IAA9</i>	Production of parthenocarpic fruit	116
Tomato	<i>ARF7</i>	Production of parthenocarpic fruit	117
Tomato	<i>MBP21</i>	Generation of "jointless" fruit stem	118
Tomato	<i>GAI</i>	Generation of dwarf tomato plants	119
Tomato	<i>BOP1, BOP2, BOP3</i>	Early flowering with simplified inflorescences	120
Tomato	<i>SP, SP5G, CLV3, WUS, GGP1</i>	Introduction of traits associated with morphology, flower and fruit production, and ascorbic acid synthesis	121
Tomato	<i>SP, OVATE, MULT, FAS, CycB</i>	Introduction of traits associated with morphology, flower number, tomato size and number, and lycopene synthesis	122
Tomato	<i>SP5G</i>	Generation of loss of day-length-sensitive tomato plants	123
Cucumber	<i>WIP1</i>	Generation of gynoecious plant	124
Groundcherry	<i>SP, SP5G, CLV1</i>	Introduction of traits associated with morphology, flower production, and fruit size	125
Kiwifruit	<i>CEN4, CEN</i>	Generation of a compact plant with rapid terminal flower and fruit development	126

OpenPlant Synthetic Biology Research Centre

OpenPlant is a joint initiative between the University of Cambridge, John Innes Centre and the Earlham Institute, funded by the BBSRC and EPSRC as part of the UK Synthetic Biology for Growth programme.

Synthetic Biology offers the prospect of reprogrammed biological systems for improved and sustainable bioproduction. While early efforts in the field have been directed at microbes, the engineering of plant systems offers even greater potential benefits. Plants are already cultivated globally at low cost, harvested on the giga-tonne scale, and routinely used to produce the widest range of biostuffs, from fibres, wood, oils, sugar, fine chemicals, drugs to food.

There is an urgent need to improve our ability to reprogram crop metabolism and plant architecture in the face of global threats from new pathogens, climate change, soil degradation, restricted land use, salinity and drought. The next generation of DNA tools for "smart" breeding of crop systems should be shared - to promote global innovation and equitable access to sustainable bioeconomies.



OpenPlant is: (i) developing new tools and methods for plant synthetic biology, (ii) providing mechanisms for open sharing of standardised resources, (iii) applying these tools to world-leading projects in trait development, and (iv) facilitating interdisciplinary exchange, outreach and international development. The initiative promotes interdisciplinary exchange, open technologies and responsible innovation for improvement of sustainable agriculture and conservation. Further details of the vision and working principles of the OpenPlant initiative can be found on the [VISION](#) page.



The Earlham BIO Foundry



<https://www.youtube.com/watch?v=wYfqVhWAbIU>

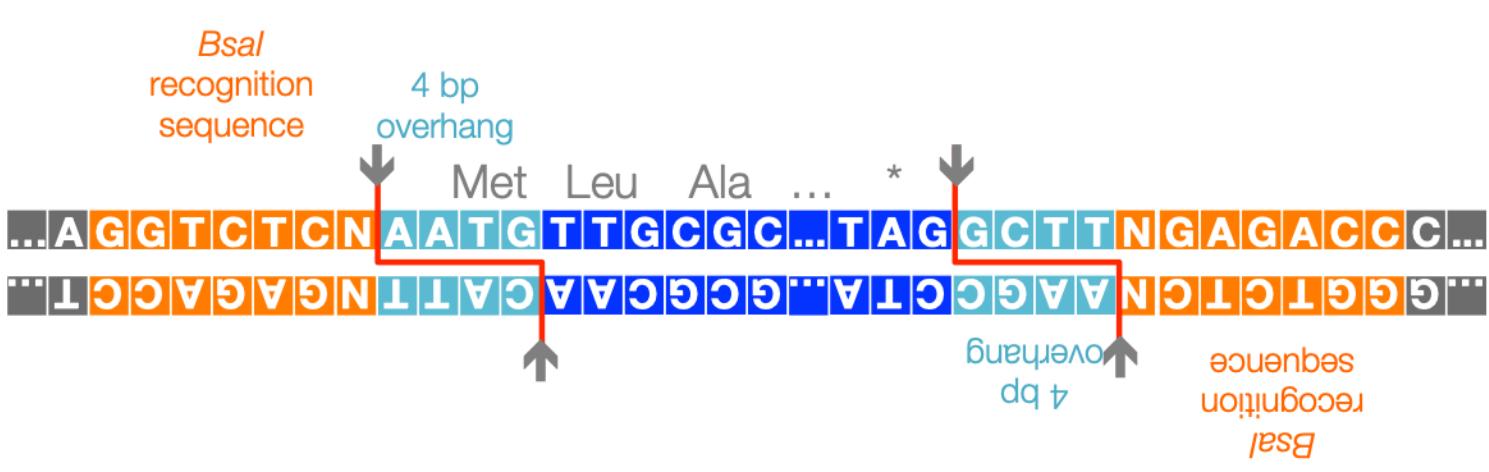


Figure 1. The cleavage sites of the Type IIs enzymes used in Golden Gate cloning are downstream of the recognition site and can be composed of any sequence. After ligation, no scar will exist between adjacent fragments.

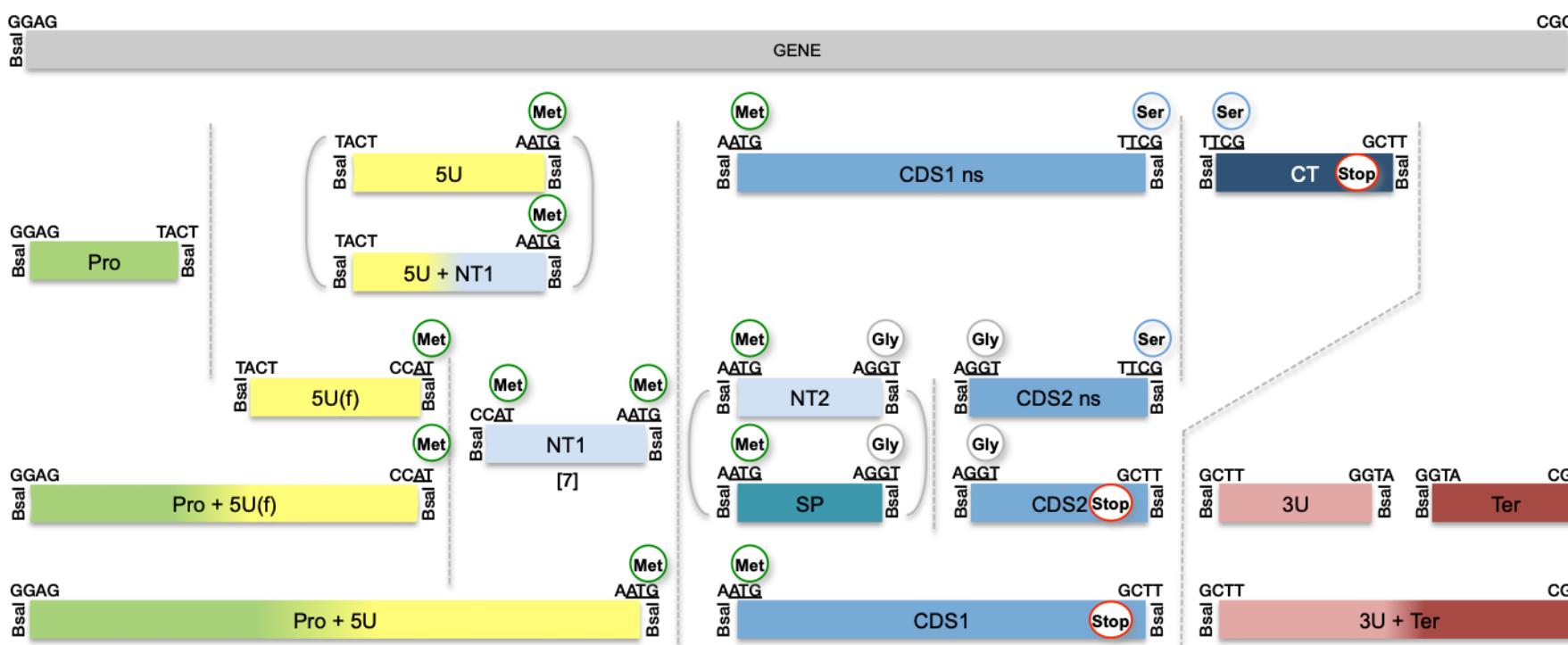


Figure 2. A modular version of Golden Gate Cloning (MoClo) has been developed in which specified, standard overhangs are used for predefined-parts of basic genetic grammar. This simplifies the rules for assembly and allows laboratories to exchange standard modules.

