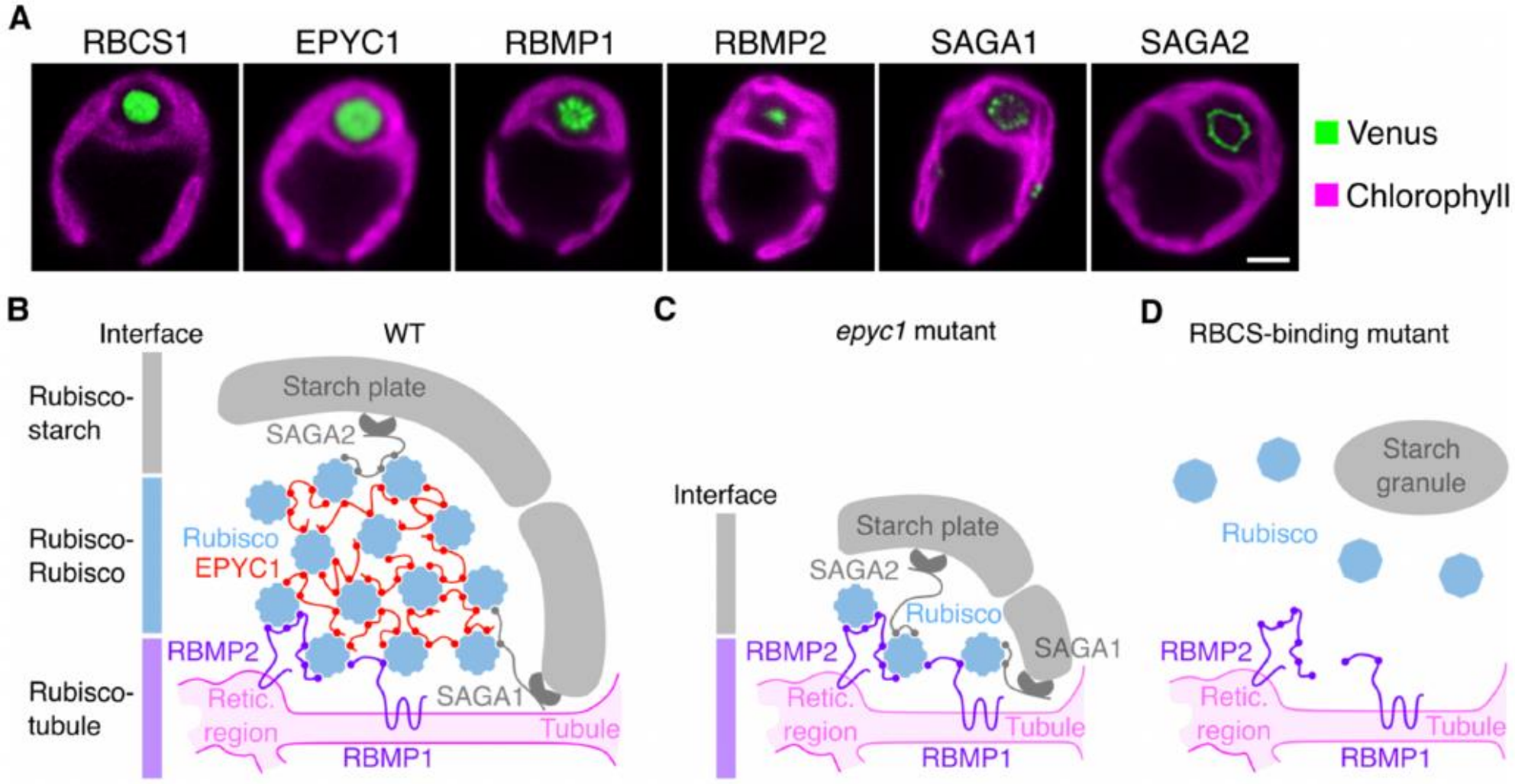
- 06/05: 8.30 – 10.30 (Synthetic Biology)
- 7/05: 14.30 – 15.30
- 14/05: 14.30 – 15.30
- 20/05: 8.30 – 10.30
- 27/05: 8.30 – 10.30
- 11/06: 14.30 – 16:30 (final test)

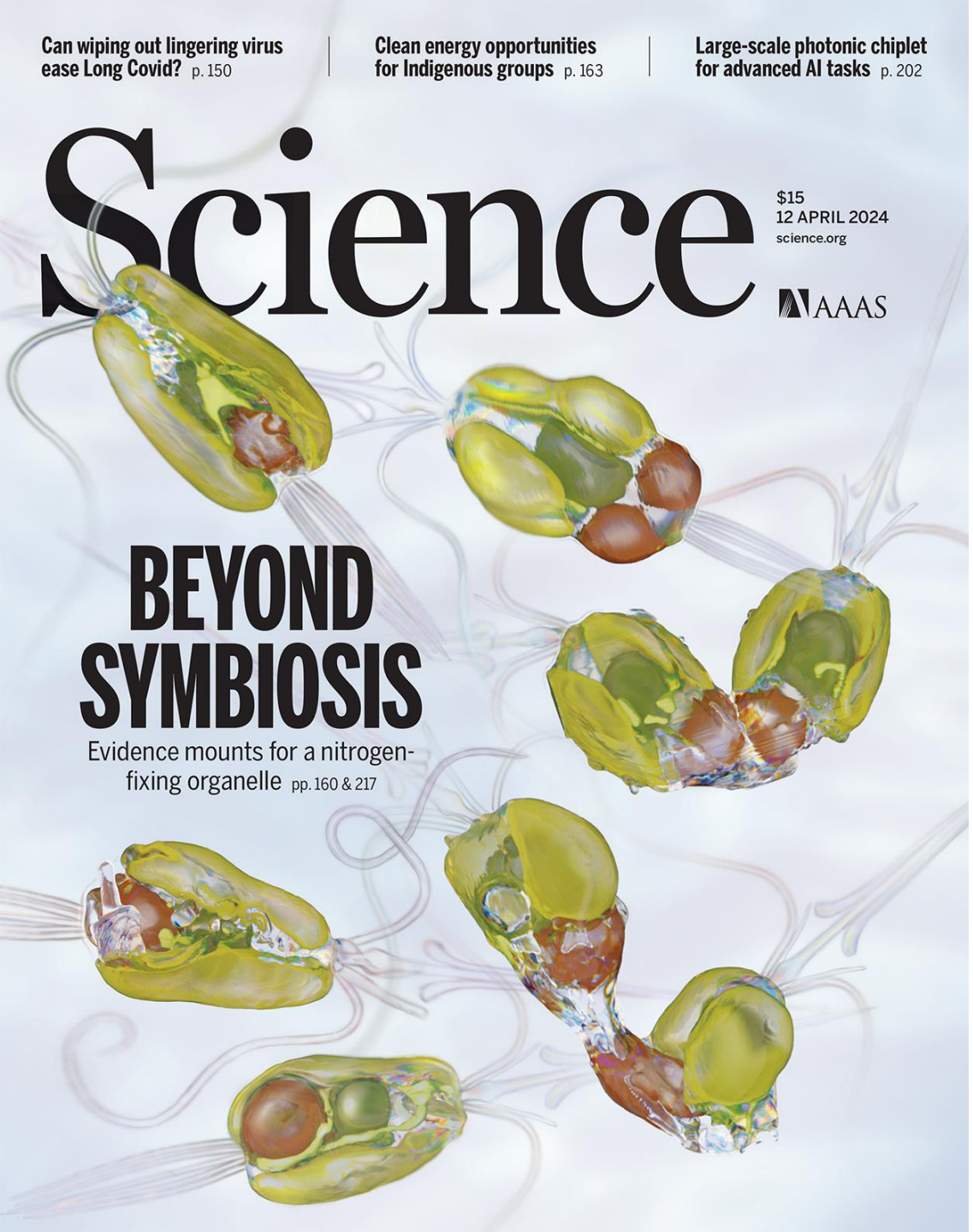
Assembly of the algal CO_2-fixing organelle, the pyrenoid, is guided by a Rubisco-binding motif

Moritz T. Meyer[1], Alan K. Itakura[2†], Weronika Patena[1], Lianyong Wang[1], Shan He[1], Tom Emrich-Mills[3], Chun S. Lau[3], Gary Yates[3], Luke C. M. Mackinder[3], Martin C. Jonikas[1*].



New frontiers are coming

- the evolutionary transition from endosymbiont to organelle



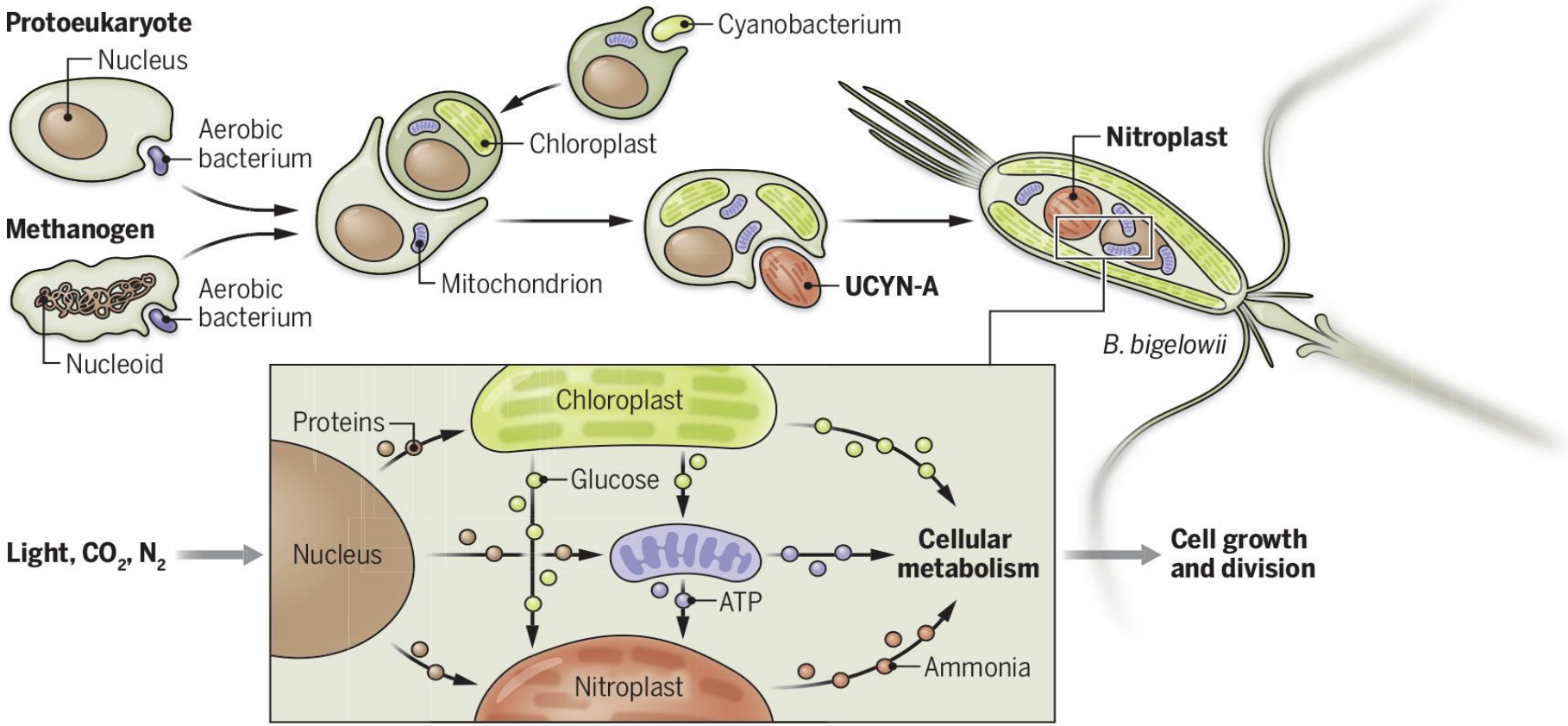
New Discovery: nitroplast

A nitrogen-fixing organelle, or “nitroplast,” has been identified in a marine alga on the basis of intracellular imaging and proteomic evidence. This discovery sheds light on the evolutionary transition from endosymbiont to organelle. The image depicts the cell architecture and synchronized cell division of the alga Braarudosphaera bigelowii with nitroplast UCYN-A (large brown spheres).

Science | AAAS

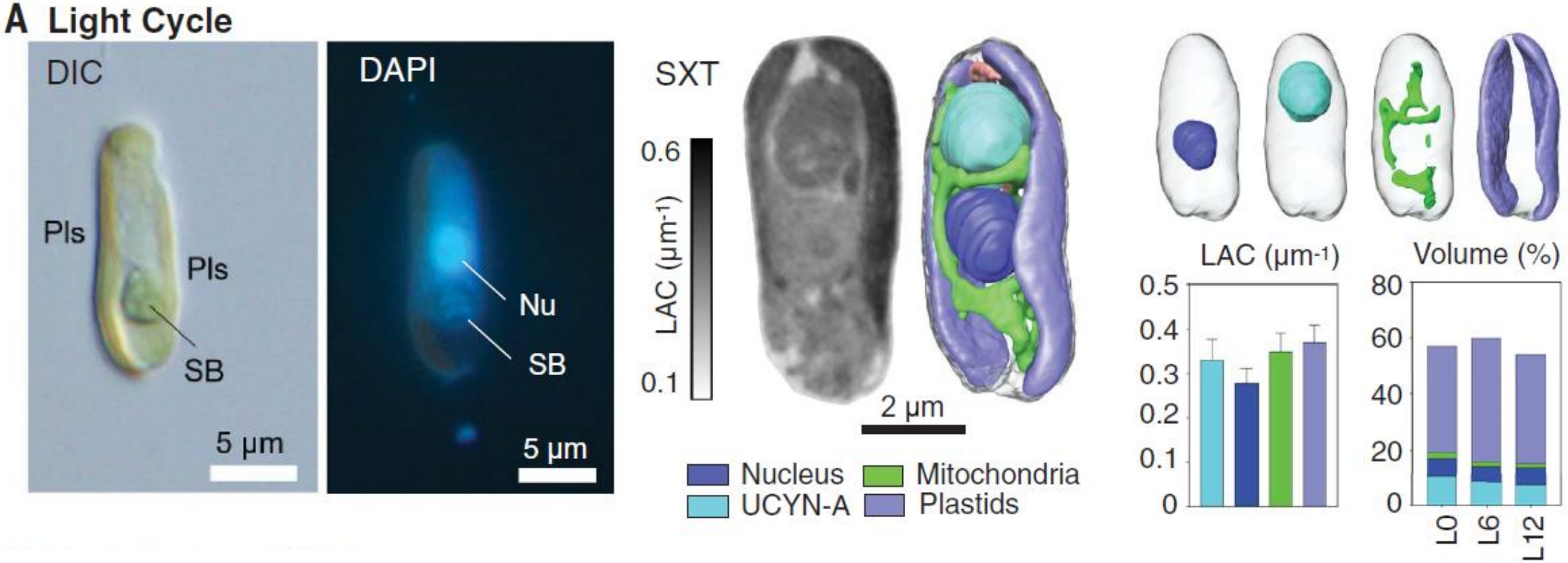
Evolution and function of the nitroplast

Multiple organelles in eukaryotic cells, including mitochondria, chloroplasts, and nitroplasts, evolved from the integration of endosymbiotic bacteria. In *Braarudosphaera bigelowii*, the chloroplast fixes inorganic carbon to produce glucose, which feeds the respiratory chain in mitochondria that produces adenosine triphosphate (ATP), which in turn fuels nitrogen fixation in the nitroplast. Glucose, ammonia, and ATP generated by the organelles, together with externally incorporated compounds (phosphorous, mineral nutrients, and vitamins), are the building blocks for cell metabolism, resulting in cell growth and division.

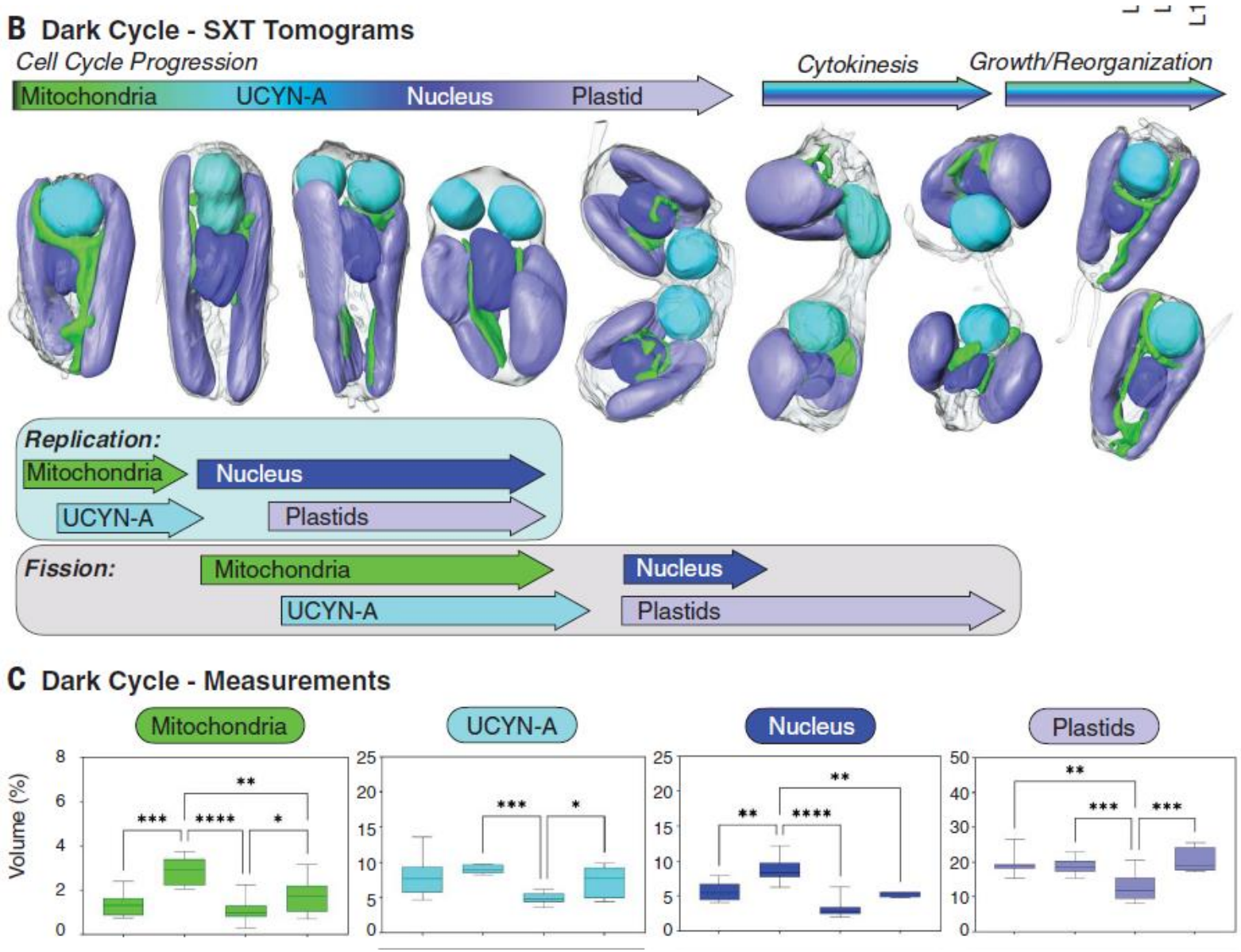


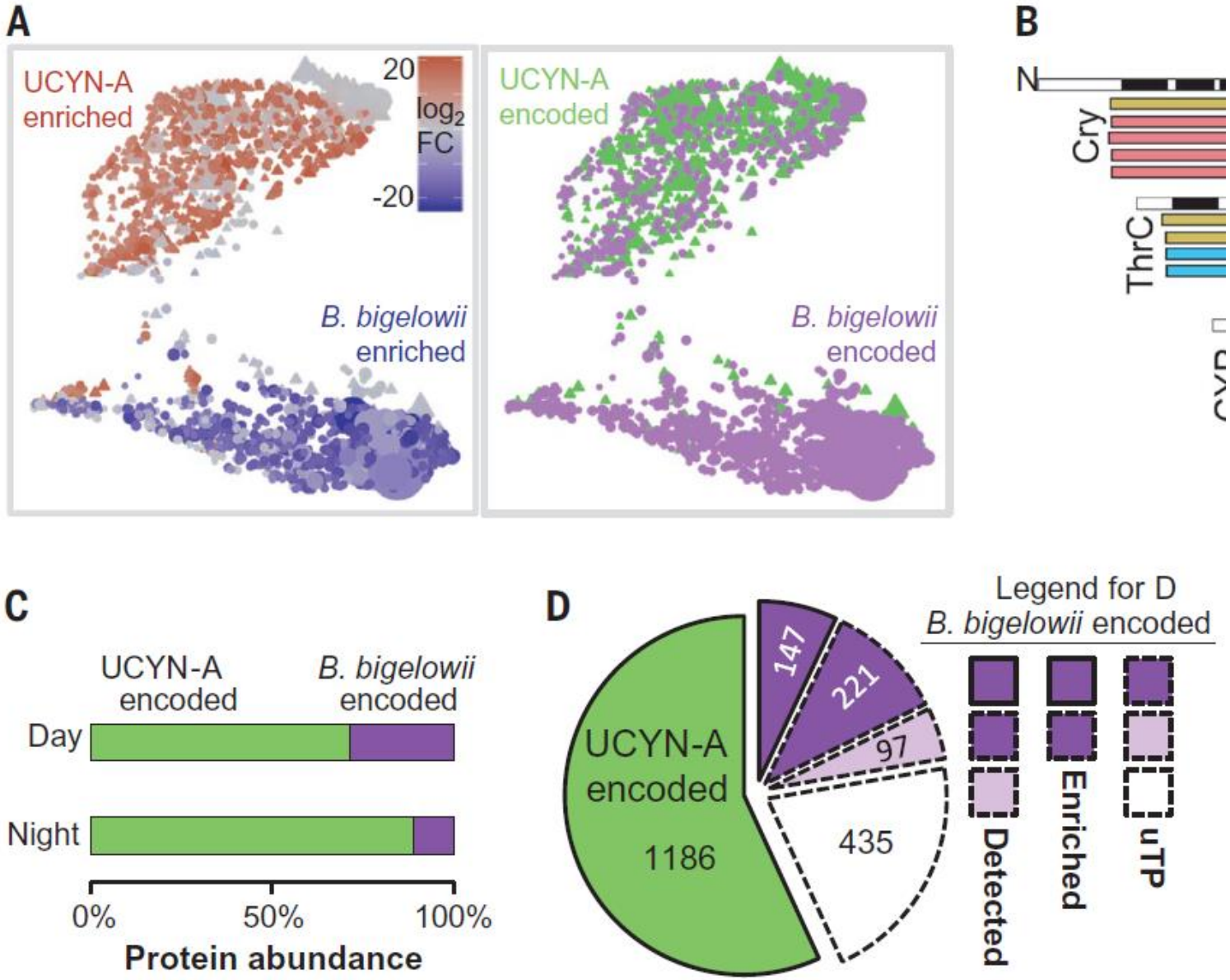
The nitroplast: A nitrogen-fixing organelle, Volume: 384, Issue: 6692, Pages: 160-161, DOI: (10.1126/science.ado8571)

Braarudosphaera bigelowii/UCYN-A lightdark cycle is highly coordinated.



Structural characterization during the light cycle
soft x-ray tomography (SXT)


B Dark Cycle - SXT Tomograms
Cell Cycle Progression
Mitochondria
UCYN-A
Nucleus
Plastid
Cytokinesis
Growth/Reorganization
Replication:
Mitochondria
Nucleus
UCYN-A
Plastids
Fission:
Mitochondria
Nucleus
UCYN-A
Plastids
C Dark Cycle - Measurements
Mitochondria
UCYN-A
Nucleus
Plastids
Volume (%)


A
UCYN-A
enriched
20
log2
FC
-20
B. bigelowii
enriched
UCYN-A
encoded
B. bigelowii
encoded
B
N
Cry
ThrC
C
UCYN-A
encoded
B. bigelowii
encoded
Day
Night
0%
50%
100%
Protein abundance
D
Legend for D
B. bigelowii encoded
147
221
97
435
UCYN-A
encoded
1186
Detected
Enriched
uTP

Origin of Chloro-Nucleo communication

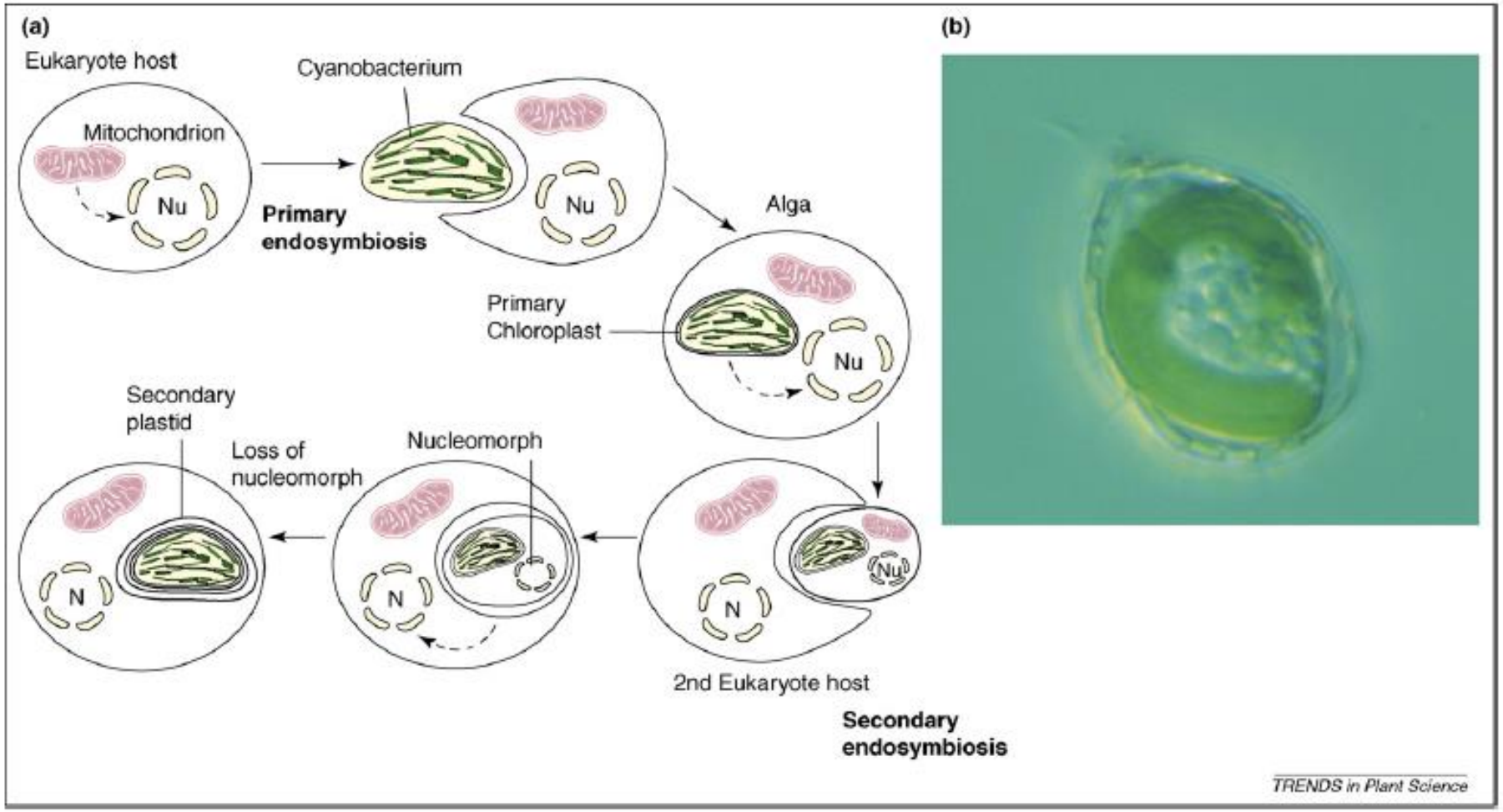
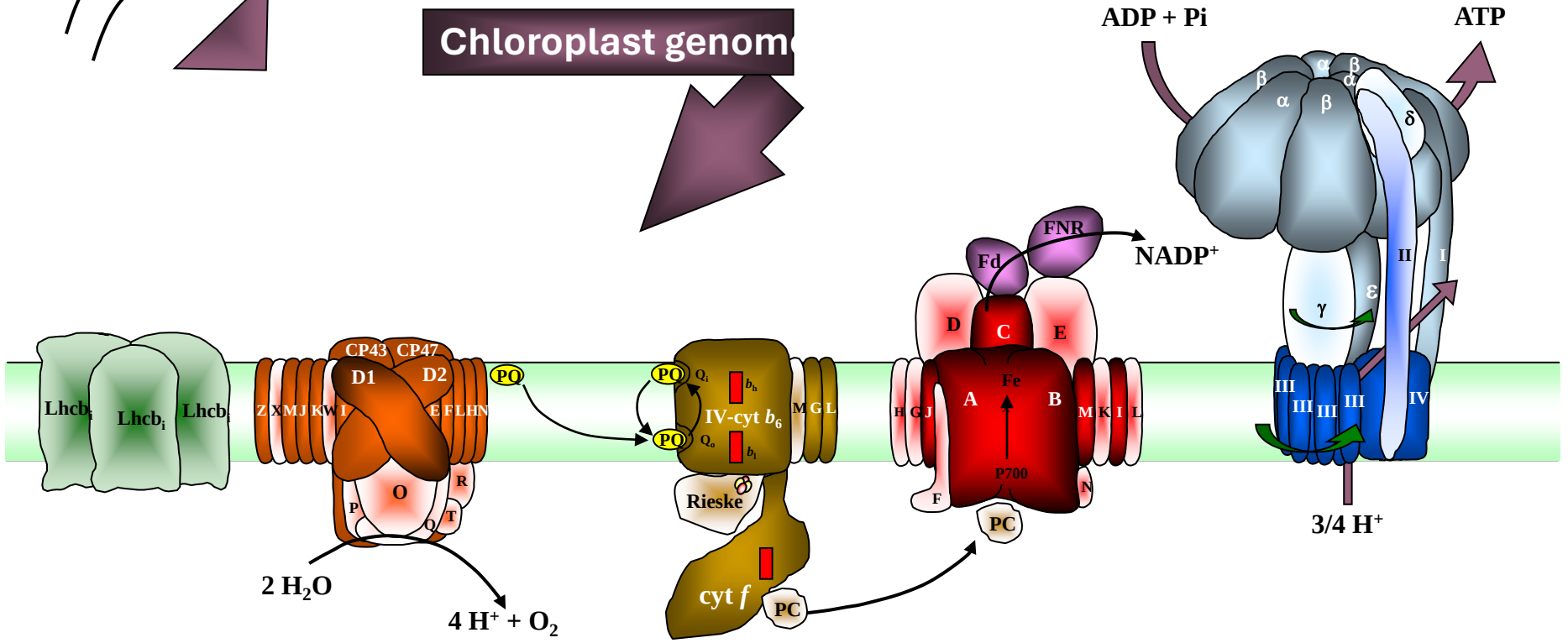


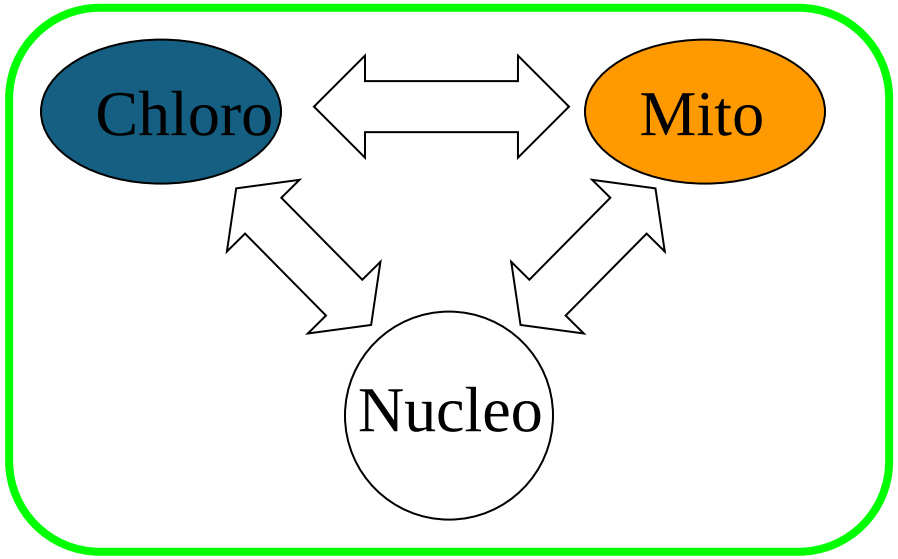
Figure 1. Origin of plastids by primary and secondary endosymbiosis. **(a)** Acquisition of a cyanobacterium by primary endosymbiosis, and subsequent secondary endosymbiotic acquisition of the resulting eukaryotic alga. The intermediate algal nucleus (Nu) forms the nucleomorph, which is subsequently reduced. N indicates the nucleus of the second eukaryote host. Broken arrows indicate gene transfer. **(b)** Photomicroscope image of a cell of *Paulinella*. Cell length is ~25 μm. The photograph shows the scales of the theca, a filopodium, and a large, dividing photosynthetic body or 'chromatophore'. Photograph kindly supplied by Birger Marin.

