NST PMS 1B

Lecture 2: From genotype to phenotype

Lecture 1: Described method for inserting DNA fragment into plant genomes

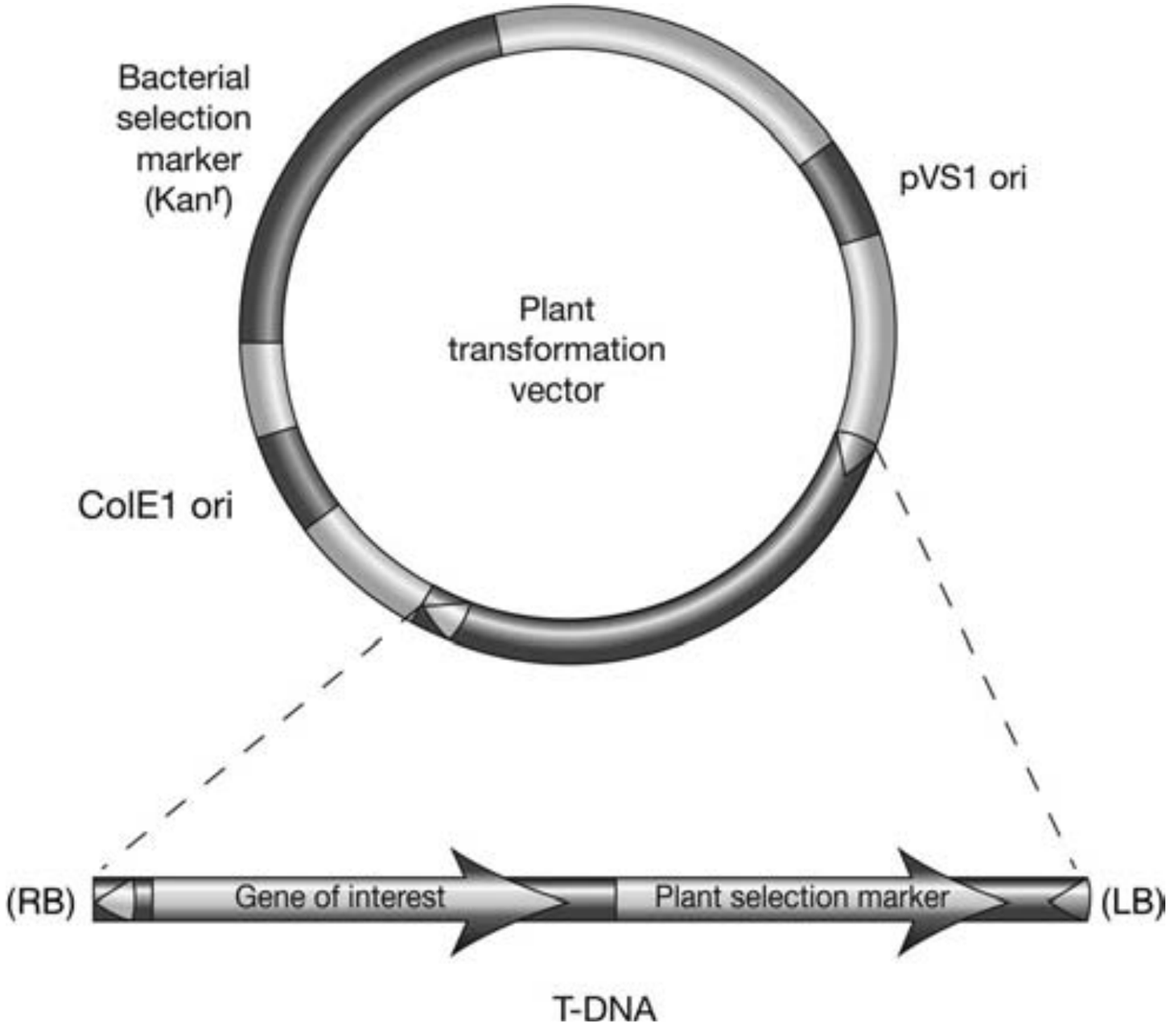


Figure 7.8. A generic plant binary vector with two origins of replication, the pVS1 ori for propagation in Agrobacterium and the ColE1 ori for propagation in *Escherichia coli*. The backbone of the vector contains an antibiotic resistance gene for bacterial selection (kanamycin resistance), and the T-DNA contains a plant selectable marker and the gene of interest (GOI).

Lecture 2: How do you manoeuvre between plant genotype and phenotype?

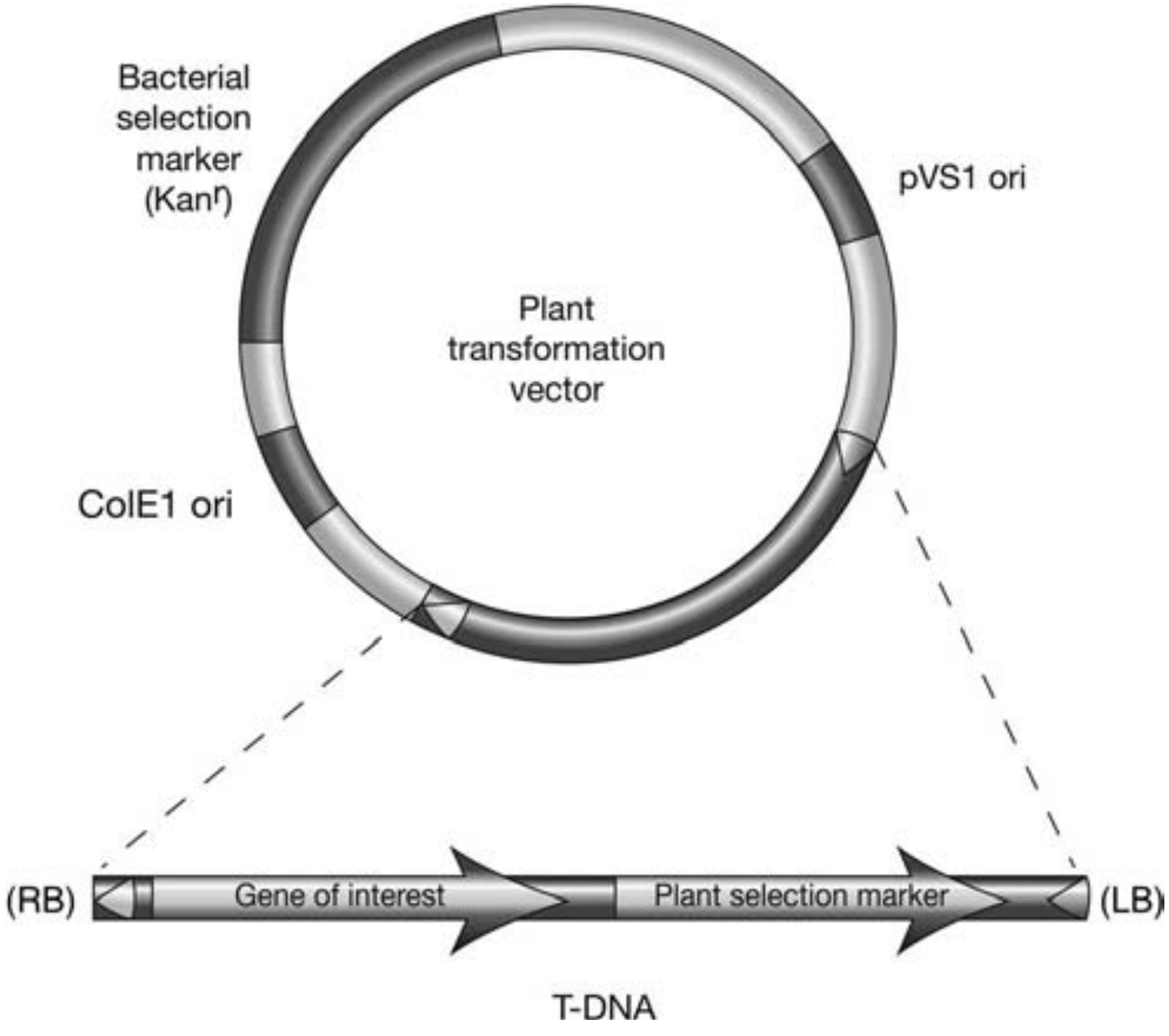
(i) Gene design

(ii) Single gene traits

(iii) Reporter genes

…from DNA to visualising the plant

How do you build a synthetic gene?



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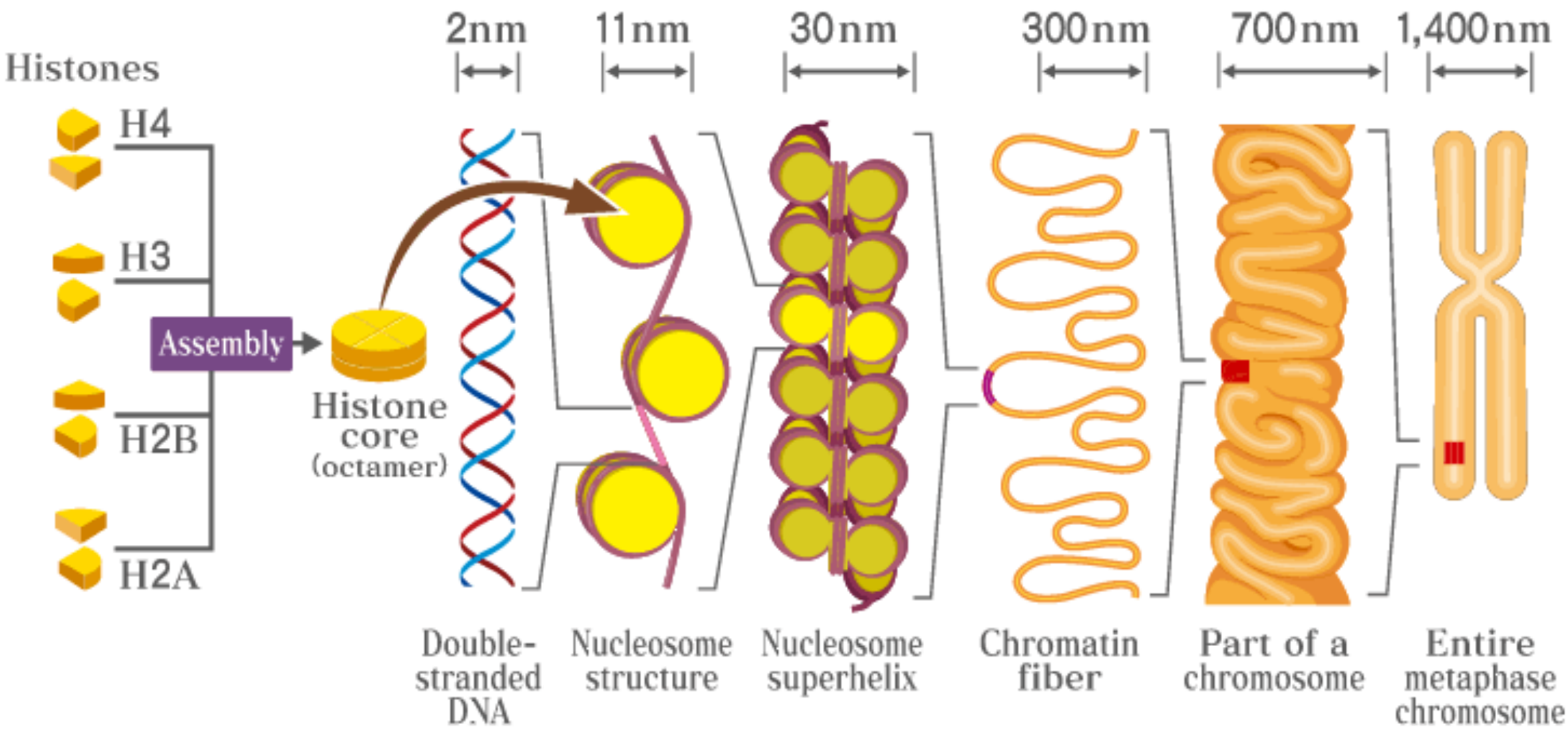
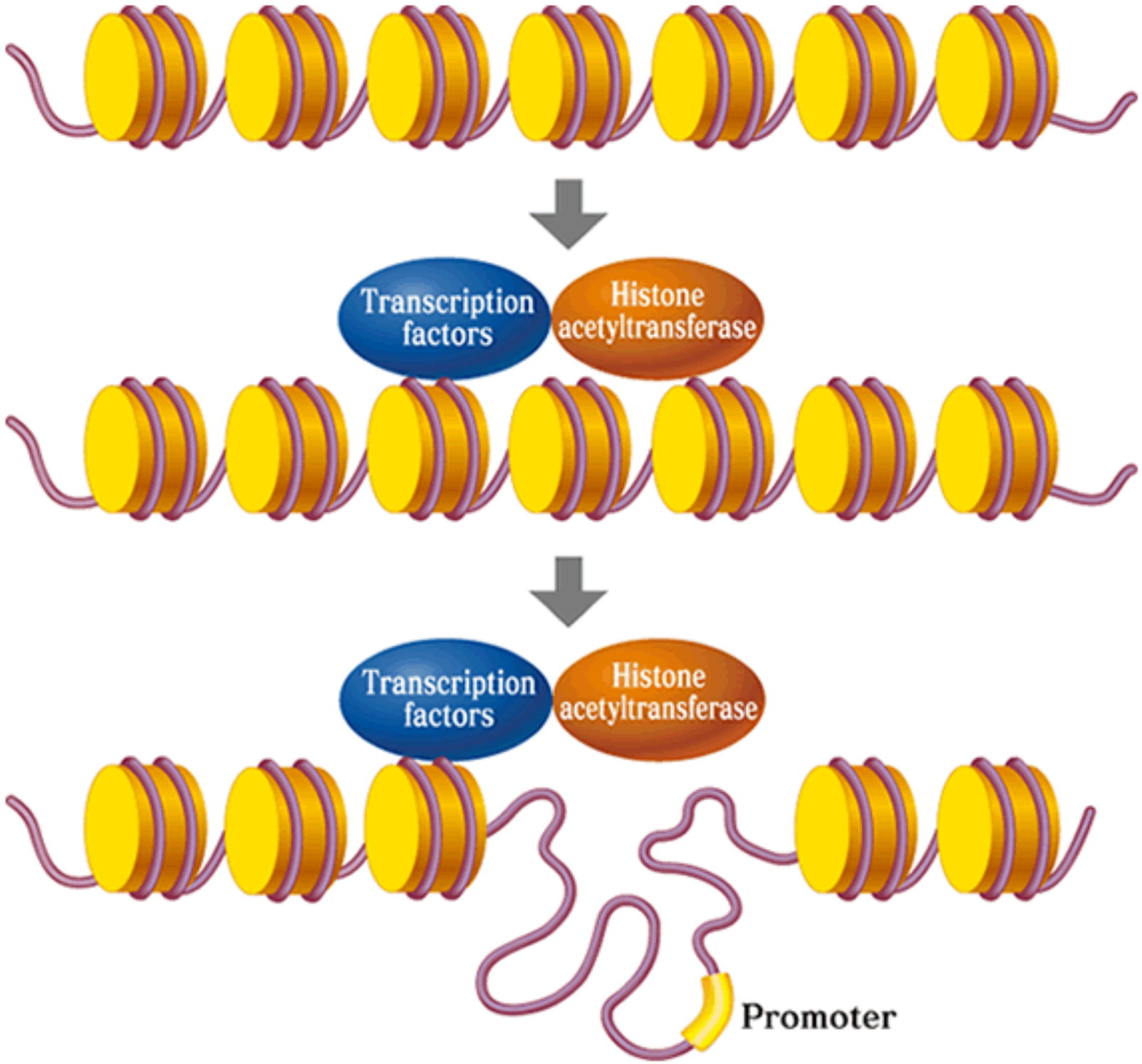
Plant genomes are organised hierarchically



When a foreign gene is inserted into a plant genome, it can inherit properties of the local chromatin

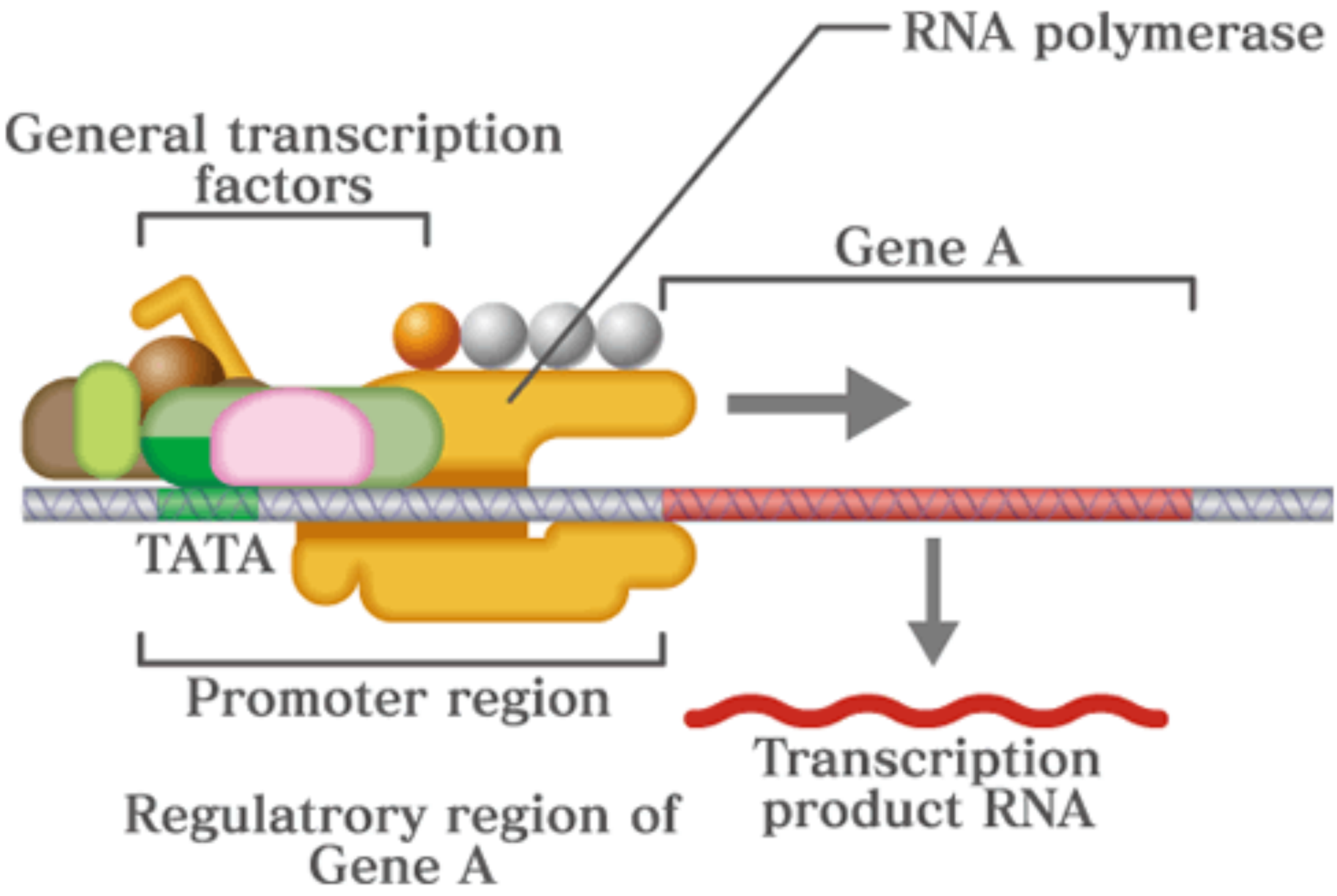


Rules for design of synthetic genes

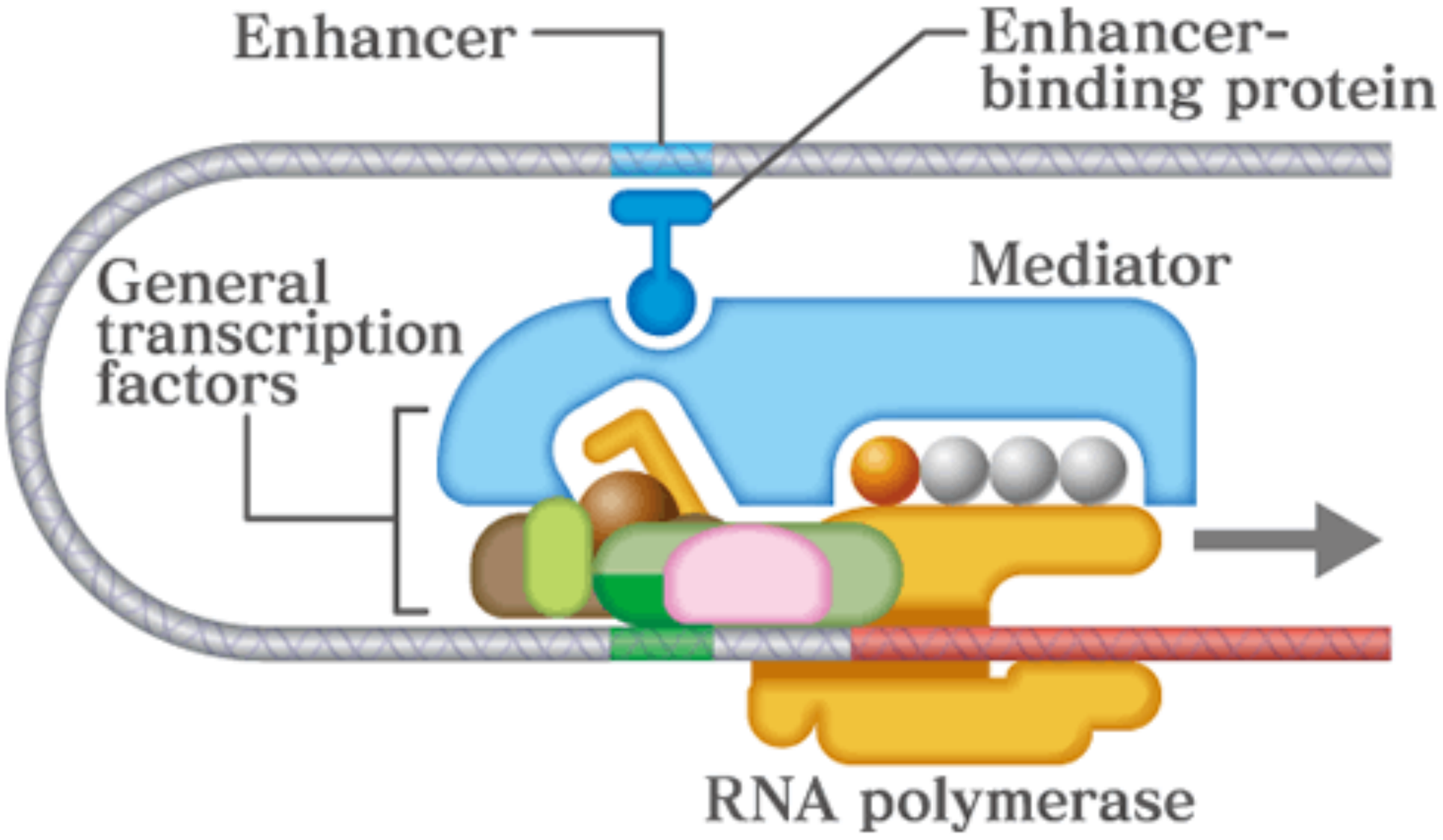
1. Specific sequences provide a key for interaction between DNA and host proteins, which ensure regulated conversion into RNA and protein.
These sequences are crucial for design of properly regulated synthetic genes.