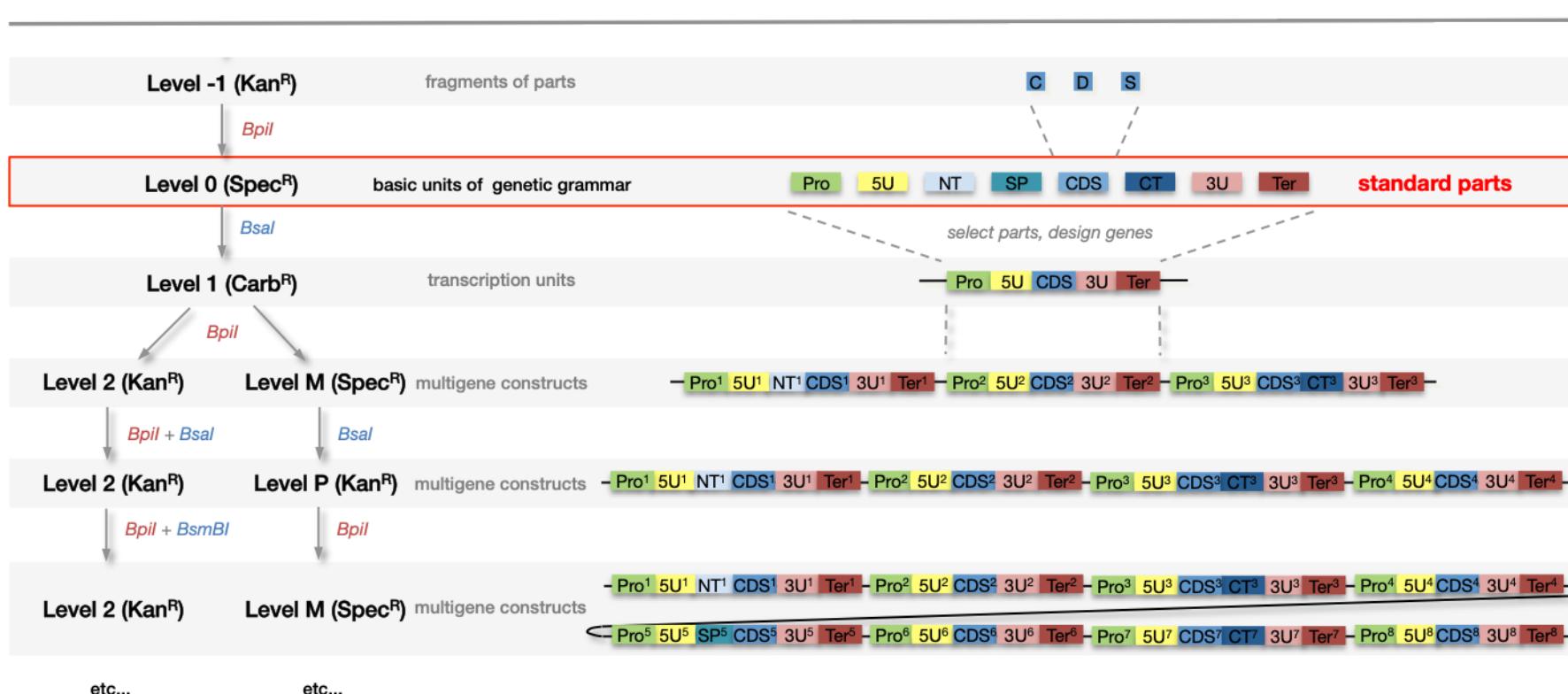


Figure 3. The Golden Gate MoClo Assembly Standard: Standard (Level 0) parts are assembled from single or multiple sequences either directly or via intermediate (Level -1) fragments. Level 0 parts are assembled into Level 1 acceptor backbones to make complete transcriptional units. Multigene constructs can be made by assembling level one constructs in Level 2, M, or P acceptor backbones.

A Golden Gate Modular Cloning Toolbox for Plants

The Sainsbury Laboratory



Carola Engler,[†] Mark Youles,[‡] Ramona Gruetzner,[§]
Tim-Martin Ehnert,[†] Stefan Werner,[†] Jonathan D. G. Jones,[‡]
Nicola J. Patron^{*‡} & Sylvestre Marillonnet^{*§}



[†] Icon Genetics GmbH, Weinbergweg 22, Biozentrum Halle, 06120 Halle, Germany, [‡] The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK, [§] Leibniz-Institut für Pflanzenbiochemie, Weinberg 3, 06120 Halle, Germany

Appendix: The Golden Gate MoClo Plant Parts Kit contains 96 standard parts:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pICH41373 plant virus Pro	pICH45180 plant Pro	pICH51277 plant + virus Pro + 5U	pICH45234 plant Pro + 5U	pICH87611 plant + virus Pro + 5U	pICH78133 chloroplast TP 5U + NT1	pICSL30008 antigenic NT1	pICSL50007 antigenic CT	pICSL50004 reporter CT	pICH49477 reporter CDS1	pICSL80016 reporter CDS1 ns	pICH44300 plant 3U + Ter
B	pICH41388 CaMV 35s plant virus Pro	pICH45125 plant Pro	pICH51288 plant + virus Pro + 5U	pICH45244 plant Pro + 5U	pICH41402 plant virus 5U	pICH78141 SP 5U + NT1	pICSL30009 antigenic NT1	pICSL50009 antigenic CT	pICSL50011 reporter CT	pICSL80004 reporter CDS1	pICSL70002 selection gene	pICH77901 bacterial 3U + Ter
C	pICH45089 plant virus Pro	pICH45131 plant Pro	pICSL12006 plant virus Pro + 5U	pICH45214 plant Pro + 5U	pICH44199 plant virus 5U	pAGM5331 nuclear TP 5U + NT1	pICSL30004 reporter NT1	pICSL50010 antigenic CT	pICSL50015 reporter CT	pICSL80001 reporter CDS1	pICSL70004 selection gene	pICH77911 bacterial 3U + Ter
D	pICH42211 bacterial Pro	pICH45152 plant Pro	pICH87633 bacterial + virus Pro + 5U	pICH87655 plant + virus Pro + 5U	pICH44222 plant virus 5U	pAGM1482 mitochondrial TP 5U + NT1	pICSL30006 reporter NT1	pICSL50012 antigenic CT	pICSL50006 reporter CT	pICH75111 reporter CDS1	pICSL70005 selection gene	pICH41432 bacterial 3U + Ter
E	pICH50581 plant Pro	pICH41551 plant Pro	pICH85281 bacterial Pro + 5U	pICH71292 plant Pro + 5U	pICH44233 plant virus 5U	pAGM5355 chloroplast TP + HIS tag 5U + NT1	pICSL30003 reporter NT1	pICSL50013 antigenic CT	pICSL80014 reporter CDS1	pICL42222 selection CDS1	pICSL70008 selection gene	pICH71431 plant 3U + Ter
F	pICH42760 plant Pro	pICSL13001 plant + virus Pro + 5U(f)	pICH88103 bacterial Pro + 5U	pICH71301 plant Pro + 5U	pICH44188 plant virus 5U	pAGM5343 SP+ HIS tag 5U + NT1	pICSL30010 reporter NT1	pICL50014 antigenic CT	pICH41531 reporter CDS1	pICH43844 selection CDS1	pICH41414 plant virus 3U + Ter	pICH71411 plant 3U + Ter
G	pICH44157 plant Pro	pICSL13002 plant + virus Pro + 5U(f)	pICH87644 plant + virus Pro + 5U	pICH71311 plant Pro + 5U	pICH44179 plant 5U	pAGM1467 HIS tag + EK 5U + NT1	pICL37431 signal peptide SP	pICSL50008 reporter CT	pICSL80005 reporter CDS1	pICH44022 silencing suppressor CDS1	pICH72400 bacterial 3U + Ter	pICH71421 plant 3U + Ter
H	pICH45173 plant Pro	pICH51266 plant + virus Pro + 5U	pICH45195 plant Pro + 5U	pICH71342 plant Pro + 5U	pAGM1479 HIS tag 5U + NT1	pICSL30005 antigenic NT1	pICL37326 signal peptide SP	pICSL50016 reporter CT	pICSL80007 reporter CDS1	pICSL80012 reporter CDS2	pICH41421 bacterial 3U + Ter	pAGT707 plant virus 5U(f)



MoClo Plant Parts Kit

(Kit #1000000047)

[Add to Cart](#)

\$350 USD + shipping

Available to academics and nonprofits only.

Depositing Labs: [Nicola Patron](#)

This modular cloning (MoClo) collection comprises 95 standardized parts to enable Golden Gate construction of multi-gene constructs for plant transformation. Thirty nine parts encode promoters and 5' untranslated regions; eleven parts encode antigenic tags; eight parts encode sub-cellular localisation signals; nineteen parts encode reporter genes; six parts encode selectable marker genes; nine parts encode terminators and 3' untranslated regions. A suppressor of silencing and two linkers are also included.

This kit consists of 1 plate, and will be shipped to most countries as bacterial glycerol stocks, 96-well format, on dry ice. Samples should be frozen at -80°C immediately upon arrival. Individual plasmids can be ordered from each plasmid page and will be shipped as bacterial stabs.



You may also like...

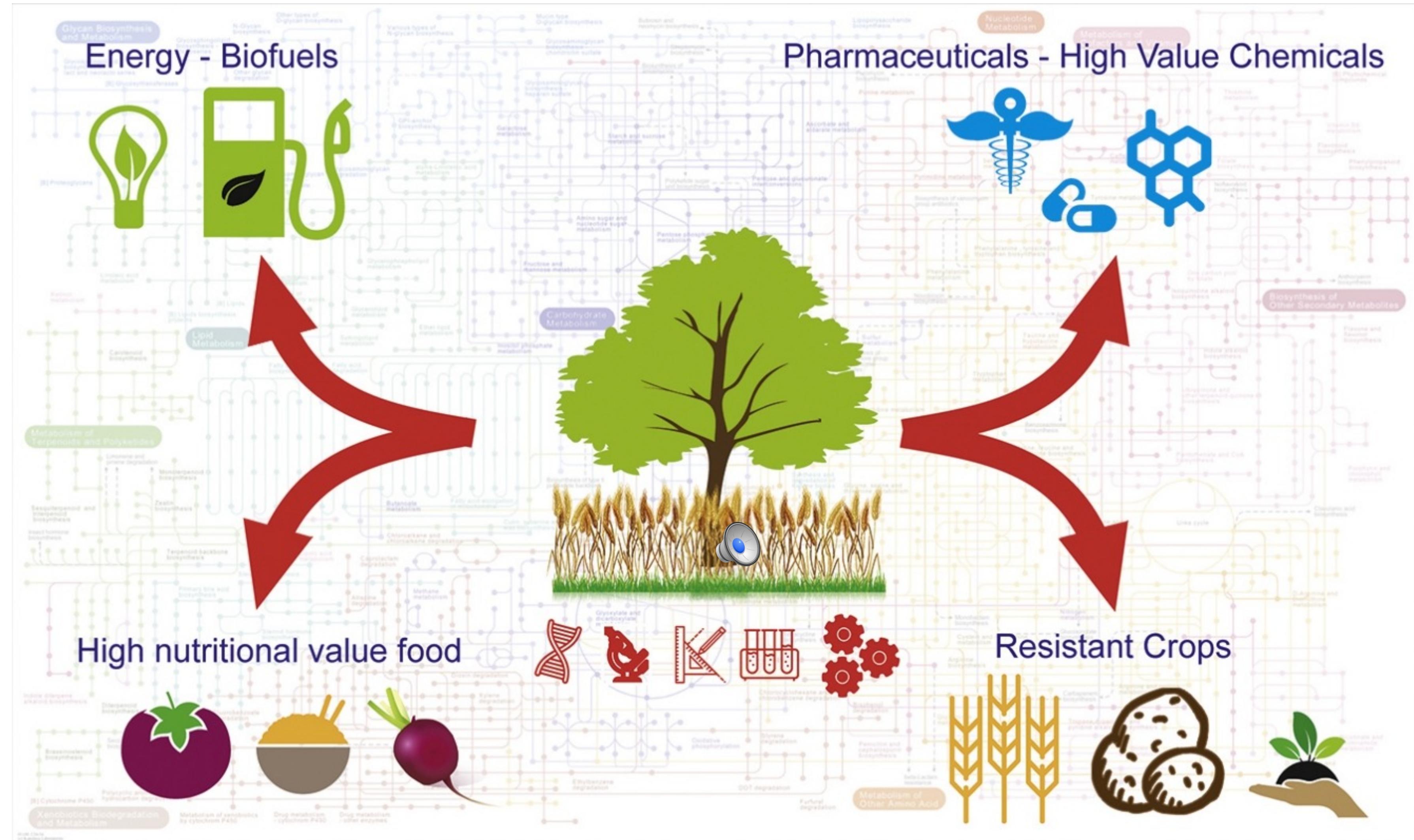
[Modular Cloning \(MoClo\) Guide](#)[MoClo Toolkit](#)[Synthetic Biology Guide](#)[CRISPR Plasmids](#)[Detailed Information](#)[Protocols & Resources](#)[Contents of Kit](#)