Nuclear genome

Chloroplast genom



Chloroplast protein regulation

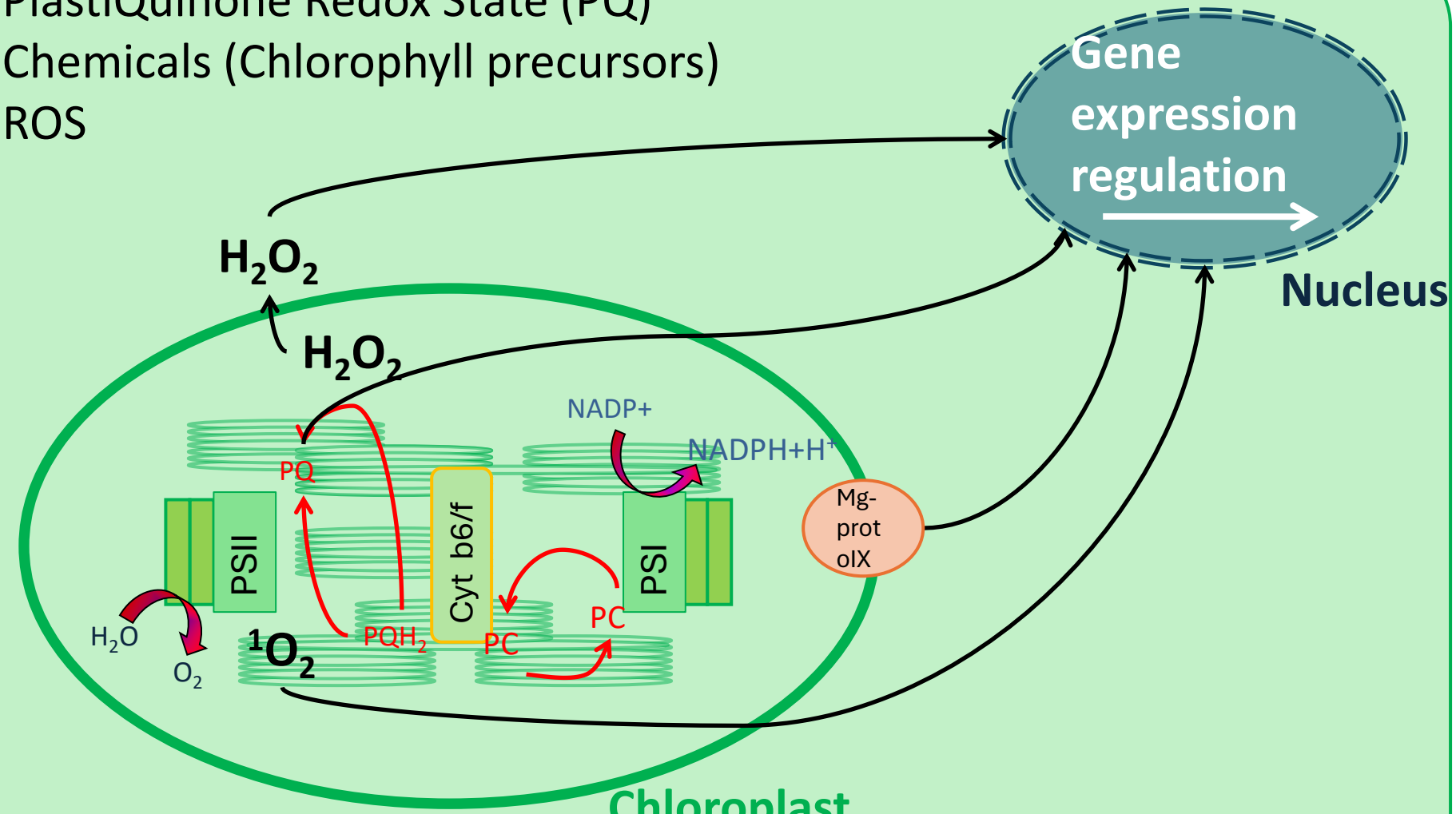


- Chloroplast genome only contains 120 genes
- Chloroplast proteome about 2500
- Synthesis of chloroplast proteins during acclimation is a concerted mechanism between plastid and nuclear genome

So what?

Which signals induce acclimation?

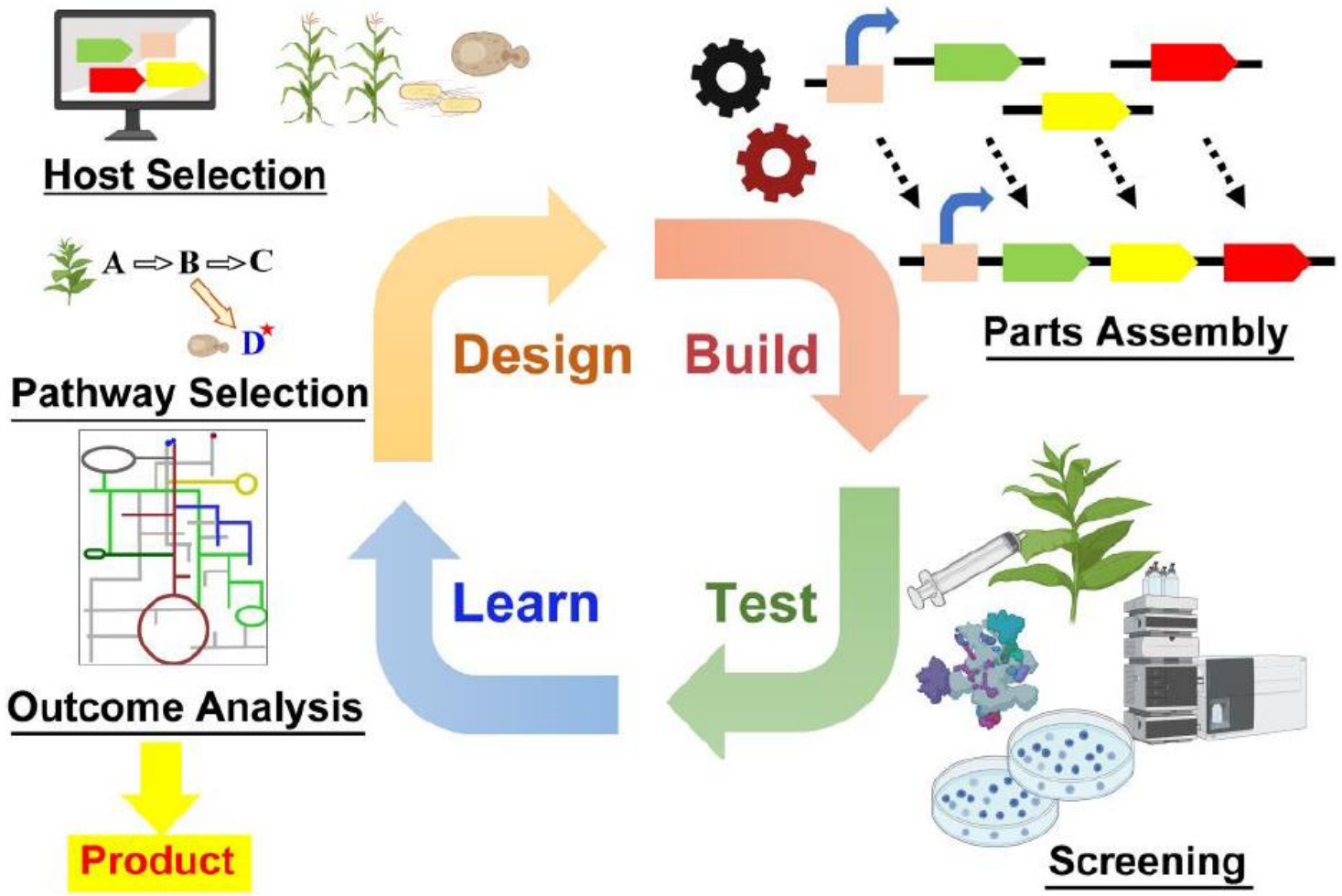
- PlastiQuinone Redox State (PQ)
- Chemicals (Chlorophyll precursors)
- ROS



Adapted from Fernandez and Strand COPB 2008

SYNTHETIC BIOLOGY

SynBio’s iconic DBTL cycle. Note that the cycle outputs products, not just information and understanding



https://doi.org/10.1093/plphys/kiad585 PLANT PHYSIOLOGY 2024: 00: 1–12

Plant Physiology®

A pilot oral history of plant synthetic biology

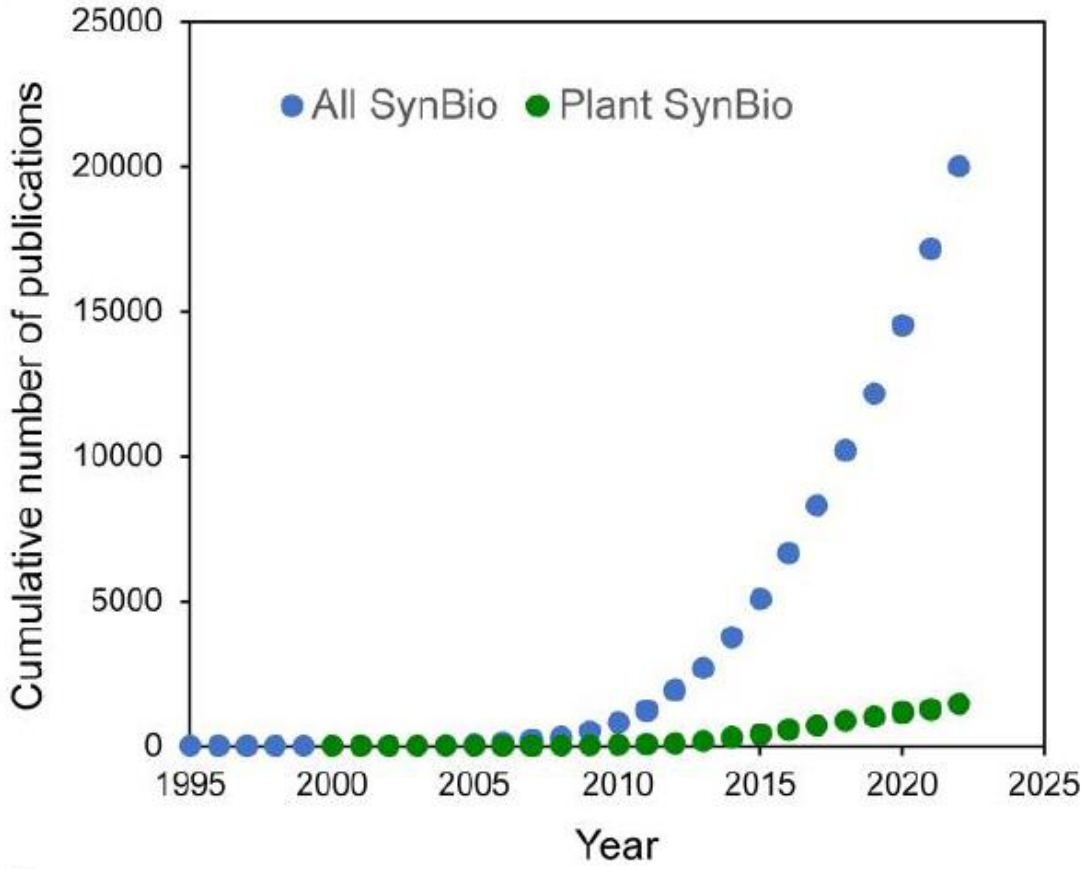
Jaya Joshi [1,*,†] and Andrew D. Hanson [2,*,†]

The whole field of synthetic biology (SynBio) is only about 20 years old, and plant SynBio is younger still. Nevertheless, within that short time, SynBio in general has drawn more scientific, philosophical, government, and private-sector interest than anything in biology since the recombinant DNA revolution. Plant SynBio, in particular, is now drawing more and more interest in relation to plants' potential to help solve planetary problems such as carbon capture and storage and replacing fossil fuels and feedstocks. As plant SynBio is so young and so fast-developing, we felt it was too soon to try to analyze its history. Instead, we set out to capture the essence of plant SynBio's origins and early development through interviews with 8 of the field's founders, representing 5 countries and 3 continents. We then distilled these founders' personal recollections and reflections into this review, centering the narrative on timelines for pivotal events, articles, funding programs, and quoting from interviews. We have archived the interview recordings and documented timeline entries. This work provides a resource for future historical scholarship.

Trends in publications in SynBio as a whole and in plant SynBio (A) and a breakdown of 2022 plant SynBio publications by category (B).

Plant SynBio publications in 2022 were 7.3% of the total.

A



B

2022 Plant SynBio publications		%
Reviews and Perspectives	76	41.5
Bioprospecting and parts characterization	37	20.2
Tools (including modeling)	31	16.9
Plant parts in heterologous host	26	14.2
Engineering in plants	10	5.5
Plant enzyme engineering	3	1.6
	Total 183	

The context of modern plant physiology

Trends in
Plant Science

CellPress

Special issue: Food security

Opinion

PlantACT! – how to tackle the climate crisis

Greenhouse gas (GHG) emissions have created a global climate crisis which requires immediate interventions to mitigate the negative effects on all aspects of life on this planet. As current agriculture and land use contributes up to 25% of total GHG emissions, plant scientists take center stage in finding possible solutions for a transition to sustainable agriculture and land use. In this article, the PlantACT! (Plants for climate ACTion!) initiative of plant scientists lays out a road map of how and in which areas plant scientists can contribute to finding immediate, mid-term, and long-term solutions, and what changes are necessary to implement these solutions at the personal, institutional, and funding levels.

Highlights

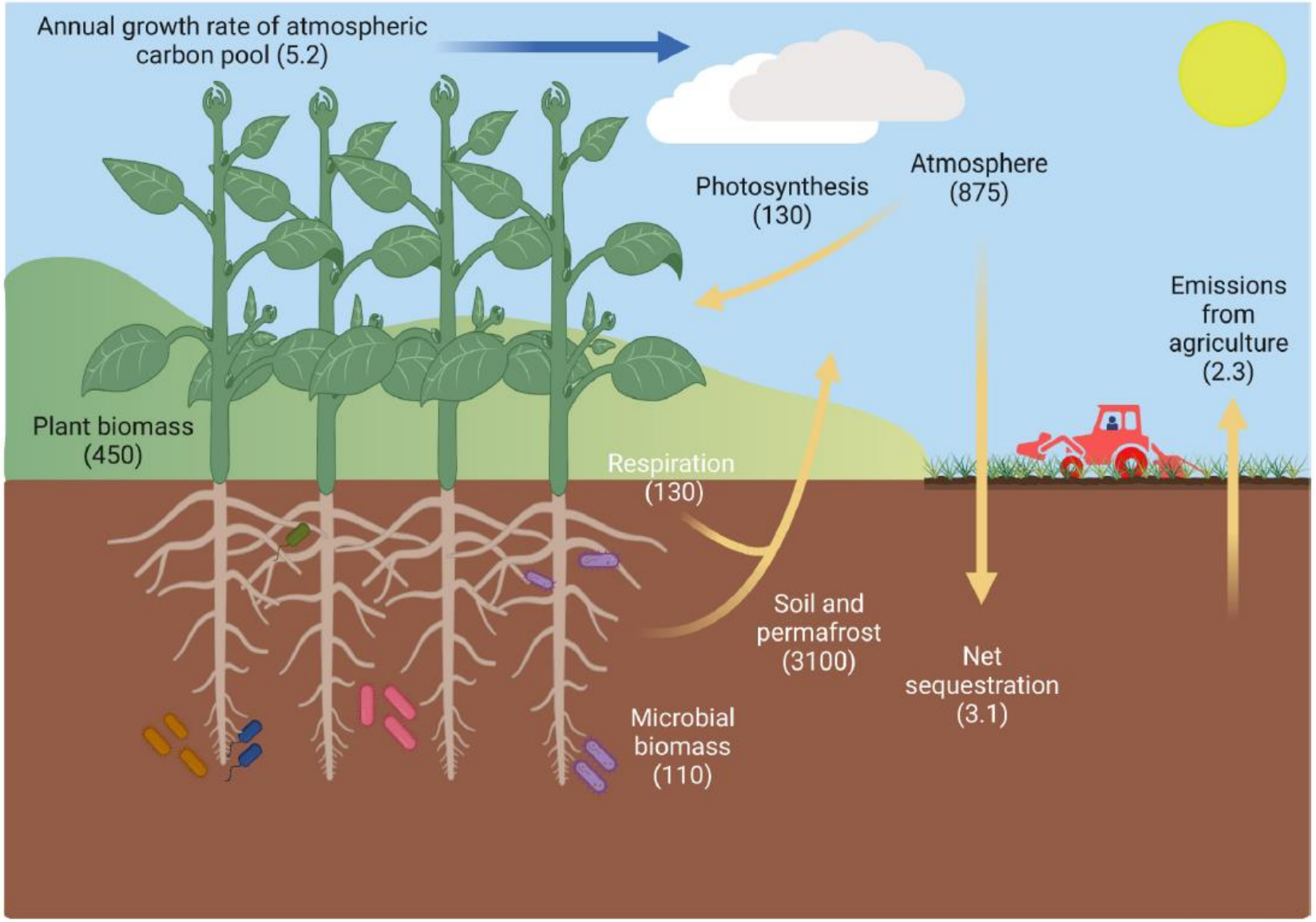
Agriculture contributes to global climate change by producing 20–25% of greenhouse gases (GHGs).

CO_2 is released from deforestation and land conversion, methane from rice paddy fields, and nitrous oxides from overfertilization.

An increasing world population requires a change in the agro food systems, including a reduction in chemical fertilizers and pesticides as well as the production and access to food.

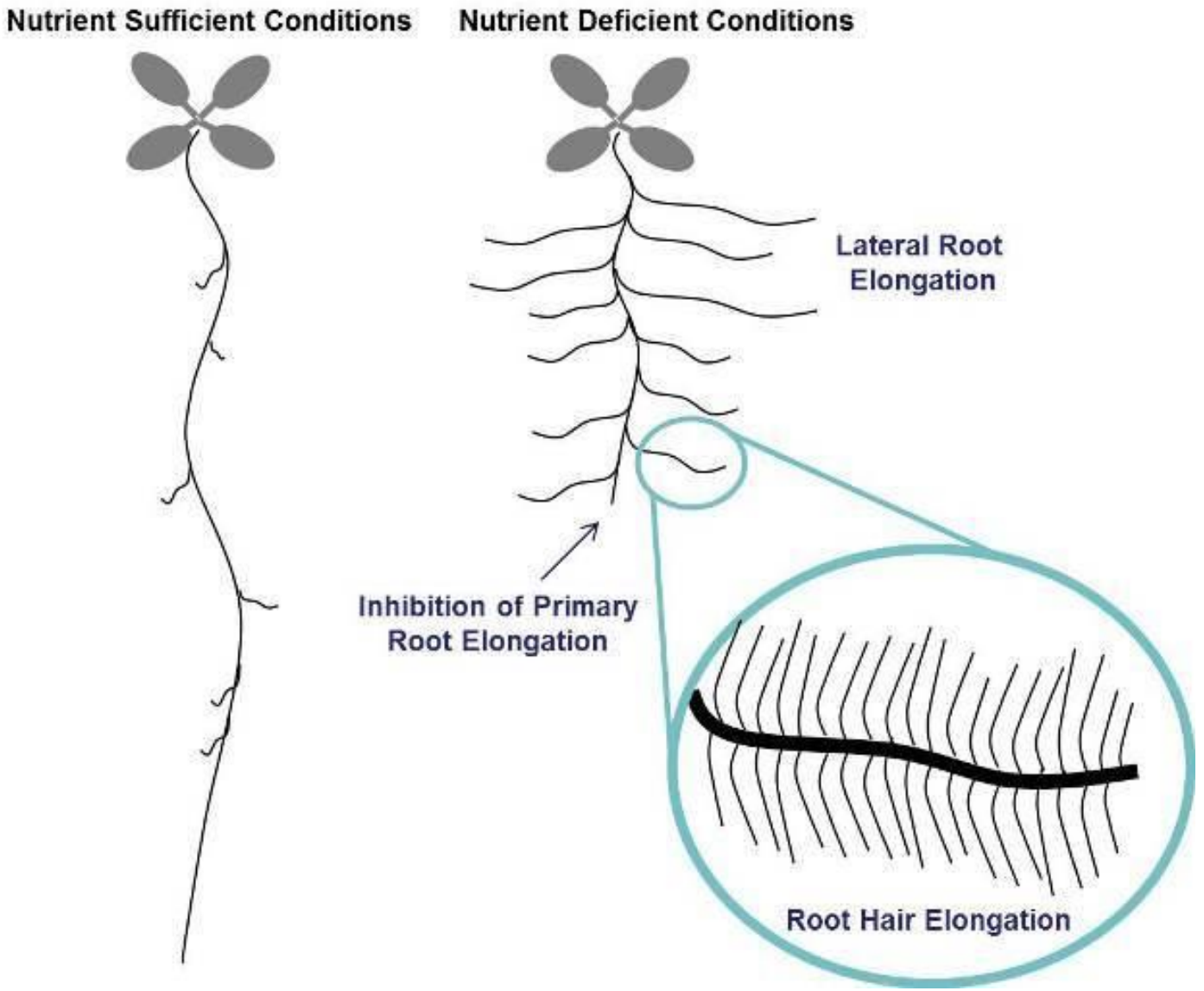
Land-based carbon fluxes

- Schematic representation of the terrestrial carbon cycle. Annual growth rate of atmospheric carbon pool (blue arrow) is the differential of emissions from fossil fuels (9.6 gigatons of carbon, Gt C), land use change (1.2 Gt C), and uptake of carbon into terrestrial (3.1 Gt C) and oceanic (2.9 Gt C) carbon pools.
- Data for carbon emissions from agriculture have been taken from the Food and Agriculture Organization of the United Nations (FAO) (www.fao.org/3/cb3808en/cb3808en.pdf). The FAO data include greenhouse gases other than CO2, converted to CO2 equivalents.



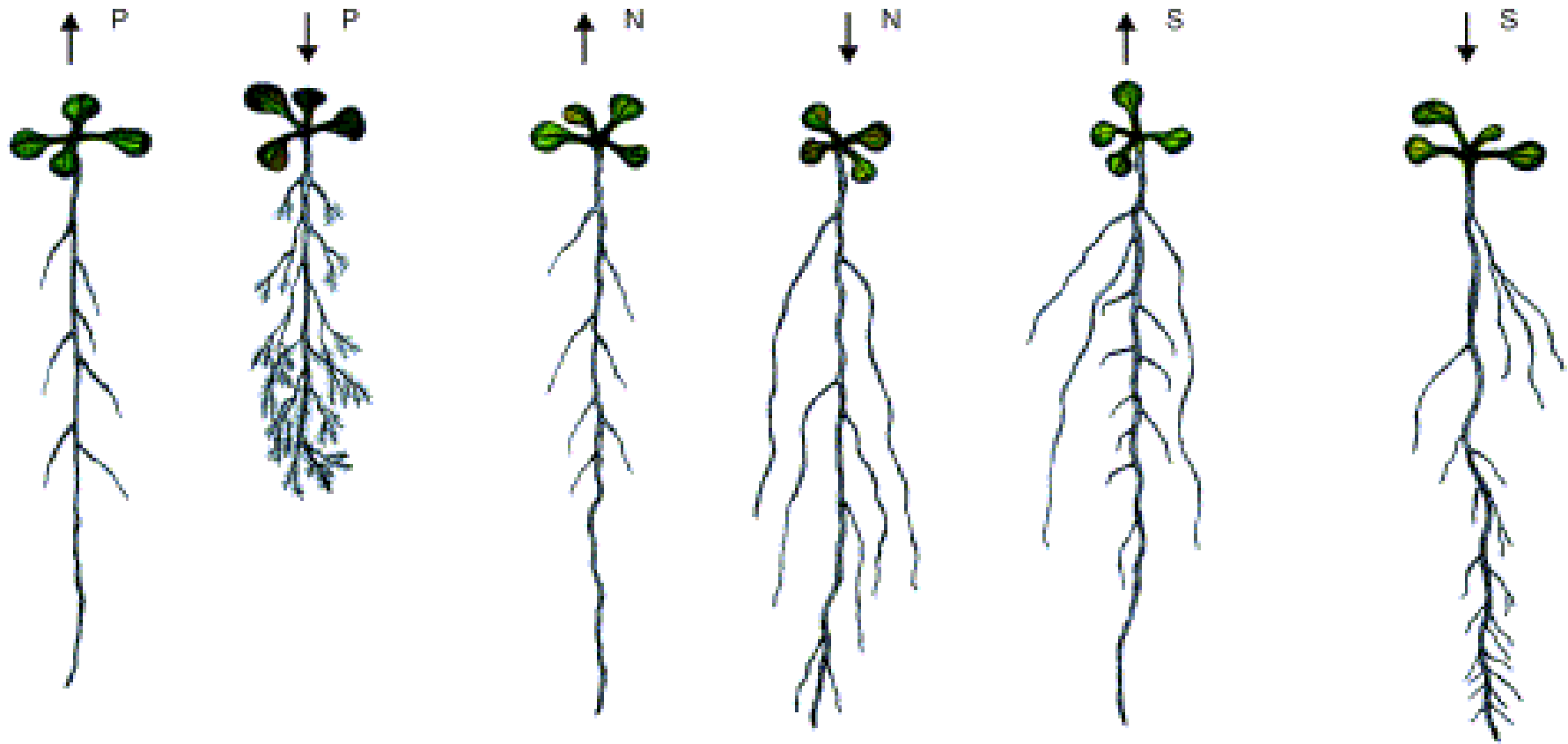
Root architecture

- Changes in root architecture, induction of root-based transport systems and associations with beneficial soil microorganisms allow plants to maintain optimal nutrient content in the face of changing soil environments.



Morgan, J. B. & Connolly, E. L. (2013) Plant-Soil Interactions: Nutrient Uptake. *Nature Education Knowledge* 4(8):2

The shape of a plant’s root system influences its ability to reach essential nutrients



Current Opinion in Plant Biology

Is there a link between mineral nutrition and photosynthesis regulation?