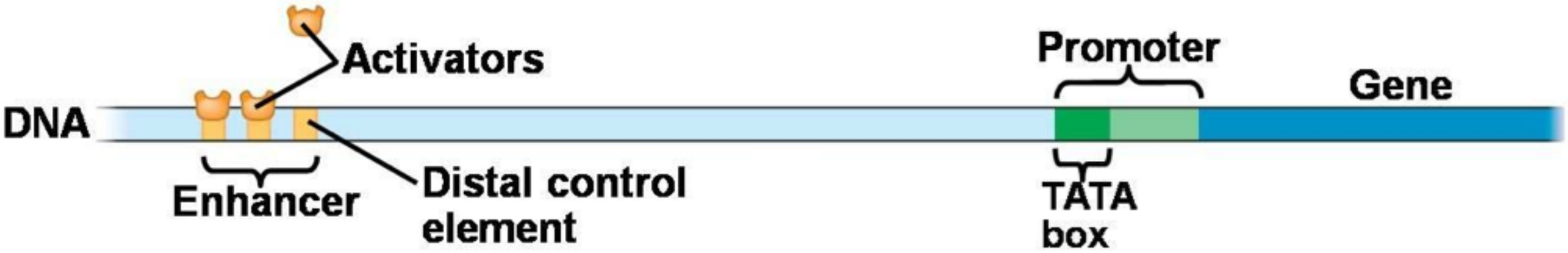
2. How do you measure and validate the behaviour of a single transgene in a genome with 10,000's of other genes being expressed?

Core promoter elements for a plant gene

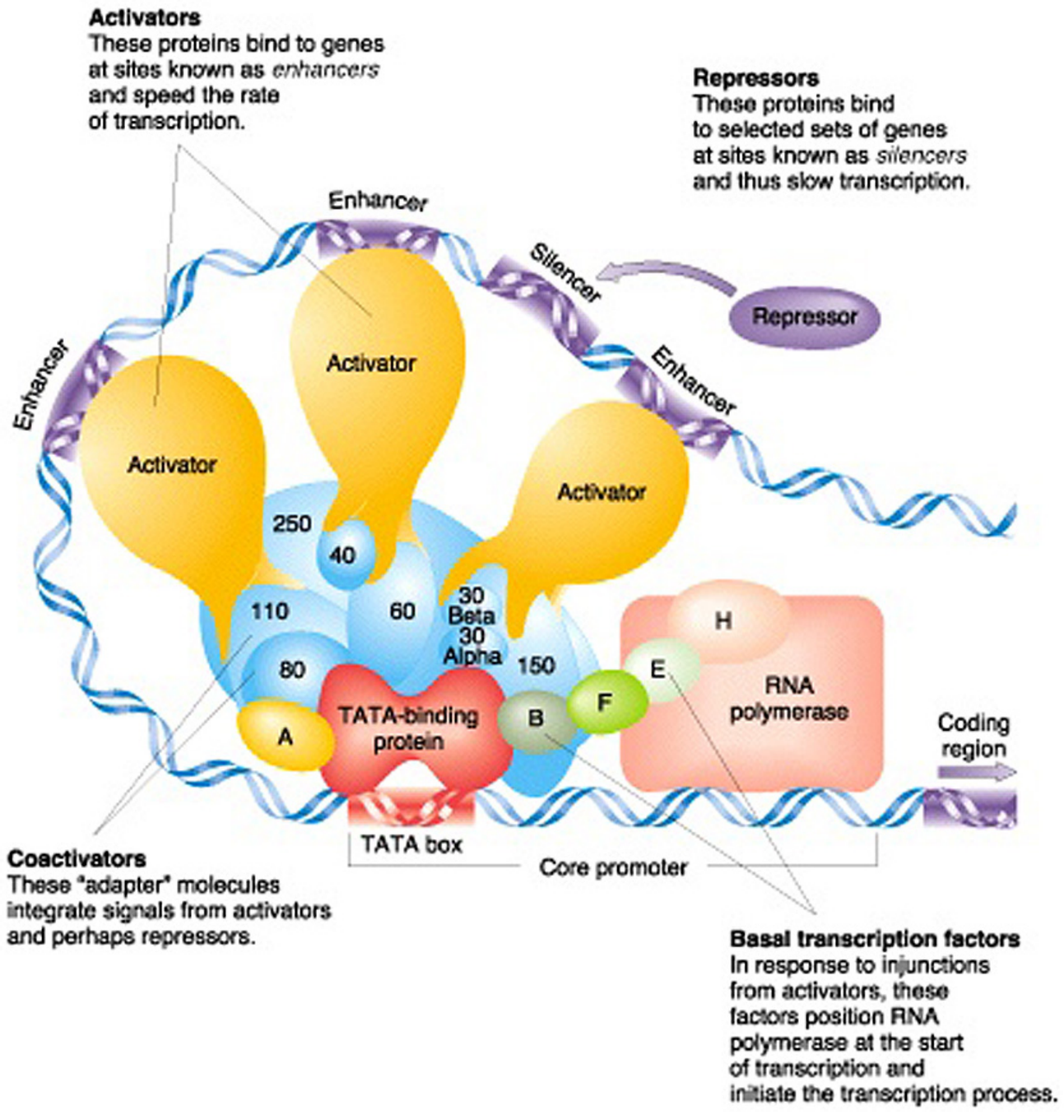


Transcription initiation requires interaction with distal promoter elements

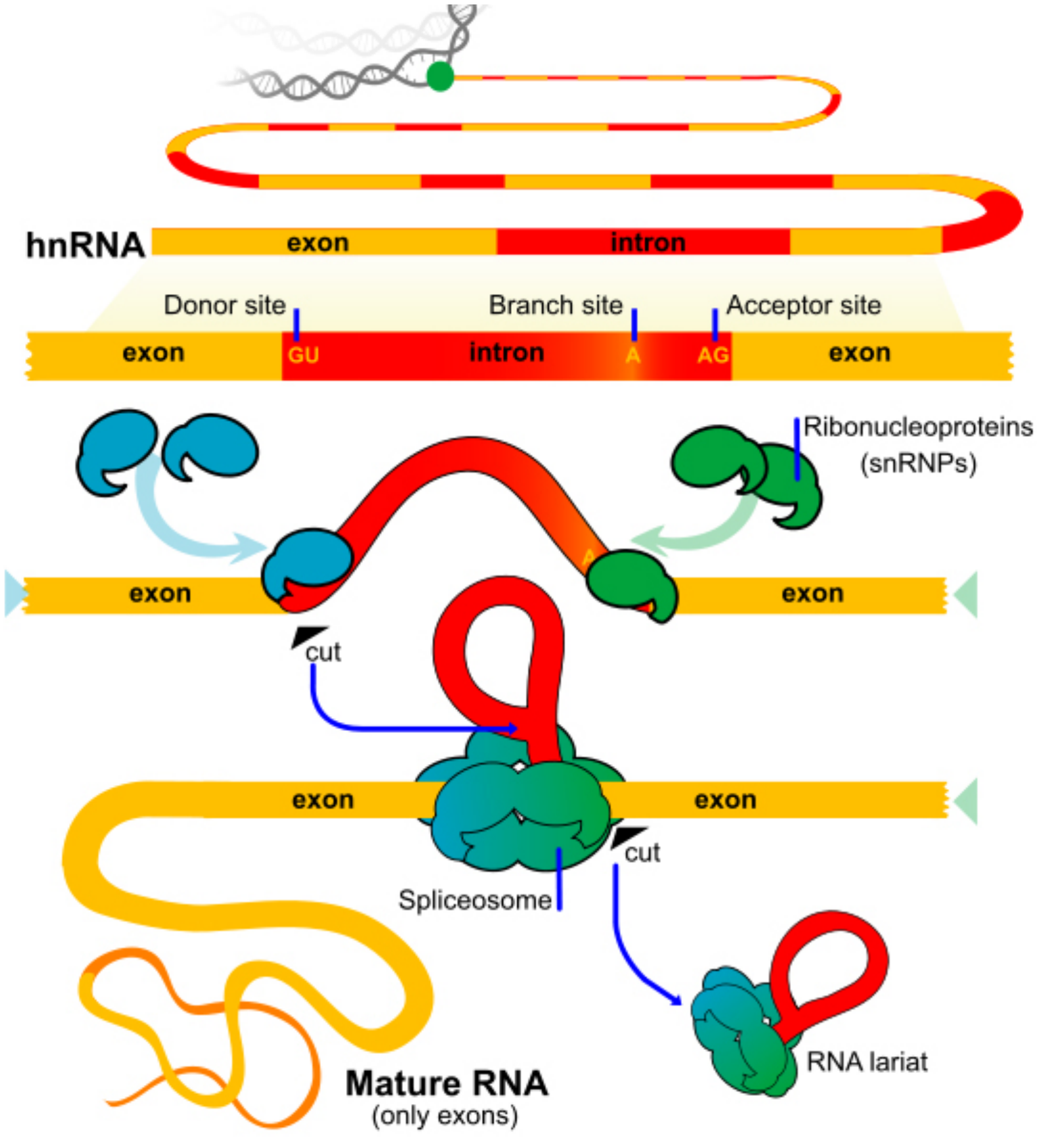




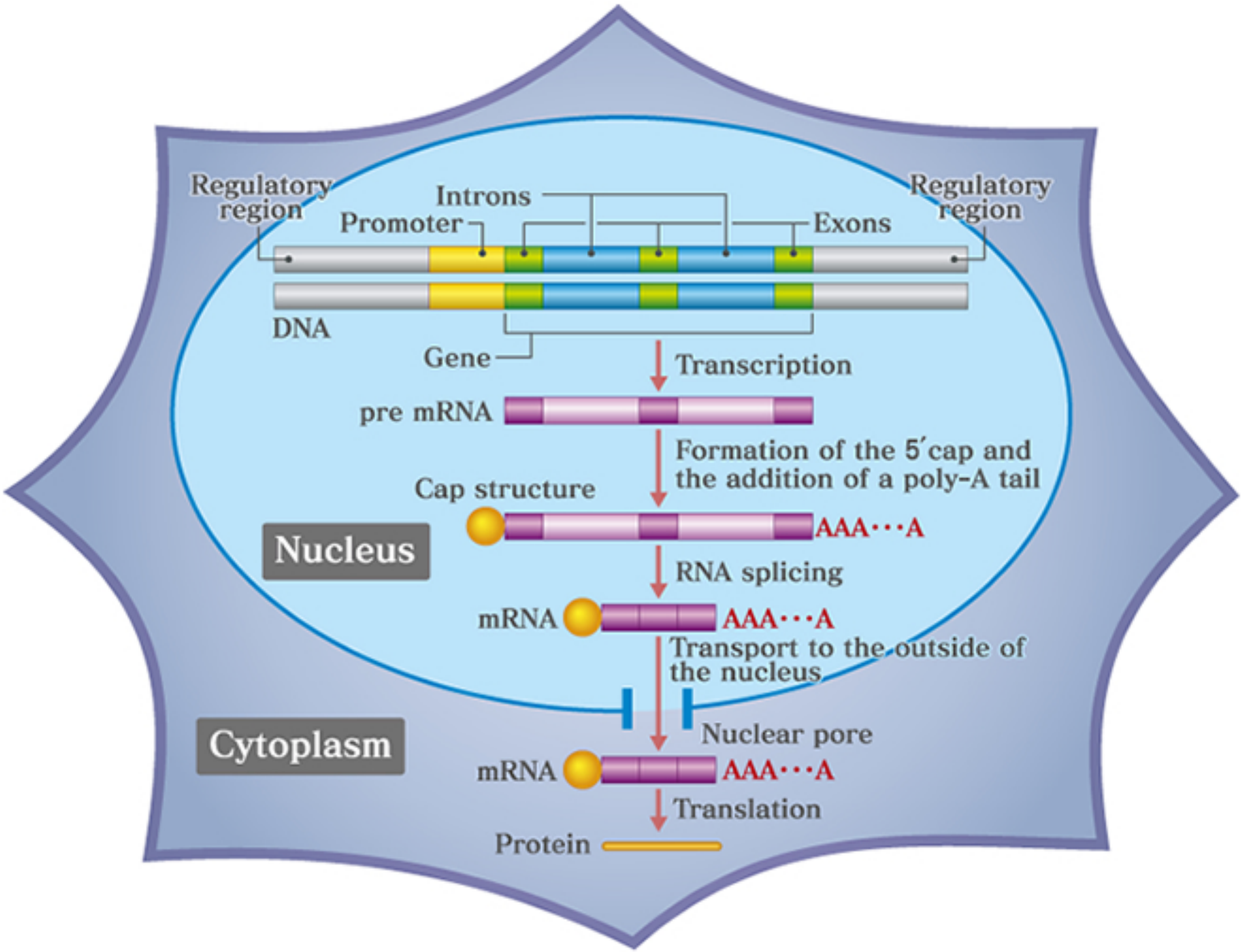
Enhancer and Silencers are position-independent distal elements for eukaryotic promoters



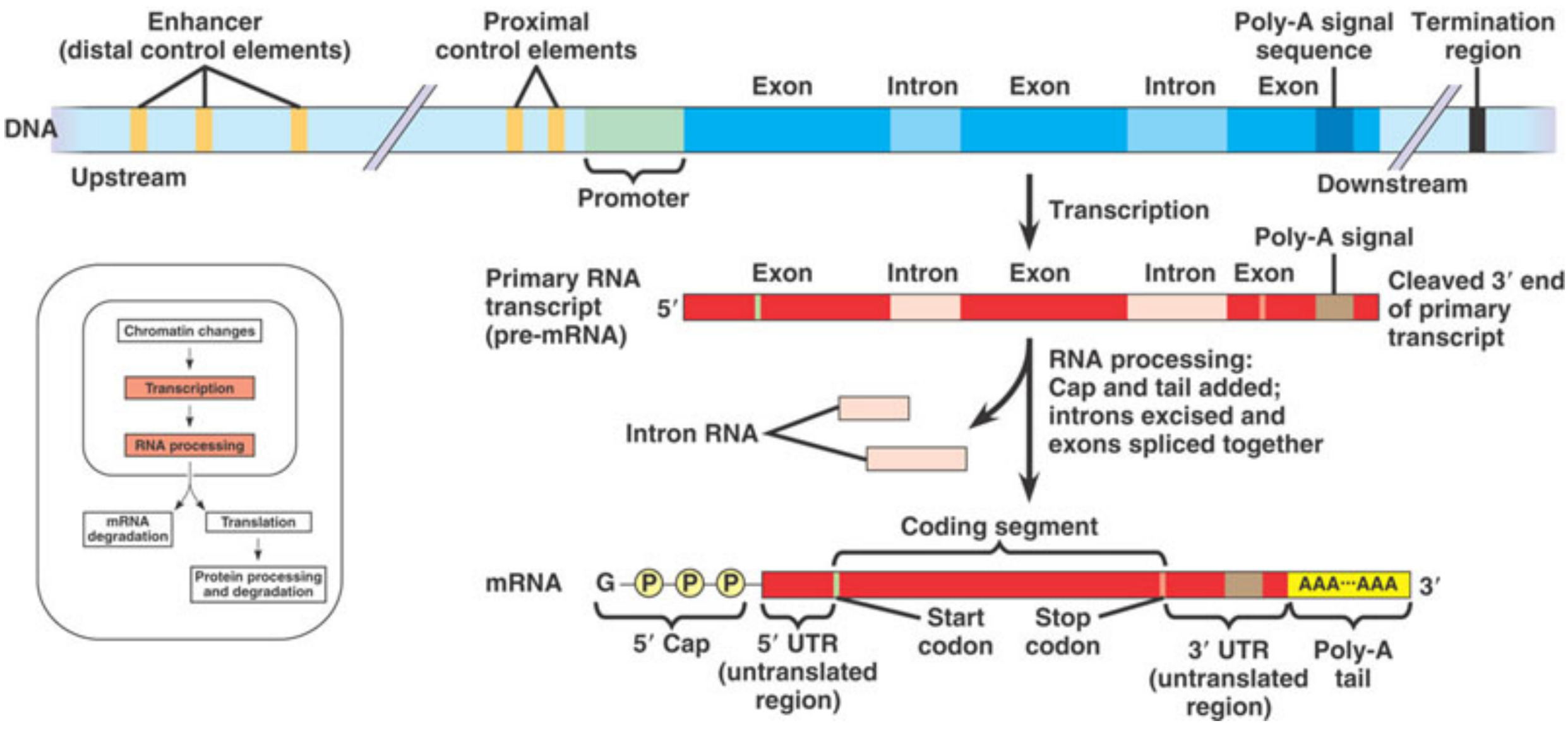
Plant genes generally contain introns (and DNA encoded signals to allow correct splicing and avoid cryptic splicing)



Processing, capping, polyadenylation and efficient translation of plant mRNAs requires appropriate DNA-encoded sequences

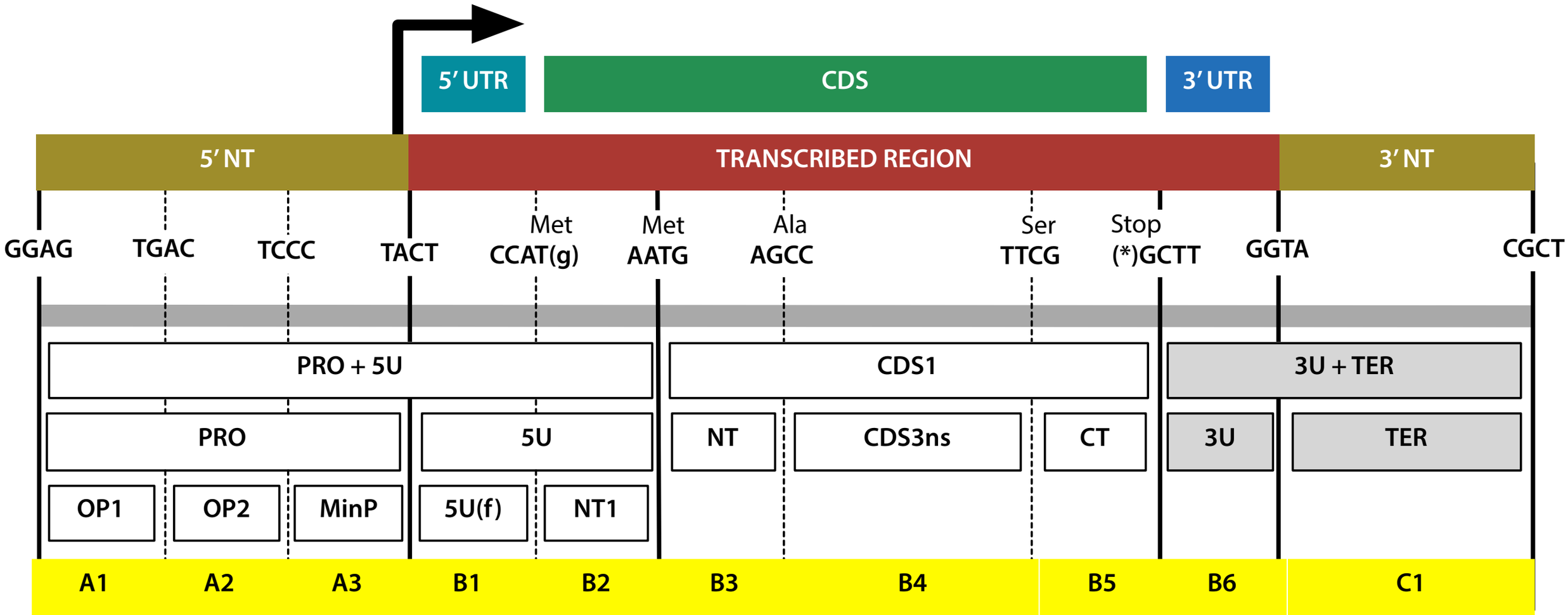


Plant gene structure



A common syntax for assembly of plant DNA parts

Based on Golden Gate standard assembly and type IIs restriction enzyme splints.