Original Publication

A Golden Gate Modular Cloning Toolbox for Plants. Engler C, Youles M, Gruetzner R, Ehnert T-M, Werner S, Jones JDG, Patron NJ, and Marillonnet S. *ACS Synthetic Biology* . 2014 Feb 20. doi: 10.1021/sb4001504. PubMed [PMID 24933124](#).

Description

This collection comprises 95 standardized parts to enable Golden Gate construction of multi-gene constructs for plant transformation. Thirty nine parts encode promoters and 5' untranslated regions; eleven parts encode antigenic tags; eight parts encode sub-cellular localisation signals; nineteen parts encode reporter genes; six parts encode selectable marker genes; nine parts encode terminators and 3' untranslated regions. A suppressor of silencing and two linkers are also included.



New developments in engineering plant metabolic pathways

Author links open overlay panel

Plant Metabolic Engineering Strategies for the Production of Pharmaceutical Terpenoids

Front. Plant Sci., 08 November 2016 | <https://doi.org/10.3389/fpls.2016.01647>

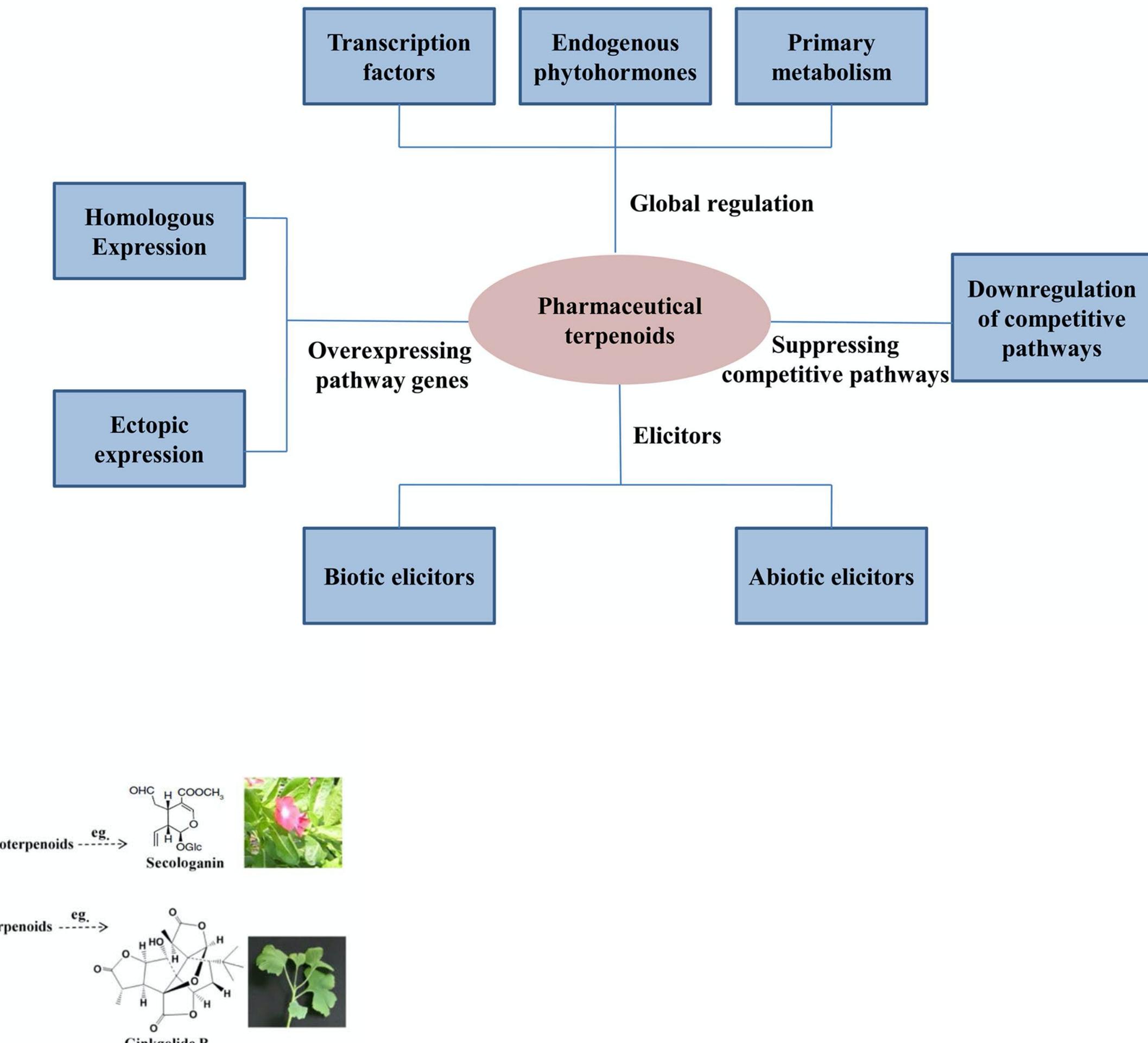
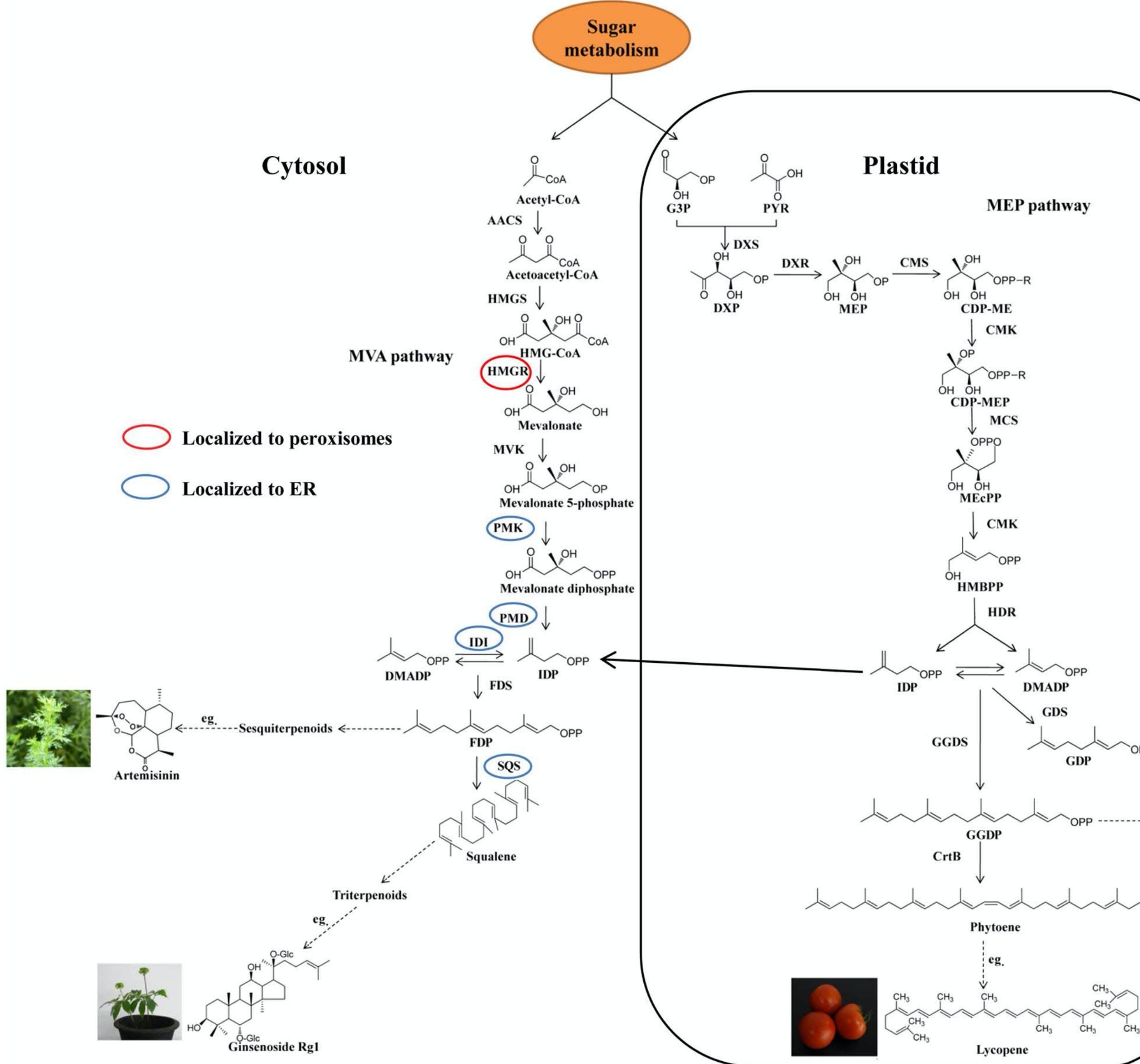