Trends in
Plant Science

CellPress

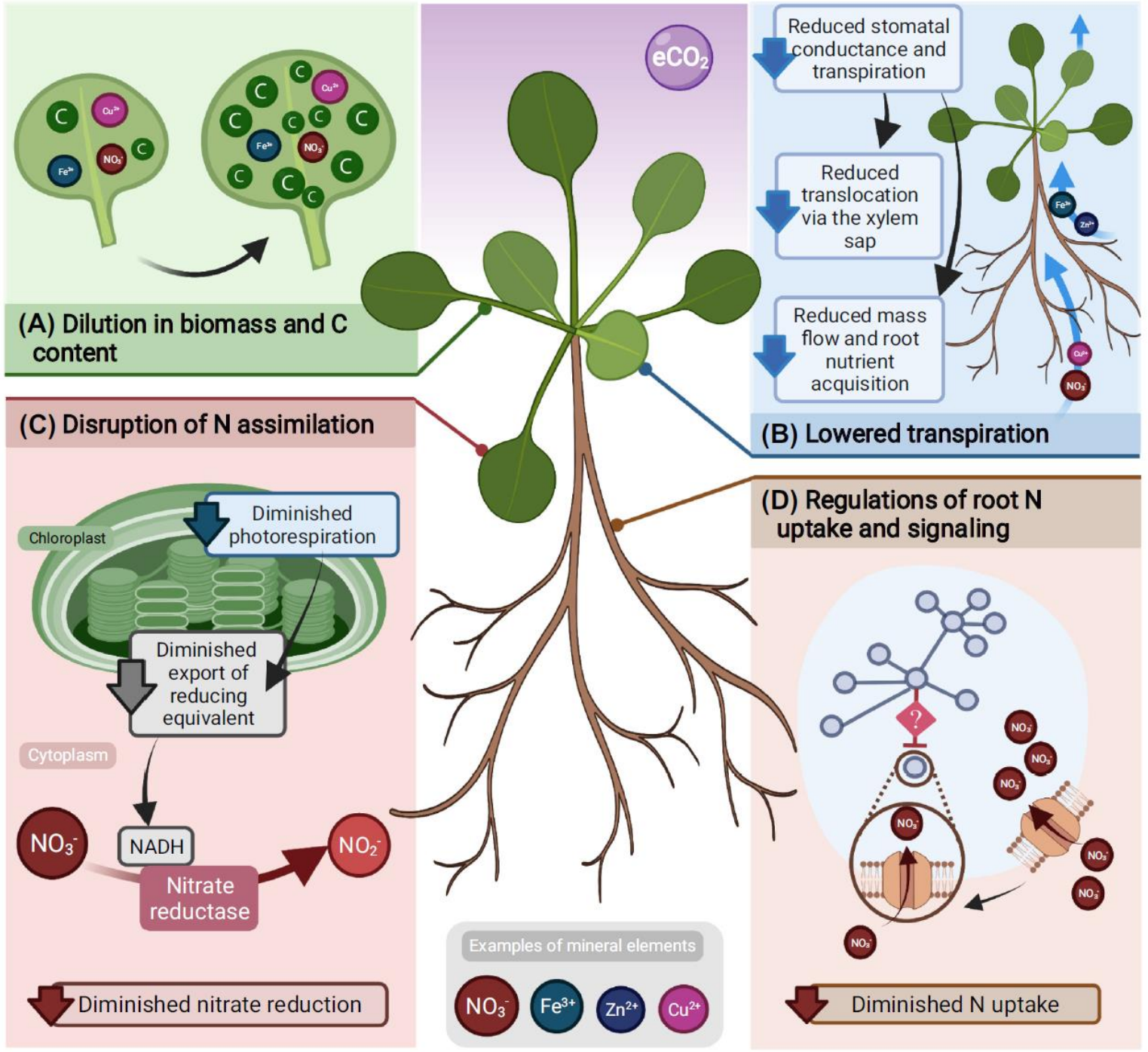
Feature Review

The decline of plant mineral nutrition under rising CO_2: physiological and molecular aspects of a bad deal

Alain Gojon,[1] Océane Cassan,[1] Liên Bach,[1] Laurence Lejay,[1] and Antoine Martin[1,*]

The elevation of atmospheric CO2 concentration has a strong impact on the physiology of C3 plants, far beyond photosynthesis and C metabolism.

In particular, it reduces the concentrations of most mineral nutrients in plant tissues, posing major threats on crop quality, nutrient cycles, and carbon sinks in terrestrial agro-ecosystems. The causes of the detrimental effect of high CO2 levels on plant mineral status are not understood.



Trends in
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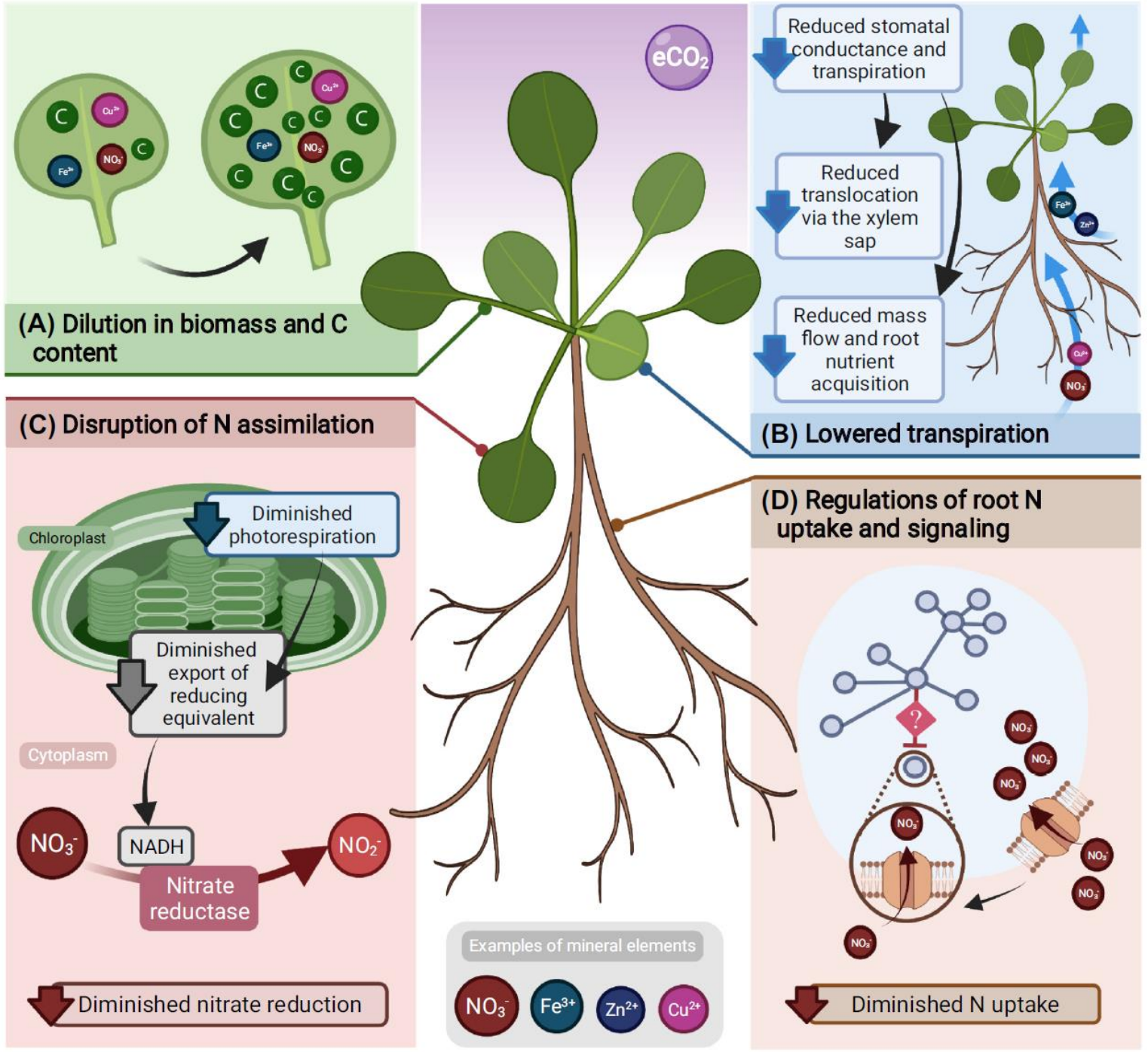
For nitrogen, this detrimental effect is associated with direct inhibition of key mechanisms of nitrogen uptake and assimilation.

B) Lowered stomata opening

C) Diminished photorespiration



Trends in Plant Science

CellPress

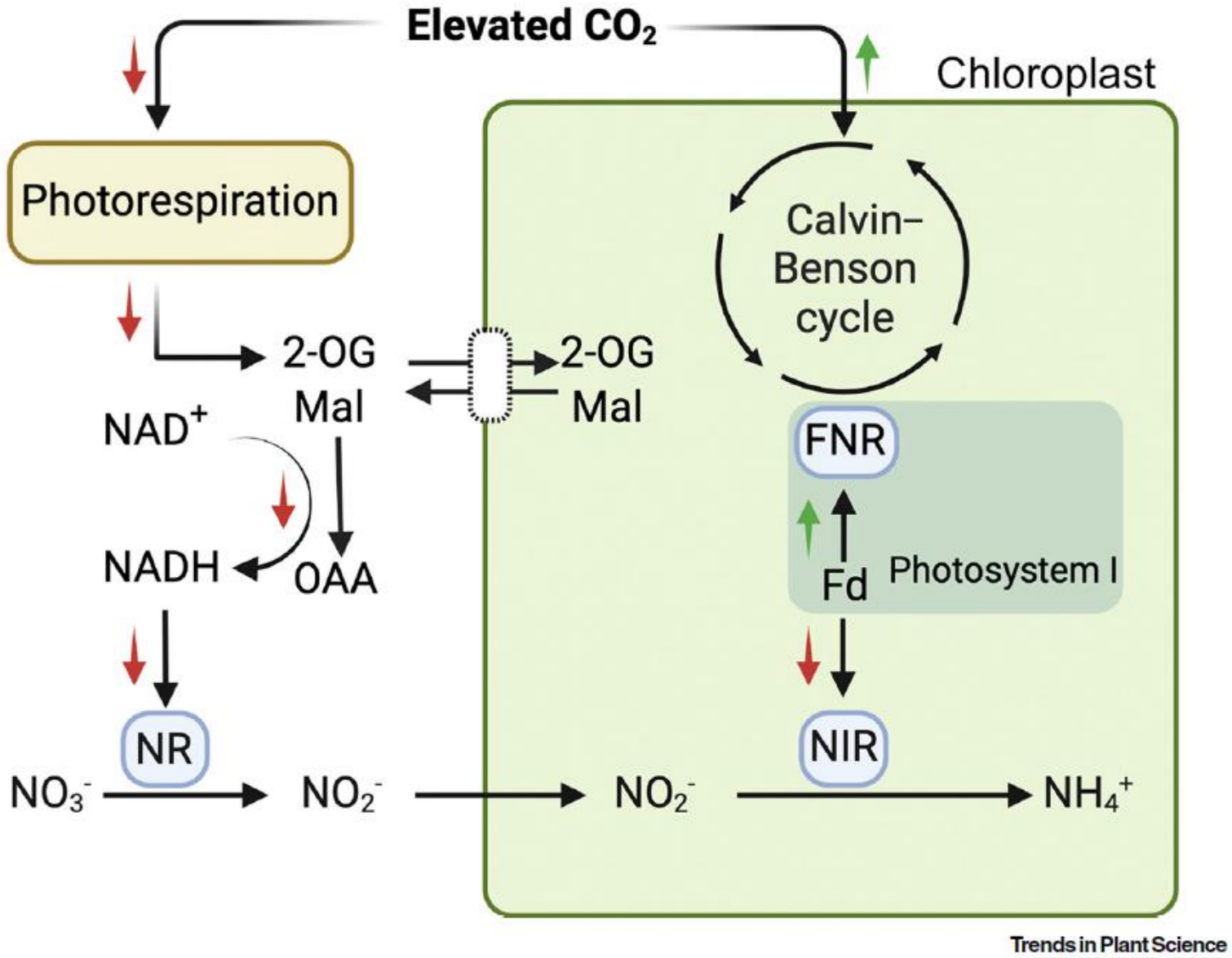
Feature Review

The decline of plant mineral nutrition under rising CO_2: physiological and molecular aspects of a bad deal

Alain Gojon,[1] Océane Cassan,[1] Liên Bach,[1] Laurence Lejay,[1] and Antoine Martin[1,*]

Metabolic pathways by which eCO2 can modify the availability of reducing power needed for the two steps of nitrate reduction.

Red and green arrows indicate the metabolic routes that can be slowed or accelerated by eCO2, respectively. eCO2 boosts the rate of the Calvin–Benson cycle, increasing the demand of reduced ferredoxin (Fd) by ferredoxin-NADPH reductase (FNR) to provide NADPH for the C fixation pathway. This can reduce the availability of Fd for nitrite reductase (NIR), which has a lower affinity than FNR for Fd. At the same time, eCO2 decreases the rate of photorespiration. The reduced production of 2-oxoglutarate (2-OG) by a lower photorespiration can decrease the export of malate (Mal) to the cytosol, which is needed to provide NADH for nitrate reductase (NR).



OAA, oxaloacetic acid.

Trends in
Plant Science

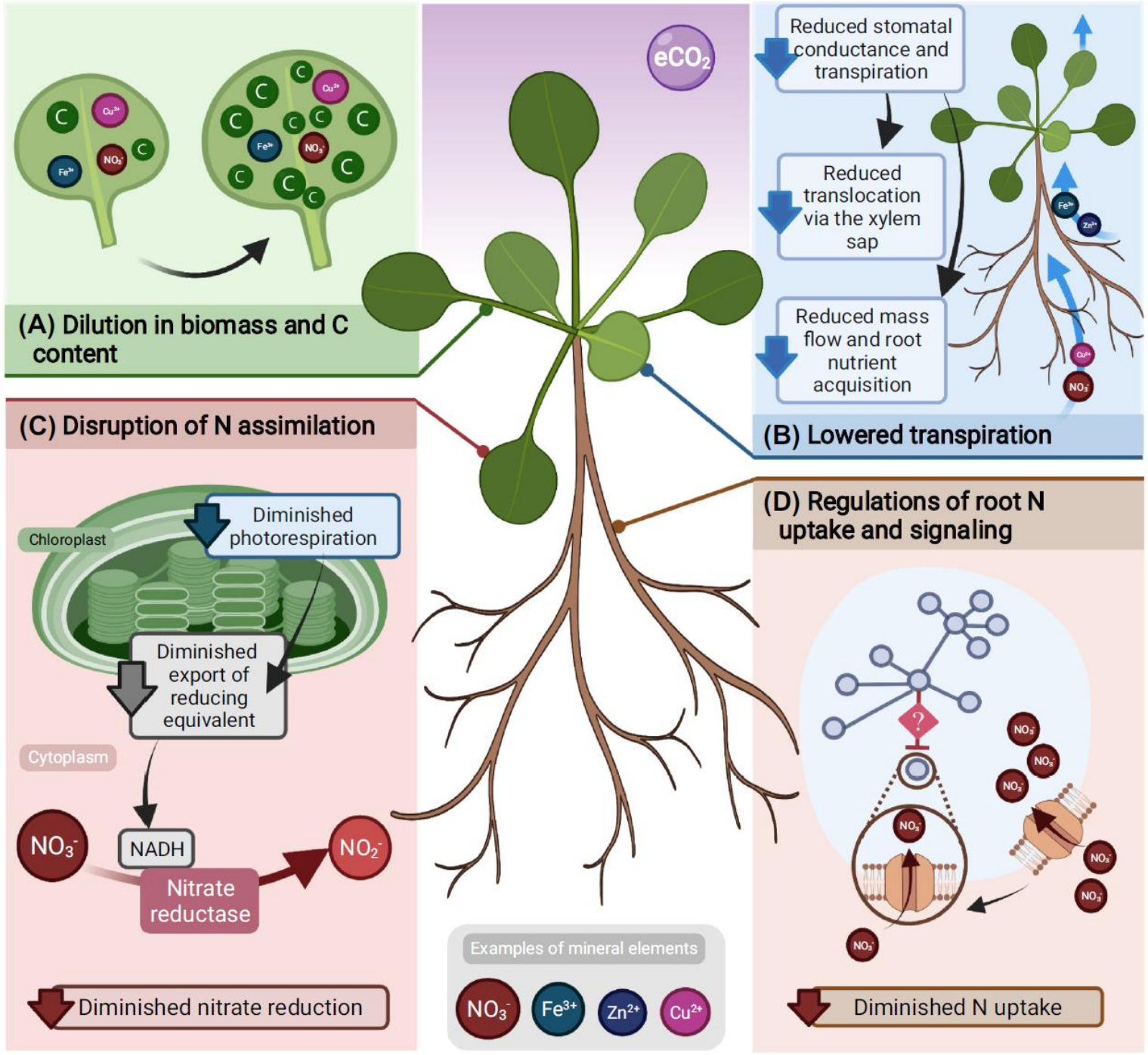
CellPress

Feature Review

The decline of plant mineral nutrition under rising CO_2: physiological and molecular aspects of a bad deal

Alain Gojon,[1] Océane Cassan,[1] Liên Bach,[1] Laurence Lejay,[1] and Antoine Martin[1,*]

Strategies for identifying genotypes that will maintain robust nutrient status in a future high-CO2 world.

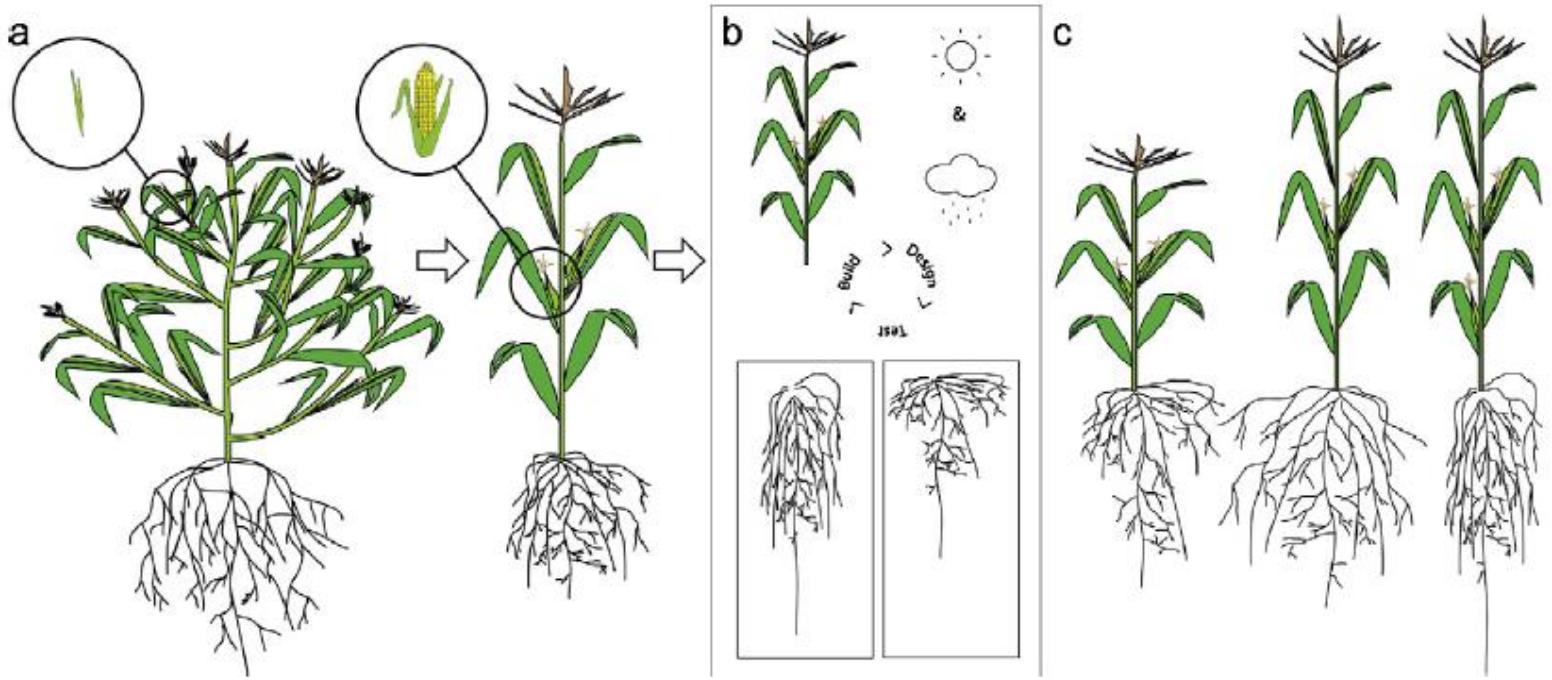


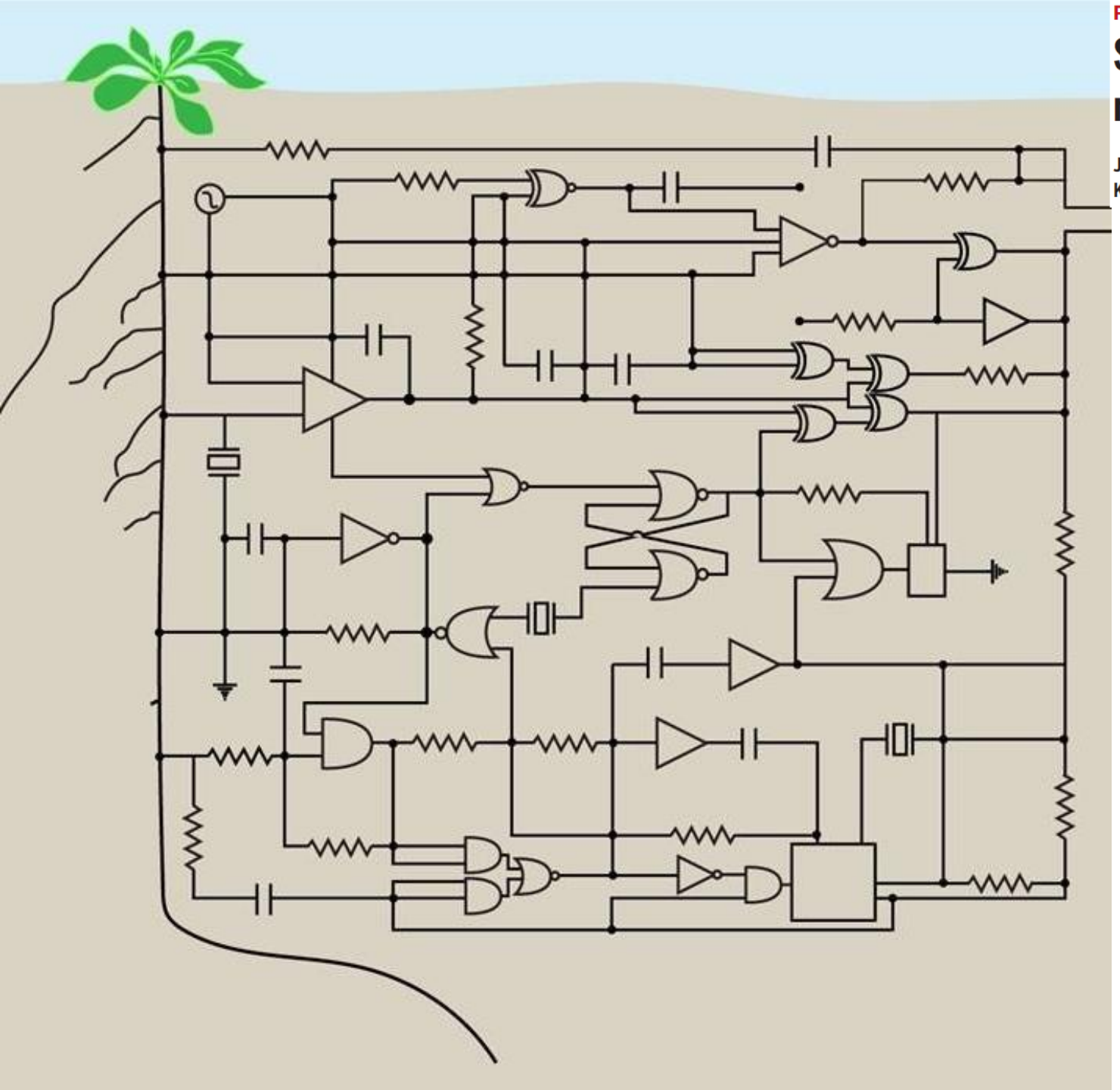
Improving genotypes

a) Domestication of teosinte to modern maize favored productivity

b) Targeted engineering of modules will allow us to produce a wide range of desired outcomes

c) Plants suited to their own environment





PLANT SCIENCE

Synthetic genetic circuits as a means of reprogramming plant roots

Jennifer A. N. Brophy[1,2]*, Katie J. Magallon[1], Lina Duan[1], Vivian Zhong[2], Prashanth Ramachandran[1], Kiril Kniazev[1], José R. Dinneny[1]*

Synthetic Genetic Circuits Reprogram Plant Roots

Image credits, Jennifer Brophy, Stanford University

The workflow

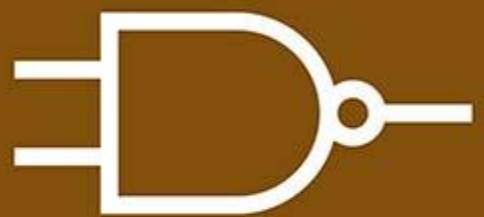
1. Controlling the activity of genes is an important step in engineering plants for improved bioenergy crops.
2. This research developed synthetic genes that can be combined to achieve specific patterns of gene expression within the plant.
3. The expression of the synthetic genes is programmed in the form of Boolean (“AND,” “OR,” and “NOT”) logic gates that work in a similar way to computer circuit boards.
4. Using the synthetic gene circuits, the researchers successfully created predictable, novel expression patterns of fluorescent proteins.
5. Finally, they used similar gene circuits to redesign root architecture by tuning the number of root branches.

Logic Gate Symbols

OR

NOR

AND



NAND

XOR

XNOR

Buffer

NOT

The impact

- To understand biological functions and design new biotechnology applications, scientists need to precisely manipulate gene expression. This is the process that converts instructions in DNA into proteins and other products that allow cells to do their jobs in an organism. Controlling specific patterns of gene expression in plants is challenging. One potential solution is synthetic genetic circuits. However, tuning circuit activity across different plant cell types has proven difficult.
- This research developed new genetic circuits that allow precise control of the root architecture. As roots are important for the uptake of water and nutrients, this approach will allow the design of tailored root architectures. This will in turn help **researchers** to engineer **bioenergy** crops with improved characteristics for growth in marginal lands.

Summary – part I

To establish synthetic gene circuits capable of predictably regulating gene expression in plants, scientists adapted **a large collection of bacterial gene regulators for use as synthetic activators or repressors of gene expression in plants**, also known as transcription factors. Using a transient expression system, the researchers demonstrated that the synthetic transcription factors and their target DNA sequences (promoters) are able to direct specific and tunable control of gene expression. They designed synthetic promoters that responded to one synthetic transcription factor to work as **simple logic gates that responded to one input**, while more complex gates required synthetic promoters that responded to multiple inputs. The research found these logic gates to control expression in predictable ways according to the specific Boolean rules encoded in the engineered genes.

Summary – part II

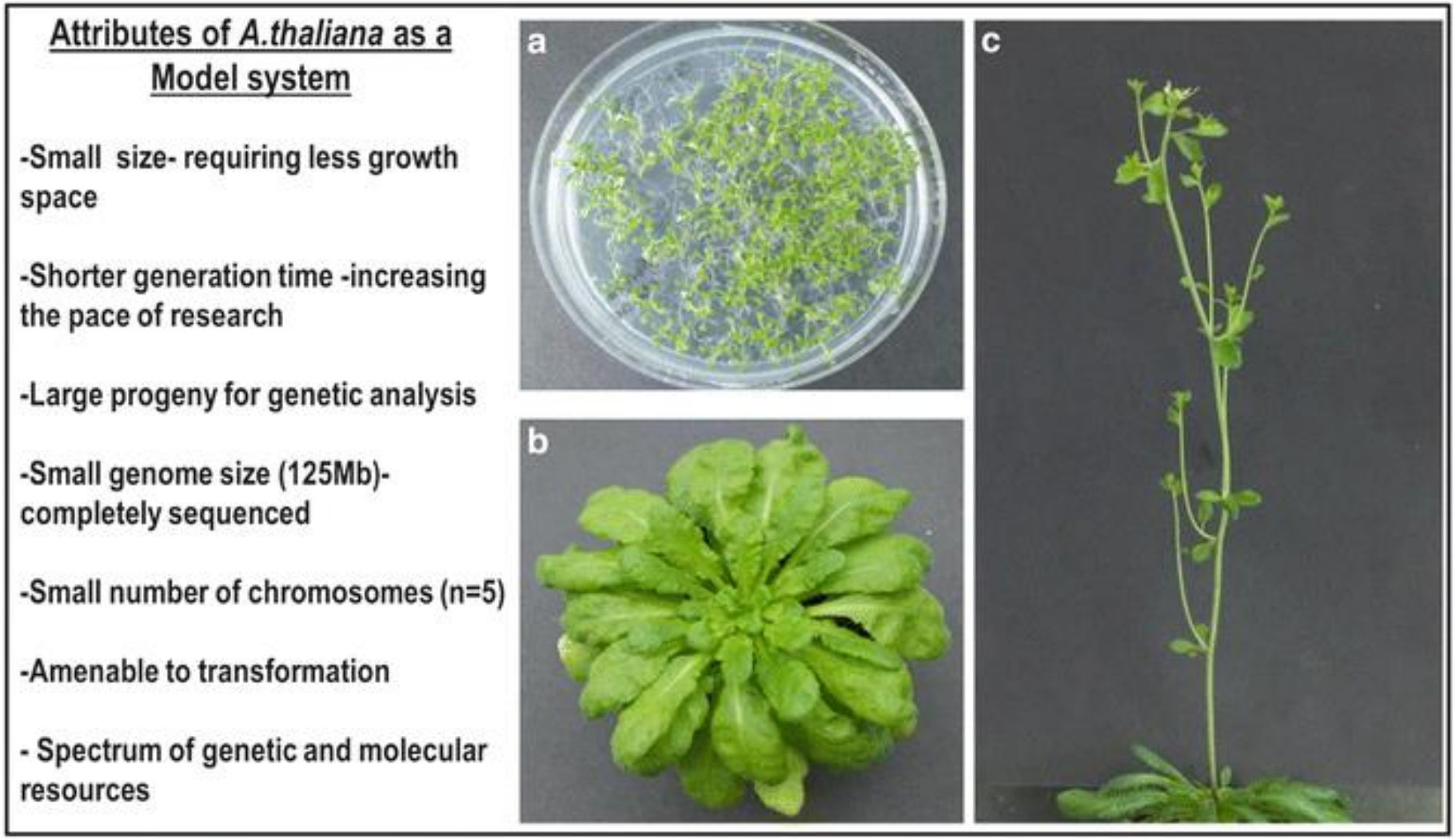
To implement synthetic gene circuits in a multicellular context, the researchers used *Arabidopsis* roots as a model system where endogenous promoters drove tissue-specific expression of the synthetic transcription factors. The gene circuits generated novel expression patterns that were the result of successfully performing logical operations. The researchers further used one of the logic gates to quantitatively control the expression of a hormone signaling regulator to tune the amount of root branching in the root system of *Arabidopsis*. These results demonstrate that it is now possible to program gene expression across plant cell types using genetic circuits, providing a roadmap to engineer more resilient bioenergy crops.