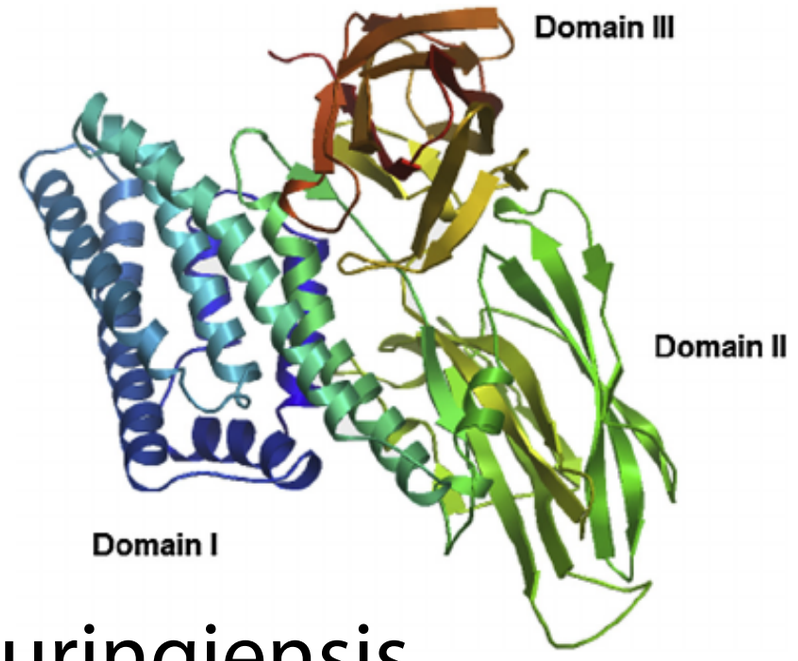
Standards for Plant Synthetic Biology: A Common Syntax for Exchange of DNA Parts. New Phytologist 208:13–9. (2015)
by Patron, Nicola; Orzaez, Diego; Marillonnet, Sylvestre; Warzecha, Heribert; Matthewman, Colette; Youles, Mark; Raitskin, Oleg; Leveau, Aymeric; Farre–Martinez, Gemma; Rogers, Christian; Smith, Alison; Hibberd, Julian; Webb, Alex; Locke, James; Schornack, Sebastian; Ajioka, Jim; Baulcombe, David; Zipfel, Cyril; Kamoun, Sophien; Jones, Jonathan; Kuhn, Hannah; Robatzek, Silke; Van Esse, H Peter; Oldroyd, Giles; Sanders, Dale; Martin, Cathie; Field, Rob; O'Connor, Sarah; Fox, Samantha; Wulff, Brande; Miller, Ben; Breakspear, Andy; Radhakrishnan, Guru; Delaux, Pierre–Marc; Loque, Dominique ; Granell, Antonio; Tissier, Alain; Shih, Patrick; Brutnell, Thomas; Quick, Paul; Rischer, Heiko; Fraser, Paul; Aharoni, Asaph; Raines, Christine; South, Paul; Ané, Jean–Michel; Hamberger, Björn; Langdale, Jane; Stougaard, Jens; Bouwmeester, Harro; Udvardi, Michael; Murray, Jim; Ntoukakis, Vardis; Schafer, Patrick; Denby, Katherine; Edwards, Keith; Osbourn, Anne; Haseloff, Jim

Single gene traits

Over a dozen genetically modified (GM) plant species have been approved for commercial production in the US, and the single-gene traits that have been genetically engineered into them fall into five categories.

Trait	Modified Plants	Gene Source
Insect resistance (Bt)	corn, cotton, potato, tomato	soil bacterium
Herbicide resistance	corn, soybeans, cotton, canola, sugarbeets, rice, flax	various bacteria, tobacco (modified)
Virus resistance	squash/zucchini, papaya, potato	plant viruses
Delayed fruit ripening	tomato	tomato, soil bacterium, or virus
Pollen control	corn, chicory, (radicchio)	soil bacterium

Pest resistance



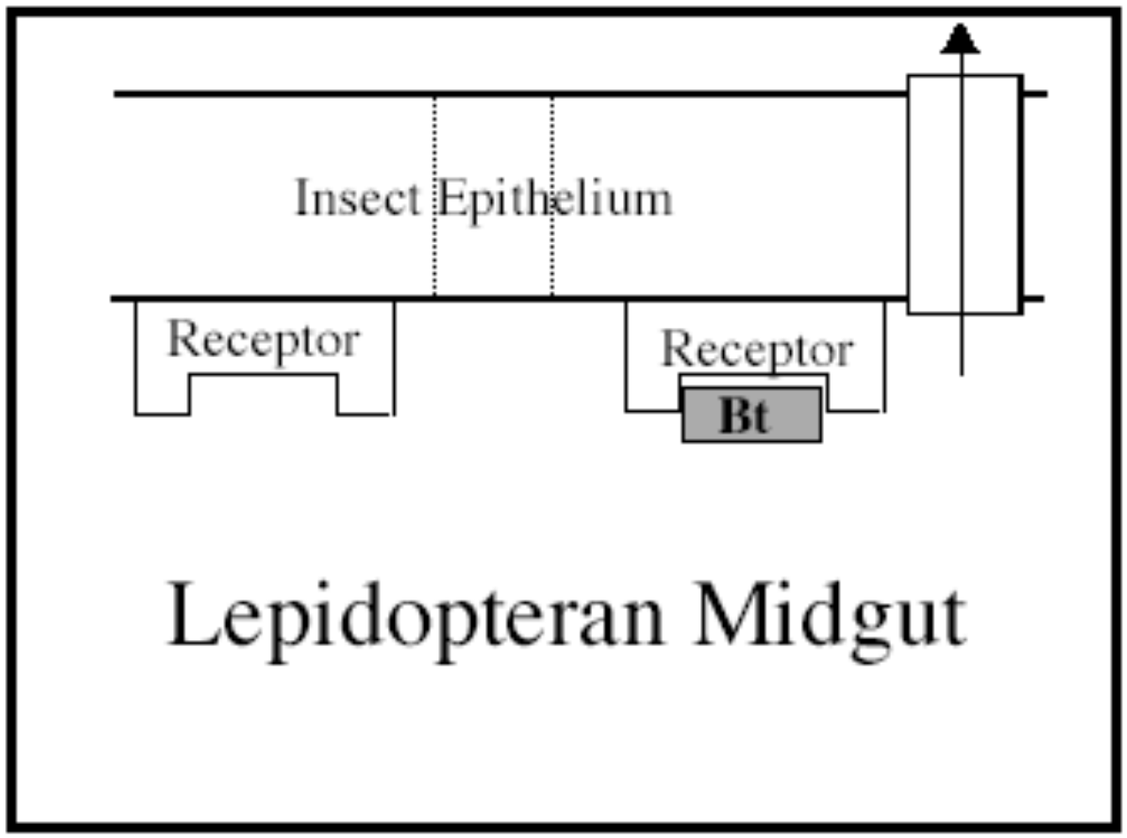
Bacillus thuringiensis (Bt) toxin

Bt toxin is a protein produced by Bacillus thuringiensis bacteria. On ingestion, and exposure to low pH and proteases in the insect gut, it binds to membrane receptors and causes water and ion leakage from epithelial cells lining the gut.

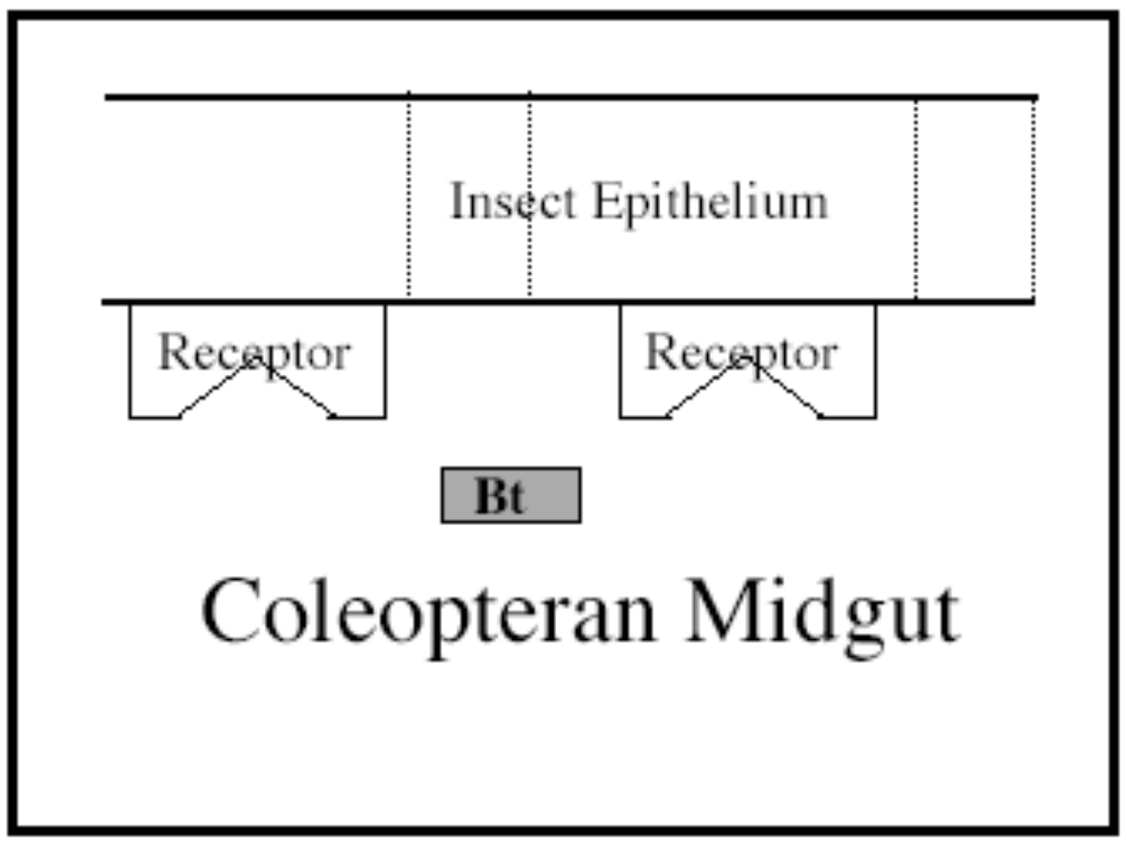
It is a highly selective toxin with no effect on mammalian cells. Bt based insecticides have been widely used in organic farming for over 50 years.

There are over 50 types of Bt toxin, each specific for different classes of insect.

Details of Bt Action



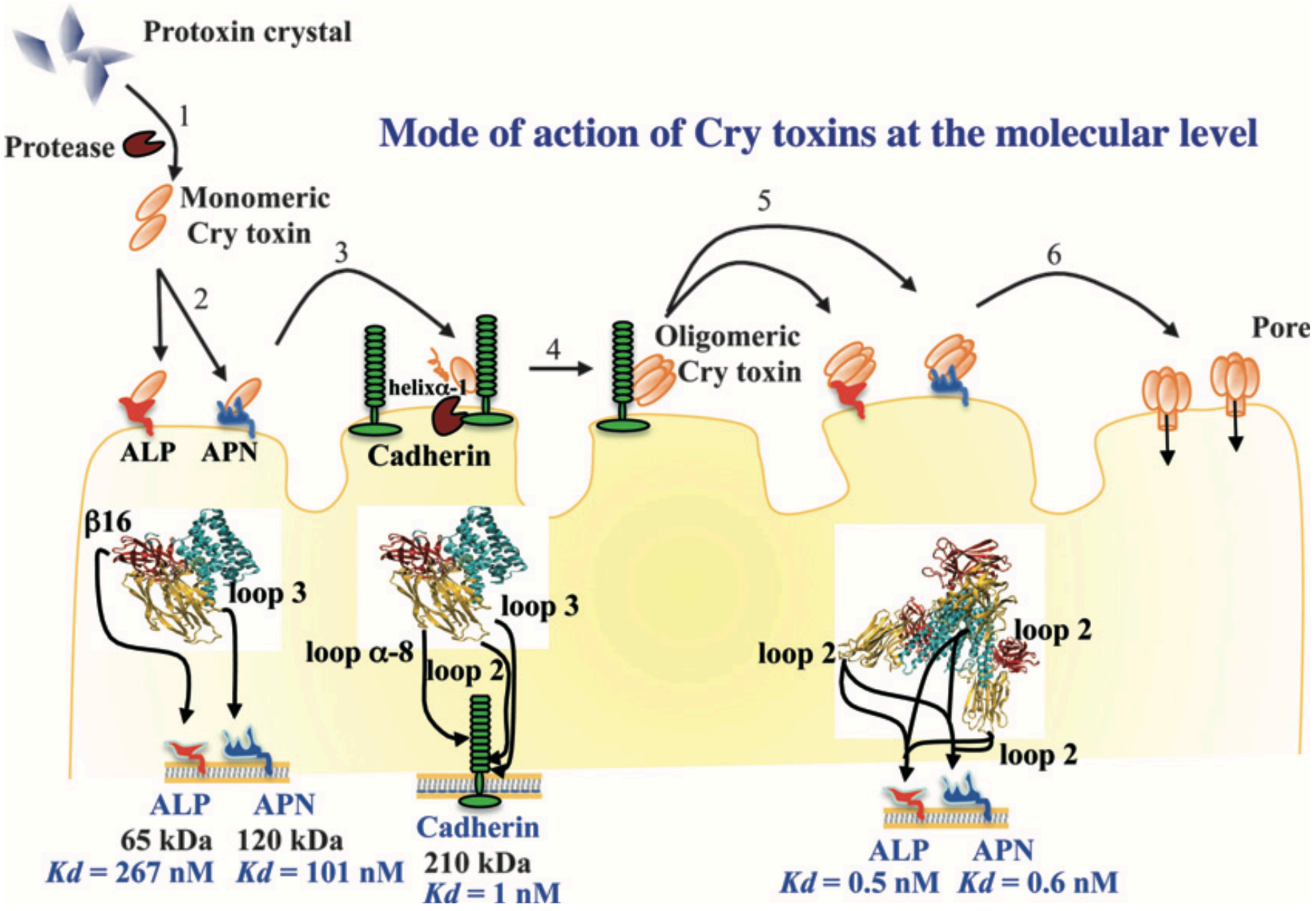
Lepidopteran Midgut



Coleopteran Midgut

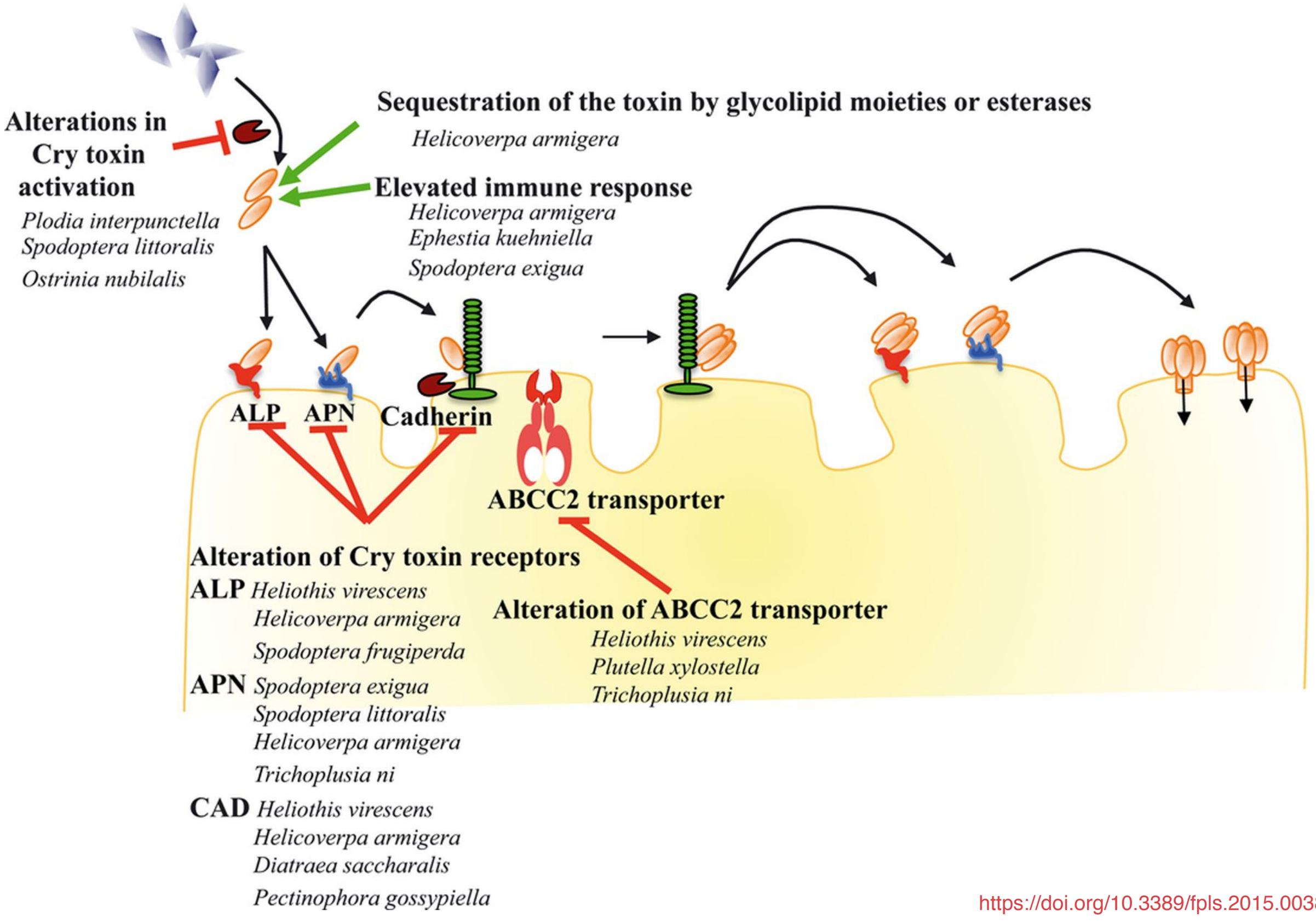
Note: This illustration is for a "lepitodoperan-specific" Bt. Other Bt proteins, specific for coleopterans, exist as well.