Table 1.
The opportunities for photosynthesis synthetic biology research and major challenges

Categories of photosynthesis engineering	Engineering options	Major challenges	Literature
Optimization and engineering of existing photosynthetic systems	Optimizing Rubisco kinetic properties Increasing Rubisco activation speed Optimizing the structure of ATP synthase Antenna size of photosystems Optimization of carbon metabolism enzymes	Chloroplast transformation in major crops No Chloroplast transformation in major crops No No	Sharwood (2017) Taylor and Long (2017) No ^a Ort and Melis (2011); Song <i>et al.</i> (2017) (Zhu <i>et al.</i> (2007); Simkin <i>et al.</i> (2015))
	Optimizing leaf anatomy Optimizing speed of non-photochemical quenching relaxation during light switching	Identifying optimal leaf anatomical features for photosynthetic efficiency No	Lundgren <i>et al.</i> (2019) Kromdijk <i>et al.</i> (2016)
	Optimization of the interaction between photosynthesis and other processes utilizing photosynthate	Optimization of photosynthate transport, storage, and utilization Releasing the feedback inhibition of photosynthate to photosynthesis	Identify limiting factors controlling source, sink, and flow Elucidate the molecular basis of inhibition of photosynthesis by photosynthate
	Engineering response of photosynthesis to phytohormones Engineering plant primary metabolism to enhance photosynthesis	Elucidate the molecular basis controlling responses of photosynthesis to phytohormones Elucidate the interaction between photosynthesis, respiration and nitrogen assimilation	Gururani <i>et al.</i> (2015) No ^b
Reconstruction of existing high-efficiency systems into current C ₃ crops	Engineering C ₄ CO ₂ -concentrating mechanism into C ₃ leaves Engineering carboxysome based CO ₂ -concentrating mechanism into C ₃ mesophyll cells Engineering pyranoid into chloroplasts of C ₃ crops Engineering crassulacean acid metabolism (CAM) into chloroplasts of C ₃ crops Developing phycobilisome Creation of the chlorophyll d and f pathway	Elucidate the molecular basis controlling Kranz anatomy Elucidate the major elements of carboxysome and chloroplast transformation in crops Elucidate the major components of pyranoid and chloroplast transformation in crops Elucidate the molecular basis controlling CAM formation Elucidate the minimal elements needed for phycobilisome construction Elucidate the metabolic basis for chlorophyll d and f synthesis	von Caemmerer <i>et al.</i> (2012); Sedelnikova <i>et al.</i> (2018) Price <i>et al.</i> (2011, 2013); McGrath and Long (2014) Mackinder <i>et al.</i> (2017) Yang <i>et al.</i> (2015) Tang <i>et al.</i> (2015); Zhang <i>et al.</i> (2017); Zhao <i>et al.</i> (2017) Blankenship and Chen (2013); Li and Chen (2015)
	Creation of new photosynthetic systems that do not exist in nature	Creation of photorespiratory bypass pathway Creation of new CO ₂ fixation pathway Creation of autotrophic <i>E. coli</i> Creation of new pathway to utilize cellulose for production of high-value product Utilizing another energy source to support CO ₂ fixation	No Chloroplast transformation in crops No Pathway design and evolution-guided engineering strategy Pathway design
	Develop composite systems by combining photosynthesis and artificial material	Artificial photosynthesis Creation of new material to enable better utilization of light and CO ₂	Material development Develop artificial material to increase light absorption or increase intercellular CO ₂ concentration
			No ^c

^a The number of helical proteins in the intramembrane F₀ complex of the ATP synthase differs between species, suggesting that there might be an optimal number of helical proteins required to gain the maximal light use efficiency in plants.

^b Respiration, photorespiration, and nitrogen assimilation interact closely with photosynthesis, and greatly influence the efficiency of photosynthesis, such as the increase of photosynthetic CO₂ uptake rate under photorespiratory conditions when NO₃⁻ was supplied as a nitrogen source (Busch *et al.*, 2018), and the supply of α-ketoglutarate by the citric acid pathway to support ammonia assimilation (Sweetlove *et al.*, 2010). Optimization of photosynthesis requires better understanding of the interaction between photosynthesis, nitrogen assimilation, and respiration, and correspondingly coordination of photosynthesis with these closely interacting processes.

^c A large fraction of incident solar energy is outside of the photosynthetic active radiation spectrum. Development of synthetic material which can convert these photons into photons that can be utilized by photosynthesis is one direction that needs be explored (Boriskina and Chen, 2014). Similarly, synthetic material capable of increasing leaf intercellular CO₂ concentration is another area in which synthetic chemistry techniques can be used to promote photosynthesis.

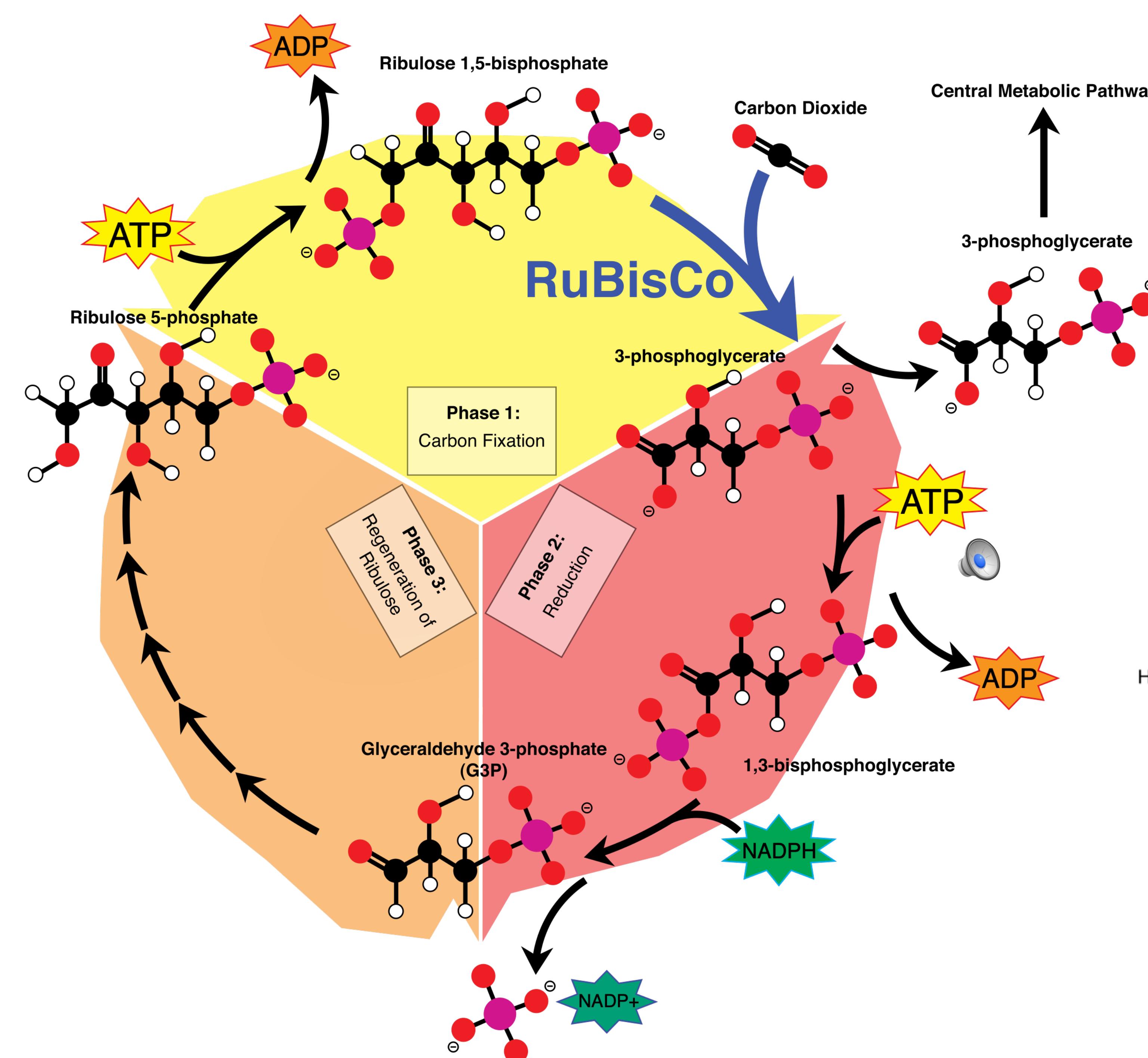
A wish list for synthetic biology in photosynthesis research

Xin-Guang Zhu , Donald R Ort, Martin A J Parry, Susanne von Caemmerer 

Journal of Experimental Botany, Volume 71, Issue 7, 6 April 2020, Pages 2219–2225,