SYNTHETIC BIOLOGY

Synthetic gene circuits take root

Complex spatial patterns of gene expression are engineered in plants to modulate root morphology

Engineering spatial transcriptional patterns in the root of the **model plant Arabidopsis thaliana** to alter its morphology.



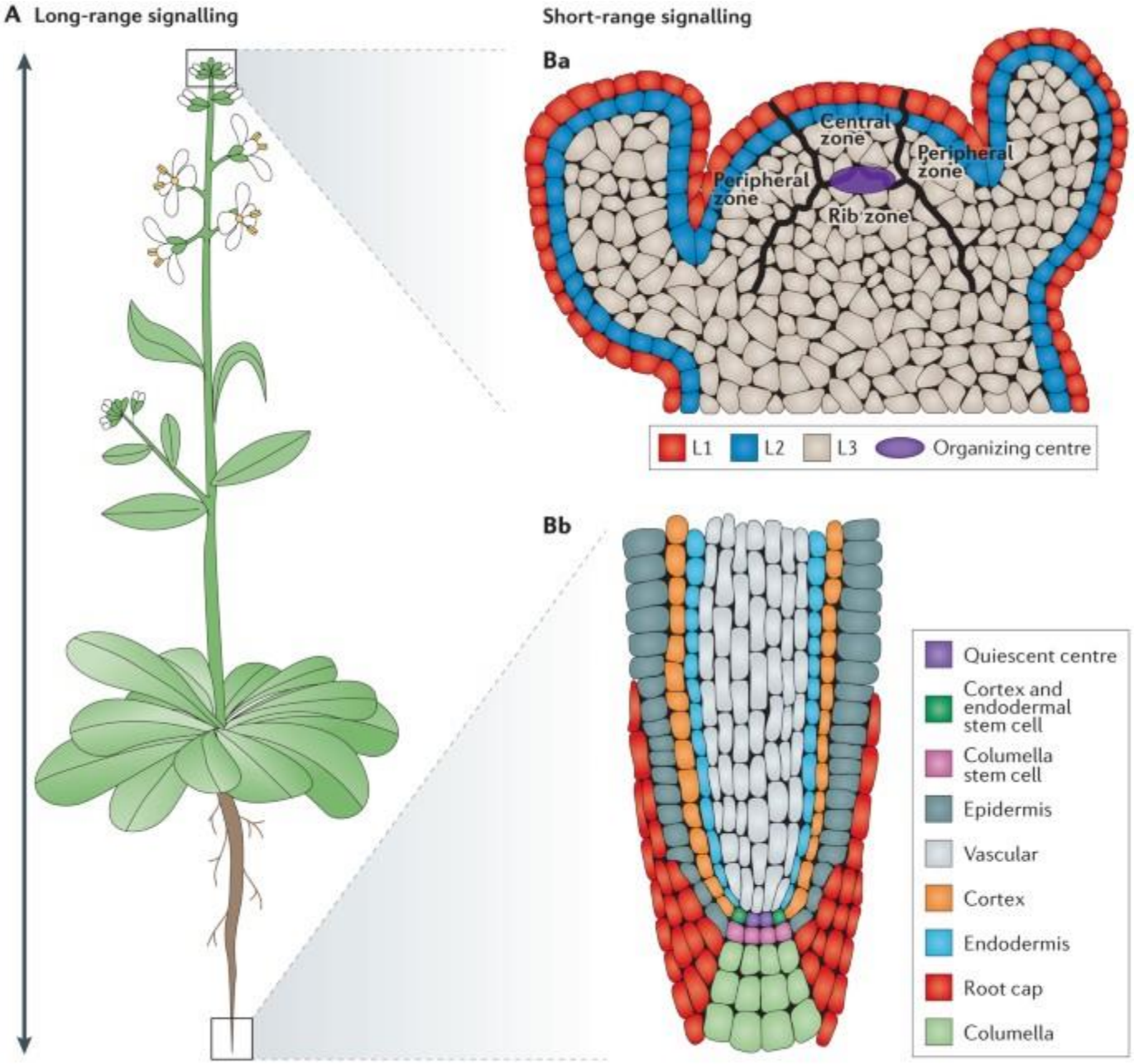
SYNTHETIC BIOLOGY

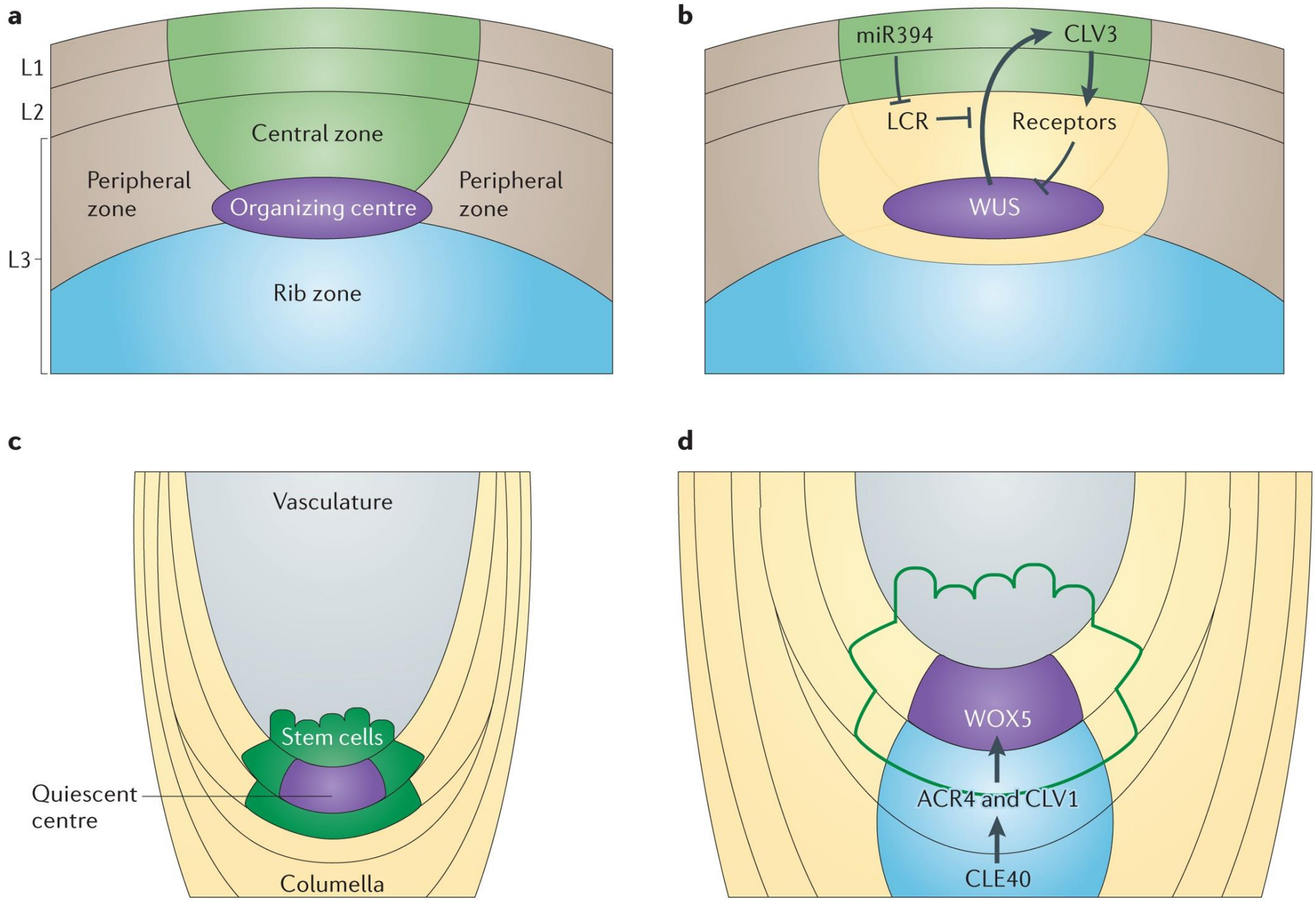
Synthetic gene circuits take root

Complex spatial patterns of gene expression are engineered in plants to modulate root morphology

A long-standing aim of synthetic biology has been to engineer genetic circuits that are able to confer prescribed spatiotemporal patterns of gene expression.

FIRST ISSUE to be solved




a
L1
L2
L3
Central zone
Peripheral zone
Peripheral zone
Organizing centre
Rib zone
b
miR394
CLV3
LCR
Receptors
WUS
c
Vasculature
Stem cells
Quiescent centre
Columella
d
WOX5
ACR4 and CLV1
CLE40

Goals

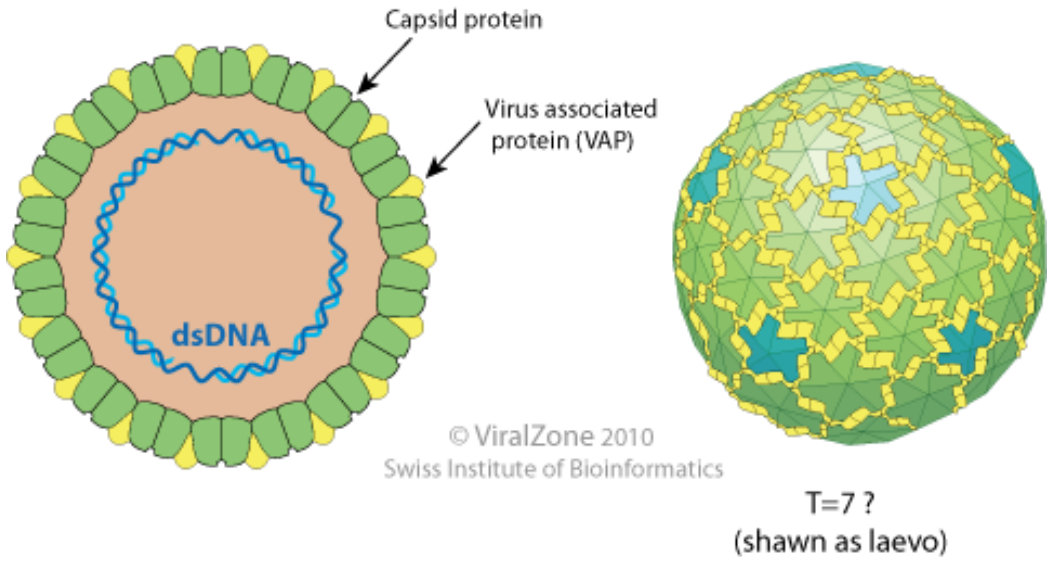
- unlock the next tier in the technology tree of translational biology
- a powerful demonstration of a predictive and quantitative basic understanding of genetic regulation in higher eukaryotes.
- This effort constitutes a milestone in the genetic engineering of a whole, fully developed multicellular organism and points to the challenges ahead.

The Cauliflower Mosaic Virus 35*S* Promoter: Combinatorial Regulation of Transcription in Plants

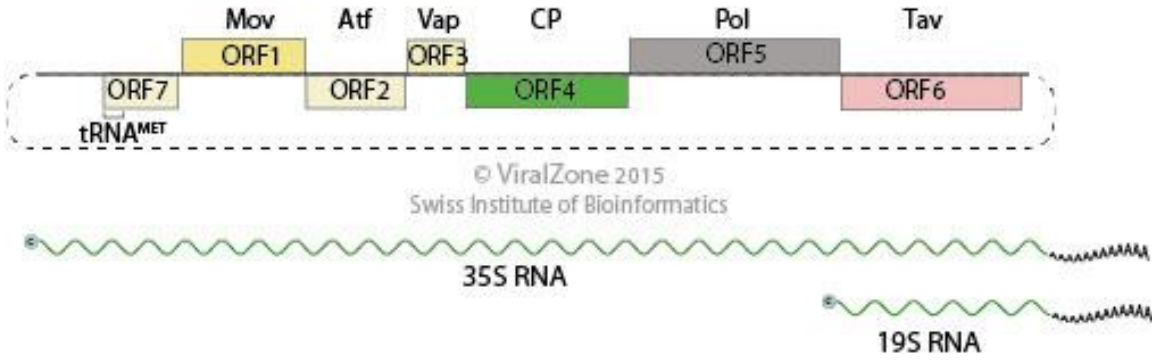
PHILIP N. BENFEY AND NAM-HAI CHUA

IMPORTANT: Species-specif

- Early studies of the cis and trans regulation of plant and animal genes showed that spatiotemporal patterns of transcription follow a combinatorial logic



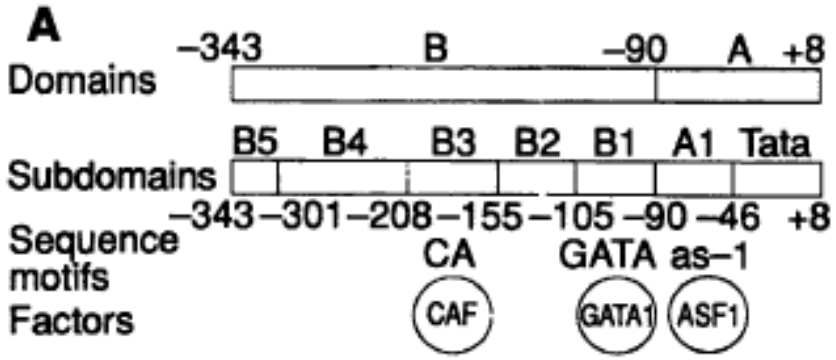
- The 35s RNA and its spliced derivatives serves as polycistronic mRNA for viral proteins.



The Cauliflower Mosaic Virus 35*S* Promoter: Combinatorial Regulation of Transcription in Plants

PHILIP N. BENFEY AND NAM-HAI CHUA

- Early studies of the cis and trans regulation of plant and animal genes showed that spatiotemporal patterns of transcription follow a combinatorial logic
- CAF – GATA1 – ASF1 trans-factors



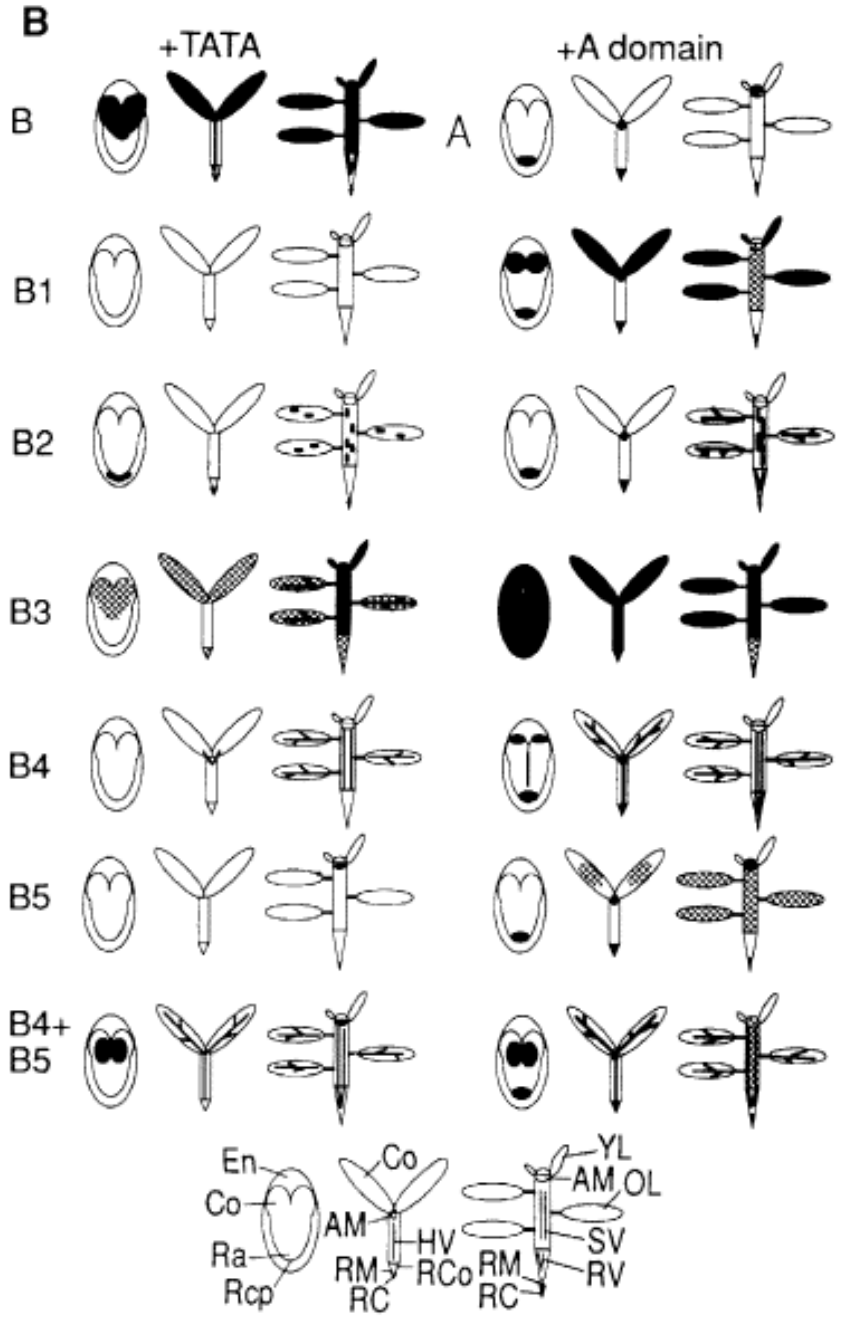
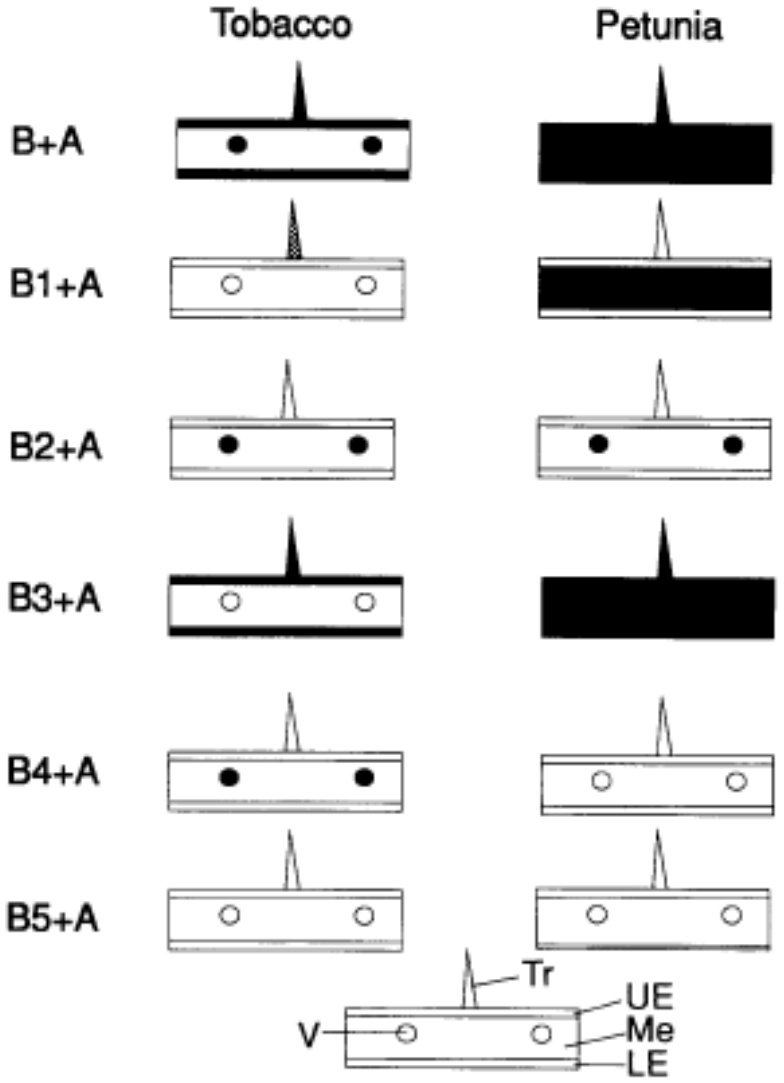


Fig. 4. Schematic representation of expression patterns conferred by 35*S* subdomains in tobacco and petunia petals. Expression conferred by the combinations of 35*S* subdomains listed on the left are shown in schematic sections through mature petals of tobacco and petunia. Only the salient features of the expression patterns are indicated. For 4 × B2 + A in petunia, the expression pattern of the single high expressing plant is shown. The cell types represented are indicated in the last row. LE, lower epidermis; Me, mesophyll; Tr, trichome; UE, upper epidermis; V, vascular tissue.



Tobacco

Petunia

- Whether a gene is expressed in a cell type depends on the combination of **DNA binding sites** in its regulatory region and whether these sites are occupied by activator or repressor **transcription factors (TFs)** present in these cells. These regulatory combinations can be described as **boolean logical operations**.
- For example, a gene may be transcribed only if its activator is present while its repressor is absent, a logic operation known as a "NIMPLY gate." (**material non-implication**). It also became clear that TF proteins themselves are modular, with DNA binding and regulatory activity being physically and functionally separated.

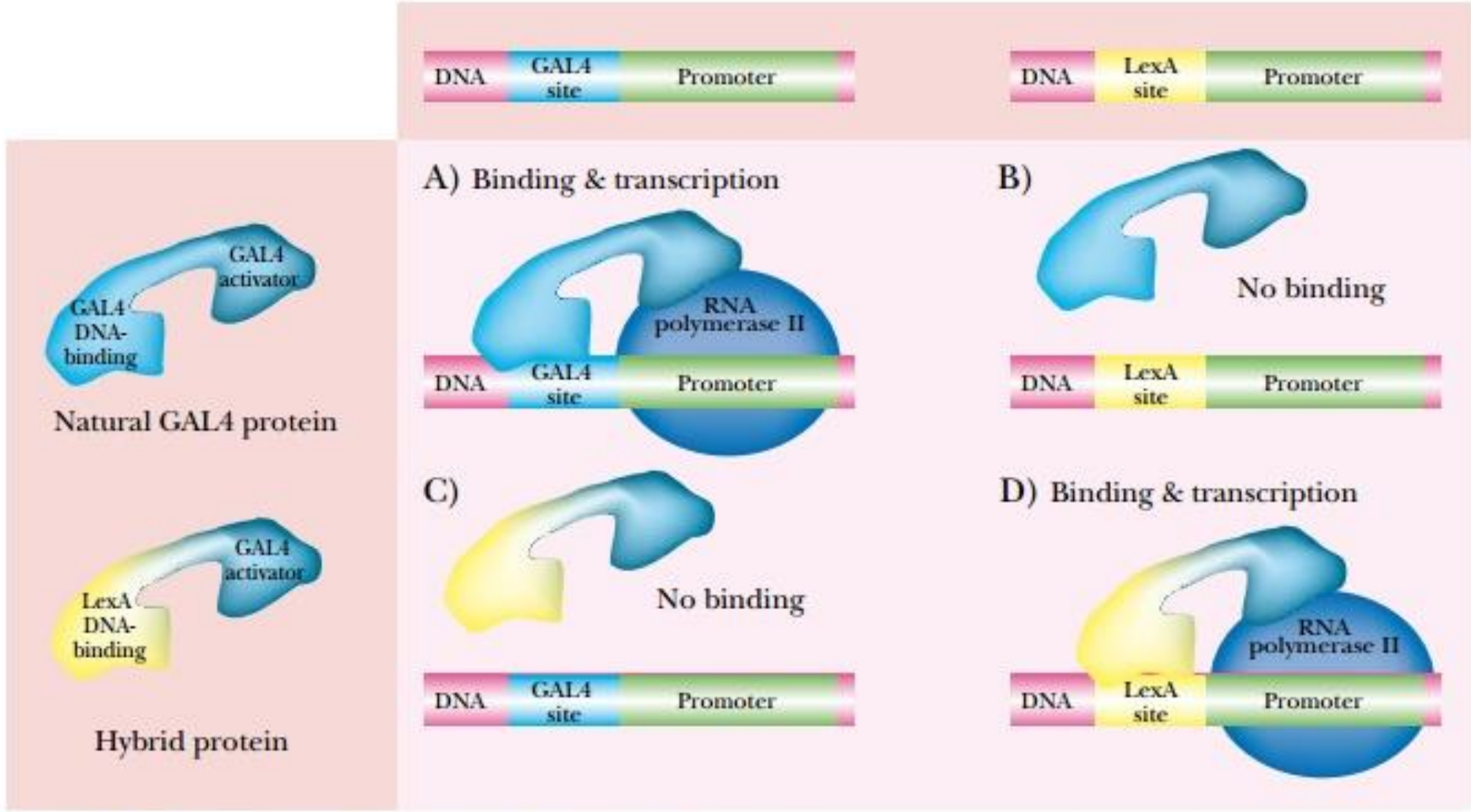


FIGURE 2.10 Transcription Factors Have Two Independent Domains
(A) One domain of the GAL4 transcription factor normally binds to the GAL4 DNA recognition sequence and the other binds the transcription apparatus. (B) If the LexA sequence is substituted for the GAL4 site, the transcription factor does not recognize or bind the DNA. (C) An artificial protein made by combining a LexA binding domain with a GAL4 activator domain will not recognize the GAL4 site, but (D) will bind to the LexA recognition sequence and activate transcription. Thus, the GAL4 activator domain acts independently of any particular recognition sequence. It works as long as it is held in close contact with the DNA.

- Indeed, there persists a humbling gap between our understanding of the endogenous genetic circuitry that controls gene expression patterns in animals and plants and our ability (or lack thereof) to engineer these patterns
- LIMITATION: engineering synthetic TFs and the synthetic promoters responsive to them can take multiple iterations of testing and optimization, whereas the turnaround time to generate a transgenic plant or animal is several months.

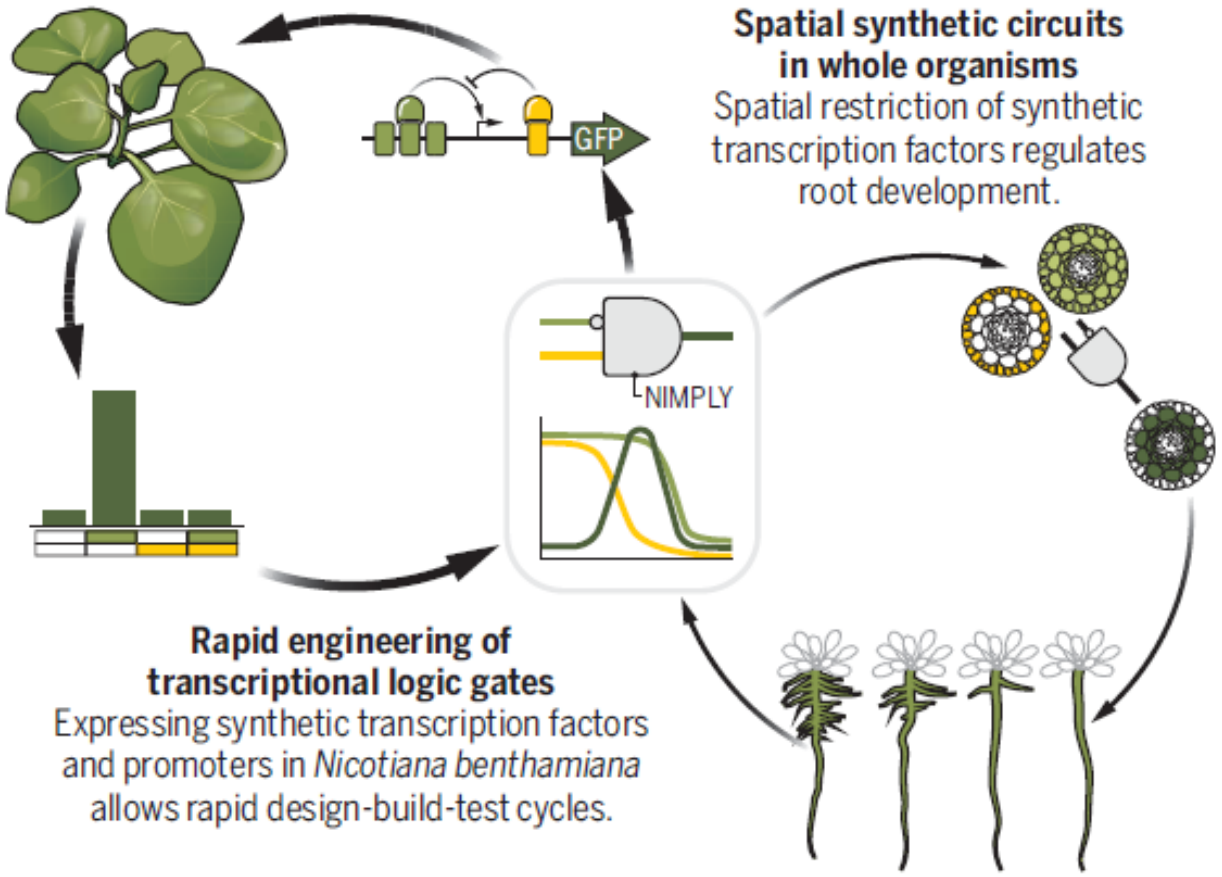
SECOND ISSUE to be solved

- It is possible to transiently express multiple transgenes in the leaves of the tobacco relative *Nicotiana benthamiana* and measure the circuit performance using green fluorescent protein (GFP) fluorescence in just 2 days

Synthetic transcriptional logic gates

Logic gates such as NIMPLY can be implemented at the transcriptional level to engineer biological computation. Brophy *et al.* developed a library of different synthetic transcription factors and promoters. These tools were optimized and then used to drive root development in *Arabidopsis thaliana*.