Mode of action of Cry toxins at the molecular level



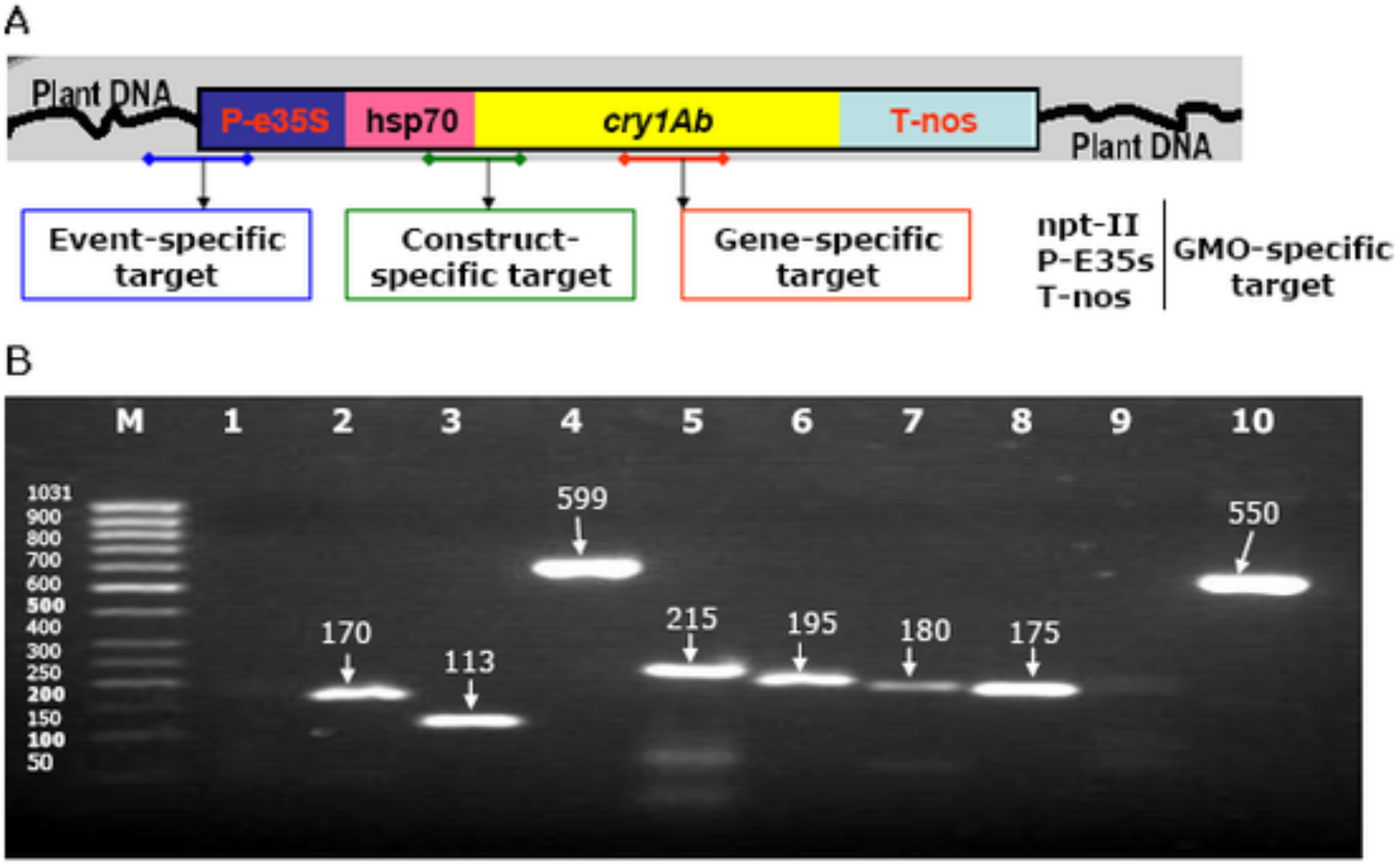
Ears of Corn: The top is GMO (Bt transgenic), and the bottom is non-GMO. The Asian corn borer has caused damage to the ear, resulting in fungal growth (mold) and sprouting. These varieties were grown in the Philippines. (Source: Food for Thought Blog)

Mechanism of action (and resistance) for Bt toxin (Cry)



https://doi.org/10.3389/fpls.2015.00381

DNA structure of a commercial Bt toxin gene



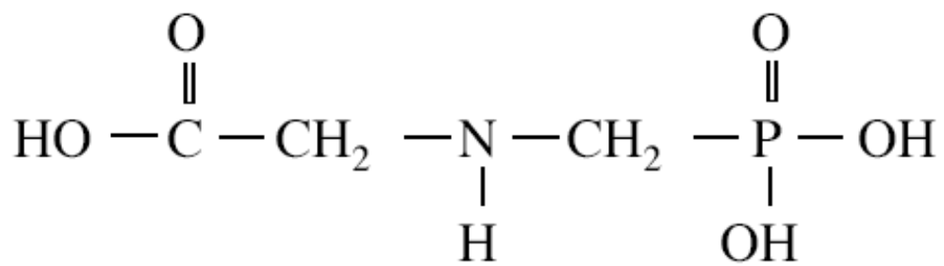
Assessment of cry1Ab transgene cassette in commercial Bt corn MON810: gene, event, construct and GMO specific concurrent characterization

Chandra K. Singh , Abhishek Ojha , Suchitra Kamle & Devendra N. Kachru

Protocol Exchange (2007) doi:10.1038/nprot.2007.440

Herbicide resistance

Glyphosate (Roundup)



Mode of Glyphosate Action

Glyphosate inhibits the shikimate pathway enzyme EPSPSase, an enzyme that acts late in that pathway. The pathway is responsible for, among other things, the biosynthesis of aromatic amino acids: phenylalanine, tyrosine and tryptophan. This pathway is also responsible for biosynthesis of such diverse plant compounds as phytoalexins, plastoquinone, alkaloids, cinnamate, coumarin and flavonoids

Mode of Glyphosate Lethality

Glyphosate rapidly moves to apical areas of the plant and inhibits protein synthesis. Cessation of growth happens almost immediately after the herbicide reaches the apical areas. Plants stop growing and many plant tissues and parts slowly degrade due to impaired protein synthesis. Symptomology on plants usually develops very slowly, with gradually increasing chlorosis, yellowing, and necrosis. Death ultimately results from dehydration and desiccation.

Mechanism of herbicide resistance

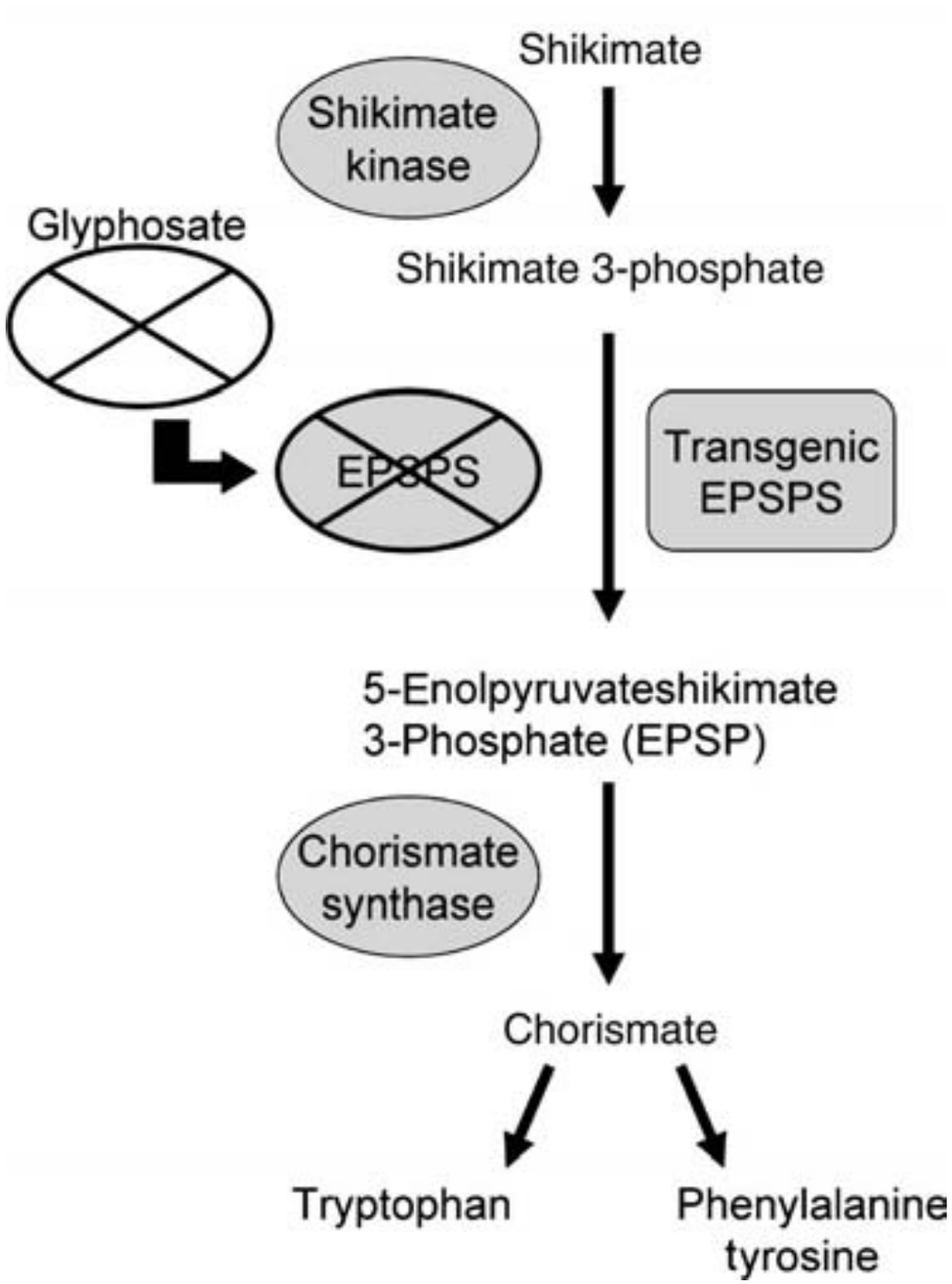


Figure 8.1. Resistance to glyphosate in RoundUp Ready™ plants is engineered by expressing a form of the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase (EPSPS) enzyme that is resistant to the herbicide. In the absence of this transgenic enzyme, glyphosate inhibits the plant EPSPS and ultimately blocks the synthesis of chorismate, the branchpoint precursor to the essential aromatic amino acids: tryptophan, phenylalanine, and tyrosine. The transgenic EPSPS is unaffected by glyphosate, and can carry out the synthesis of EPSP leading to chorismate production.

No-till farming using herbicide resistant crops

DuPont Crop Protection
Glyphosate-Resistant Waterhemp Trial[1]

Untreated

DuPont PRE herbicide followed by glyphosate POST

*DuPont PRE herbicide followed by glyphosate + dicamba POST**

Multiple herbicide resistance genes

New varieties contain two herbicide-tolerant traits – one for glyphosate and one for dicamba herbicides. The addition of dicamba tolerance provides farmers with tools to manage glyphosate resistant and tough-to-control broadleaf weeds such as waterhemp, marestail, Palmer amaranth, giant ragweed, kochia and others.

https://www.pioneer.com

Stacking of transgenic traits in hybrid corn

Here's how the corn hybrid naming system works:

G 11 U58 - 3111 A

A B C D E

A "G" indicates Golden Harvest.

B Last two digits of relative maturity number.

C Existing Garst hybrid numbering.

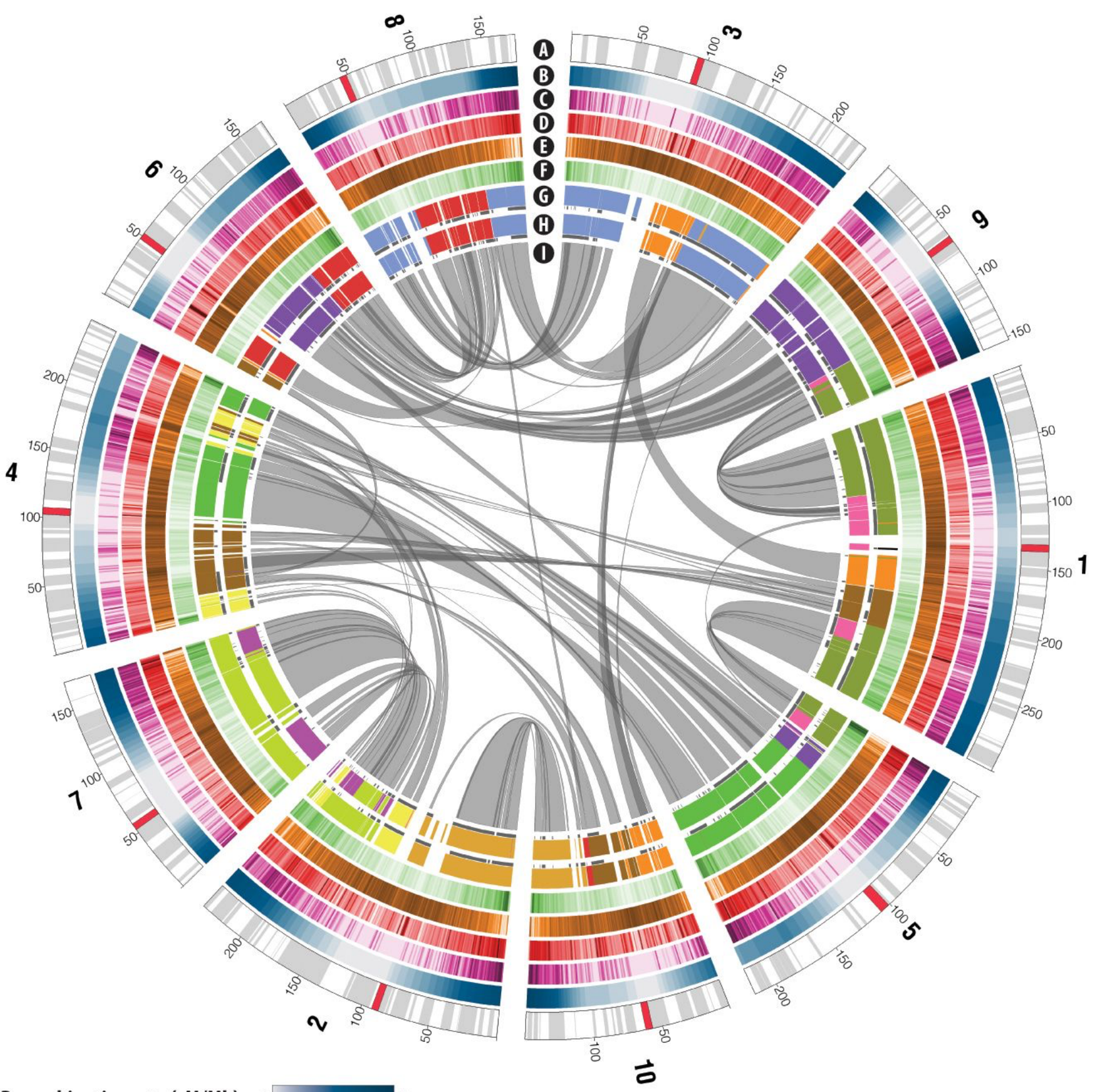
D Separates the genetic and trait portions.

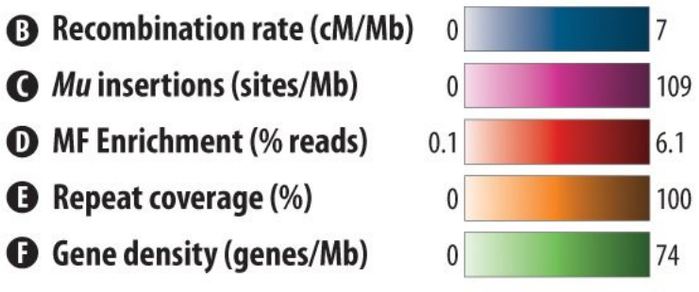
E From Agrisure traits naming system.

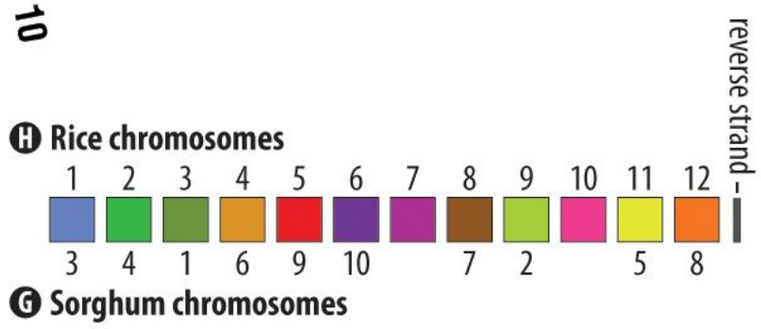
- First number represents Herbicide Tolerance Technology Series
- Second number represents number of modes of action against broad lepidopteran pests
- Third number represents number of modes of action against corn borer
- Fourth number represents number of modes of action against corn rootworm
- "A" denotes Agrisure Artesian technology

Syngenta

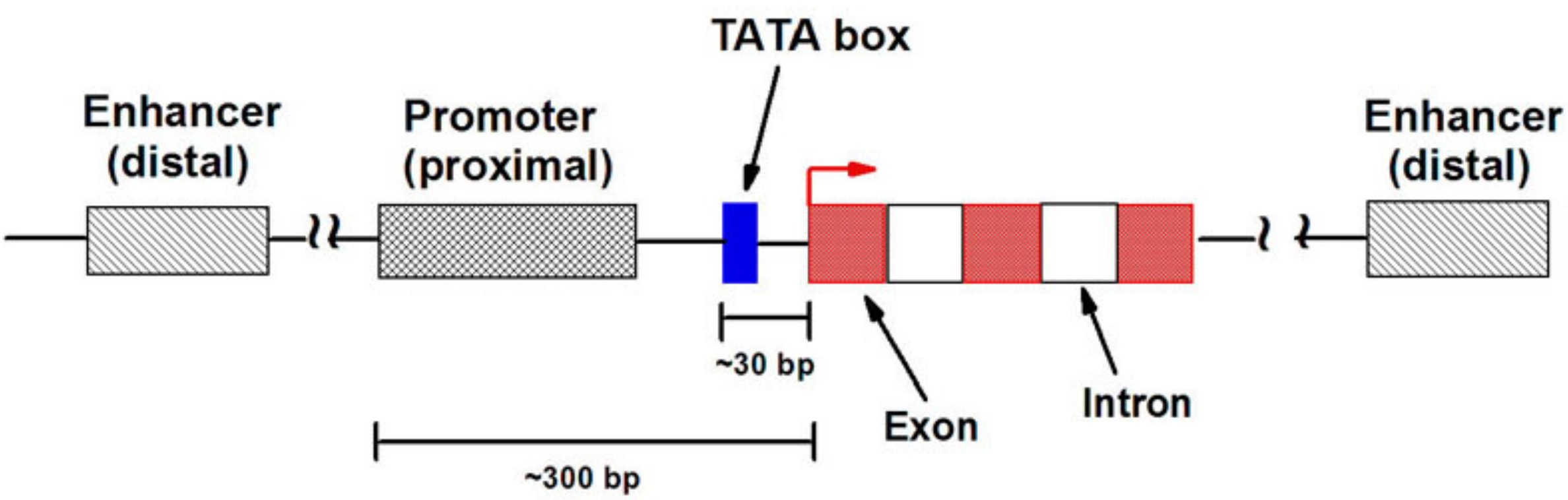
Maize genome
10 chromosomes
2.4 Gbp
32,000 genes







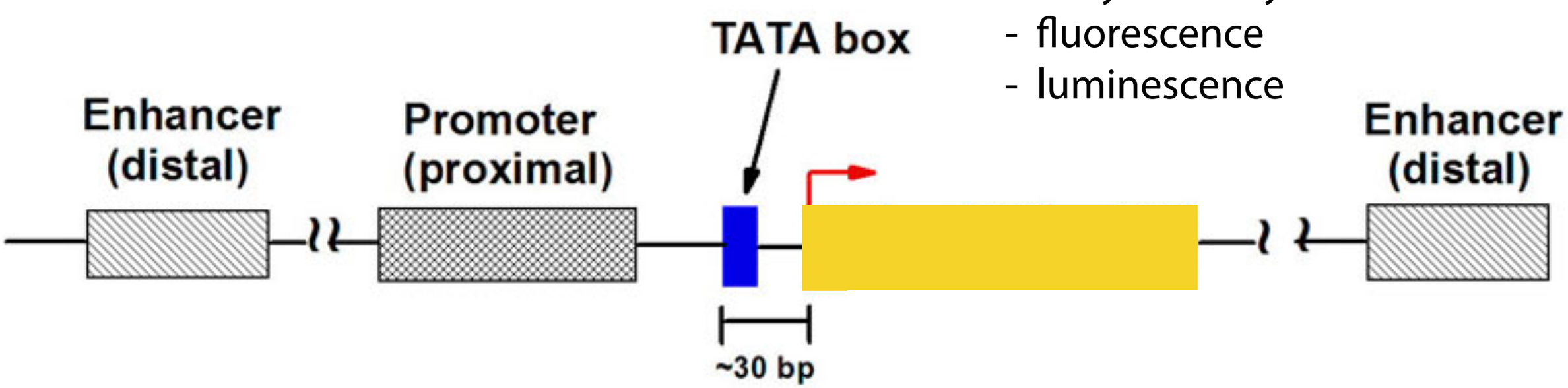
How can the activity of an individual gene be visualised?