<https://doi.org/10.1093/jxb/eraa075>

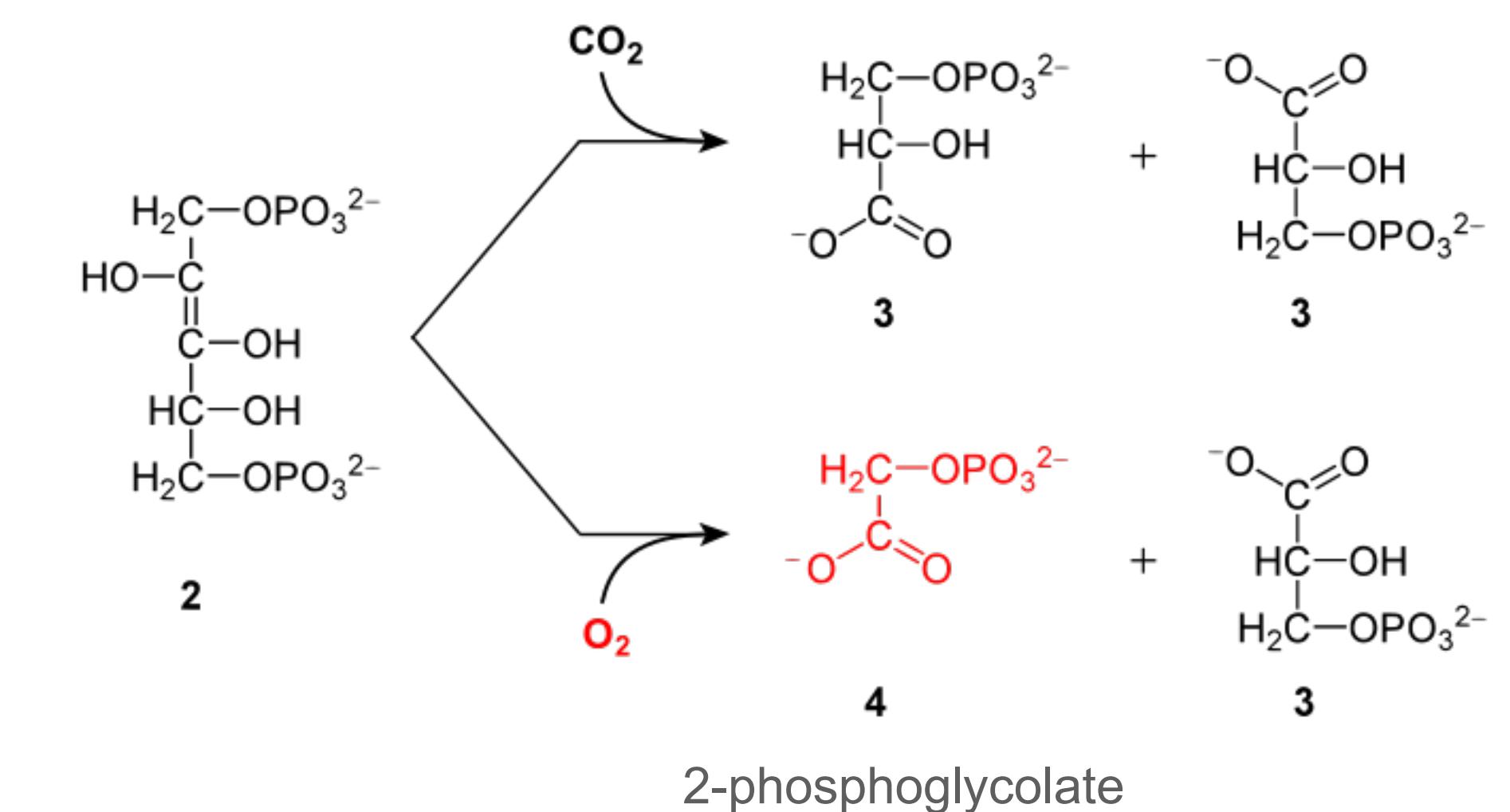
Published: 15 February 2020



the effective time-averaged catalytic rate of Rubisco is $\approx 0.03 \text{ s}^{-1}$ on land

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

RuBisCo CO₂ fixation reaction



2-phosphoglycolate

RuBisCo oxygenase reaction

Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field

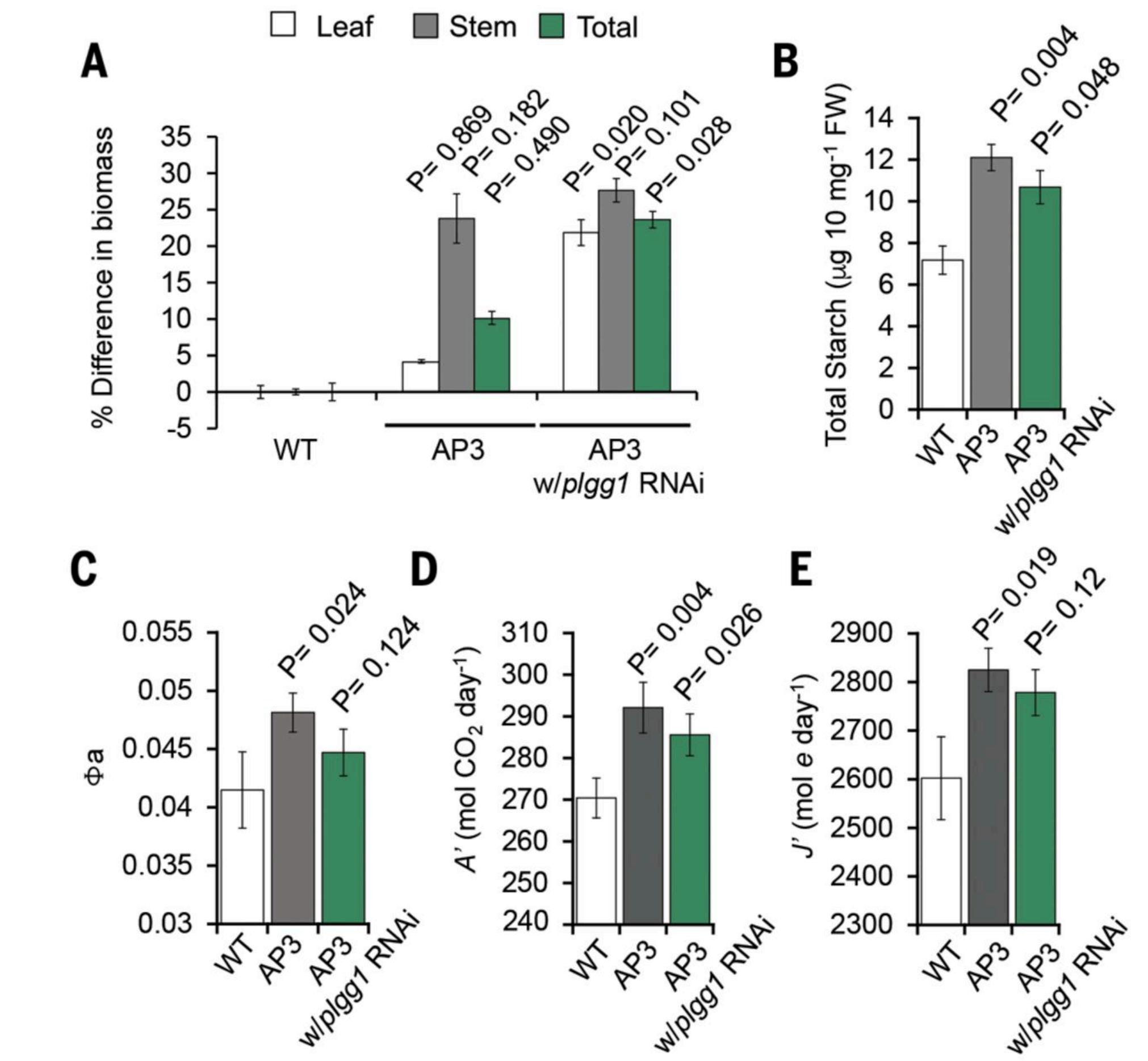
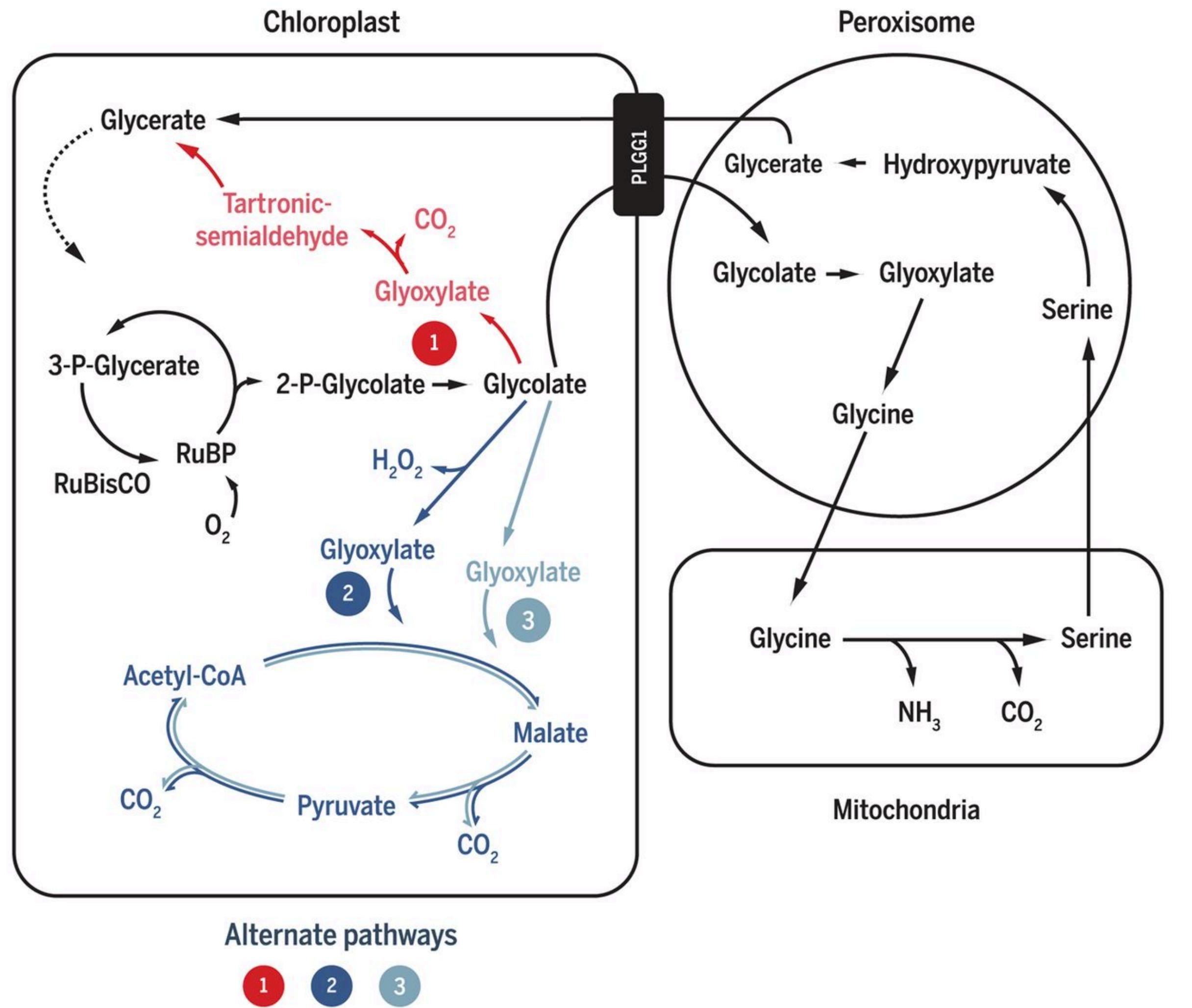
Paul F. South, Amanda P. Cavanagh, Helen W. Liu, Donald R. Ort