GFP, green fluorescent protein



- Using this platform, they developed a library of synthetic TFs based on bacterial DNA binding domains and eukaryotic activation and repression domains. Combined with synthetic promoters that bear bacterial DNA binding sites, these form an impressive collection of parts that can perform all major logical operations

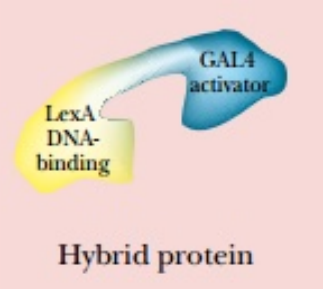
Constructing synthetic genetic circuits in plants

PLANT SCIENCE

Synthetic genetic circuits as a means of reprogramming plant roots

Jennifer A. N. Brophy[1,2]*, Katie J. Magallon[1], Lina Duan[1], Vivian Zhong[2], Prashanth Ramachandran[1], Kiril Kniazev[1], José R. Dinneny[1]*

- Generation of a collection of synthetic transcriptional regulators.
- Design of:



transcriptional activators [1. composed of bacterial DNA binding proteins, 2. VP16-activation domains, and 3. SV40 nuclear localization signals (NLSs)]

+

synthetic repressors that rely on steric hindrance to achieve repression (composed of only DNA binding proteins and NLSs)

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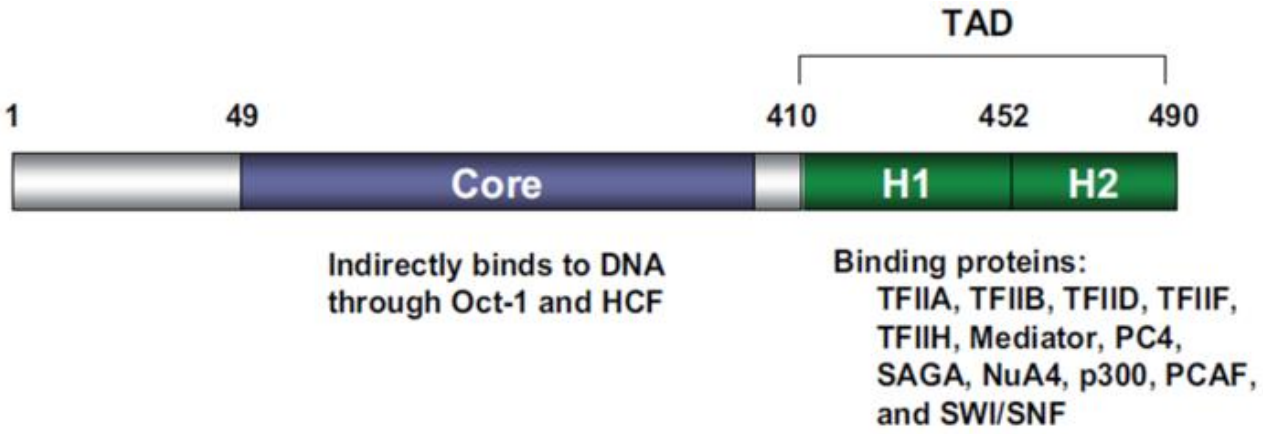
- Generation of a collection of synthetic transcriptional regulators.
- Design of transcriptional activators [1, composed of bacterial DNA binding proteins, 2. VP16-activation domains, and 3. SV40 nuclear localization signals (NLSs)].
- Virus Protein 16 (VP16) is a transcription factor encoded by the UL48 gene of Herpes simplex virus-1 (HSV-1).
- Transactivation domain (TAD) of VP16 is one of the most efficient TADs. It is fused to host transcription factors to increase their activity.



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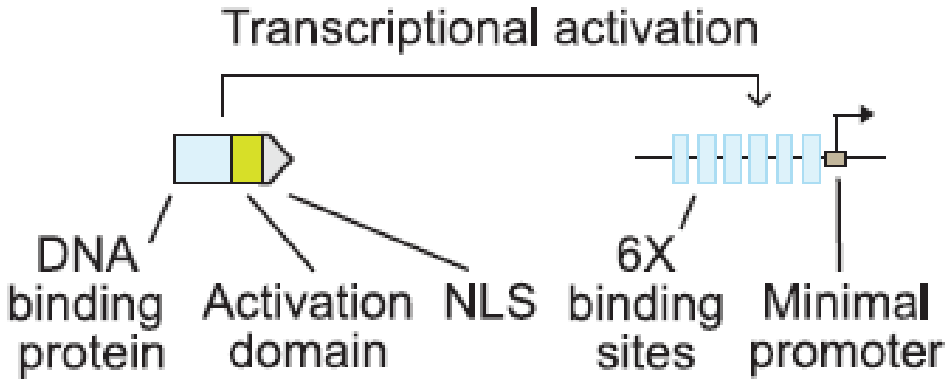
- Generation of a collection of synthetic transcriptional regulators.
- Design of transcriptional activators [1. composed of bacterial DNA binding proteins, 2. VP16-activation domains, and 3. SV40 nuclear localization signals (NLSs)]
- SV40 large T antigen (Simian Vacuolating Virus 40 TAg) is a hexamer protein that is a dominant-acting oncoprotein derived from the polyomavirus SV40.

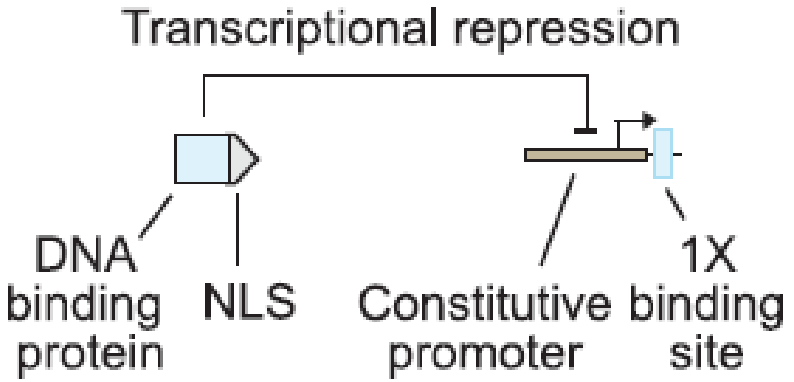
$NLS_{SV40\ T\text{-}ag}$ 125-132 **PKKKRKV**

- Schematics of the synthetic transcriptional activators built to control gene expression in plants. Small bent arrow denotes the transcription start site.



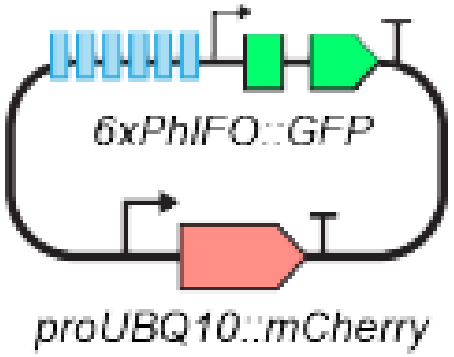
- Similar to previous synthetic promoter designs, activatable plant promoters were created by fusing six copies of the DNA sequence (operator) bound by these transcription factors (TFs) to a minimal plant promoter [positions –66 to +18 of the cauliflower mosaic virus (CaMV) 35S promoter]

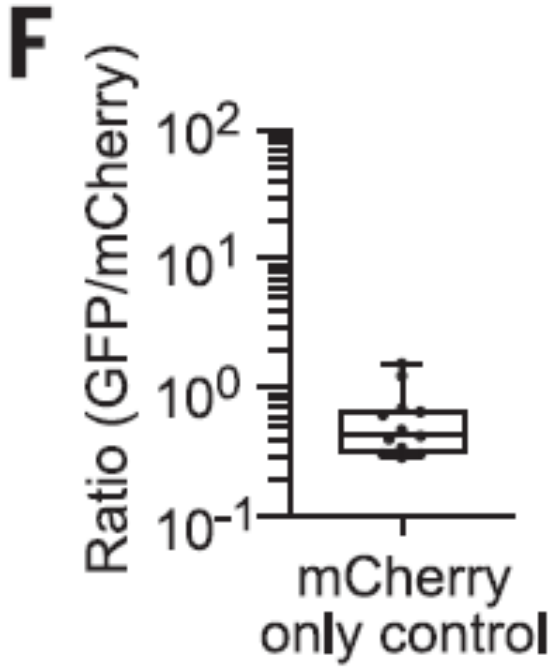
- Schematics of the synthetic transcriptional repressors built to control gene expression in plants. Small bent arrow denotes the transcription start site.

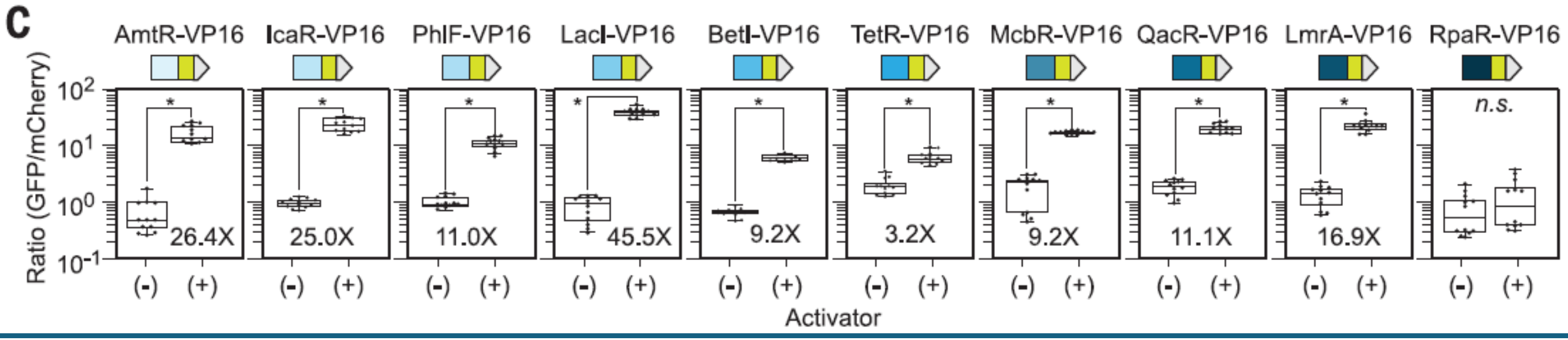


- Repressible promoters were built by placing one operator sequence at the 3' end of a full-length CaMV 35S promoter (Fig. 1B). This design was selected to avoid disrupting 35S promoter activity when adding operators.

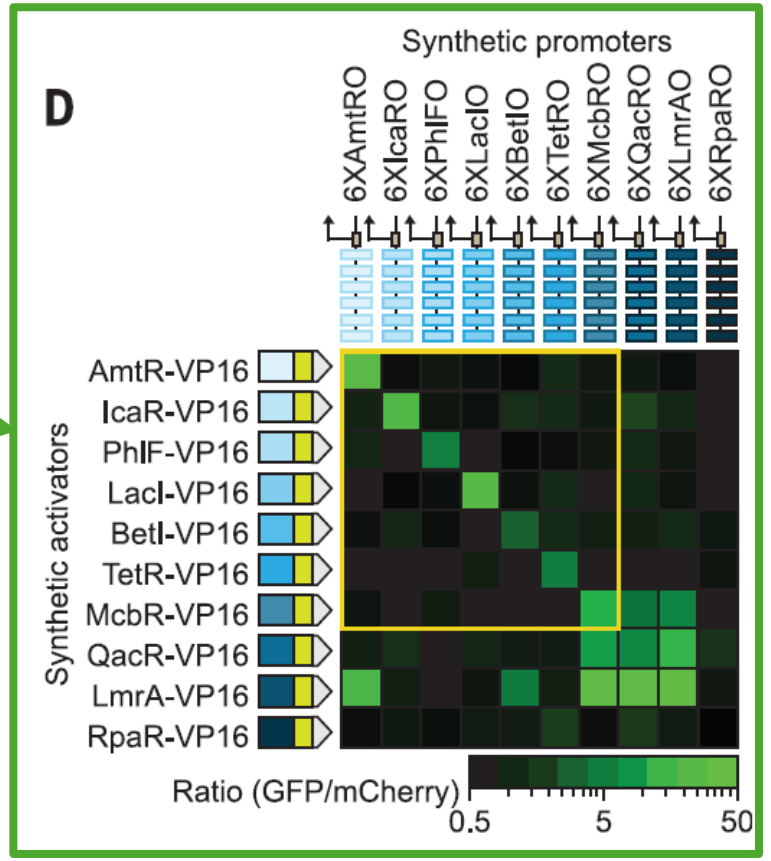
- mCherry only control
- To enable quantitative measurements of gene expression, they used the synthetic promoters to drive expression of green fluorescent protein (GFP) and normalized GFP expression to a constitutively expressed mCherry encoded on the same T-DNA. They also introduced an **intron** to GFP to prevent Agrobacterium from expressing the reporter and confounding fluorescence measurements

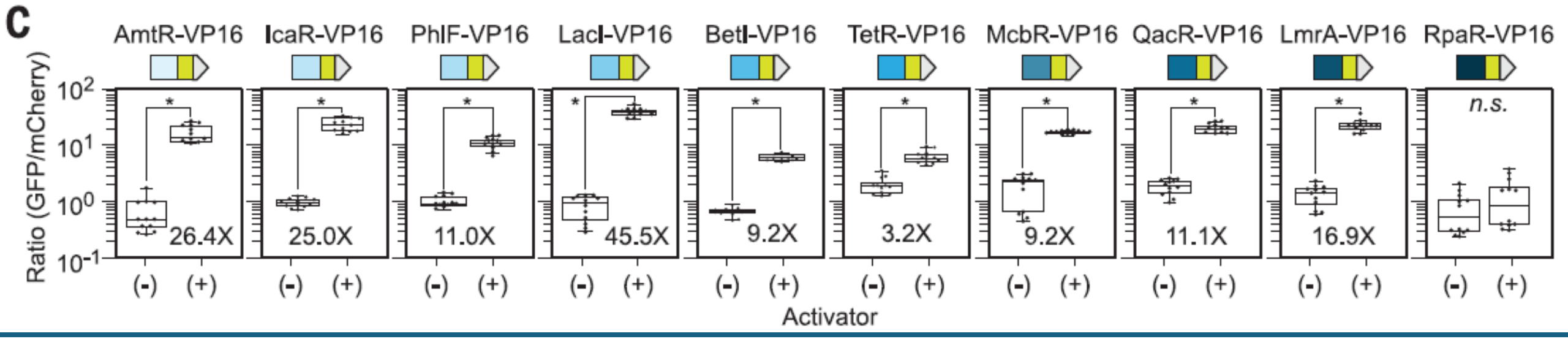




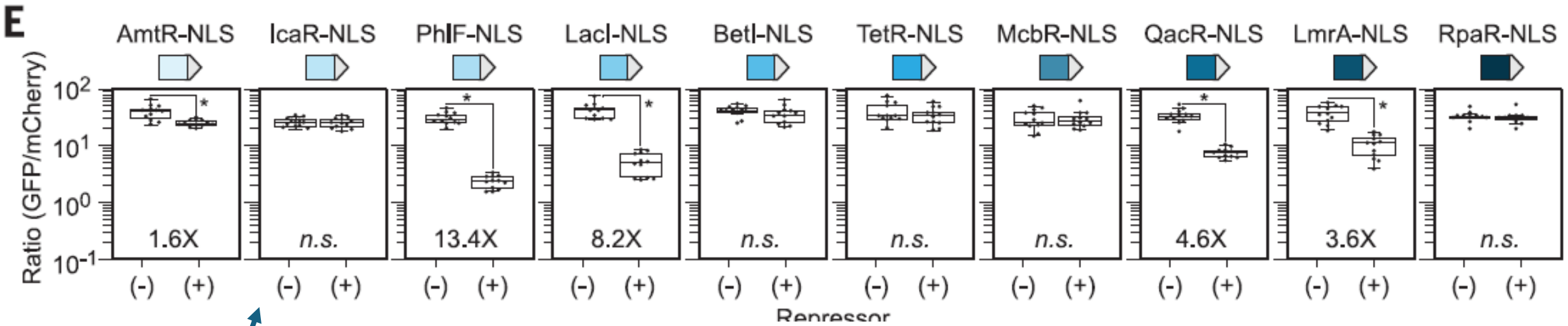


- Activity and specificity of the activator system
- 9/10 were active
- 7 were also specific for the promoter





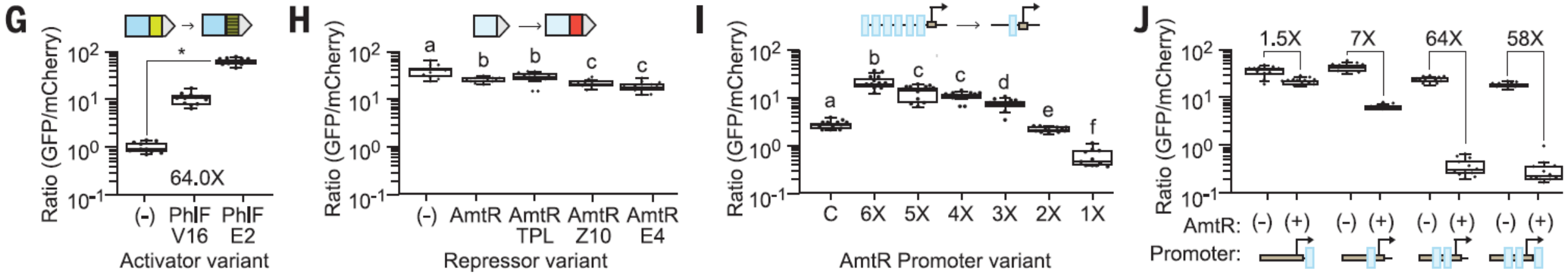
- AmtR, the master regulator of nitrogen control in Corynebacterium glutamicum



- Activity of the repressor system

- Only 4 worked more than 2x

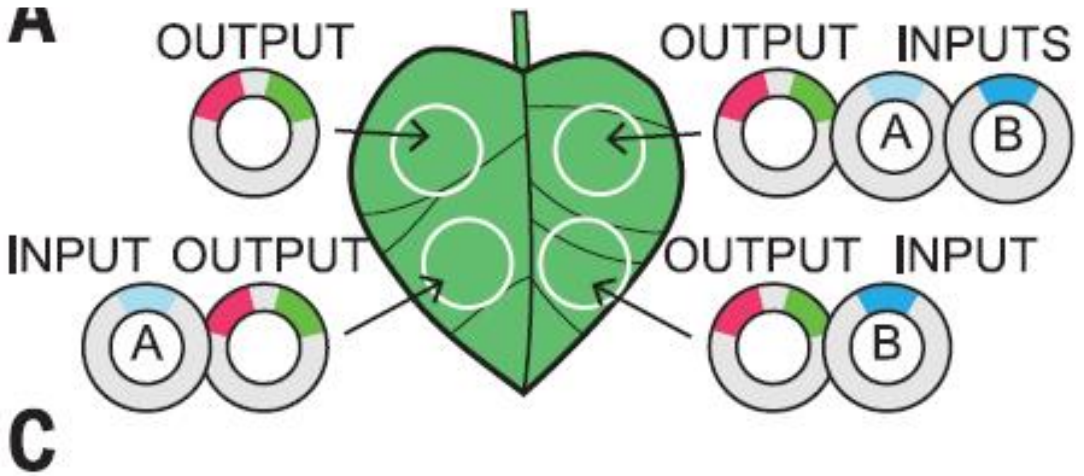
Module optimization



- Arabidopsis ETHYLENE RESPONSE FACTOR2 (ERF2AD) instead of VP16
- adding a repressor domain to the AmtR-based synthetic repressor either had no effect or only modestly increased repression
- Changing the number of TF binding sites in the AmtR-activatable promoter resulted in a collection of promoters of varying strength.
- Adding a second operator between the TATA box and the transcription start site further improved repression and dynamic range

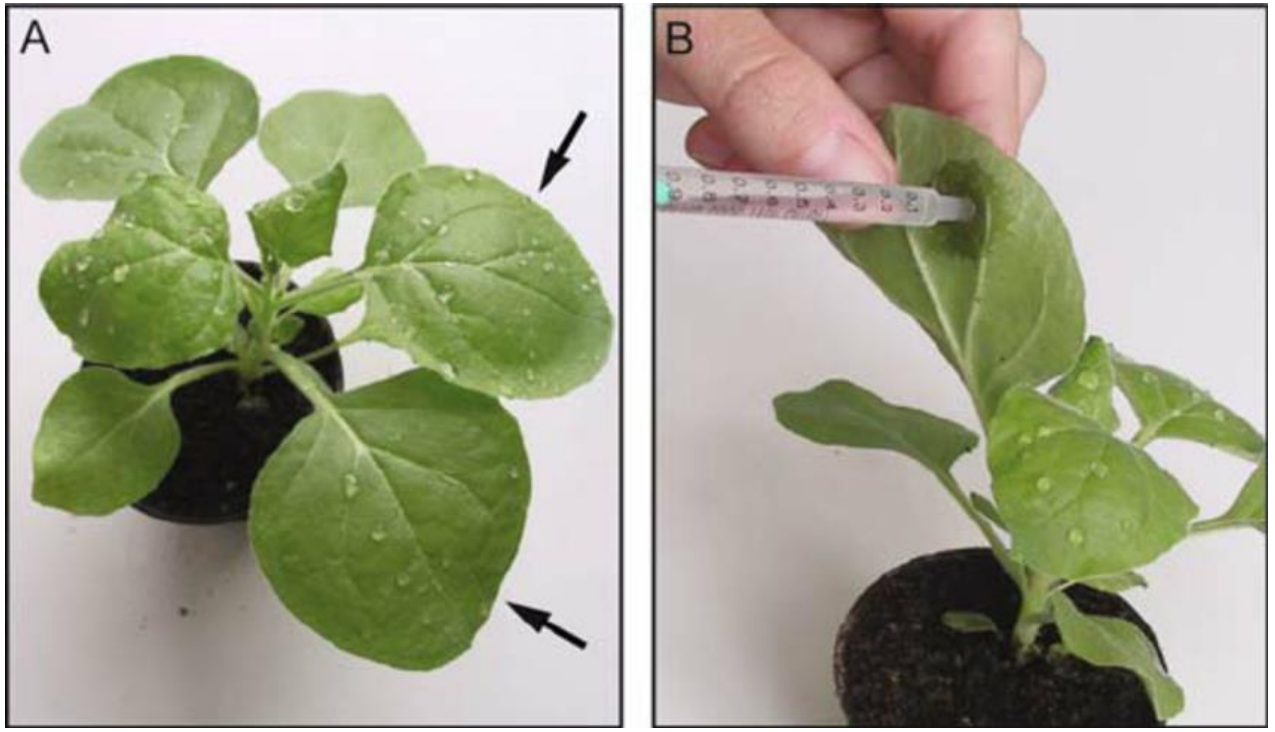
Boolean logic operations

- Synthetic TFs built with the AmtR and PhlF DNA binding proteins, which demonstrated strong activation and repression in our initial TF designs, served as the inputs to all circuits, and GFP served as the output.
- Circuit activity was measured in N. benthamiana leaves, which were infiltrated with multiple Agrobacterium strains, each containing one plasmid that encoded either an input TF or the output

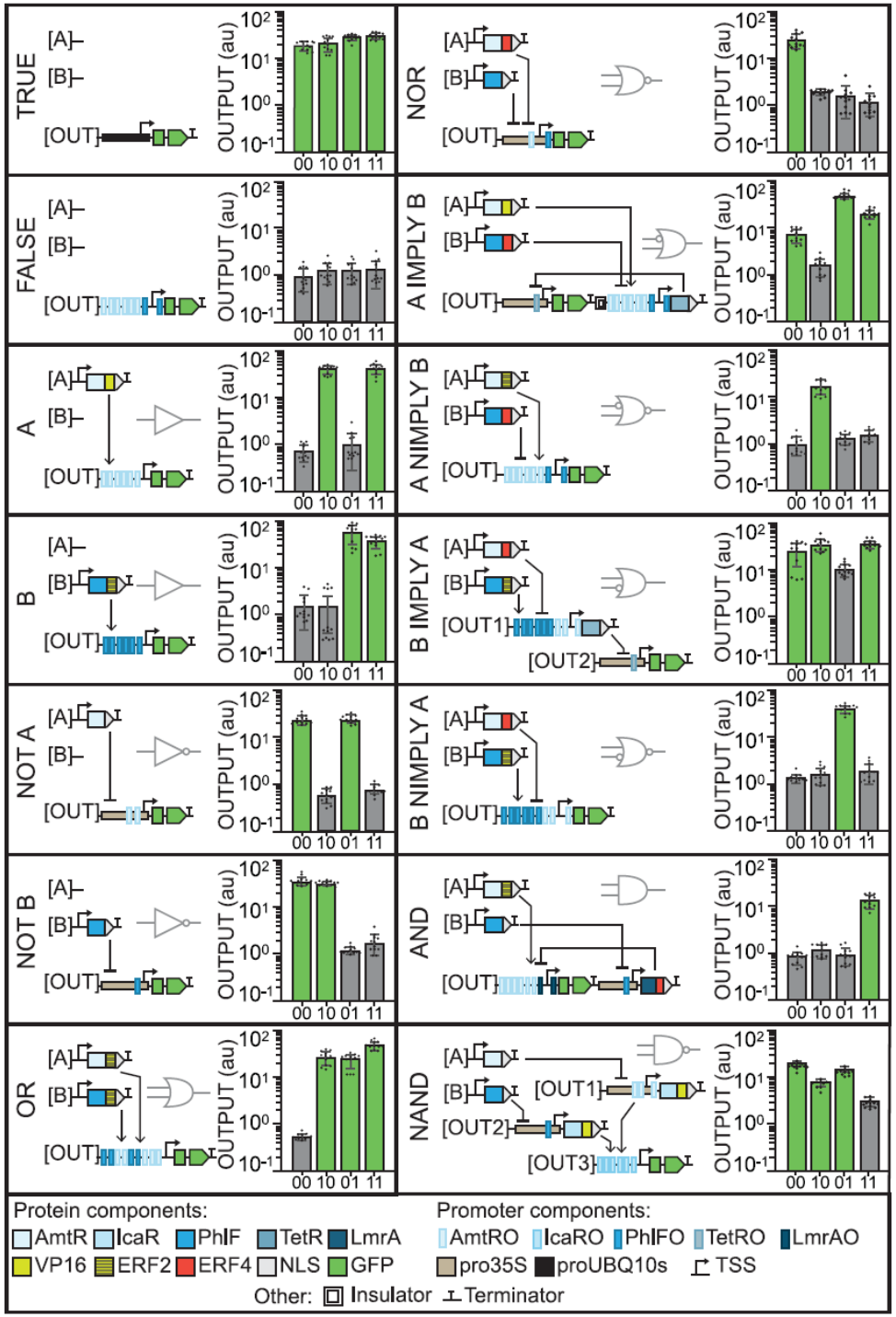


Nicotiana benthamiana

- Most circuits involved several design-build-test cycles, which were facilitated by the rapid N. benthamiana–based assays and the modular nature of the synthetic biology parts generated here.