Reporter genes: markers for gene expression

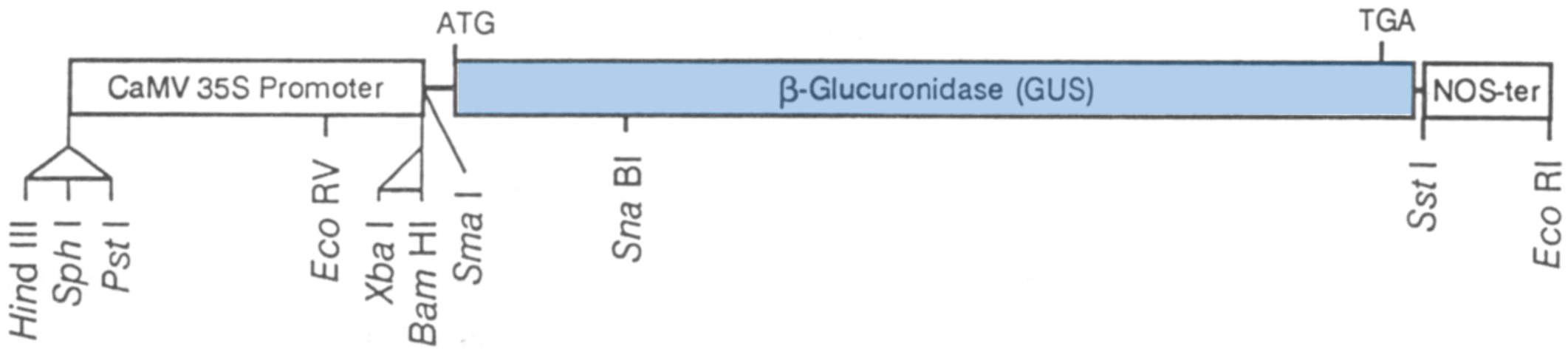
gene product

- enzyme assay
- fluorescence
- luminescence



ß-glucuronidase
fluorescent protein
luciferase

Synthetic GUS gene for plant transformation



pBI221 The CaMV 35S promoter-GUS-NOS-ter portion of pBI121 was cloned into pUC19 to produce pBI221.

ß-glucuronidase (GUS) is a glycolytic enzyme from *E. coli* without a counterpart in most plant cells. Specific histochemical staining can be used to indicate the presence of the expressed gene product.

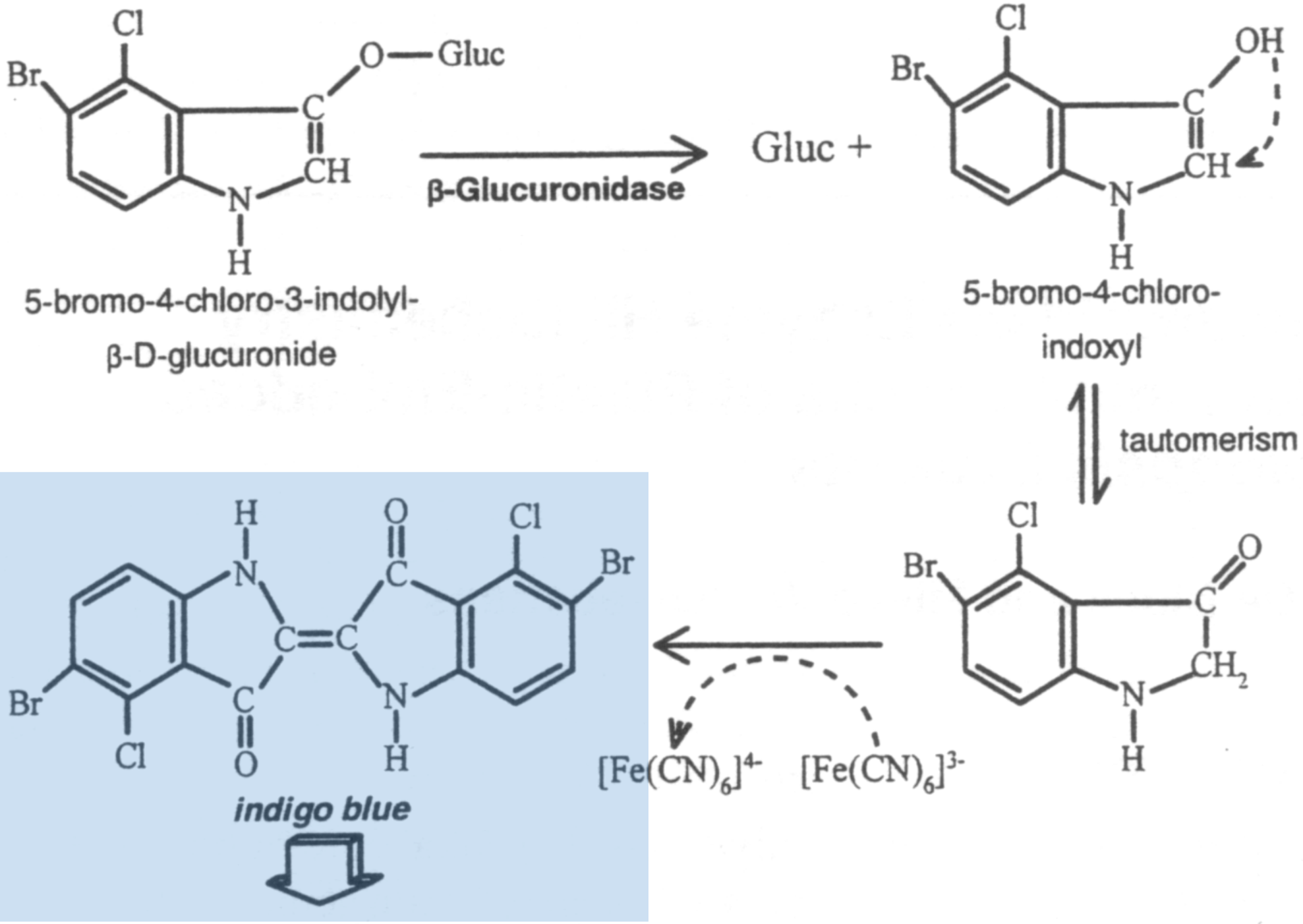
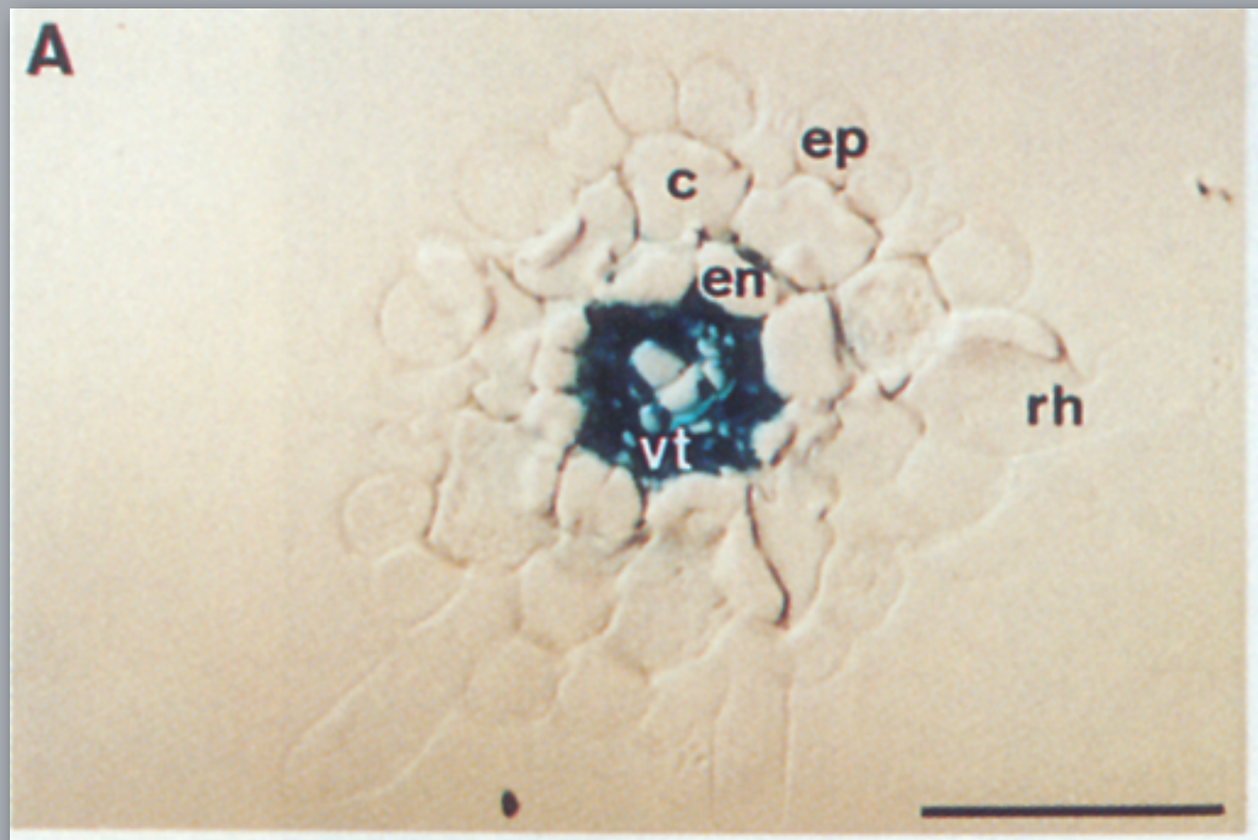


Fig. 1. Chemistry of X-Gluc reaction. Hydrolyzation of X-Gluc by the β-glucuronidase enzyme results in a reactive indoxyl molecule. Two indoxyl molecules are oxidized to indigo blue; ferri(III)cyanide enhances the dimerization.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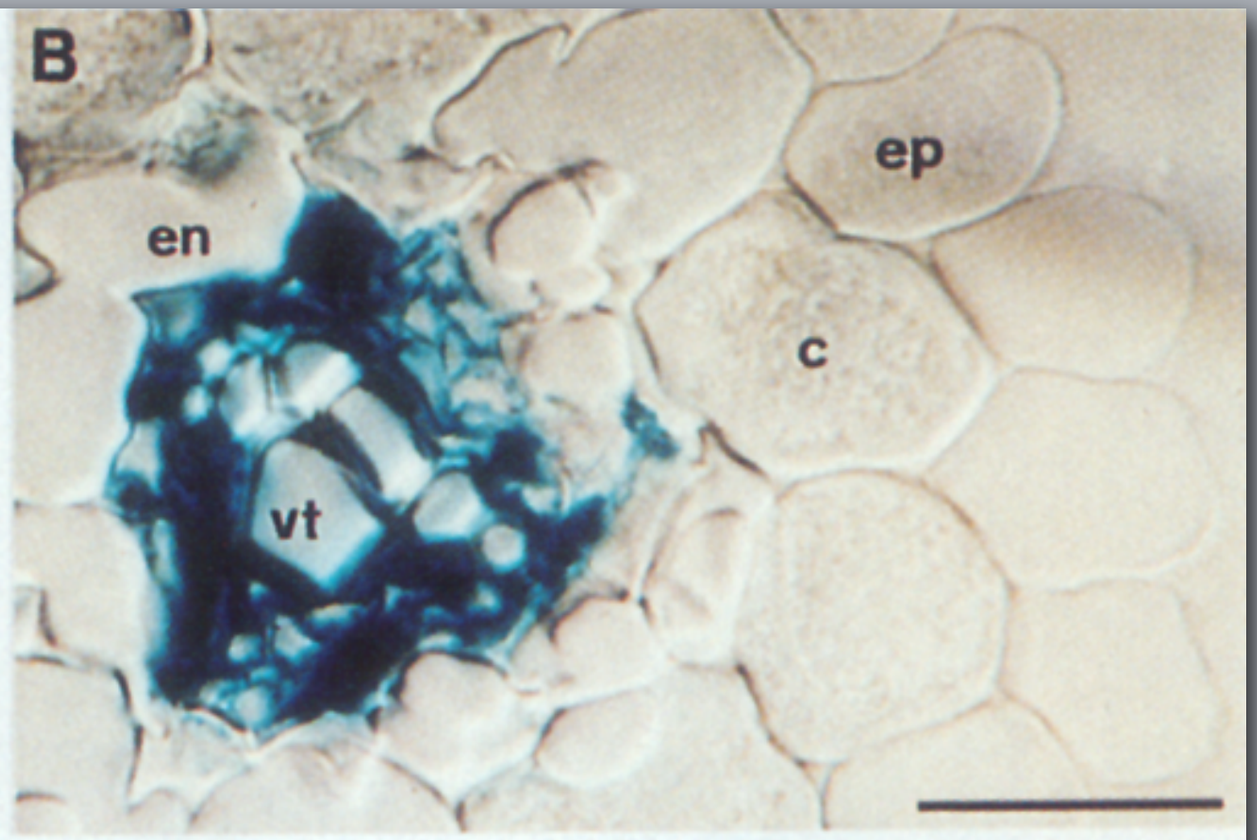
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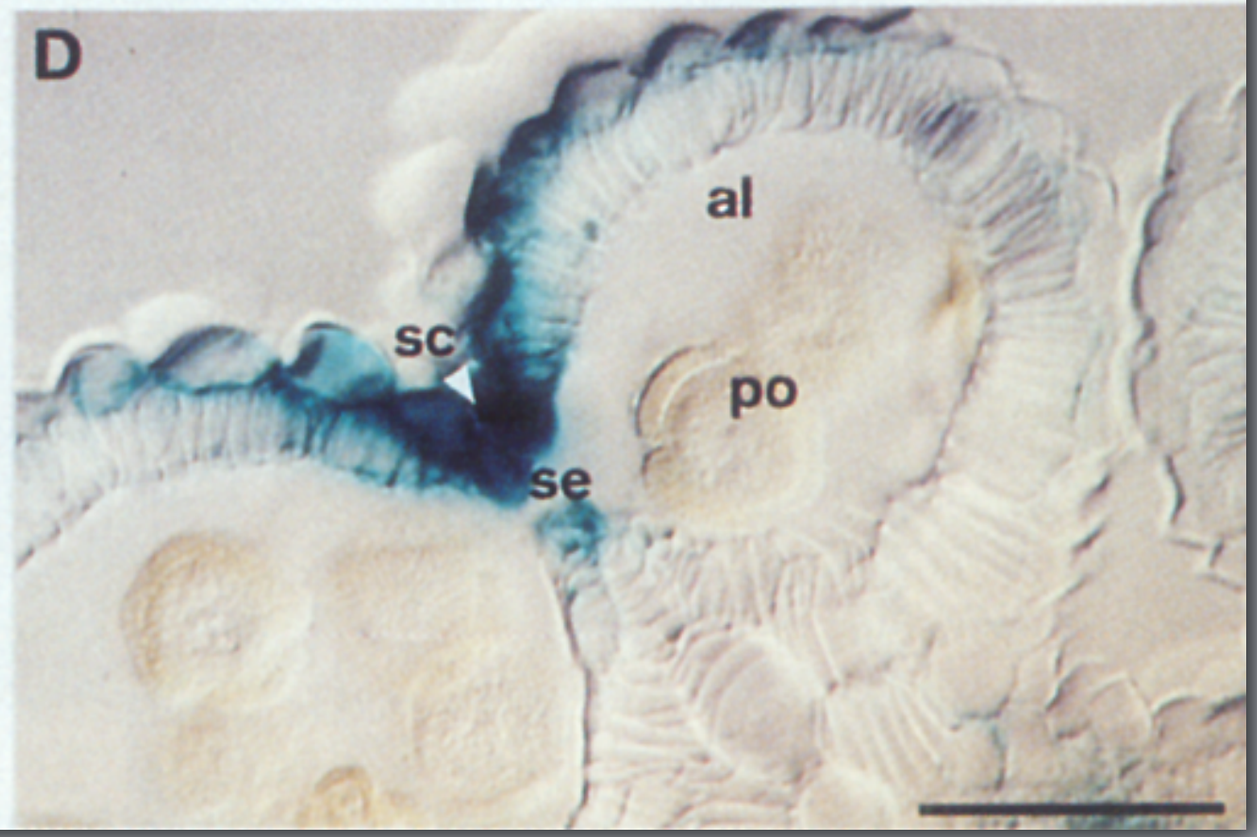
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The Bioluminescence of Green Fluorescent Protein in *Aequorea victoria*

Aequorea victoria is known for its naturally occuring green fluorescence around the ring of its bell, in large thanks to the prescence of two fluorescent proteins: Aequorin and Green Fluorescent Protein (GFP).

The process begins when calcium ions bind to the chemiluminescent Aequorin, a protein with lu in the middle of each of its two subunits. Once the calcium is bound, the chromophores begin to emit blue light at 470nm.

Aequorin:

Ca 2+

excited state

Chromophore

Coelenterazine: a luciferin compound that fluoresces upon oxidation by calcium

Blue light is emitted at **470 nm**

The light emitted by Aequorin's chromophore causes the chromophore of GFP to become excited.

GFP

GFP contains a naturally built chromophore consisting of a special sequence of 3 amino acids: Serine, Tyrosine, and Glycine. When the chromophore receives blue light from aequorin, it becomes excted and emits its very own green light.

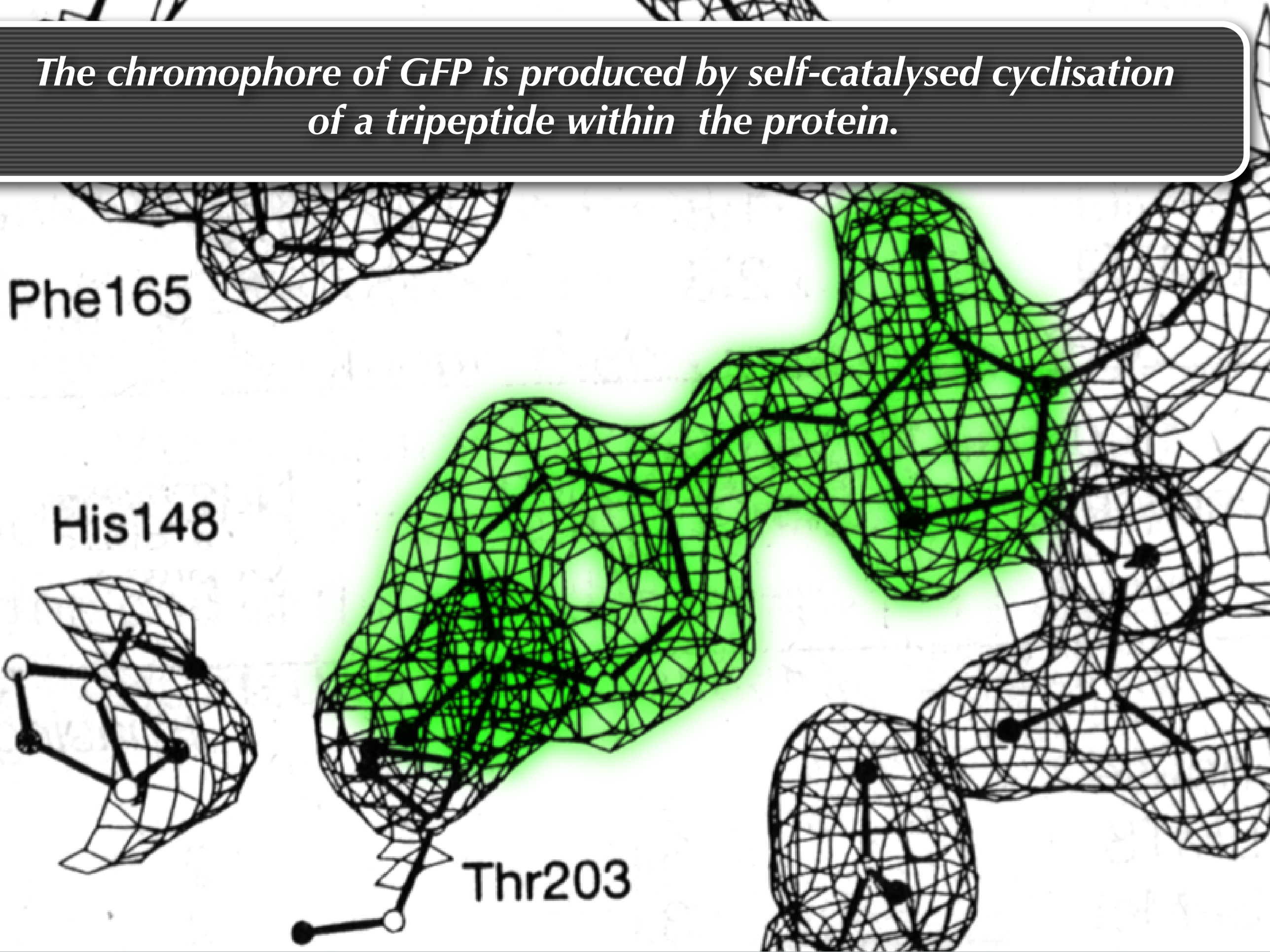
GFP Chromophore

GFP - excited state

Green fluorescent protein (GFP) was isolated from bioluminescent jellyfish.