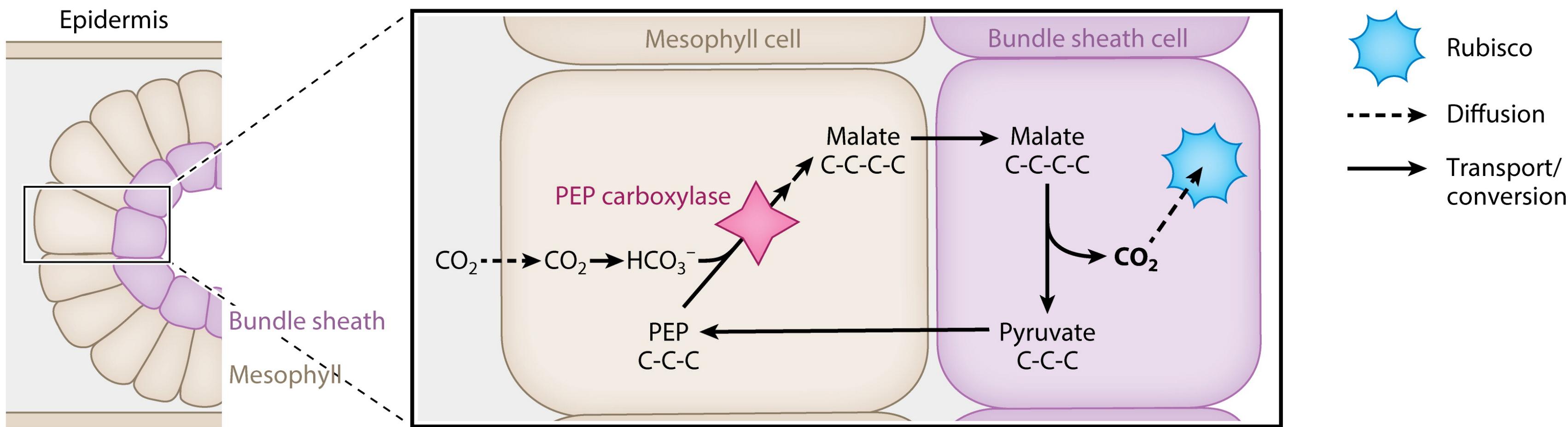
Science 04 Jan 2019:

Vol. 363, Issue 6422, eaat9077

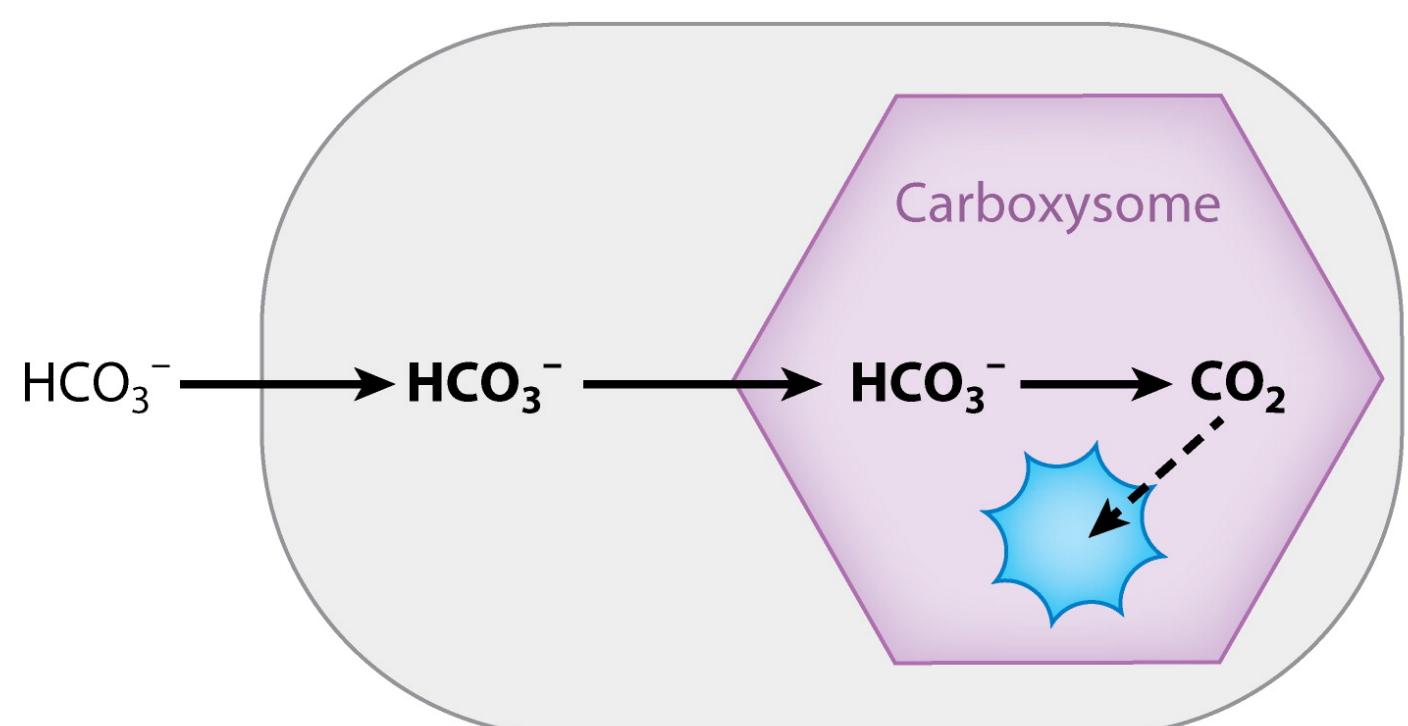
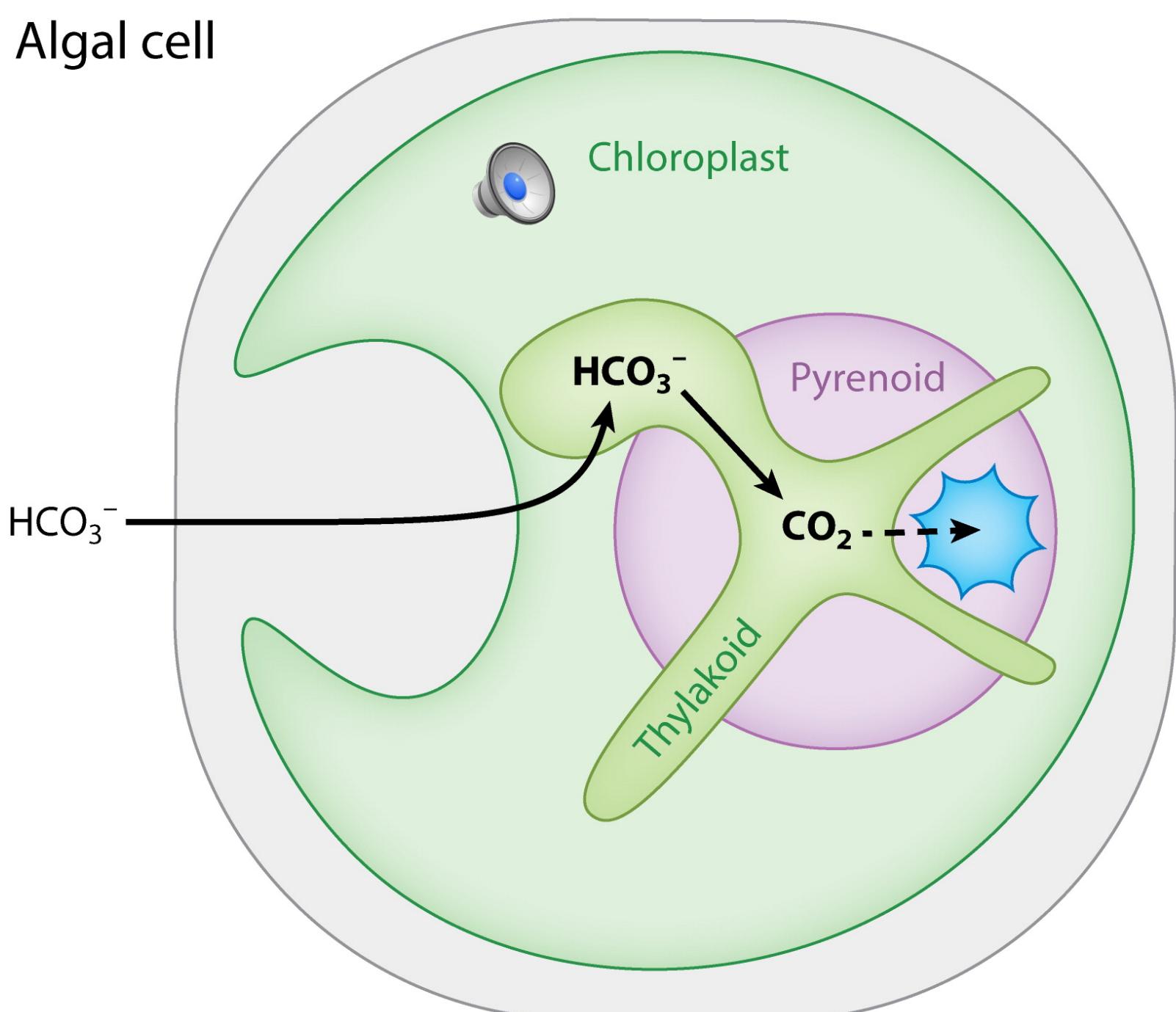
DOI: 10.1126/science.aat9077