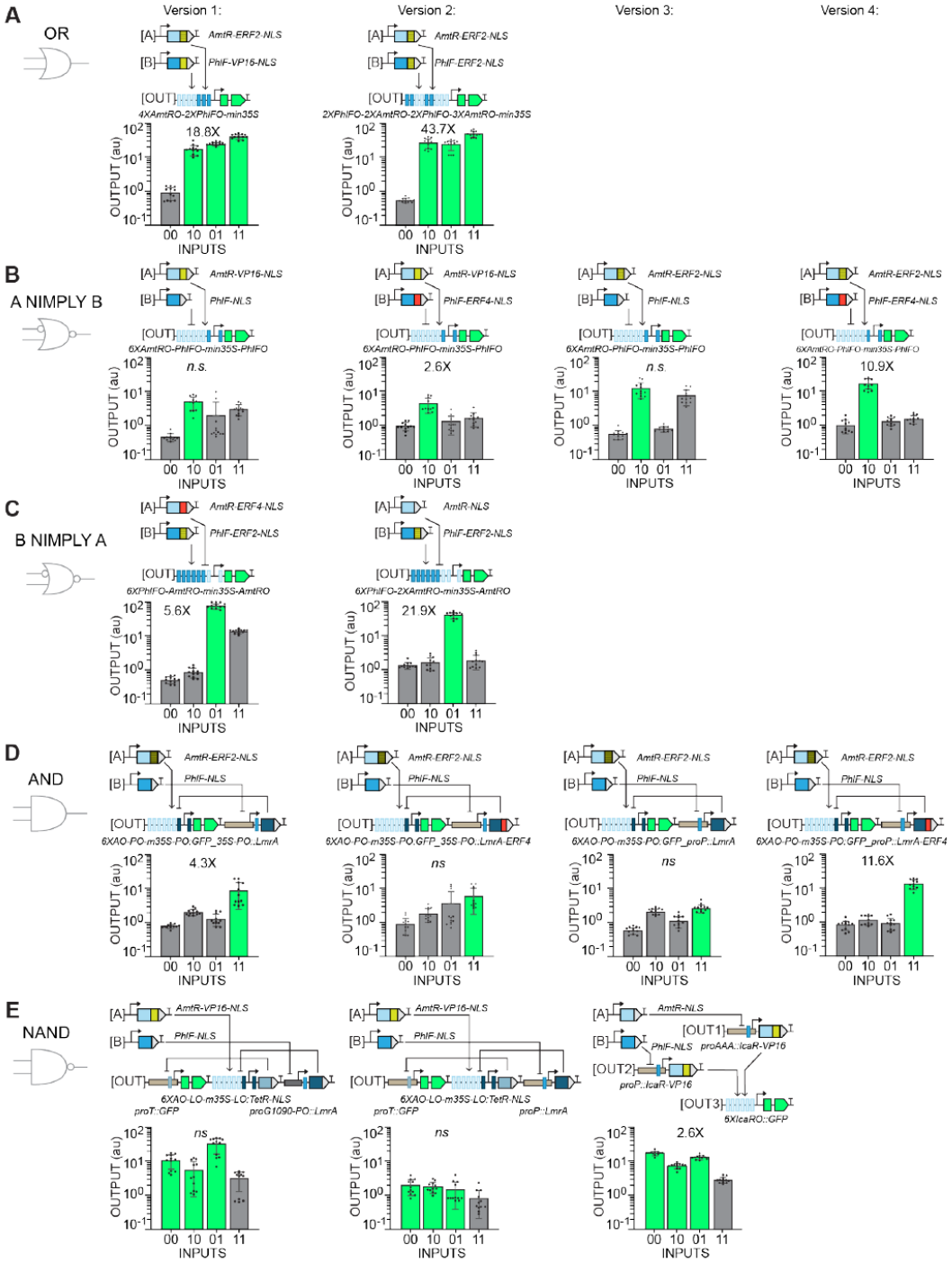


- Gate behavior in N. benthamiana. Bar charts show output of each circuit, reported as the ratio of GFP to mCherry. Green bars indicate gate states that should be ON; gray bars indicate states that should be OFF to implement correct logic.
- Data are mean and SD of 12 leaf punches collected from three leaves infiltrated and measured on different days. Dots show individual data points.
- ERF4RD, to prevent synthetic activators from initiating transcription at composite promoters (1-1 state, A NIMPLY B)

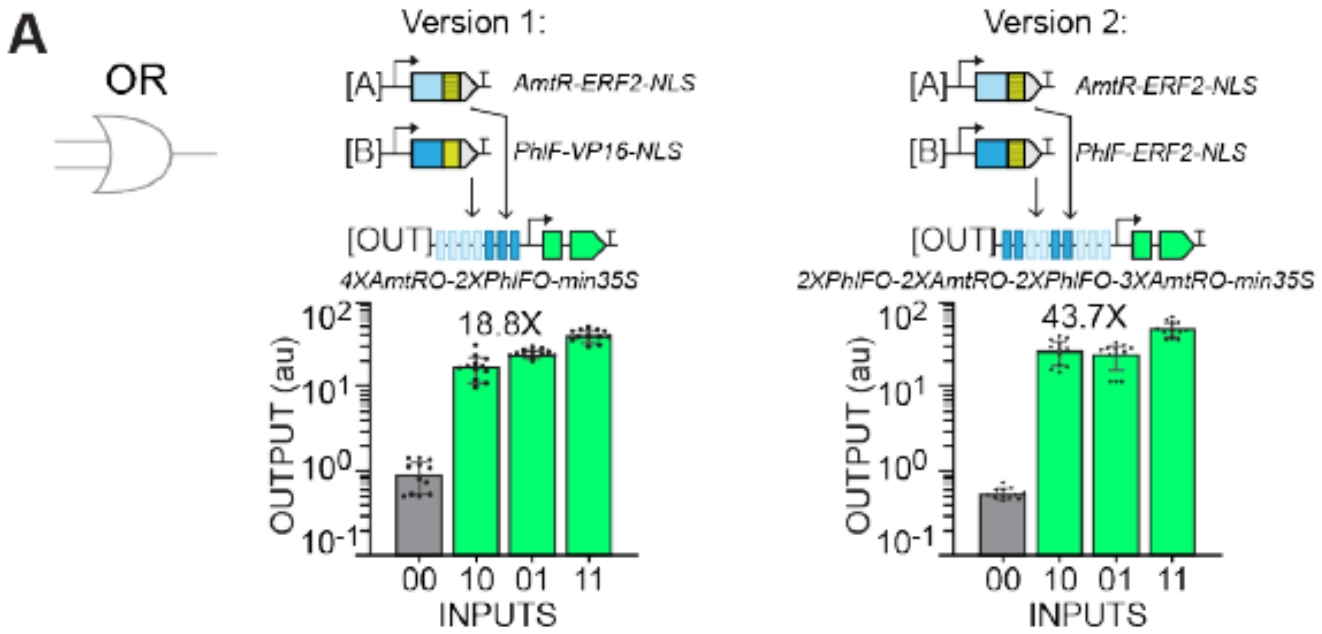


Optimization is needed (different versions)

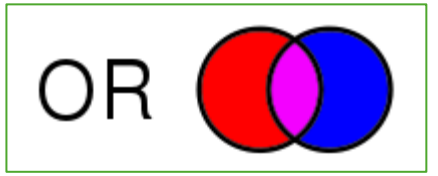
- To create functional circuits, both the promoter architecture and the synthetic TFs needed to be optimized
- We found that the arrangement of operators in the OR promoter affected fold change, with the best design containing alternating pairs of operators



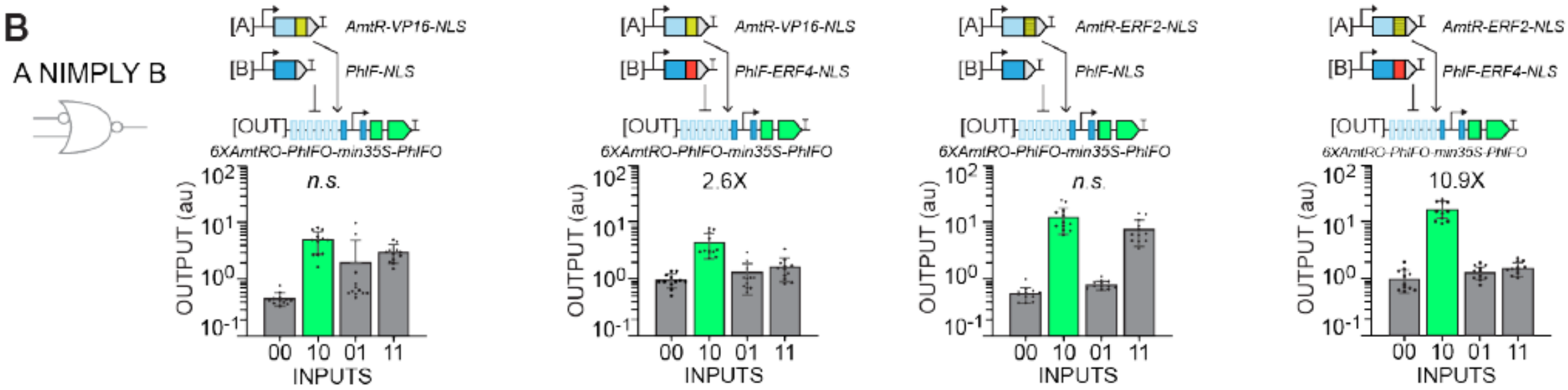
Tuning logic behaviour in N. benthamiana



- OR gate variants. The number and location of AmtR and PhlF operators were varied in the output promoter.

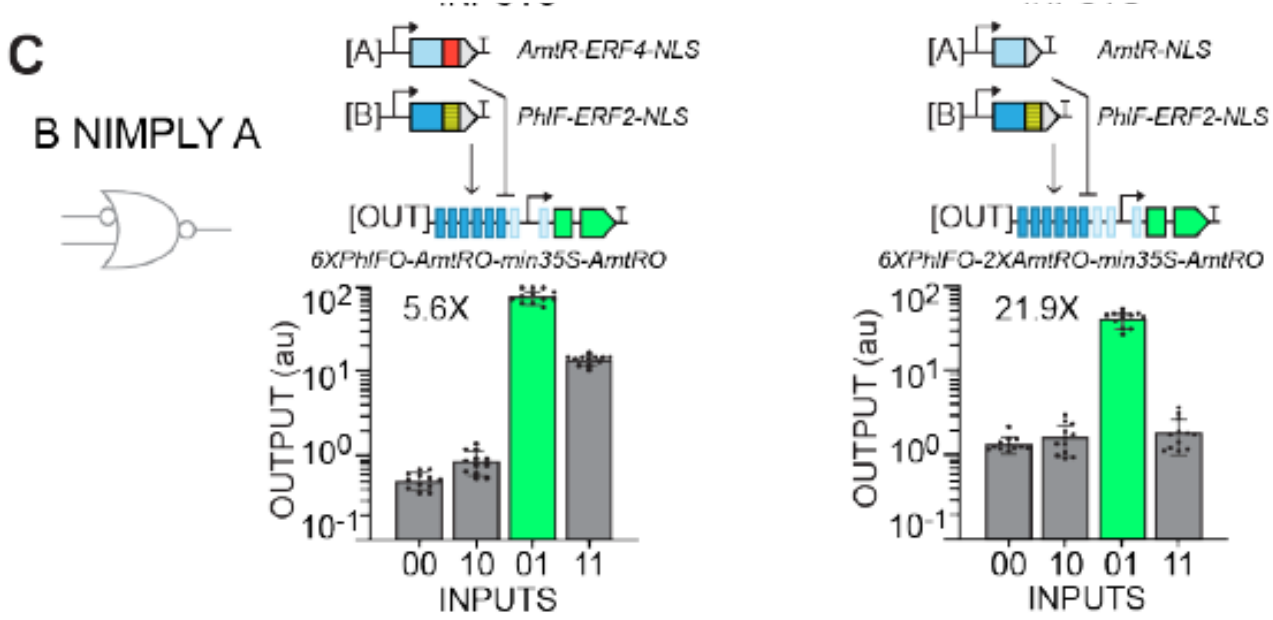


Tuning logic behaviour in N. benthamiana



- A NIMPLY B gate variants. Different combinations of AmtR- and PhlF-based synthetic TFs were tested.

Tuning logic behaviour in N. benthamiana



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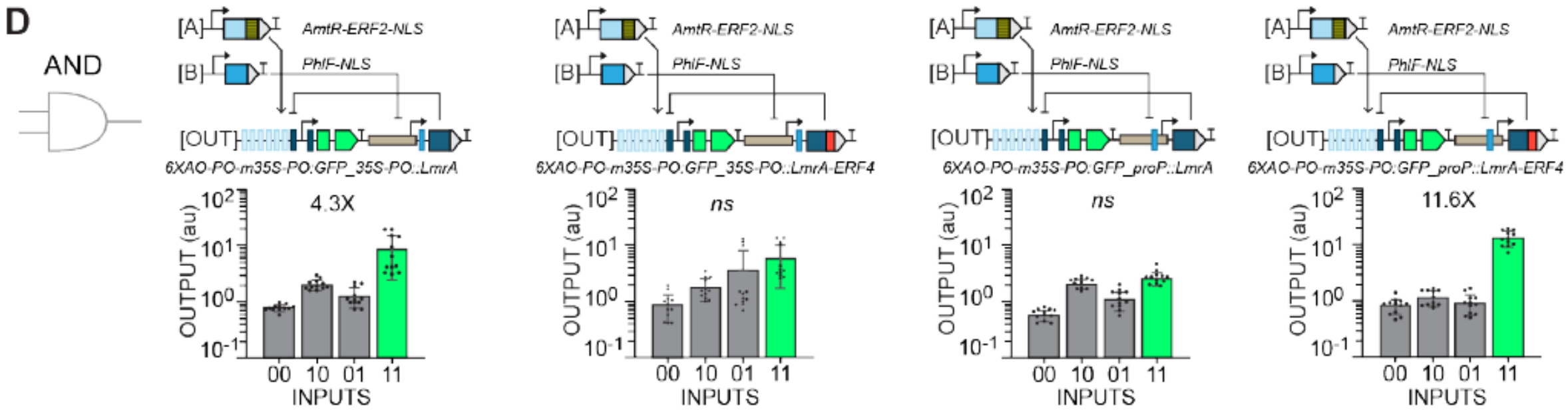

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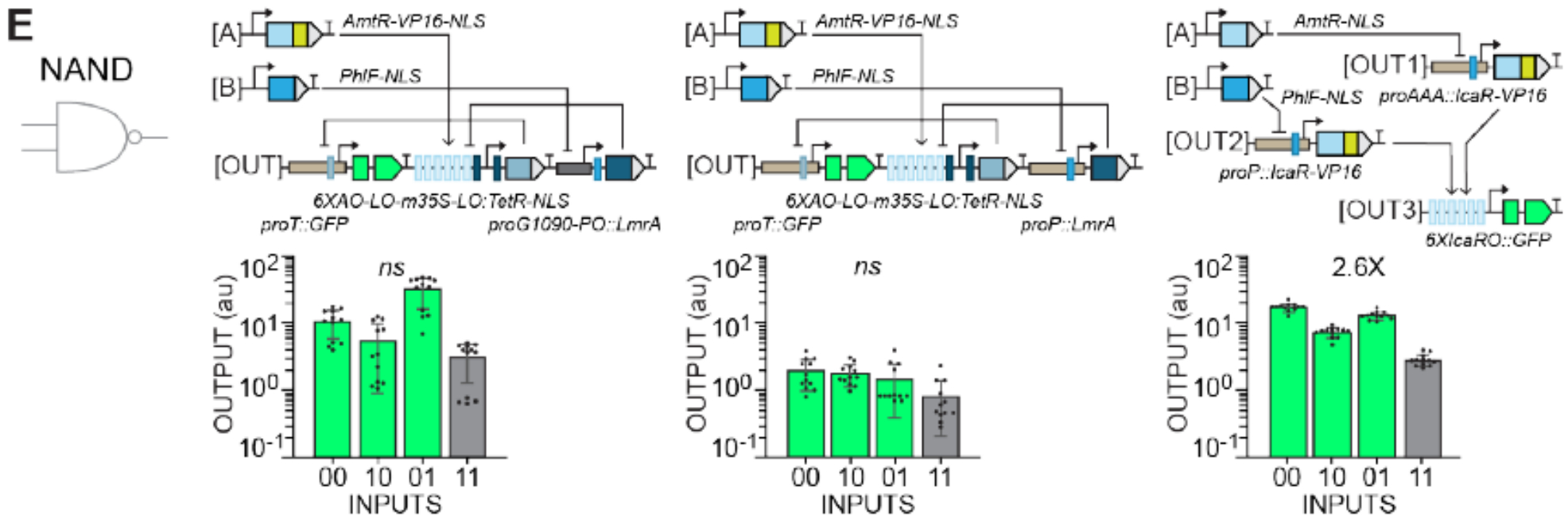
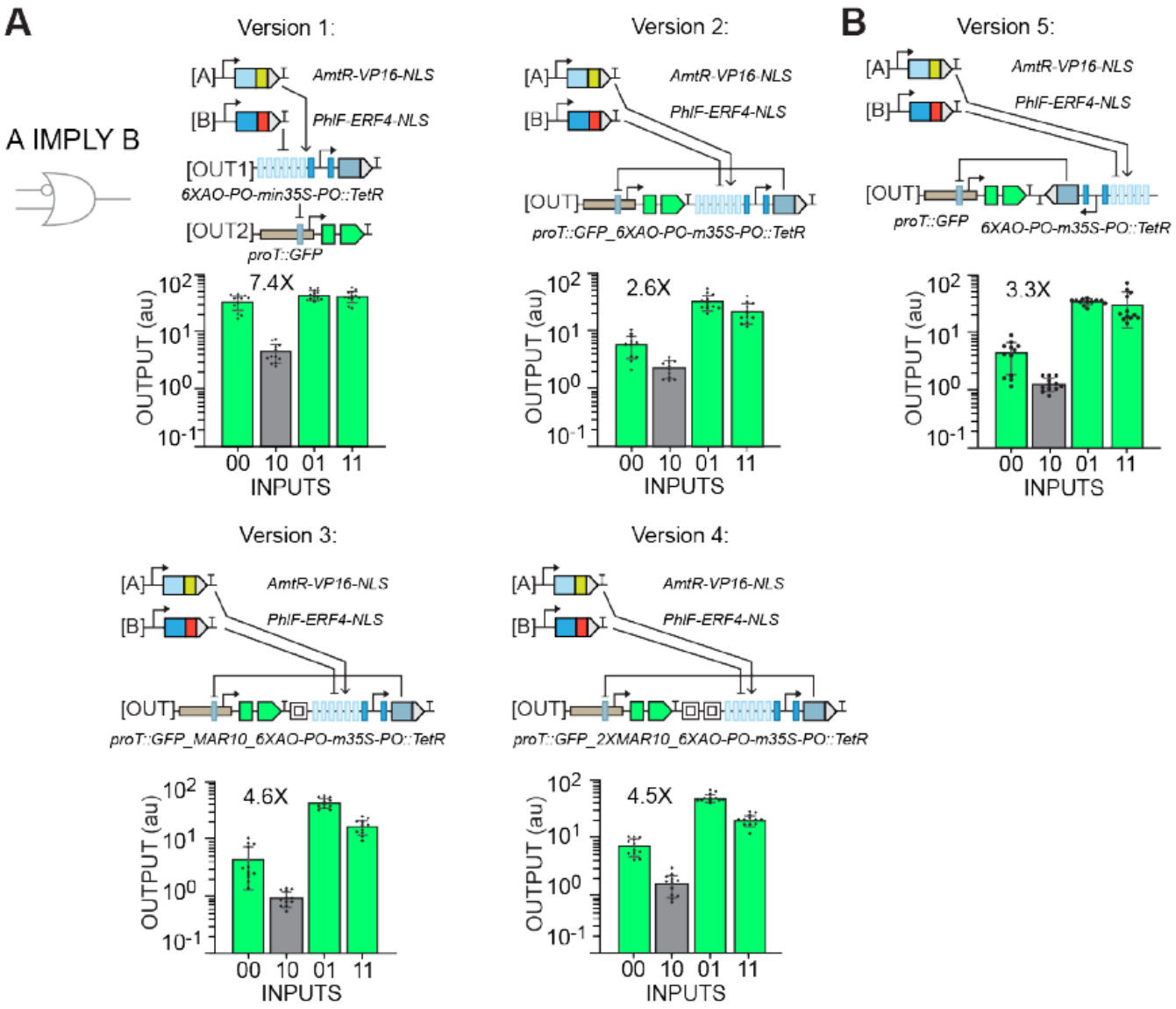
Tuning logic behaviour in N. benthamiana



- A NIMPLY B gate variants. Different combinations of AmtR- and PhlF-based synthetic TFs were tested.

Tuning logic behaviour *N. benthamiana*

- The A IMPLY B gate, which worked well when its output genes were encoded on separate plasmids, had an erroneously reduced “no input” state when both output genes were encoded on the same plasmid
- (A) Arabidopsis MATRIX ATTACHMENT REGION 10 (AtMAR10) was tested as an insulator between expression cassettes on the output promoter

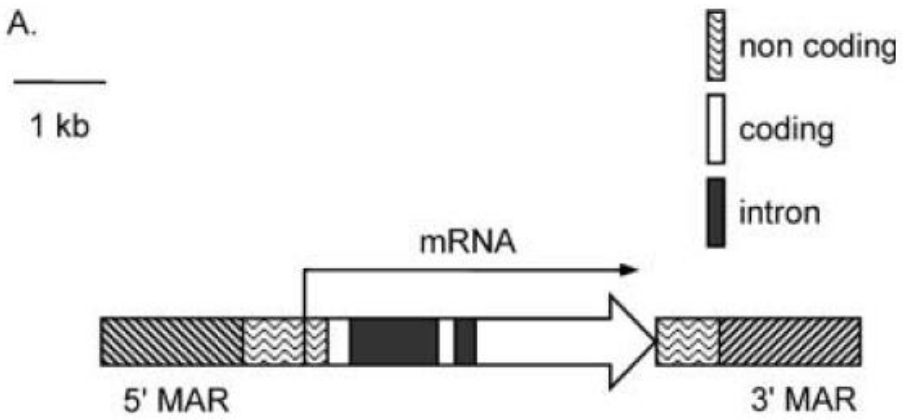


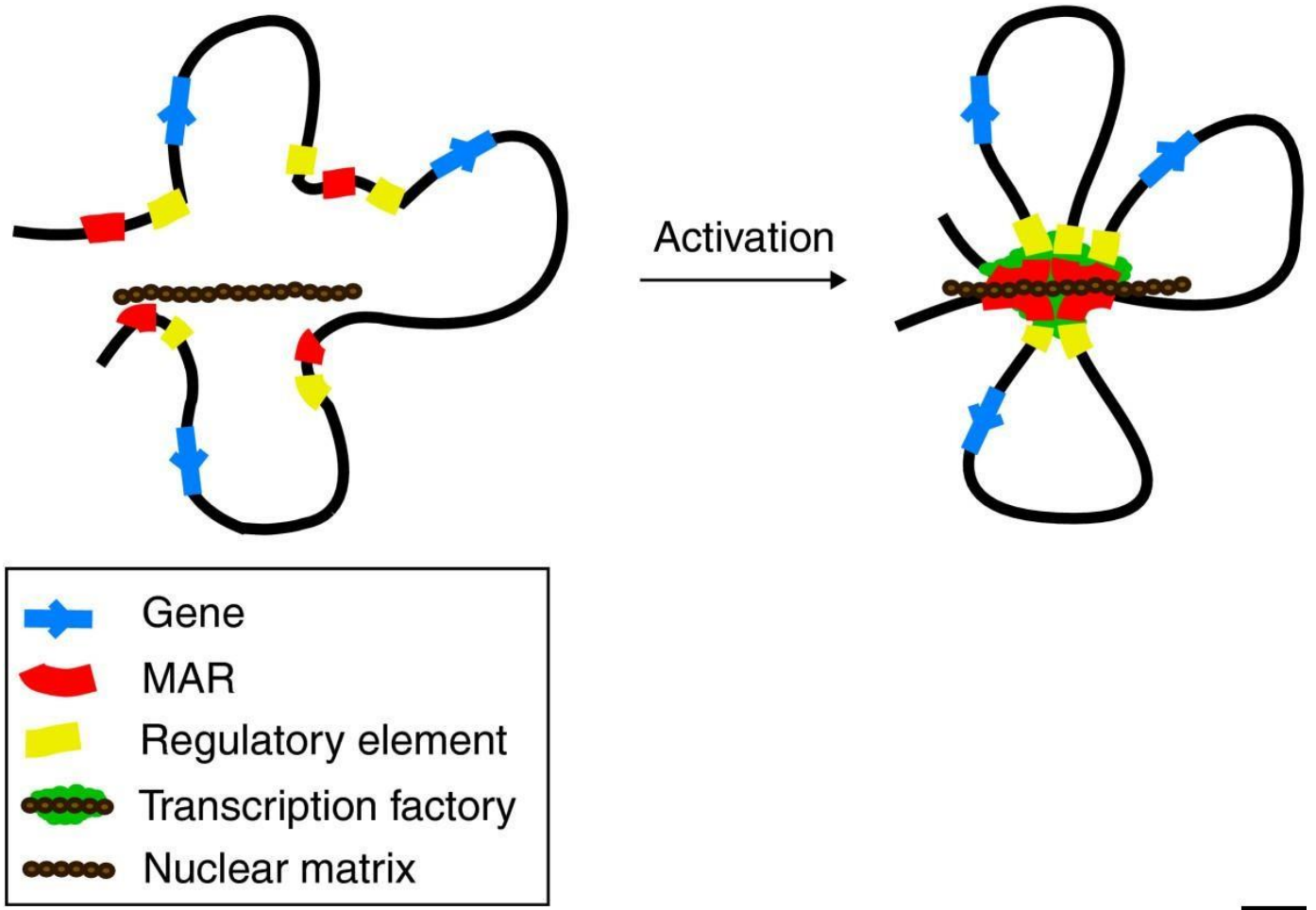
Mol Genet Genomics (2002) 266: 891–898
DOI 10.1007/s00438-001-0613-x

ORIGINAL PAPER

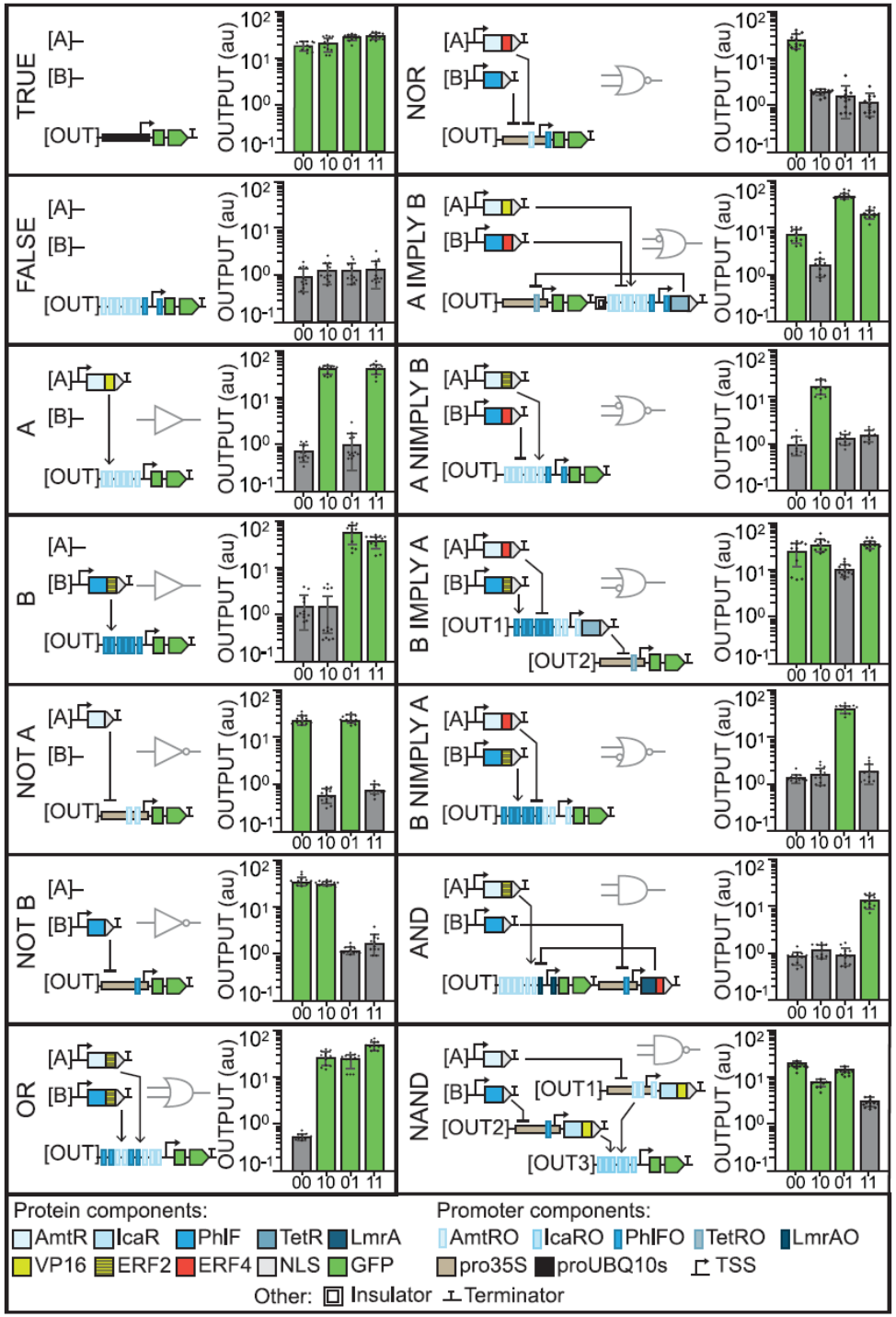
R. Holmes-Davis · L. Comai

The matrix attachment regions (MARs) associated with the *Heat Shock Cognate 80* gene (*HSC80*) of tomato represent specific regulatory elements



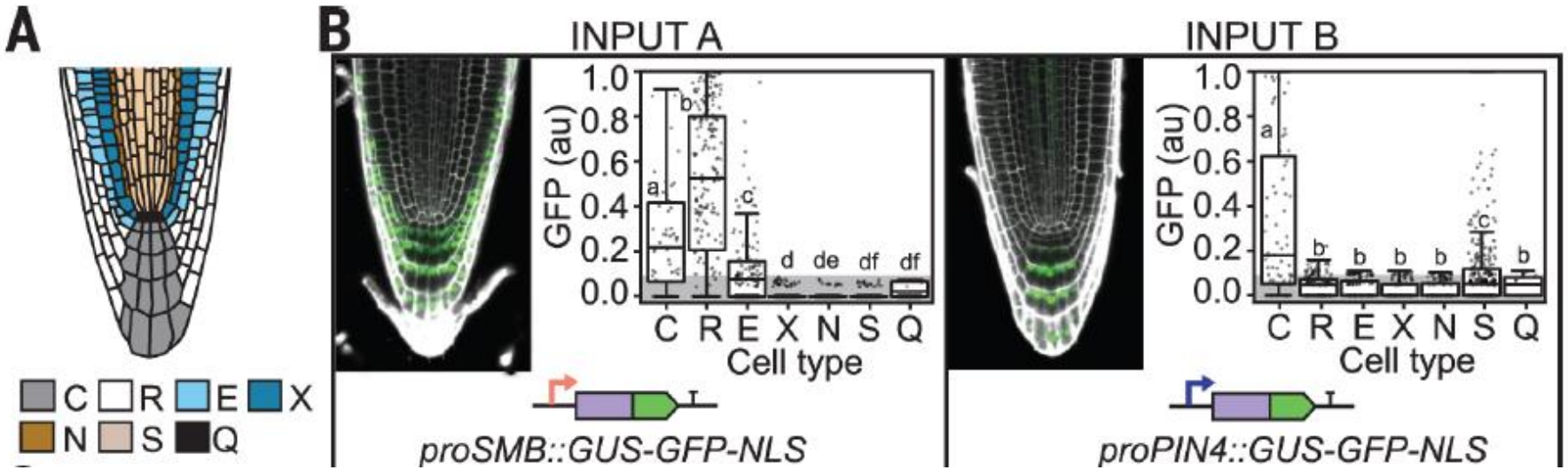


- Gate behavior in N. benthamiana. Bar charts show output of each circuit, reported as the ratio of GFP to mCherry. Green bars indicate gate states that should be ON; gray bars indicate states that should be OFF to implement correct logic.
- Data are mean and SD of 12 leaf punches collected from three leaves infiltrated and measured on different days. Dots show individual data points.
- ERF4RD, to prevent synthetic activators from initiating transcription at composite promoters (1-1 state, A NIMPLY B)
- the other IMPLY gate (B IMPLY A) was built with two output plasmids that were co-delivered to plant cells



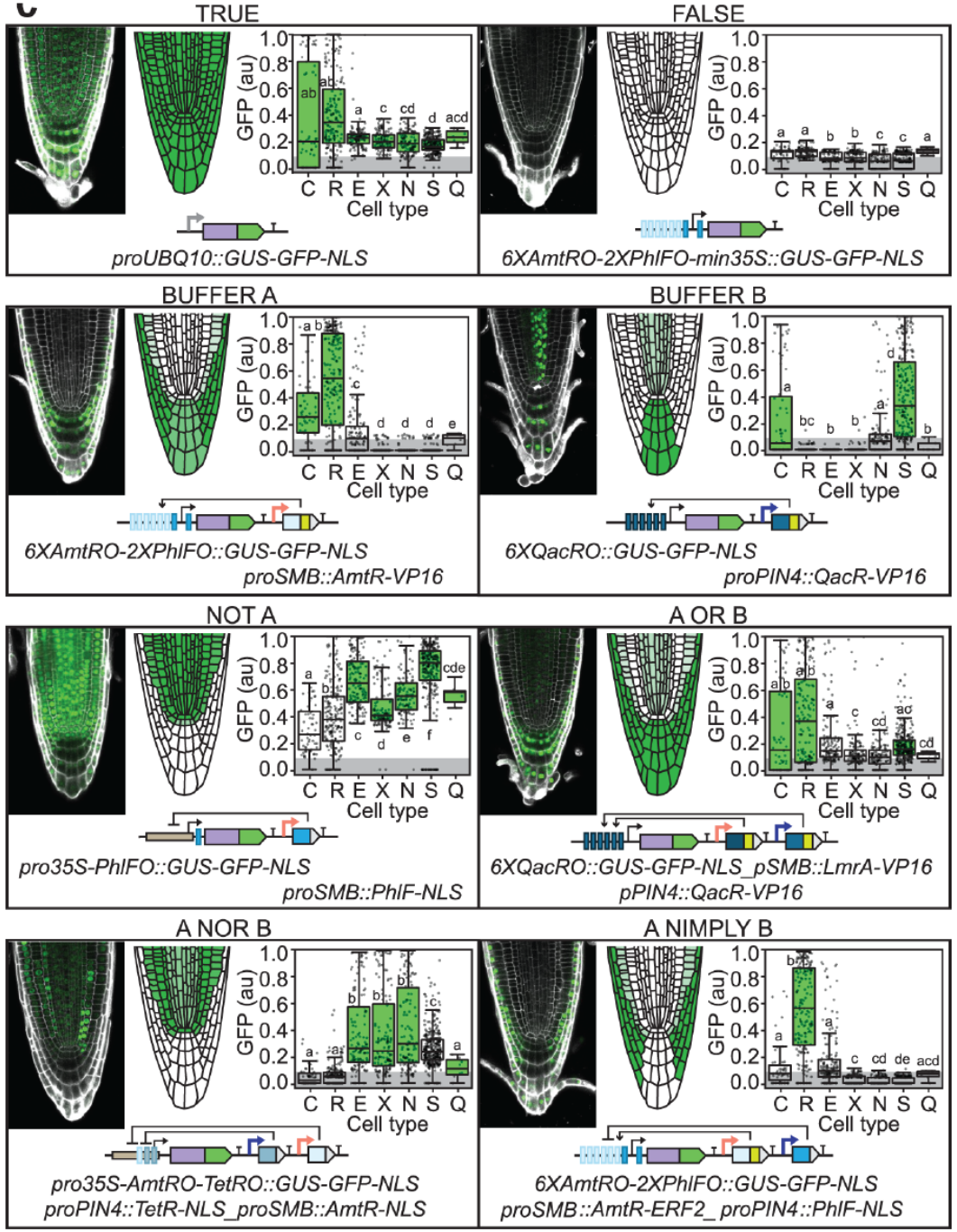
input promoters + logic gates

- Functional plant circuits were transferred to themodel plant A. thaliana to test their capacity to generate specific spatial patterns of gene expression across root tissues. The tissue-specific promoters of SOMBRERO (proSMB, expressed in the entire root cap) and PIN-FORMED4 (proPIN4, expressed in columella, root cap,and stele) were used to drive expression of ourinput TFs



Patterning gene expression using logic gates.