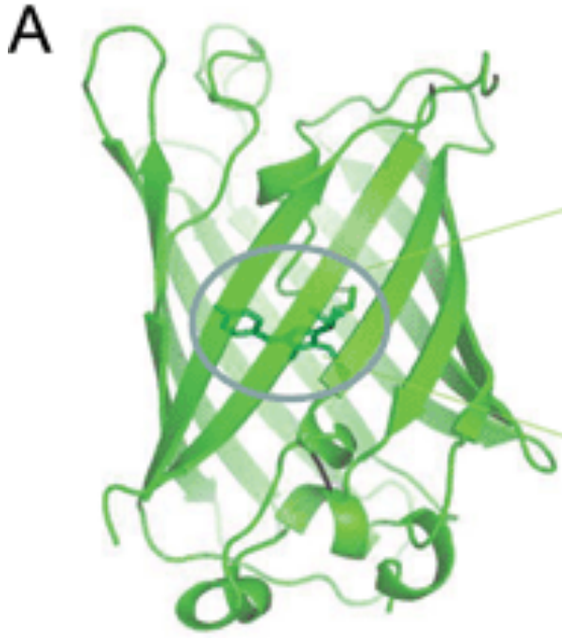
3D structure of green fluorescent protein

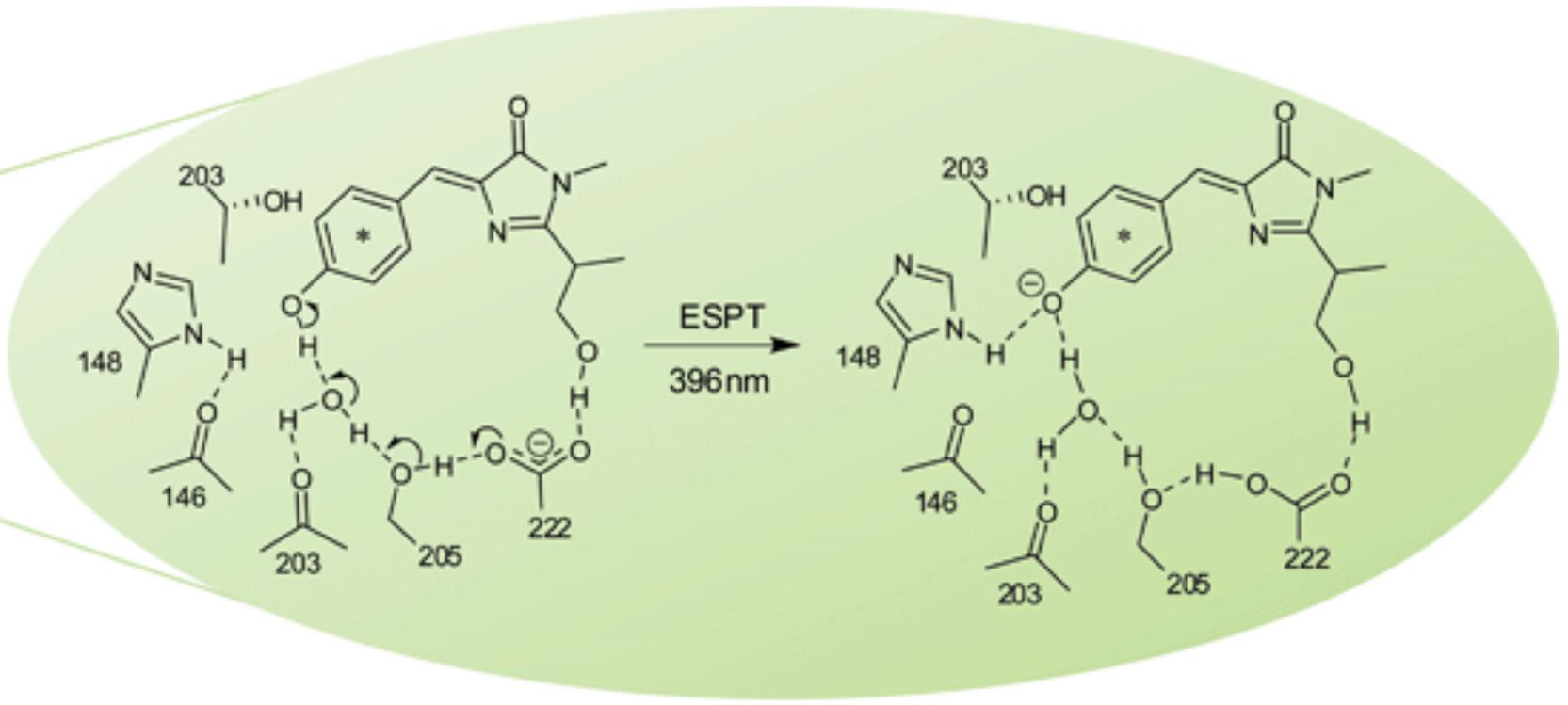
The chromophore of GFP is produced by self-catalysed cyclisation of a tripeptide within the protein.



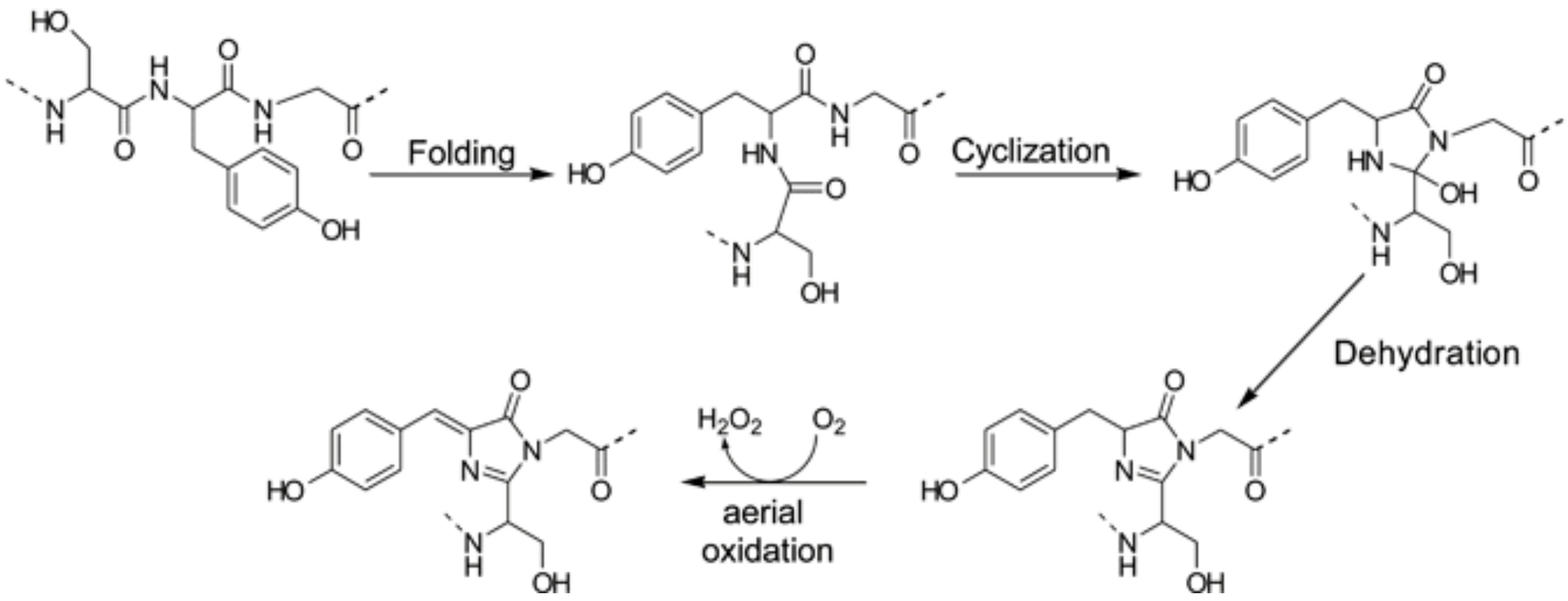
Autocatalytic maturation of the peptide chromophore in GFP