Photorespiration is required in C3 plants to metabolize toxic glycolate formed when ribulose-1,5-bisphosphate carboxylase-oxygenase oxygenates rather than carboxylates ribulose-1,5-bisphosphate. Depending on growing temperatures, photorespi-

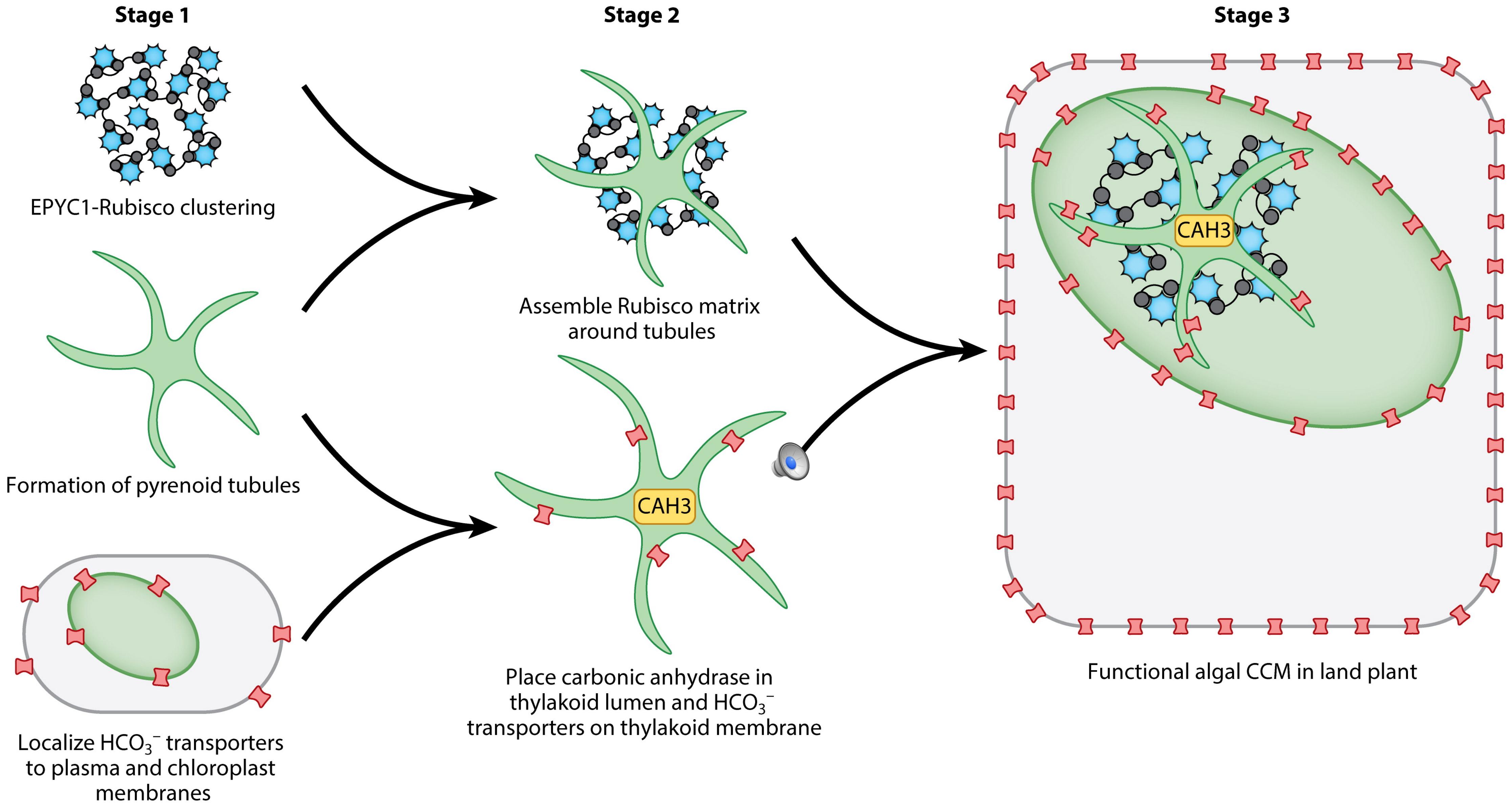


a C₄ leaf**CO₂-concentrating mechanisms**

C₄ plants, cyanobacteria, and algae concentrate CO₂ around the enzyme Rubisco using different strategies

b Cyanobacterial cell**c Algal cell**

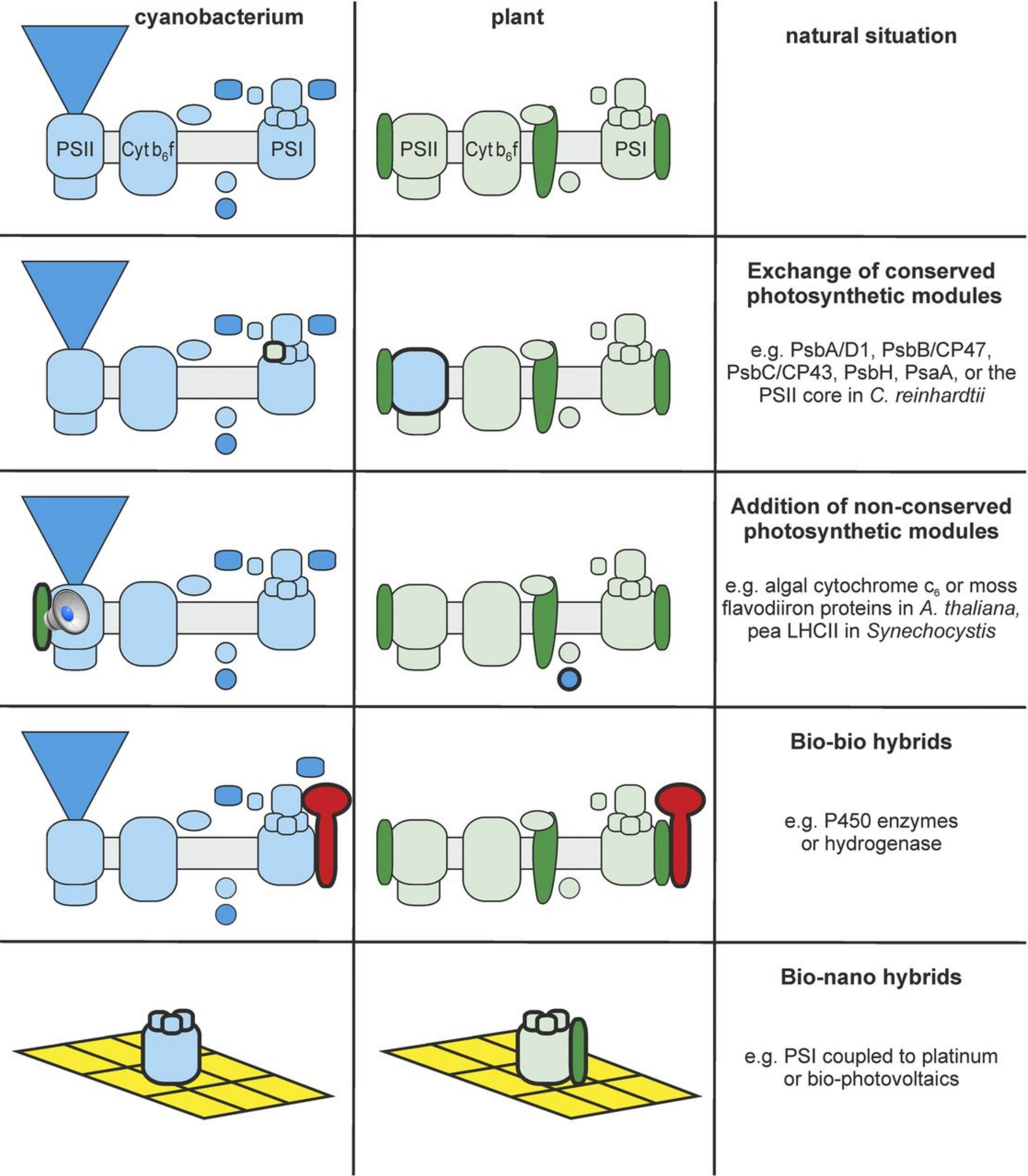
(a) In the mesophyll cells of C₄ plants, HCO₃⁻ is added to the three-carbon molecule phosphoenolpyruvate (PEP) by the enzyme PEP carboxylase, generating the four-carbon molecule malate or aspartate. The malate or aspartate is then decarboxylated to form the three-carbon molecule pyruvate and release CO₂ in bundle sheath cells, where Rubisco is expressed. The biophysical CCMs of (b) cyanobacteria and (c) algae actively transport HCO₃⁻ into the cell, then convert it to CO₂ at a site of clustered Rubisco, which is either the (b) carboxysome or (c) pyrenoid.