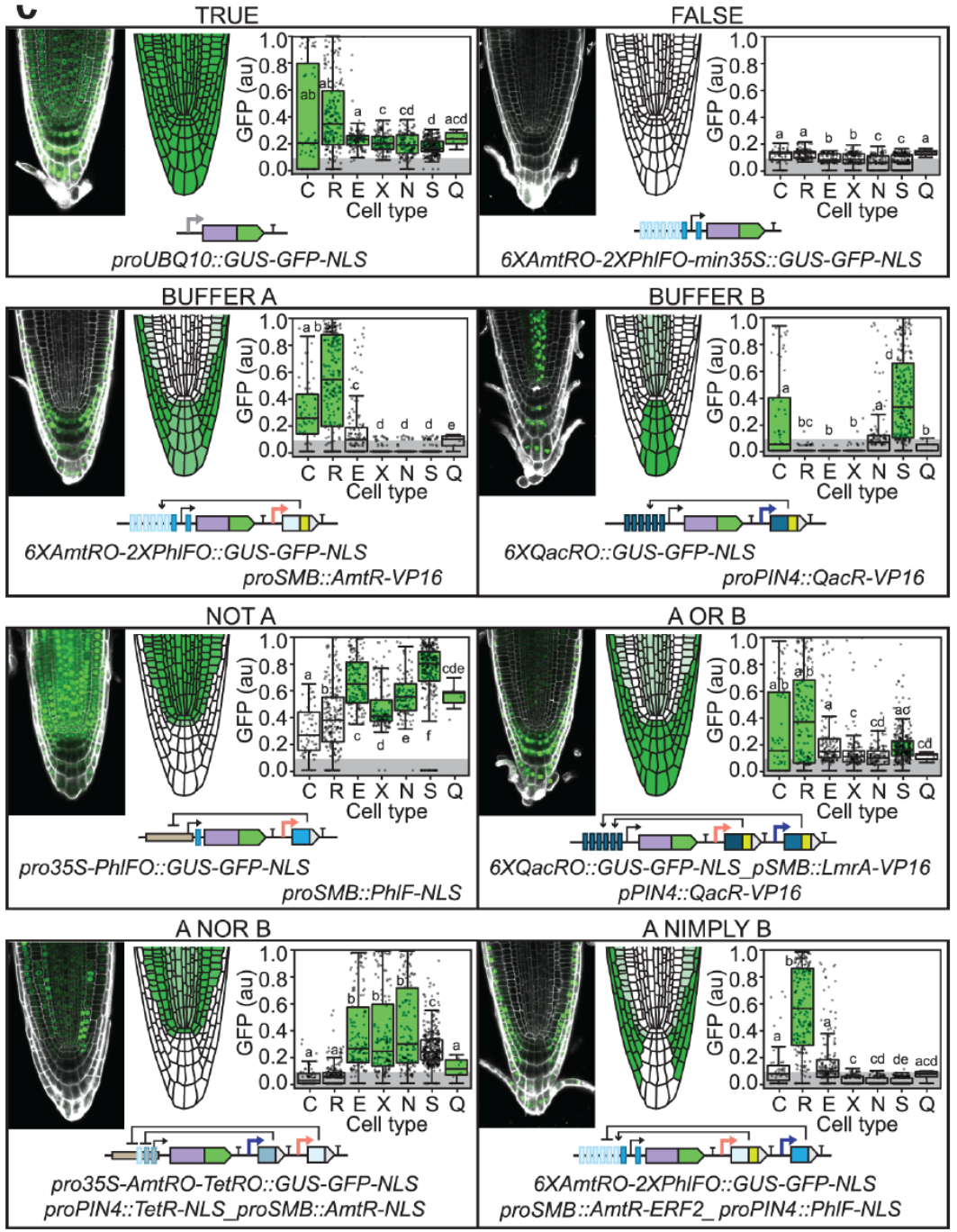
- Patterning gene expression using logic gates.
- Output of each circuit is nuclear localized GUS-GFP fusion protein. In all panels, T-DNA schematics show the identity and arrangement of circuit components. Root images were taken 5 days after sowing. Box-and-whisker plots show quantified reporter expression for three T2 plants from a single transgenic line. Green boxes indicate cell layers that should be ON and gray bars indicate states that should be OFF to implement correct logic. Expression levels of individual cells in each root layer are shown as black dots. Box plot hinges indicate the first and third quartiles. Letters denote significant differences in expression ($P < 0.01$, Student's two-tailed t test). Additional independent lines in fig S6.



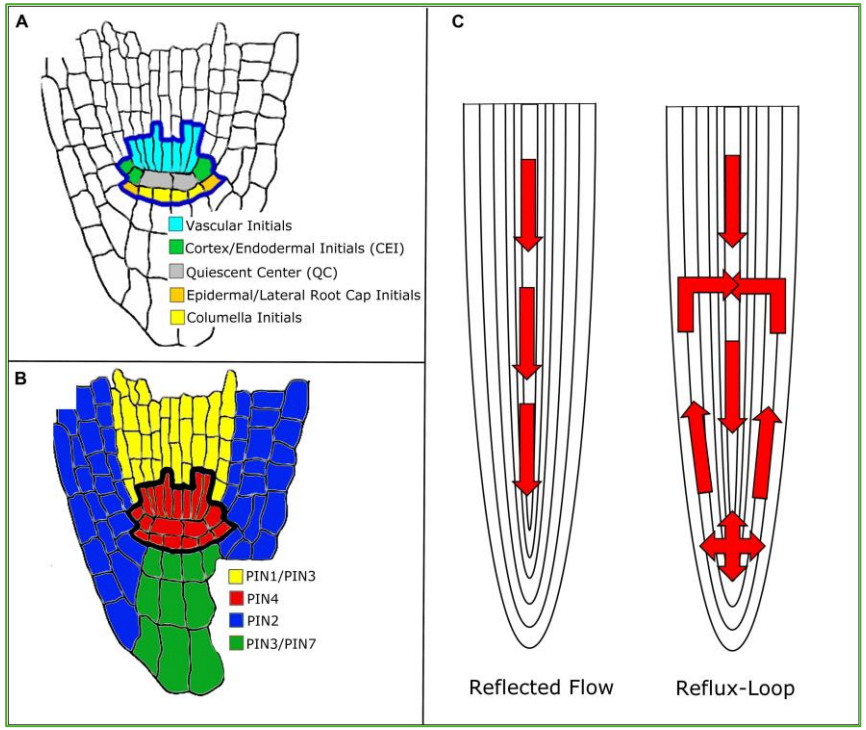
Patterning gene expression using logic gates.

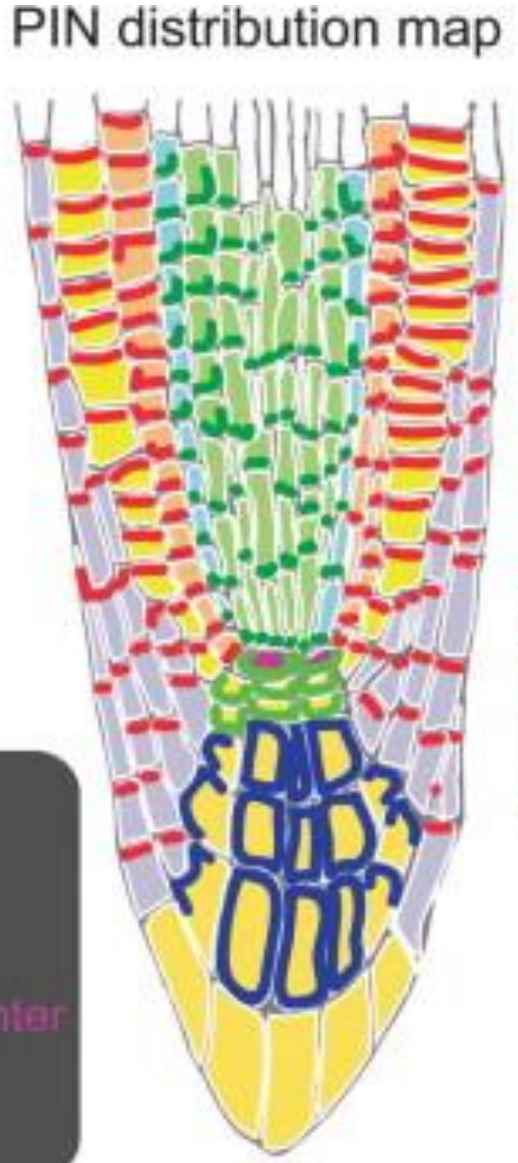
Successful gates qualitatively matched the expected expression patterns and produced a significant difference between GFP expression in tissues expected to be ON versus those expected to be OFF. By this definition of success, TRUE, FALSE, A BUFFER, and NOT

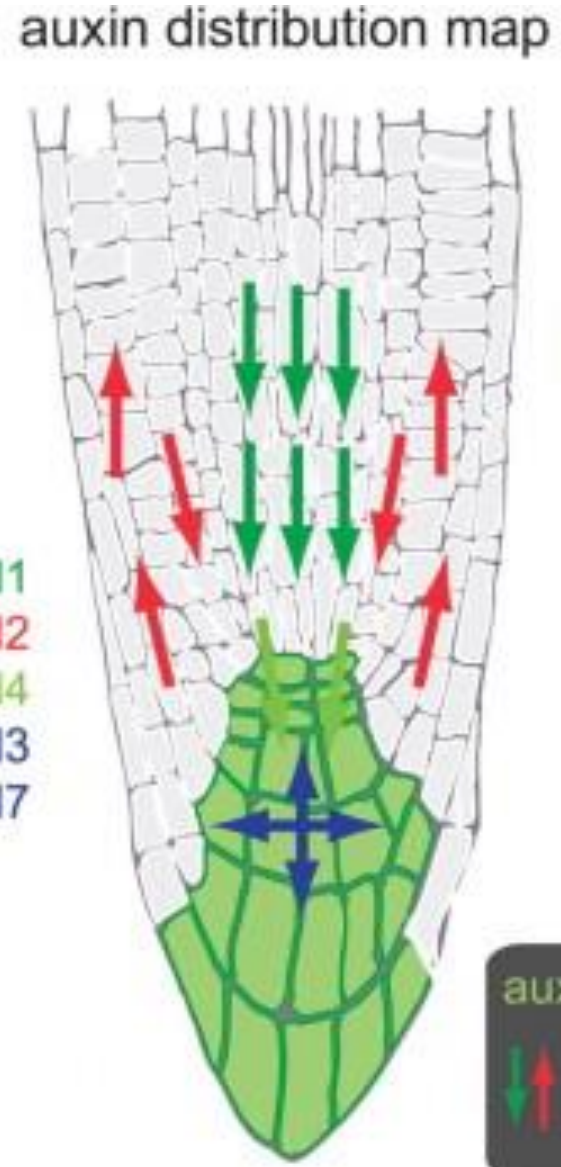
A gates were successful. For NOT A, the difference between the lowest ON (cortex) and highest OFF (root cap) states was only 1.2×; this finding suggests that further optimization may be necessary for applying the circuit in other contexts (Fig. 3C).



PIN and auxins







Epidermis
Cortex
Endodermis
Stele
Quiescent center
Columella
Lateral cap

auxin maxima

auxin flux directions

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SOMBRERO, BEARSKIN1, and BEARSKIN2 Regulate Root Cap Maturation in *Arabidopsis* [C][W]

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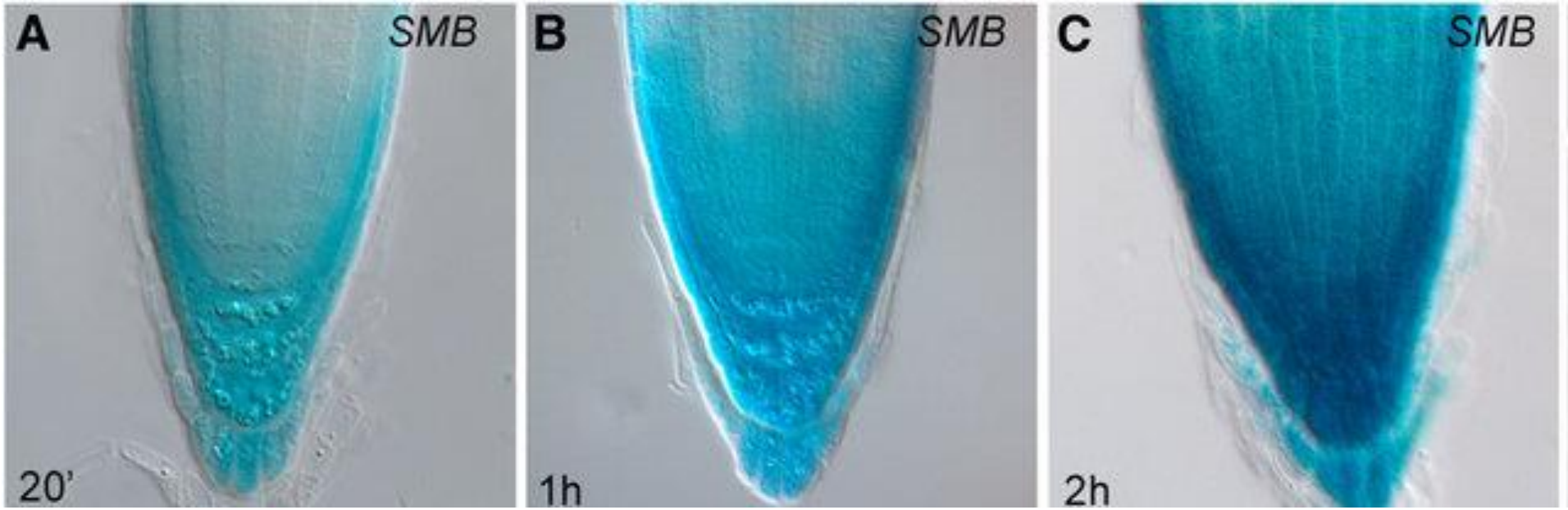
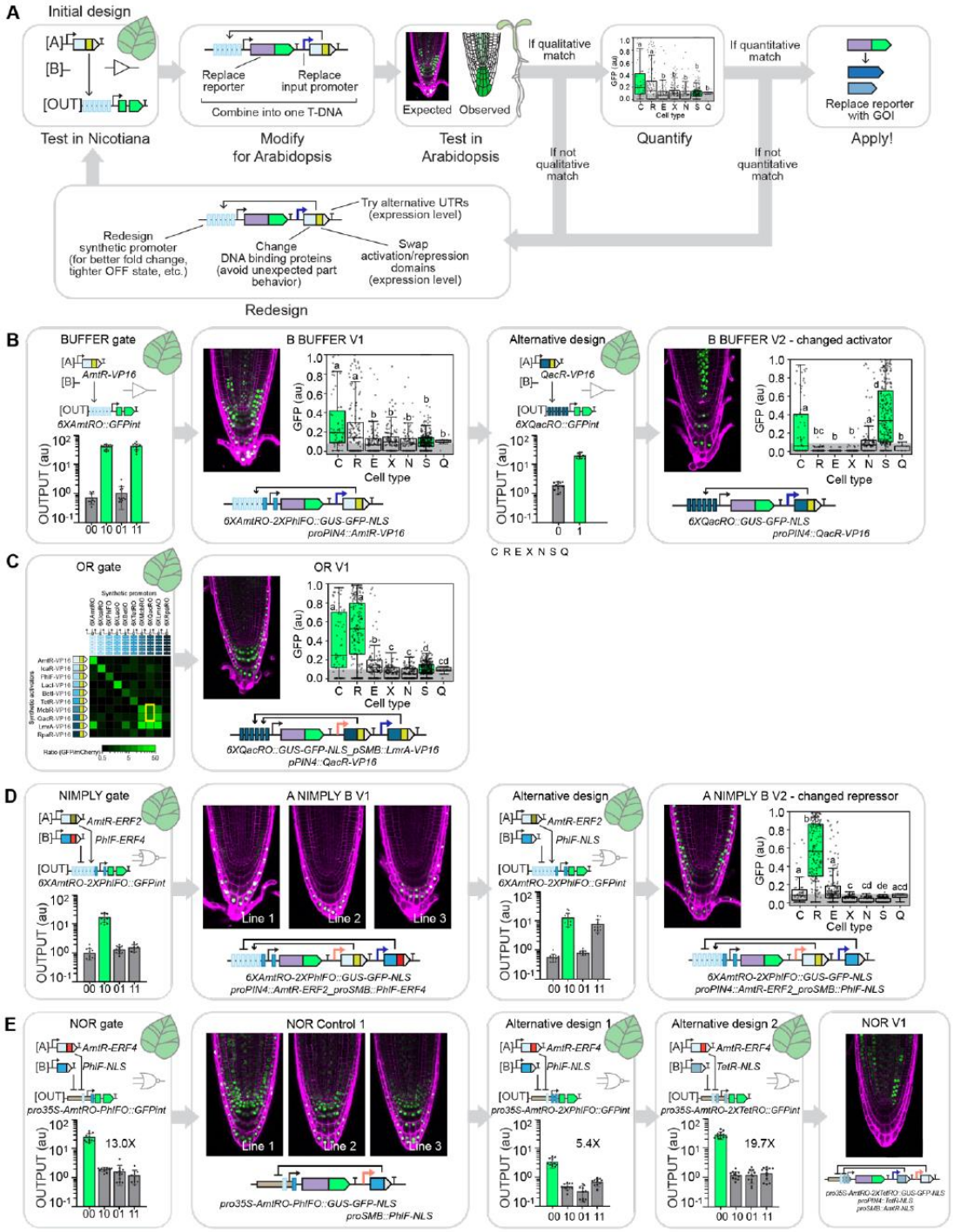
- the cellular maturation of root cap is redundantly regulated by three genes, SOMBRERO (SMB), BEARSKIN1 (BRN1), and BRN2



Tuning

The gates that did not work revealed differences in circuit behavior between *Arabidopsis* and *N. benthamiana*. For example, the first B BUFFER gate produced a spatially expanded expression pattern relative to the input promoter, with aberrant expression in the quiescent center (QC) and neighboring initial cells (figs. S5 and S6). Similarly, the A NIMPLY B pattern was incorrect in *Arabidopsis*; expression was missing in several root cap cell layers (figs. S5 and S6).



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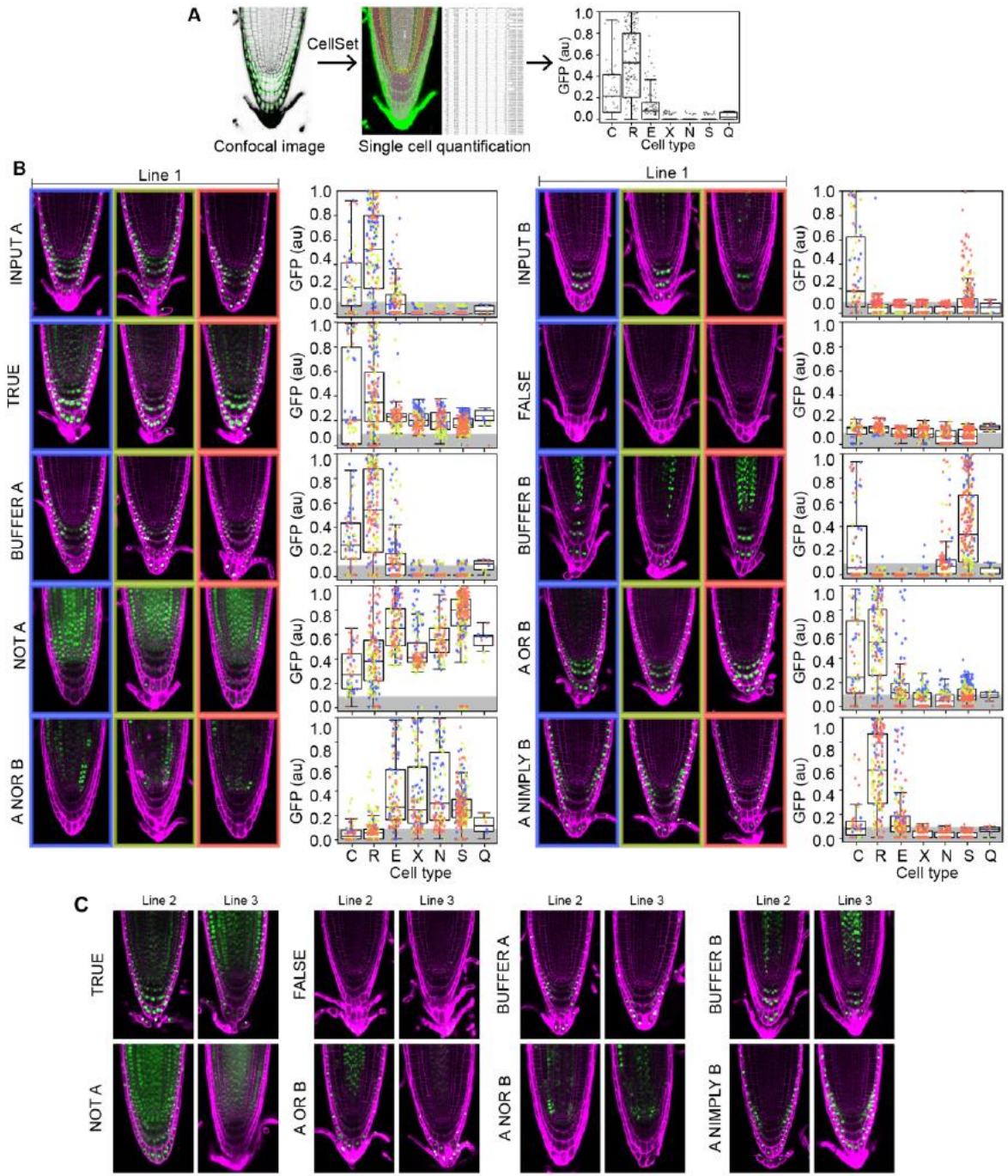
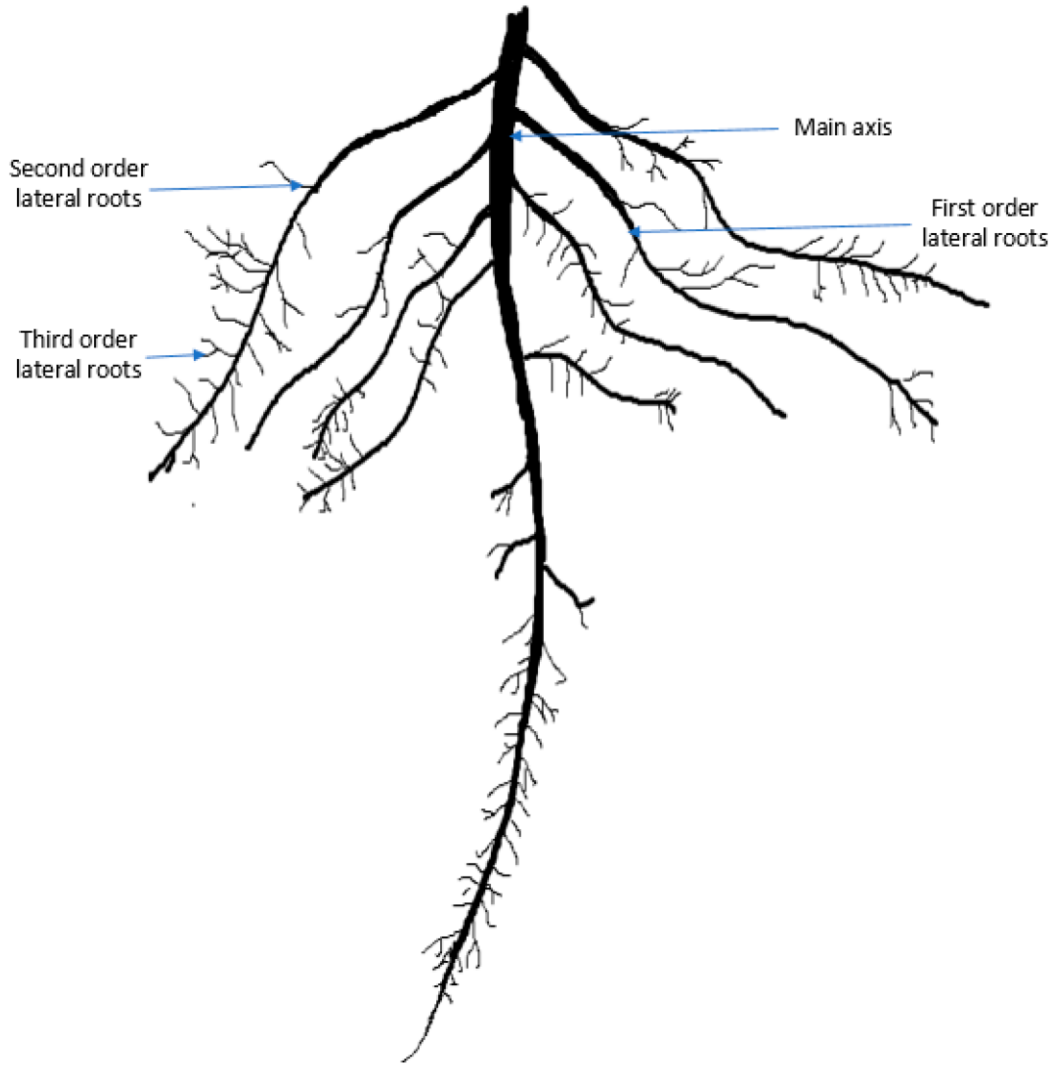


After tuning, all gates qualitatively matched the expected expression patterns (Fig. 3C). However, three had expression in a single cell layer that was either aberrantly high (B BUFFER, endodermis) or low (OR, stele; NOR, QC). Thus, quantitative analysis highlights the challenge of implementing circuits across cell types in heterogeneous tissue and additional optimization would be required for these gates to achieve significant differences across every tissue layer.