A



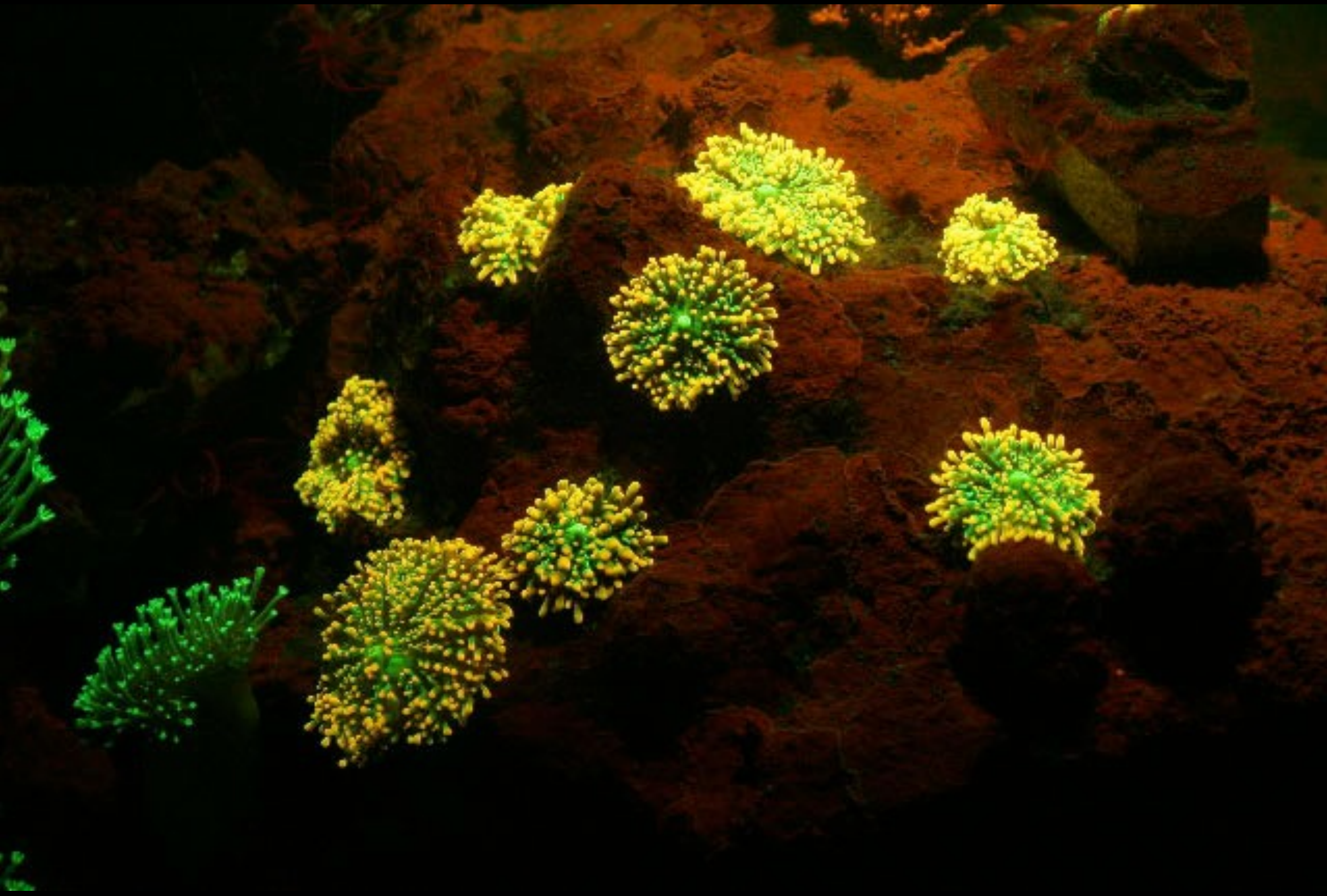


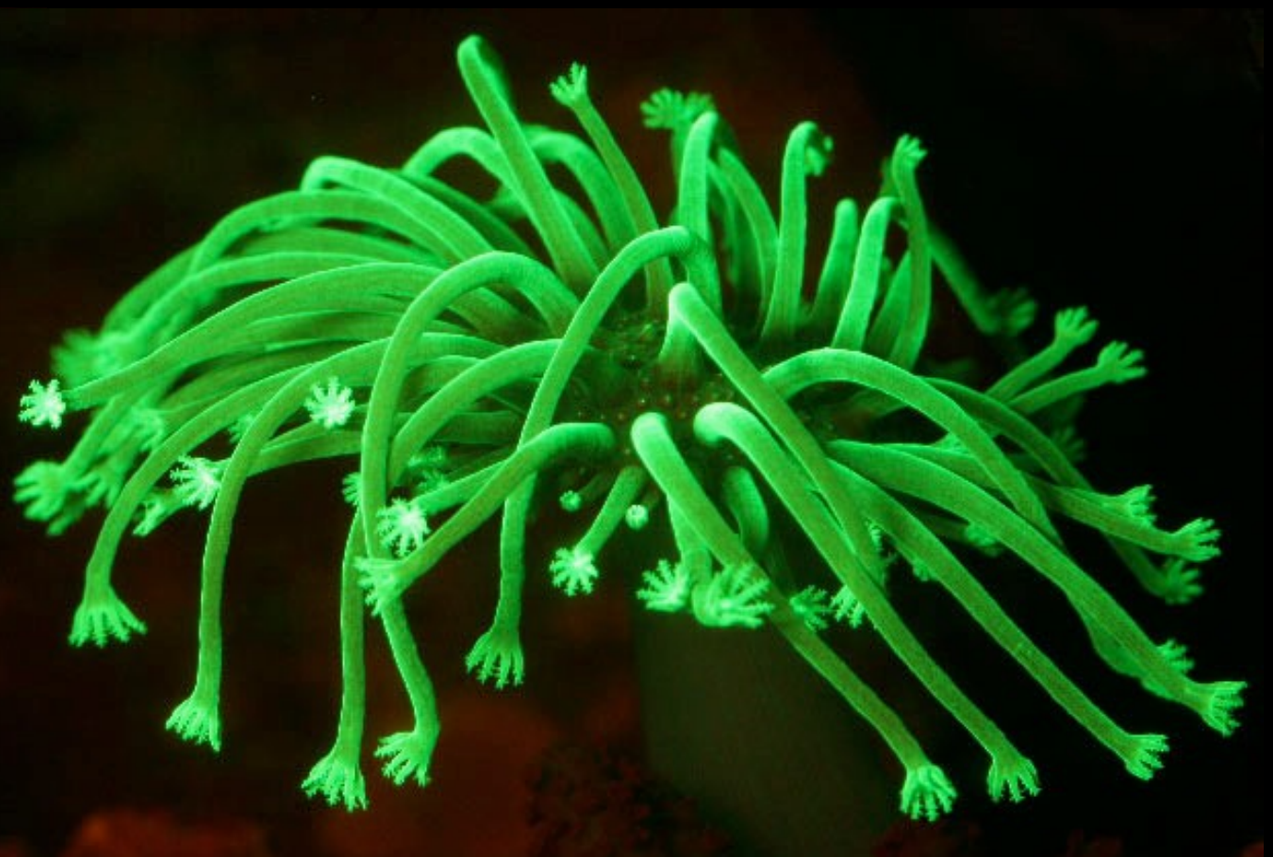
B

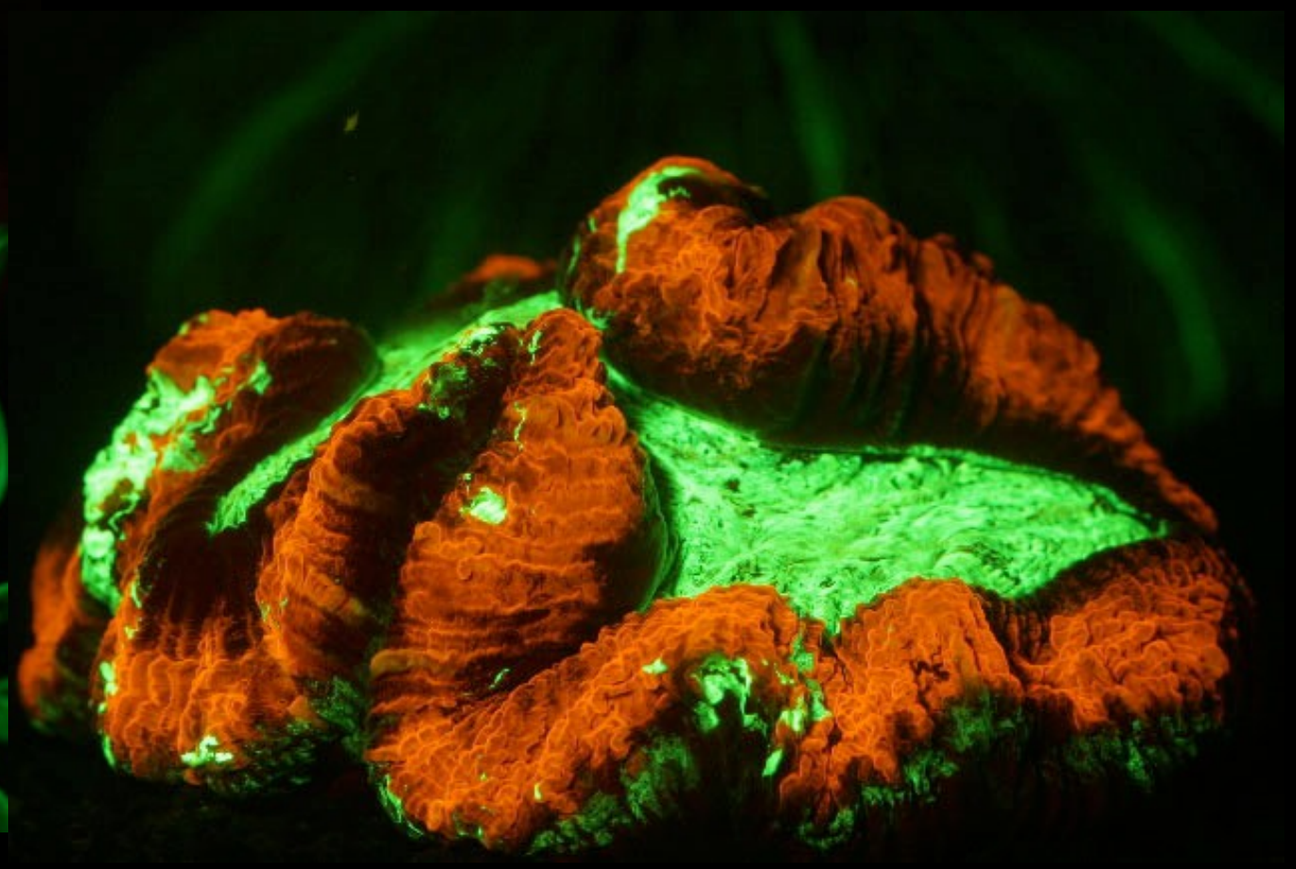


Ectopically expressed GFP undergoes spontaneous maturation

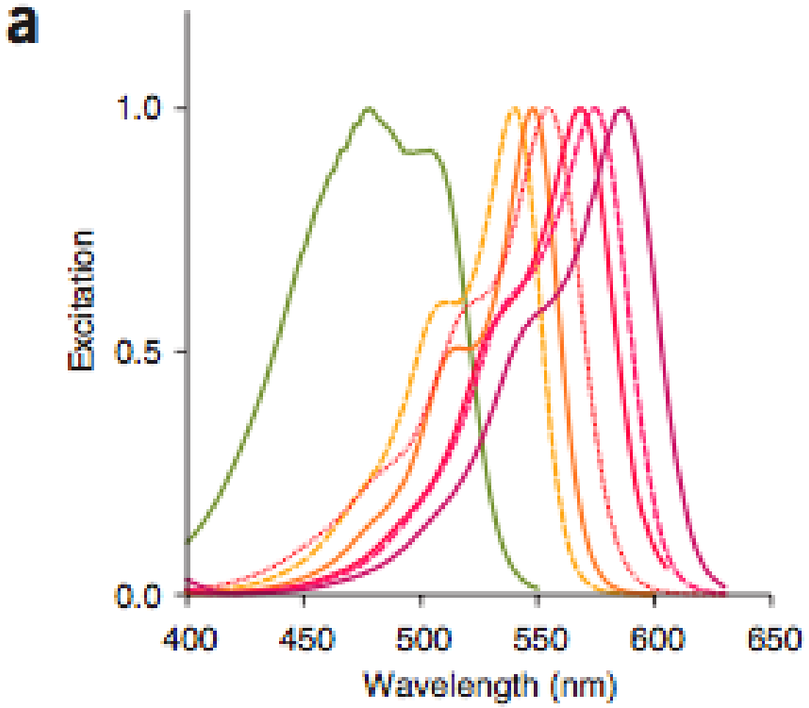
New fluorescent proteins in coral

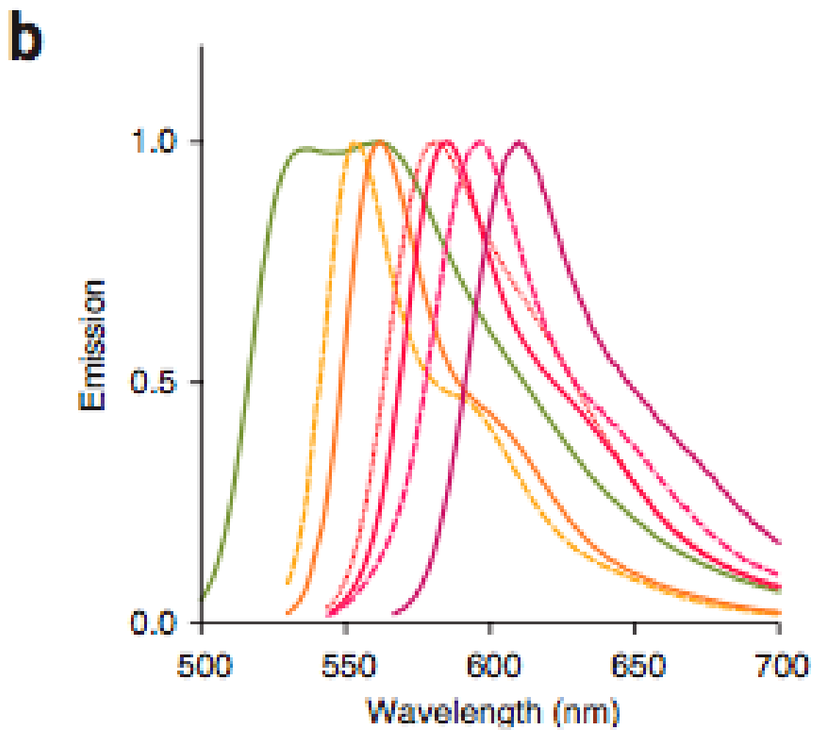


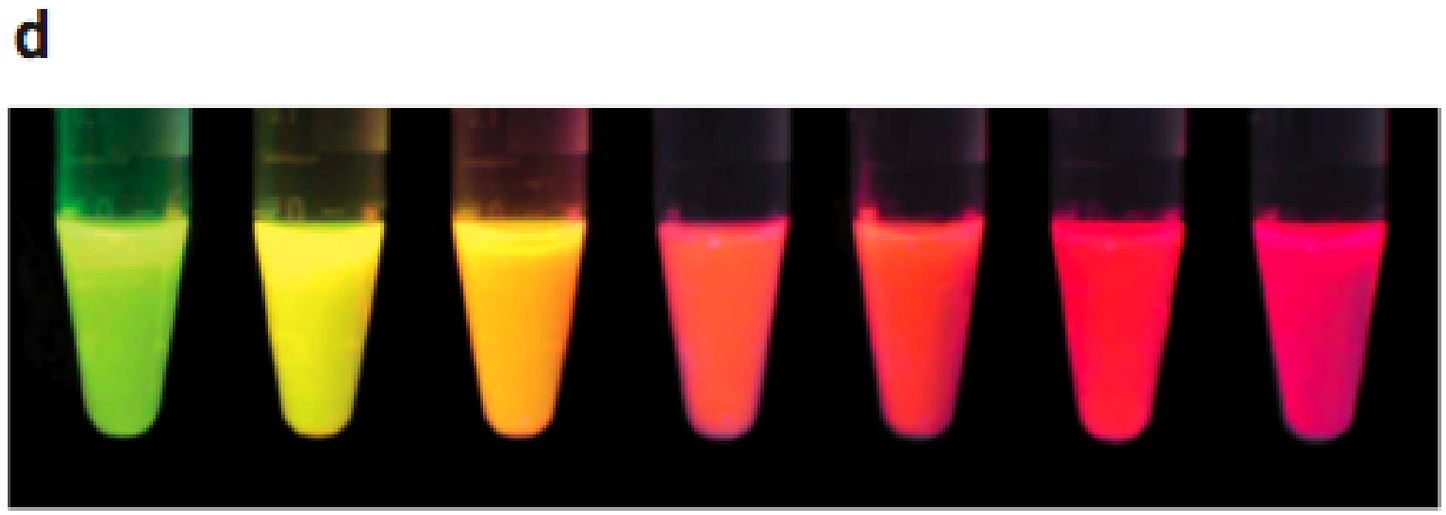




Multispectral fluorescent protein species







Chromophore Structural Motifs of Green Fluorescent Protein Variants

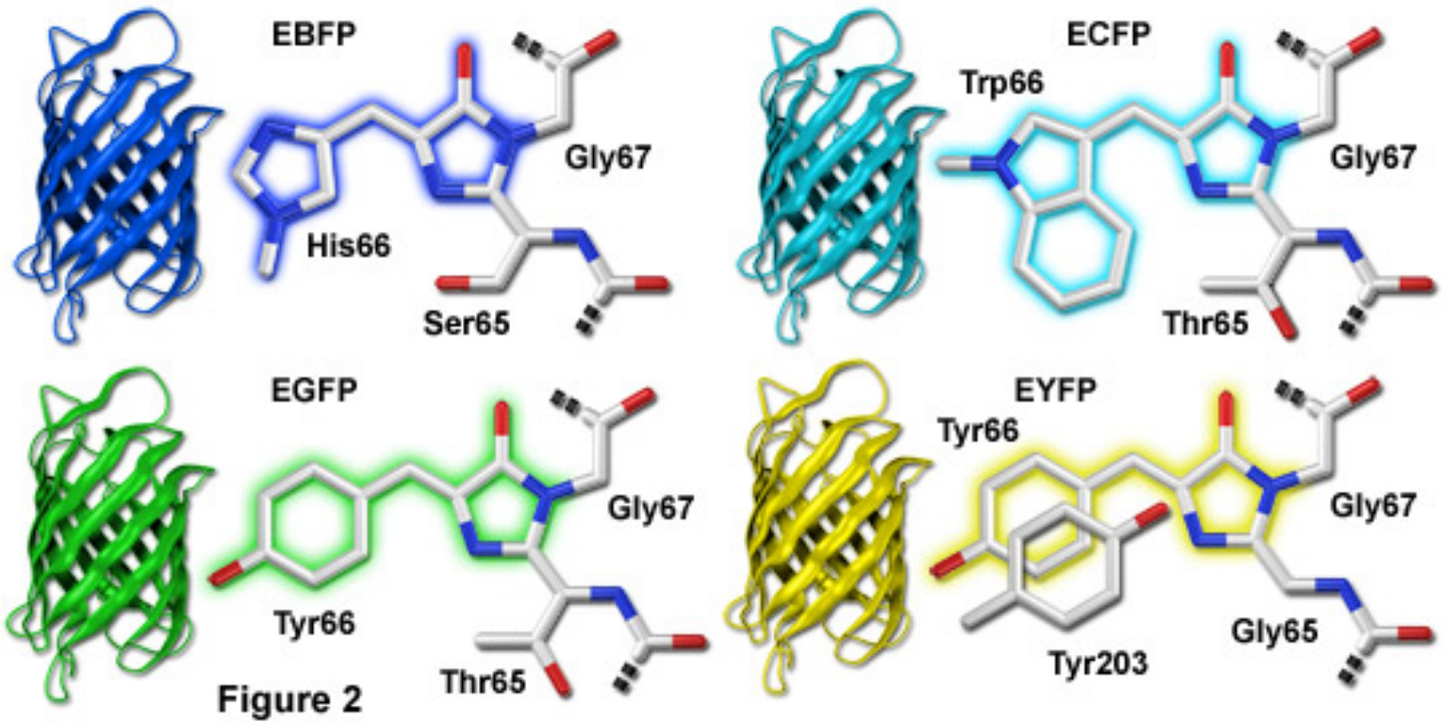
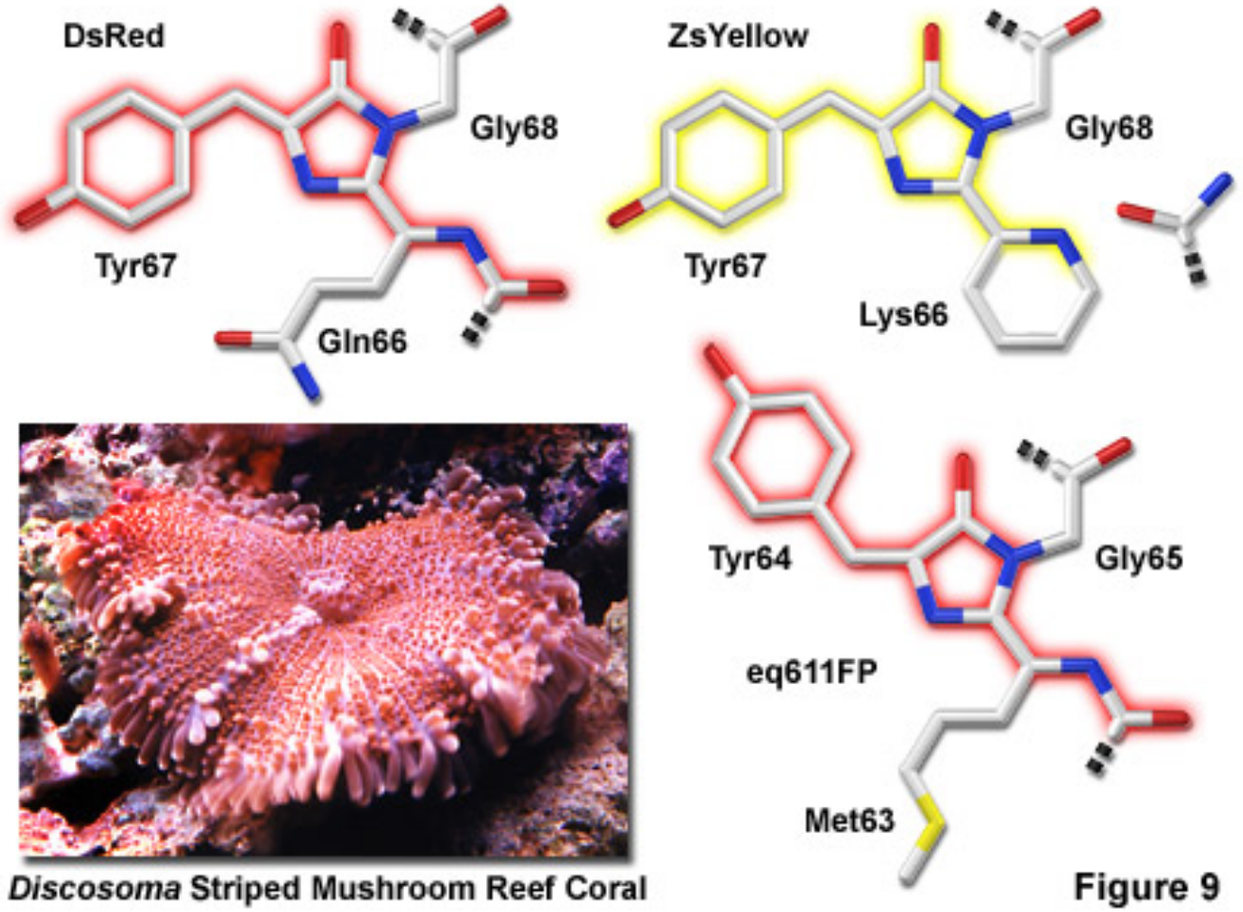