Genetic Engineering, Synthetic Biology and the Light Reactions of Photosynthesis

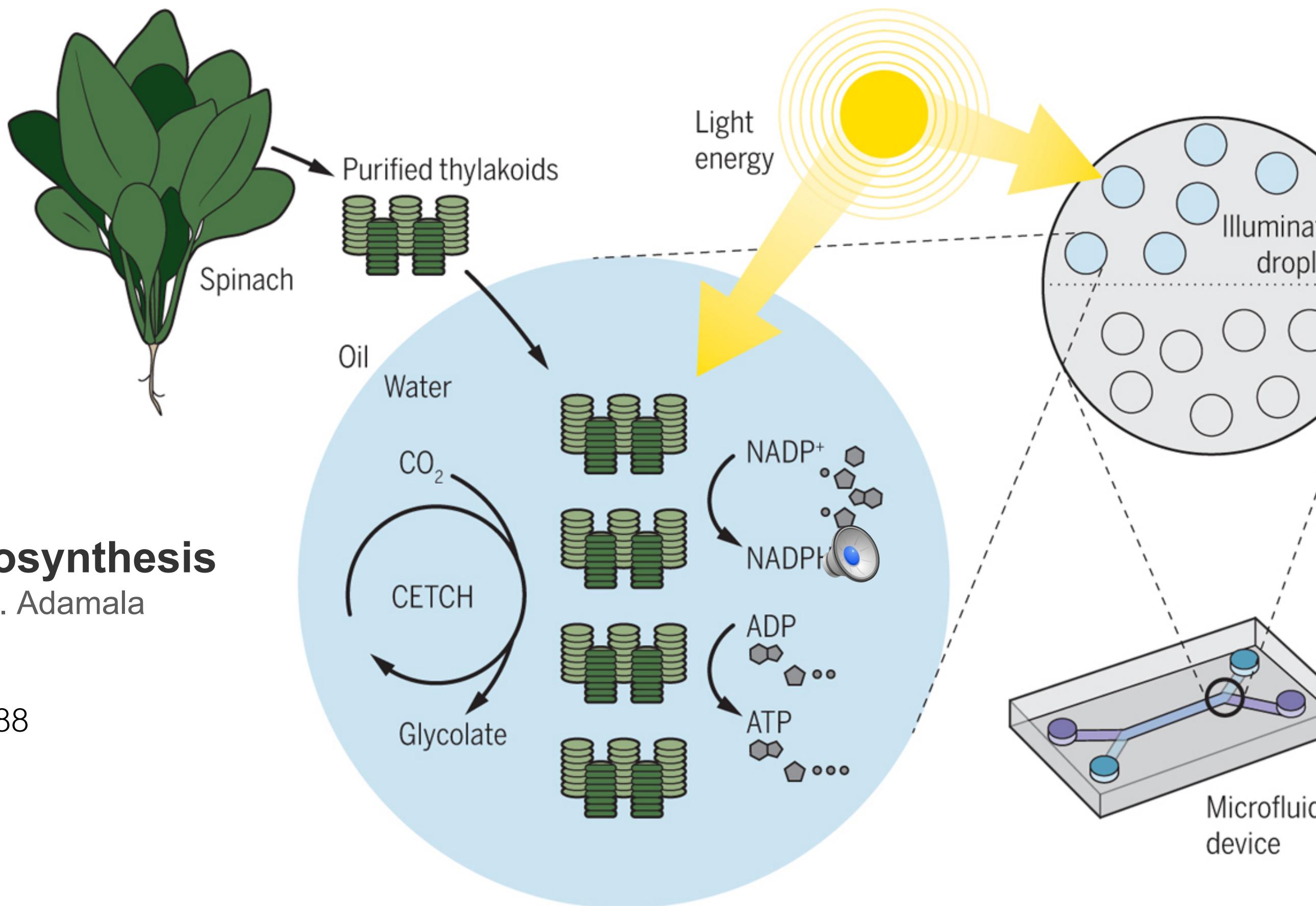
Dario Leister

DOI: <https://doi.org/10.1104/pp.18.00360>



A technological approach to semisynthetic photosynthesis

Thylakoids (where photosynthesis occurs) are purified from spinach, situated within oil-in-water droplets along with the 16 CETCH enzymes. When hit with light, the underlying reactions are triggered. This could be used in applications such as solar-powered bioreactors and engineering cells.



PERSPECTIVE SYNTHETIC BIOLOGY

Toward artificial photosynthesis

Nathaniel J. Gaut, Katarzyna P. Adamala

Science 08 May 2020

Vol. 368, Issue 6491, pp. 587-588

DOI: 10.1126/science.abc1226

REPORT

Light-powered CO_2 fixation in a chloroplast mimic with natural and synthetic parts

Tarryn E. Miller, Thomas Beneyton, Thomas Schwander, Christoph Diehl, Mathias Girault, Richard McLean, Tanguy Chotel, Peter Claus, Niña Socorro Cortina, Jean-Christophe Baret, Tobias J. Erb

Science 08 May 2020

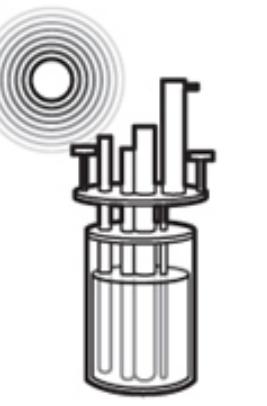
Vol. 368, Issue 6491, pp. 649-654

DOI: 10.1126/science.aaz6802

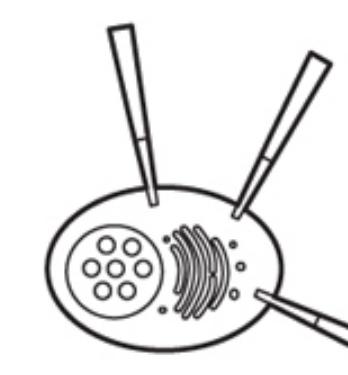
APPLICATIONS



Removing greenhouse gases

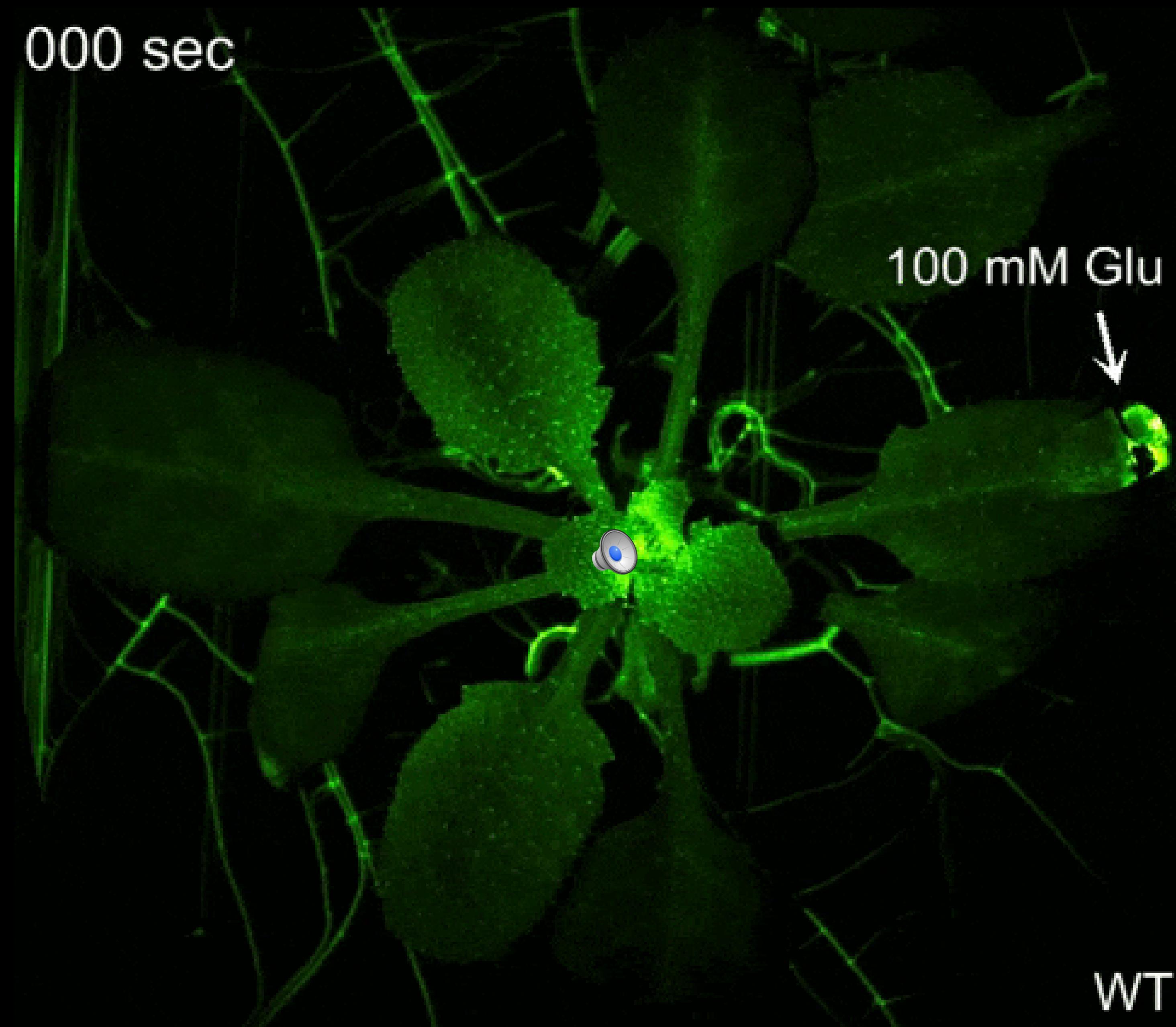


Solar-powered bioreactors



Energy-independent synthetic cells

Plants as environmental sensors



- Complete parts lists

Creative concepts

Non-legume nodulation, N-fixing plants, efficient N-use design, reduced respiratory C loss, C-conserving photorespiration, new-to-nature CO₂-fixing pathways, logic gates and circuits, rationally redesigned architectures, light-driven enzymes, synthetic plastomes, synthetic microbial communities, synthetic metabolons, synthetic microcompartments, rebuilt mitochondrial ETCs, rebuilt ATP synthases, new-to-nature biosynthetic pathways and products, energy-efficient enzyme protein-turnover rates

nature plants

Perspective | Published: 18 November 2019

Revolutionizing agriculture with synthetic biology

Eleanore T. Wurtzel [✉](#), Claudia E. Vickers [✉](#), Andrew D. Hanson [✉](#), A. Harvey Millar, Mark Cooper, Kai P. Voss-Fels, Pablo I. Nikel & Tobias J. Erb

Nature Plants 5, 1207–1210(2019) | [Cite this article](#)

Proven possibilities

Sentinel plants, smart plants, synthetic receptors, synthetic glycolate metabolism, improved phytochemicals, digestible biomass

Commercial or close

Plant pathways in microorganisms, microbiome N-fixation

- Engineering training
- Entrepreneurship
- Public–private partnerships
- Biofoundries

- Engagement with/of stakeholders