Root architecture to demonstrate how precise spatial control over gene expression may be used to engineer development

Lateral roots allow plants to radially sample soil, and the number of lateral roots that a plant generates affects its ability to search for water and essential nutrients in the environment



Root ideatypes

The close relationship between root growth and plant fitness has led to proposals of ideal root architectures for plant growth in specific environments

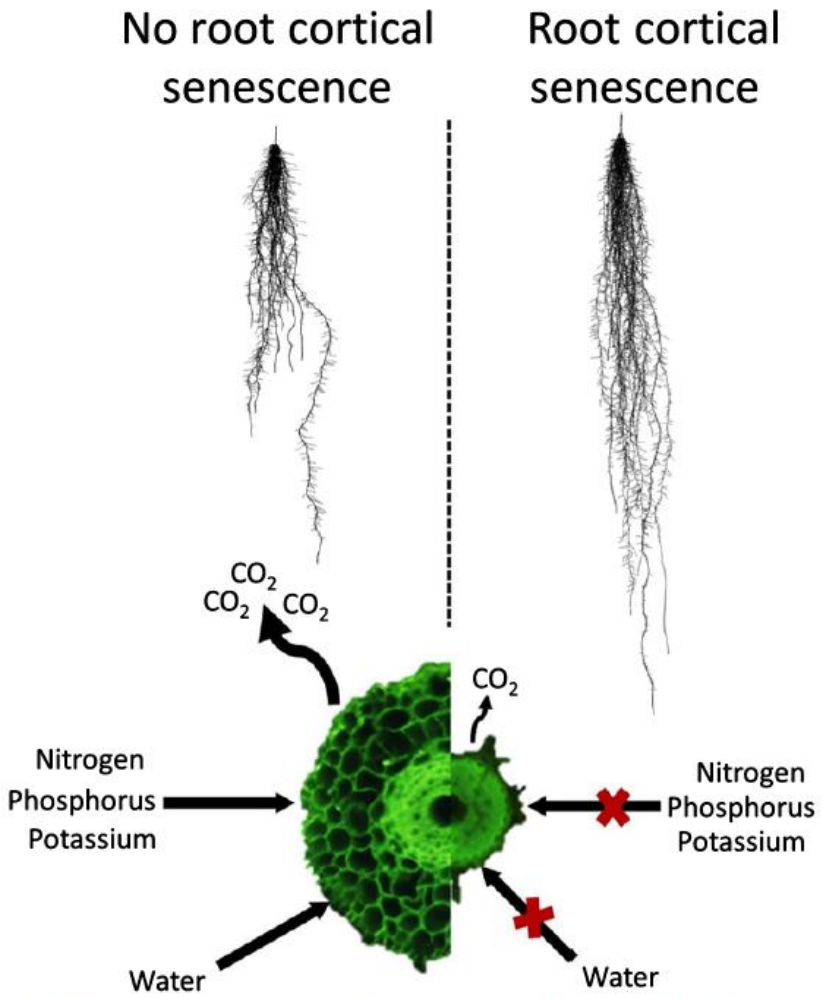


Fig. 6 Root cortical senescence (RCS) improves nutrient capture. The root cross-section image shows a barley (*Hordeum vulgare*) root with intact root cortex (left side of image) or lacking a cortex because of RCS (right side of image). RCS reduces the nutrient and respiratory costs of maintaining root tissue, permitting greater root growth, soil exploration, and nutrient capture from soils with suboptimal nutrient availability, as shown by the top images of barley root phenotypes as simulated in SimRoot (Schneider *et al.*, 2017). Reduction of radial water and nutrient transport in axial root tissue with RCS has small effects on total plant nutrient acquisition, since lateral roots, which acquire the majority of nutrients and water, do not form RCS. From Schneider & Lynch (2018).

Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture

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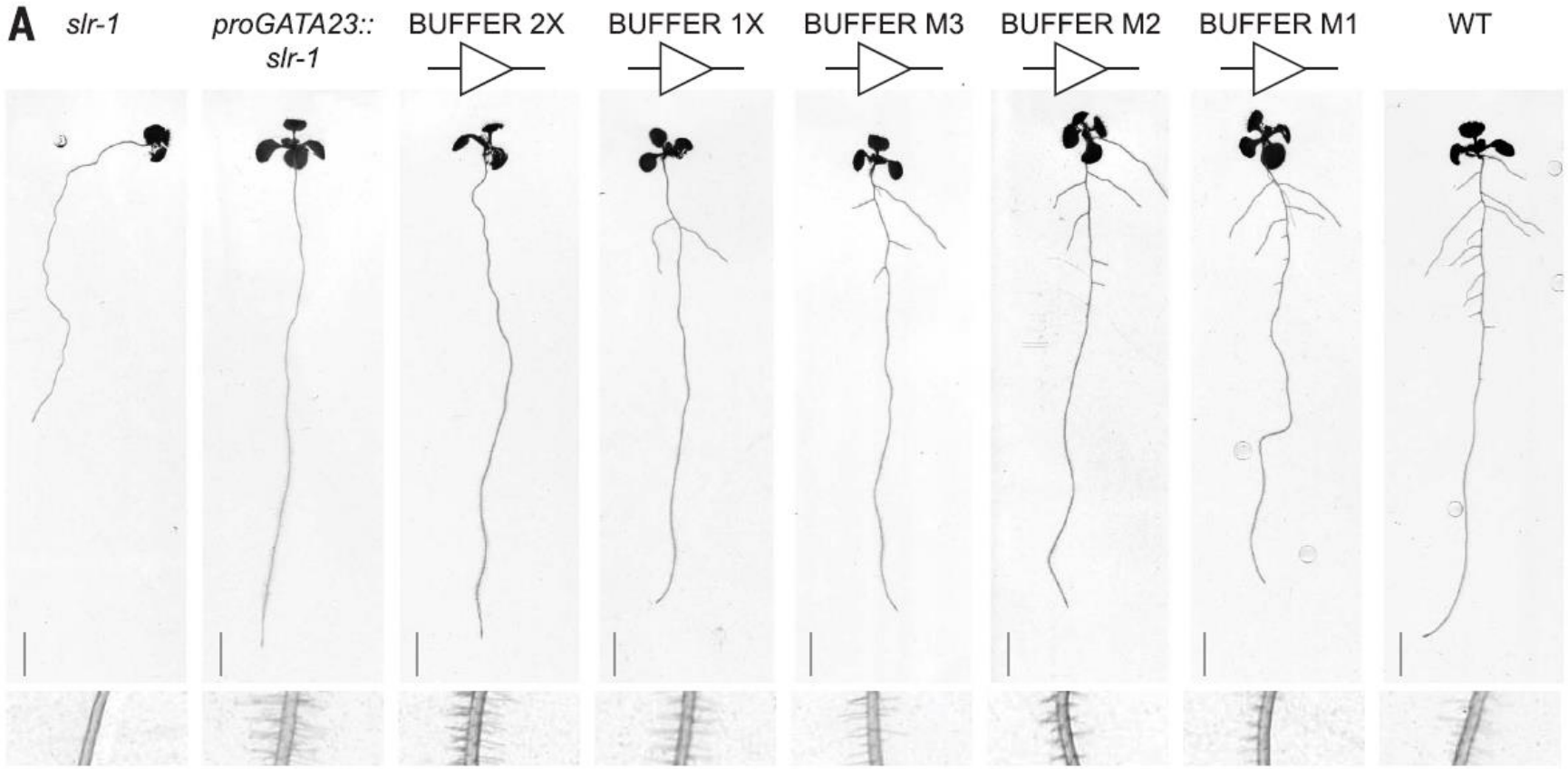
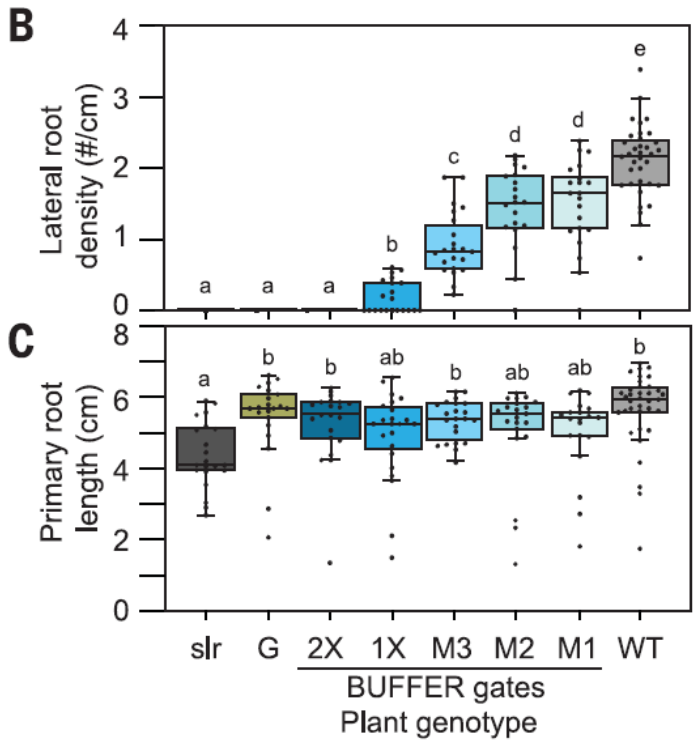
Root architecture to demonstrate how precise spatial control over gene expression may be used to engineer development





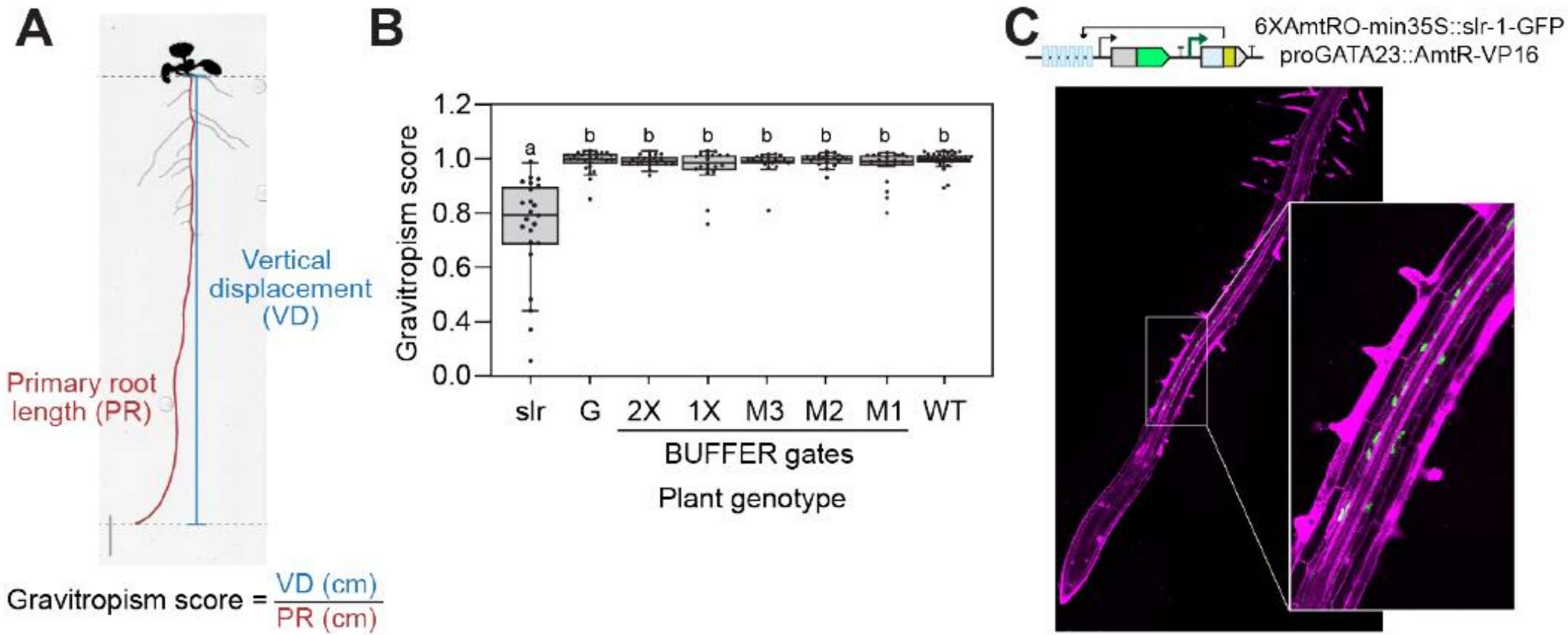
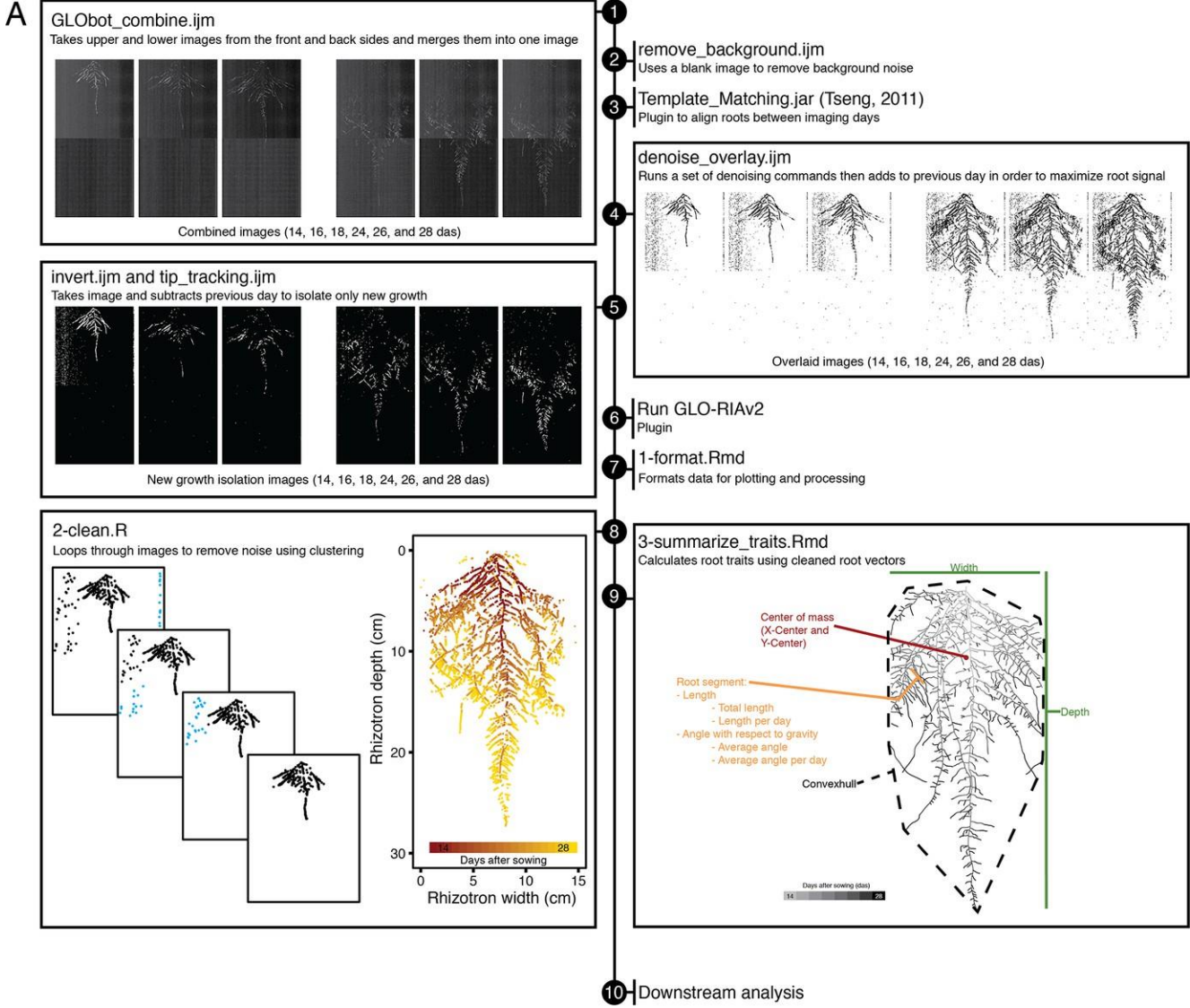
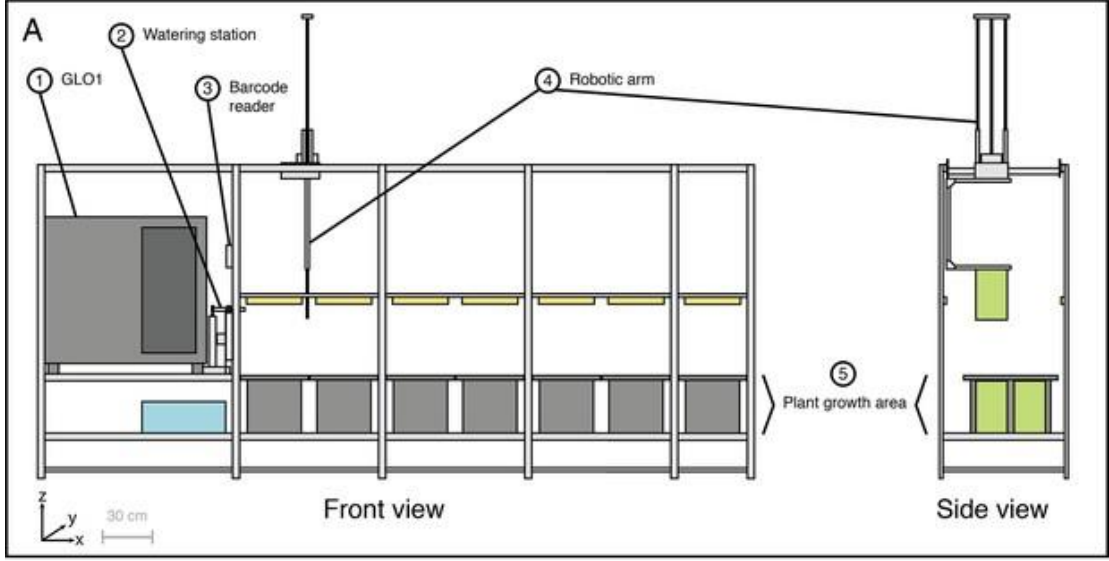


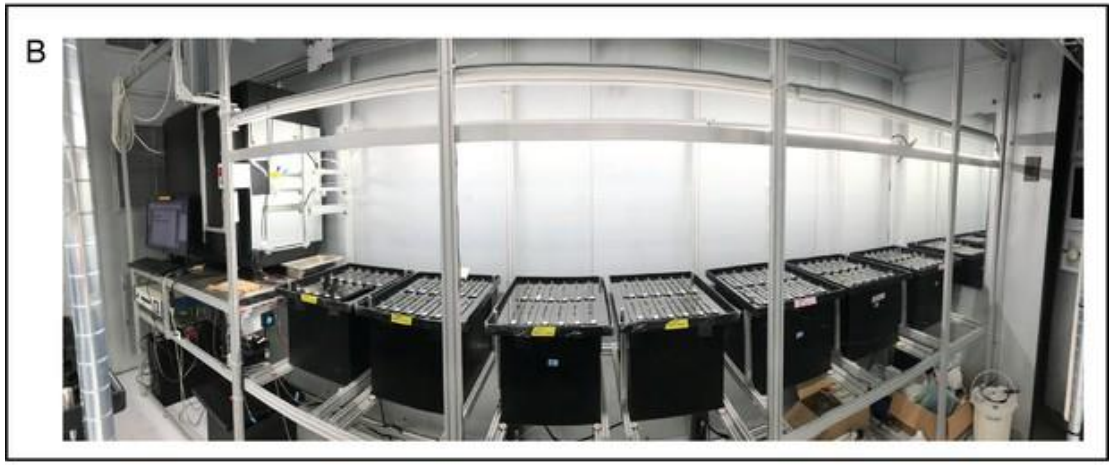
Fig. S8. Root gravitropism and *slr-1* expression in engineered plants.

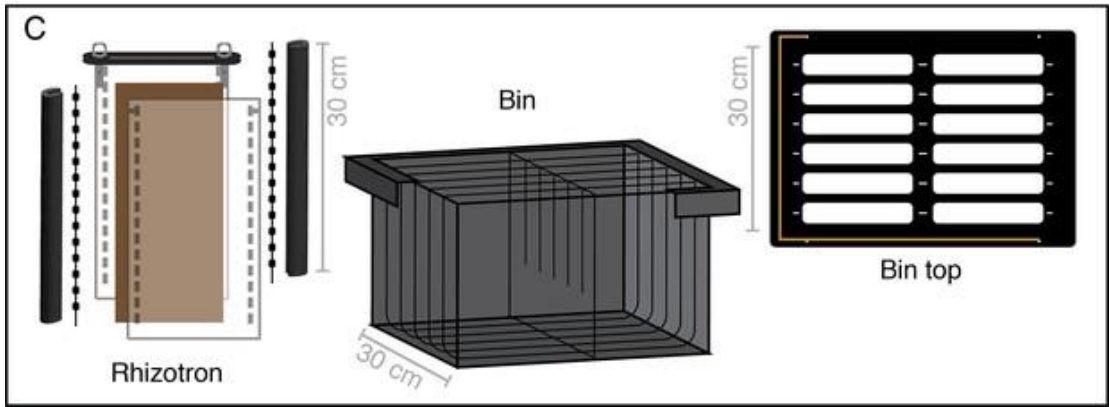
Robotics-assisted phenomics platform



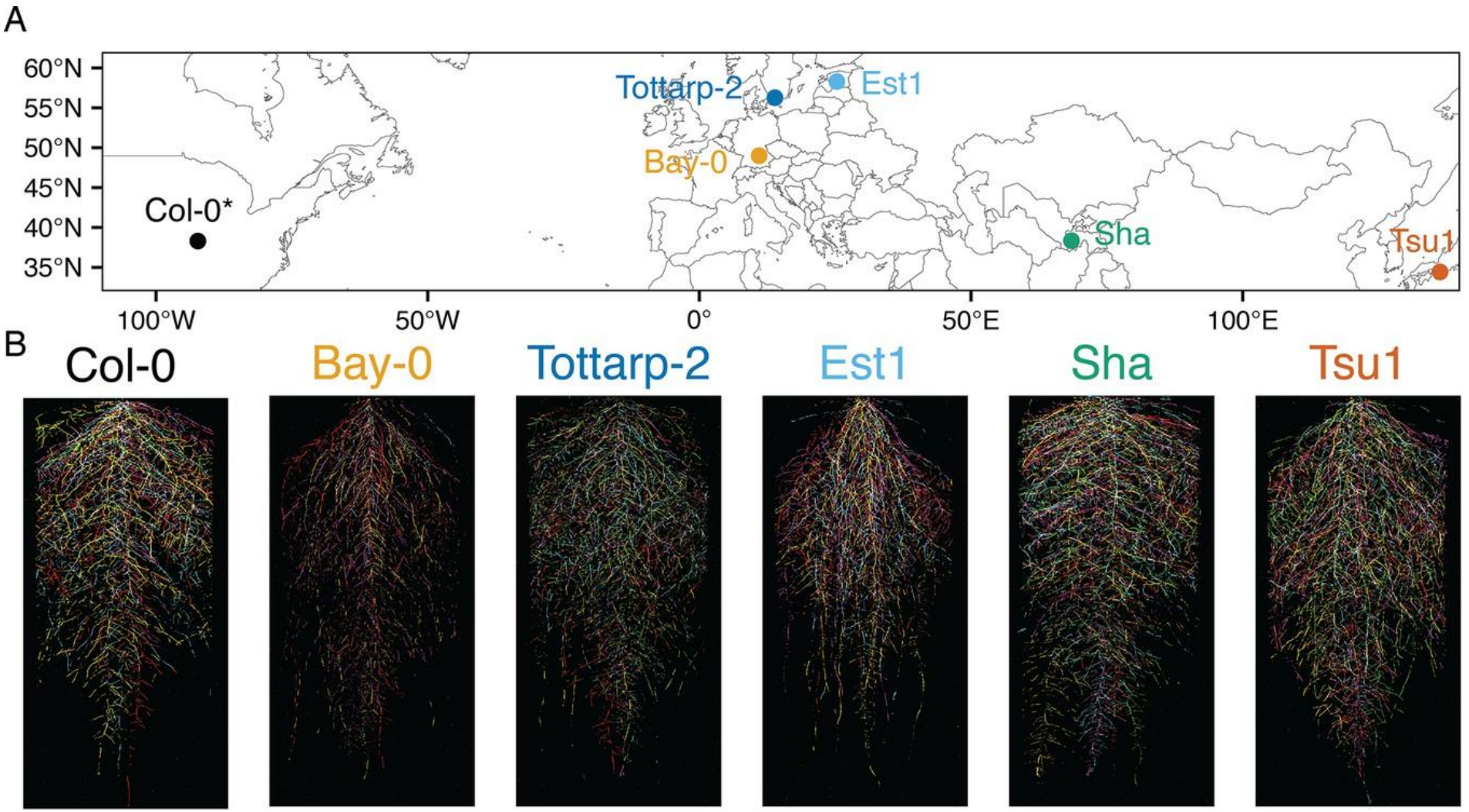
Growth and Luminescence Observatory for Roots (GLO-Roots) automation: GLO-Bot.
(**A**) Schematic of the GLO-Bot Cartesian gantry system includes: (1) The Growth and Luminescence Observatory 1 (GLO1) imaging system published in Rellán-Álvarez et al., 2015, which houses two cameras and a rotating stage for root imaging, (2) a station for general watering or treatment with diluted luciferin solution prior to imaging, (3) a barcode scanner to identify the rhizotron and load a specific watering and imaging protocol, (4) a robotic arm, which moves in the x-, y-, and z-directions and has a hook at the end to pick up rhizotrons, and (5) an area for plant growth, which can be seen in the photograph of GLO-Bot (**B**). (**C**) Automation updates required modification of the GLO-Roots growth vessel design to include a black acrylic plate and hooks for rhizotron handling as well as dividers within the growth boxes and guides along the bin top, which allow the rhizotron to hang and shield the roots from light. Copper tape along the edge of the bin top enables positioning. Gray-scale bars denote 30 cm.

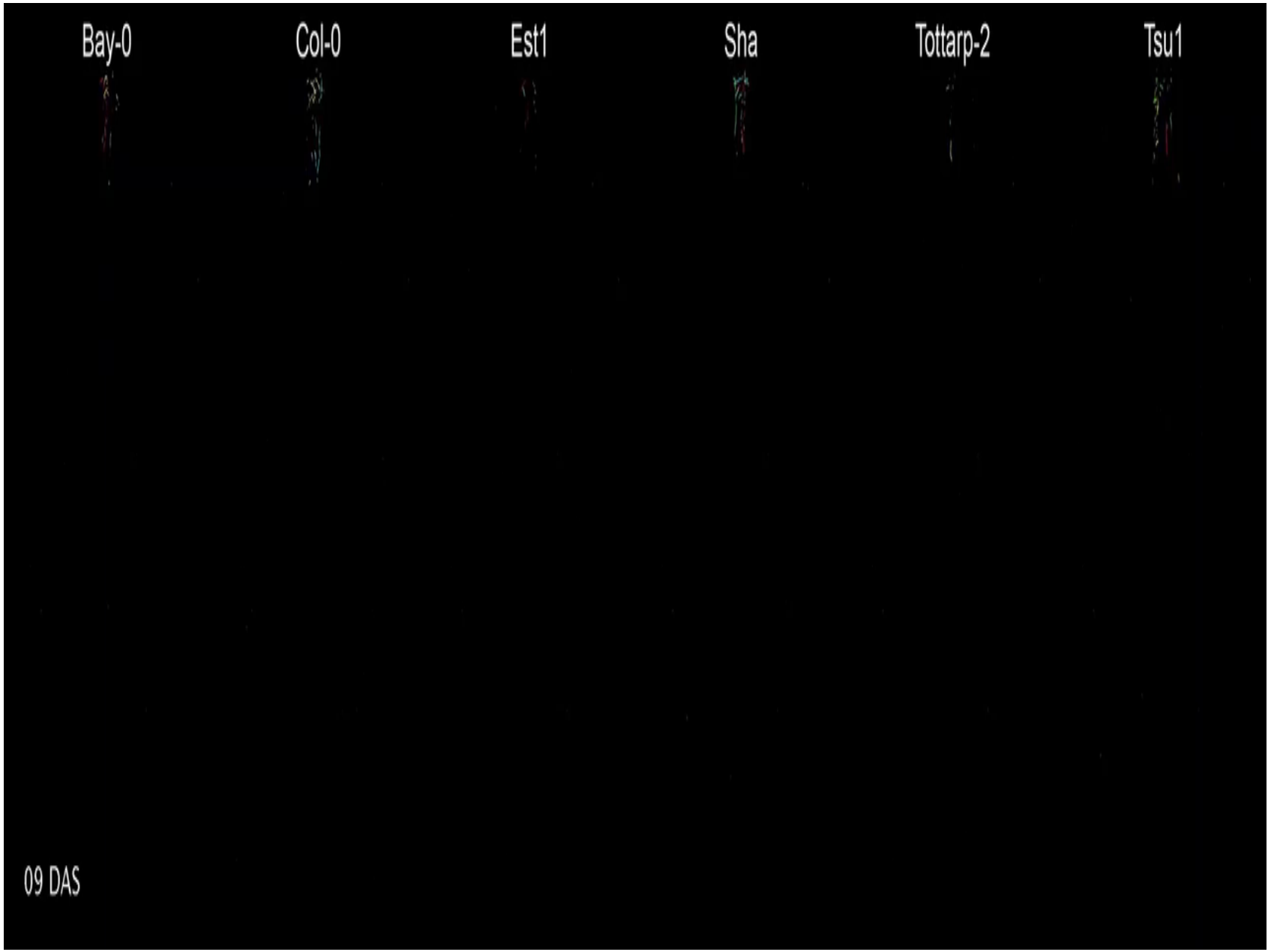






Uncovering natural variation in root system architecture and growth

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