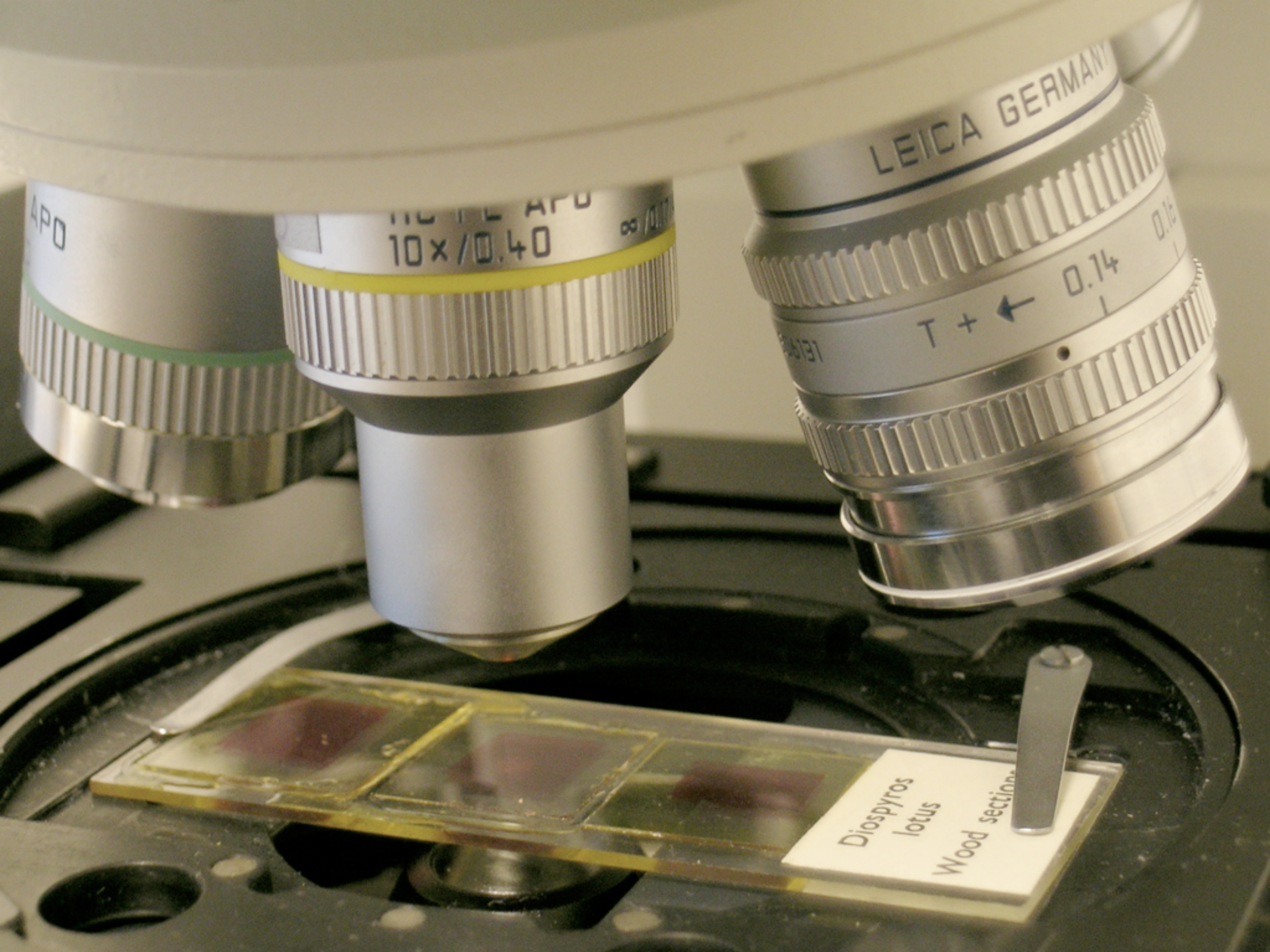
Figure 2

Chromophore Structure of Anthozoa Fluorescent Proteins



Discosoma Striped Mushroom Reef Coral

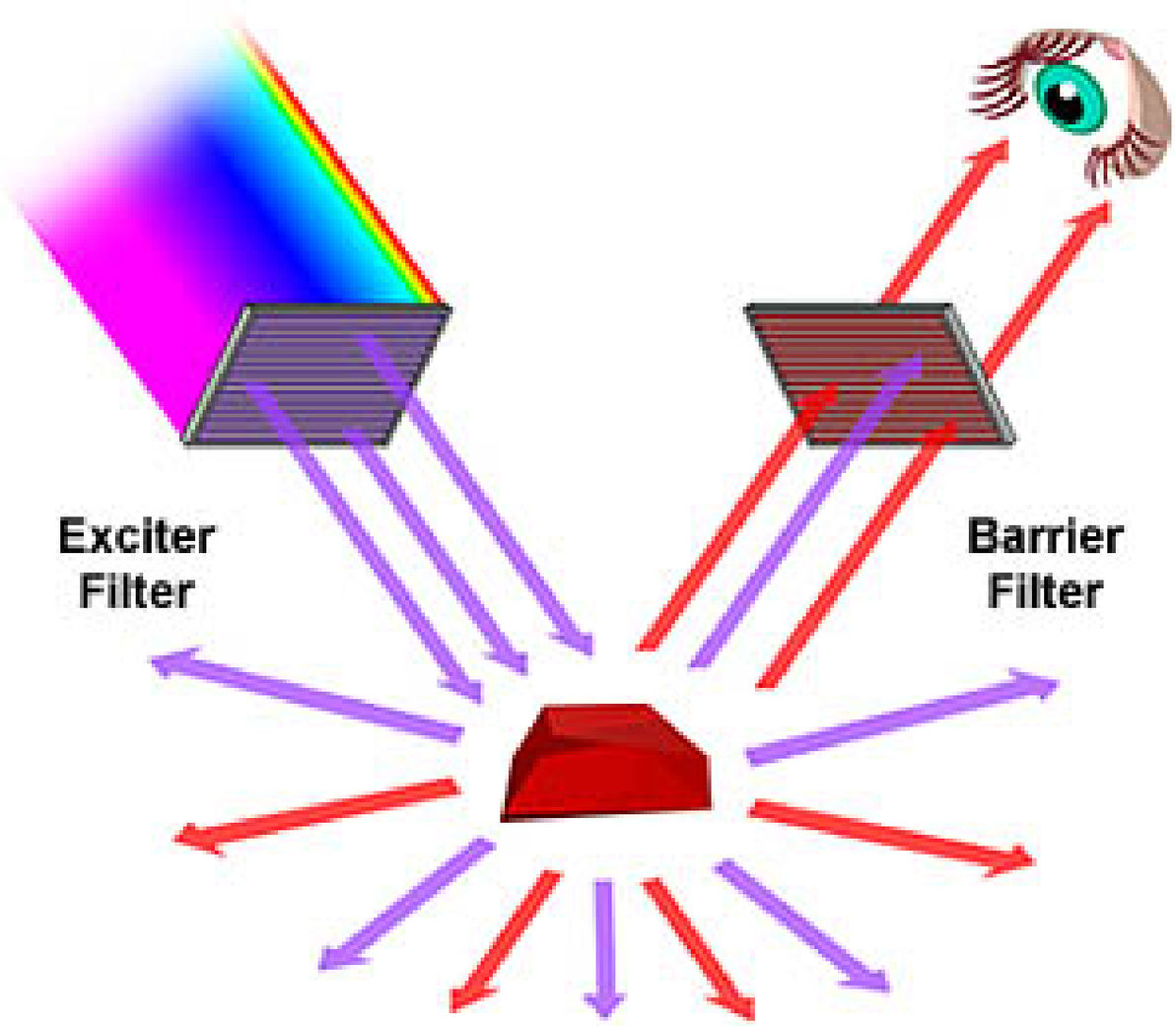
Figure 9

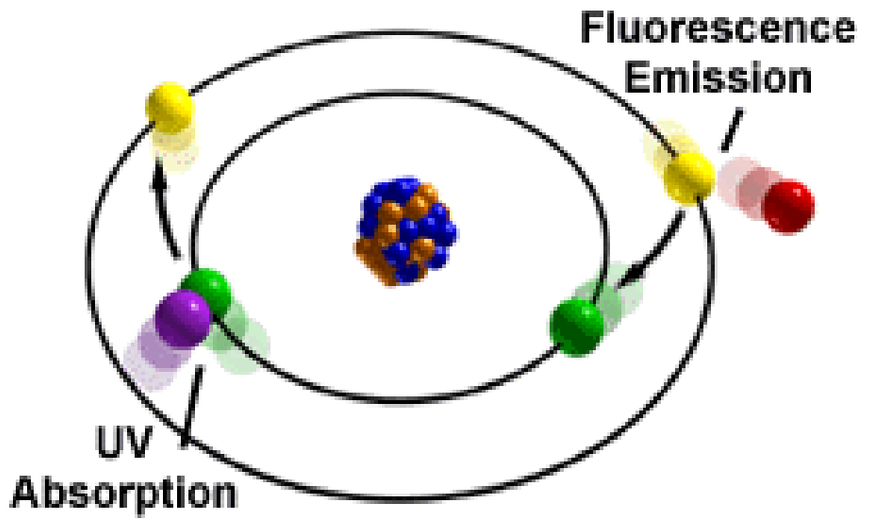

APO
10×/0.40
LEICA GERMANY
T +
0.14
Diospyros
lotus
Wood section

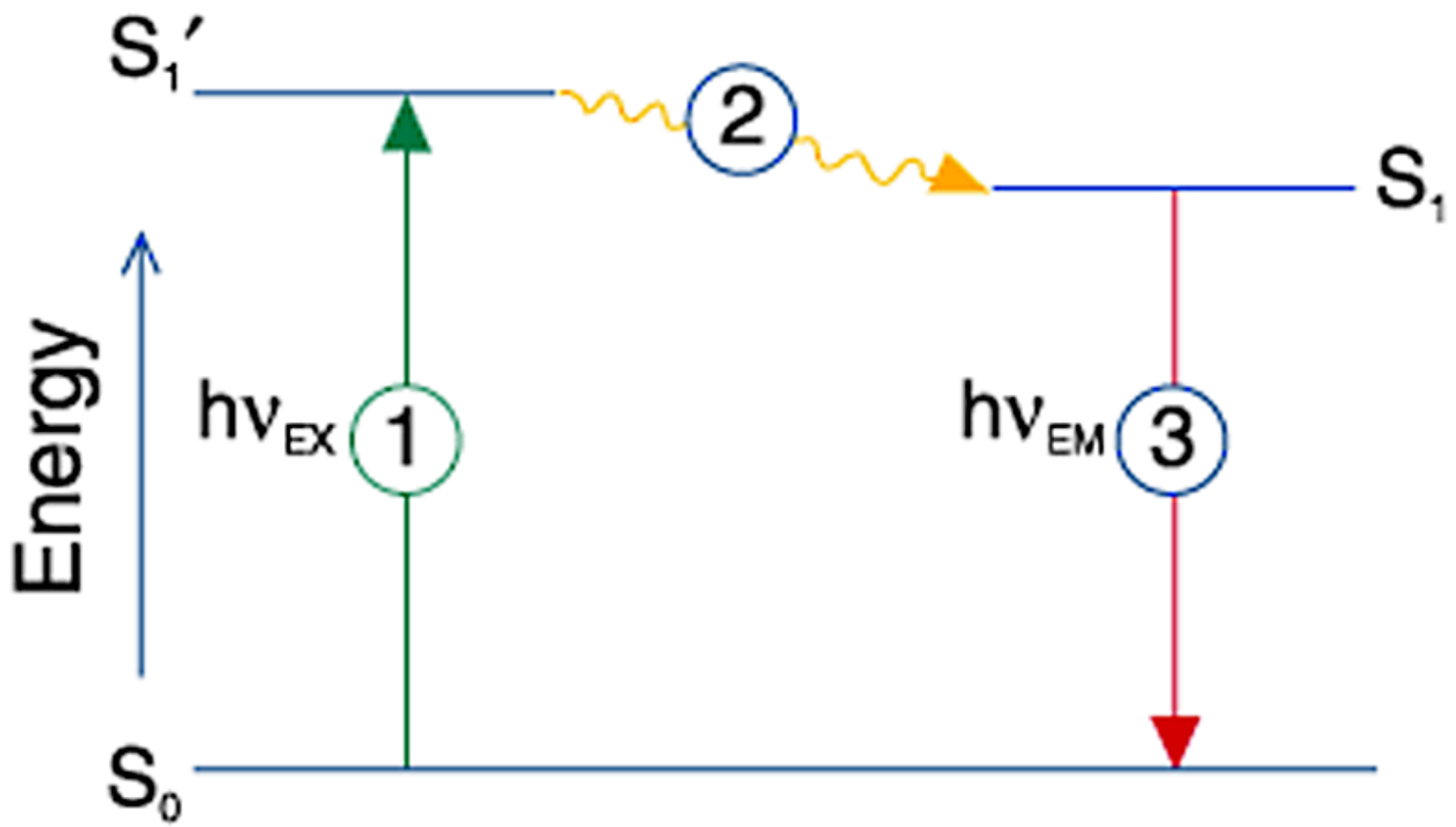
Benefits of fluorescence microscopy with FP's

New optical and computing methods allow selective, non-invasive imaging of fluorescent labels within intact cells.

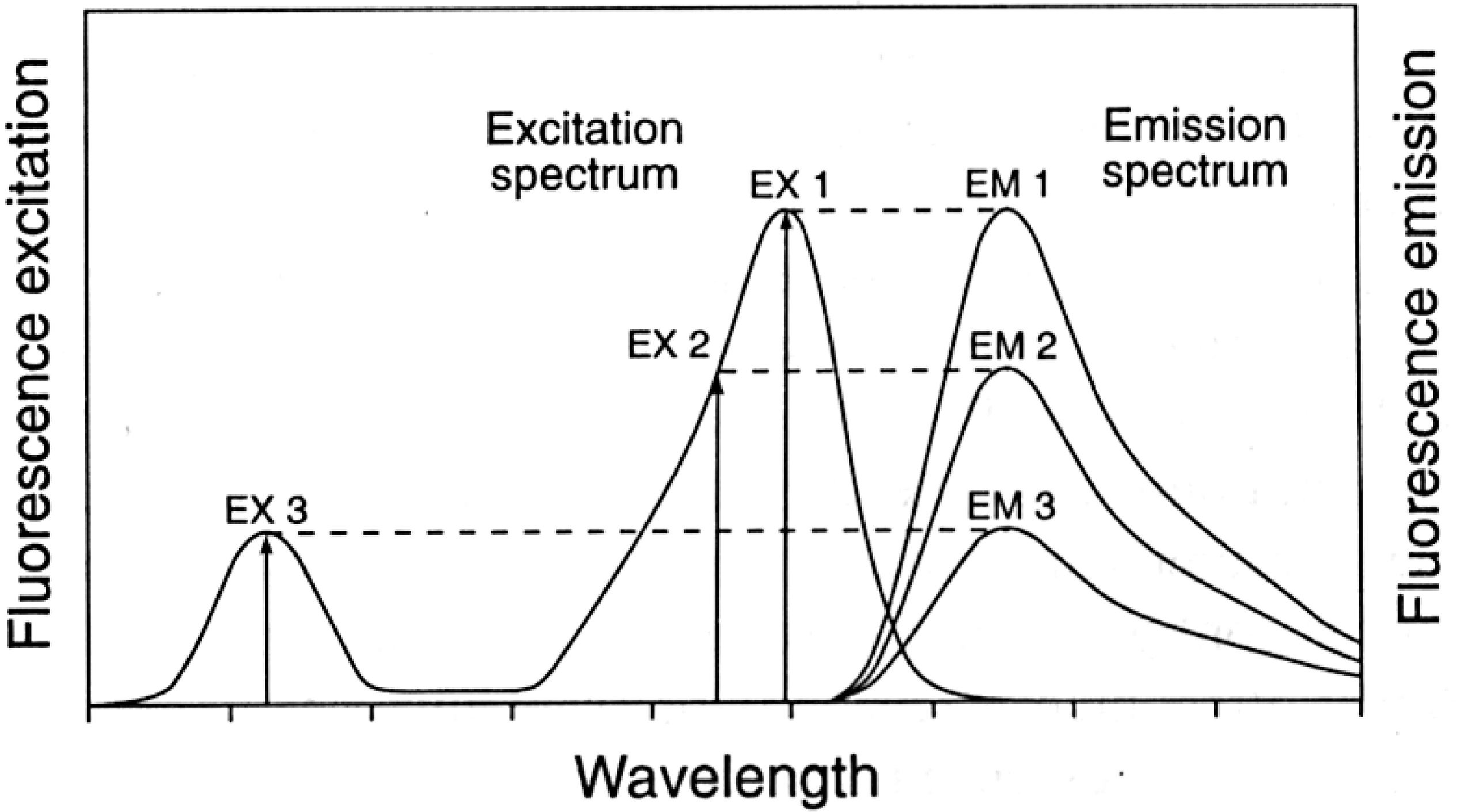
(i) Expression of fluorescent proteins allows live imaging
(ii) Fluorescent emission can be selectively filtered
(iii) Confocal imaging allows optical sectioning and 3D reconstruction



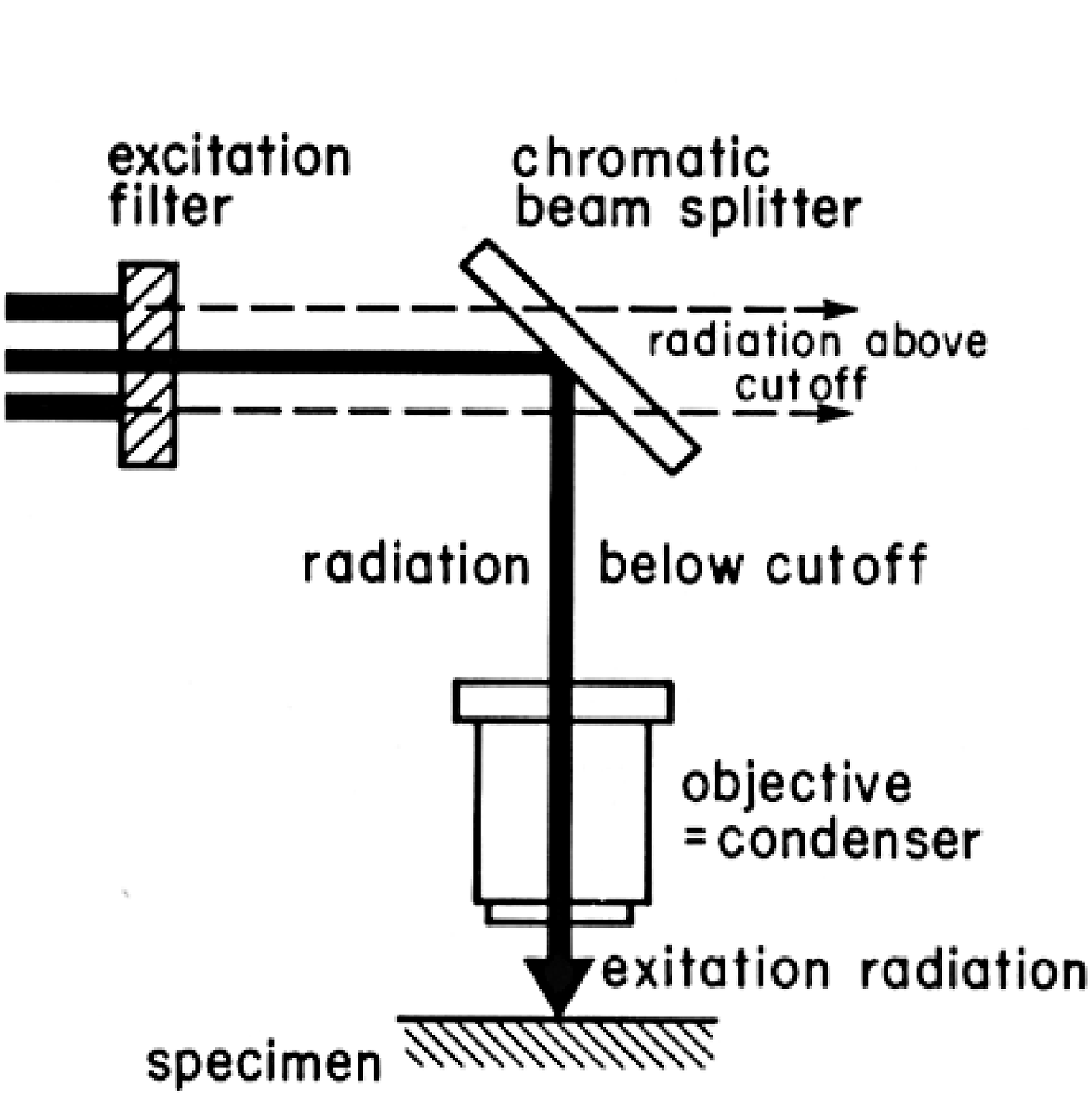


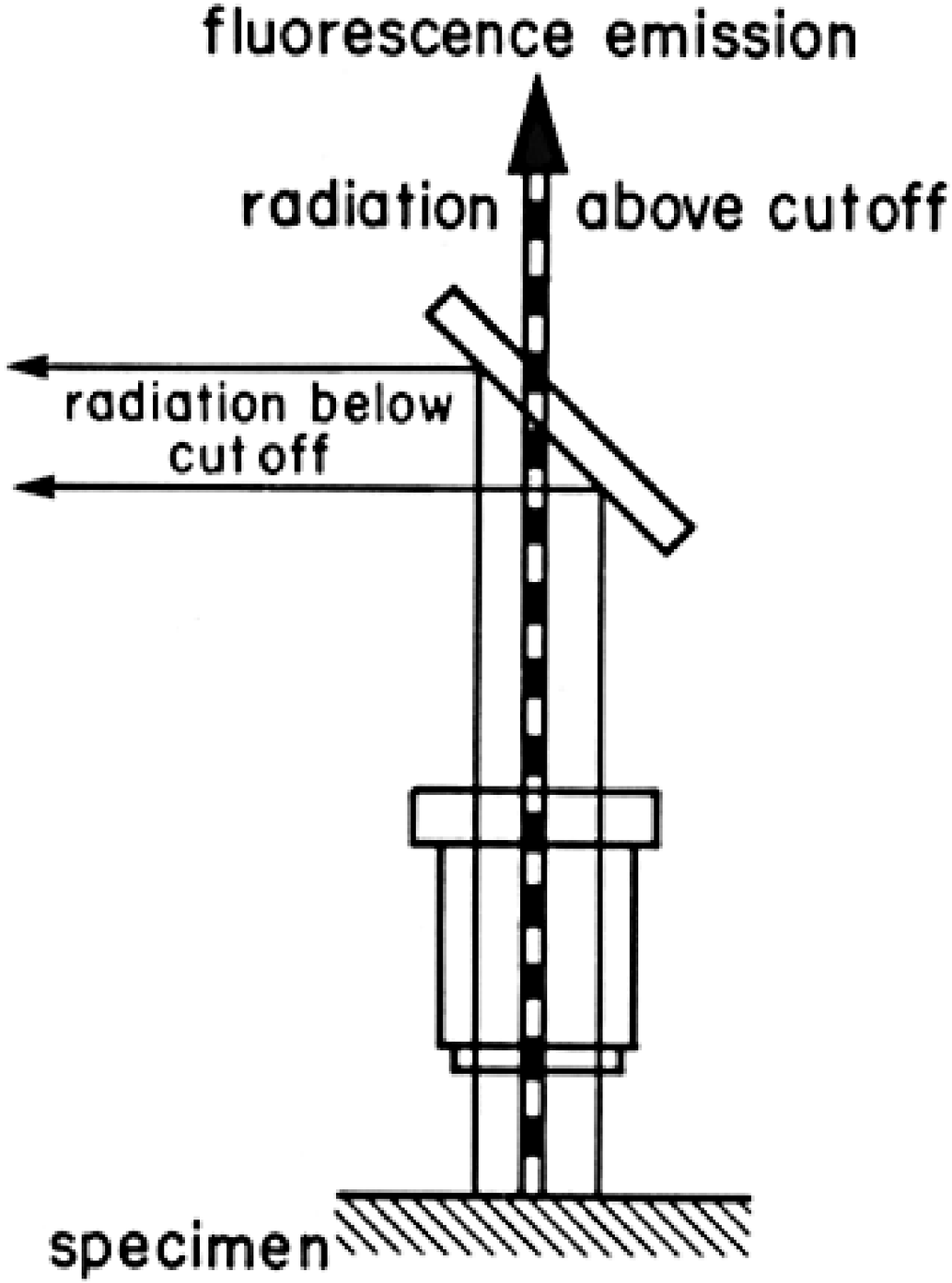


The light used for excitation of fluorescence is higher energy (shorter wavelength) than the emitted light



The light used for excitation of fluorescence is higher energy (shorter wavelength) than the emitted light





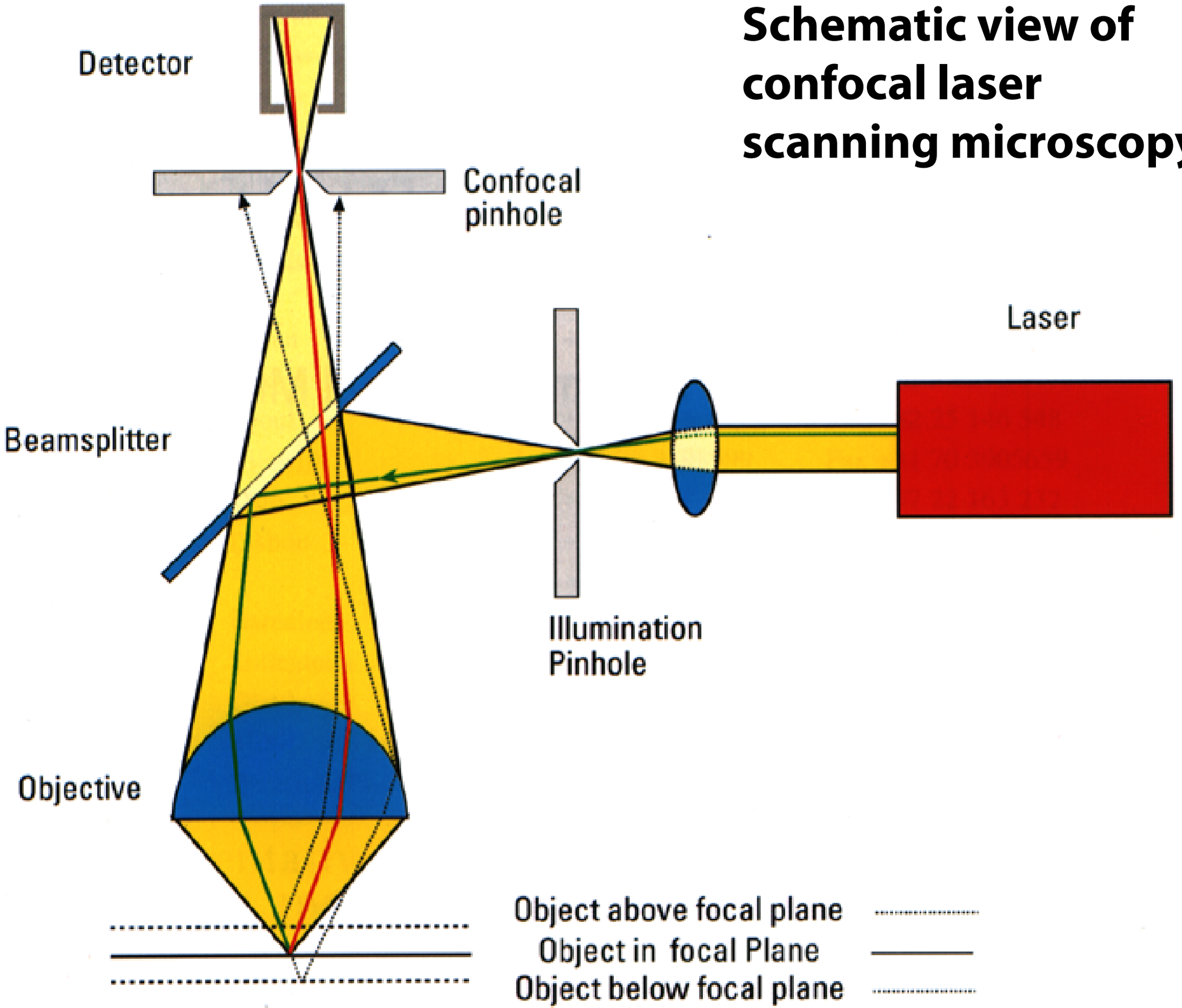
Excitation wavelengths can be separated from the emitted light using optical filters and beam splitter mirror

+35S::GFP

Arabidopsis seedlings

Arabidopsis seedling apex

Schematic view of confocal laser scanning microscopy

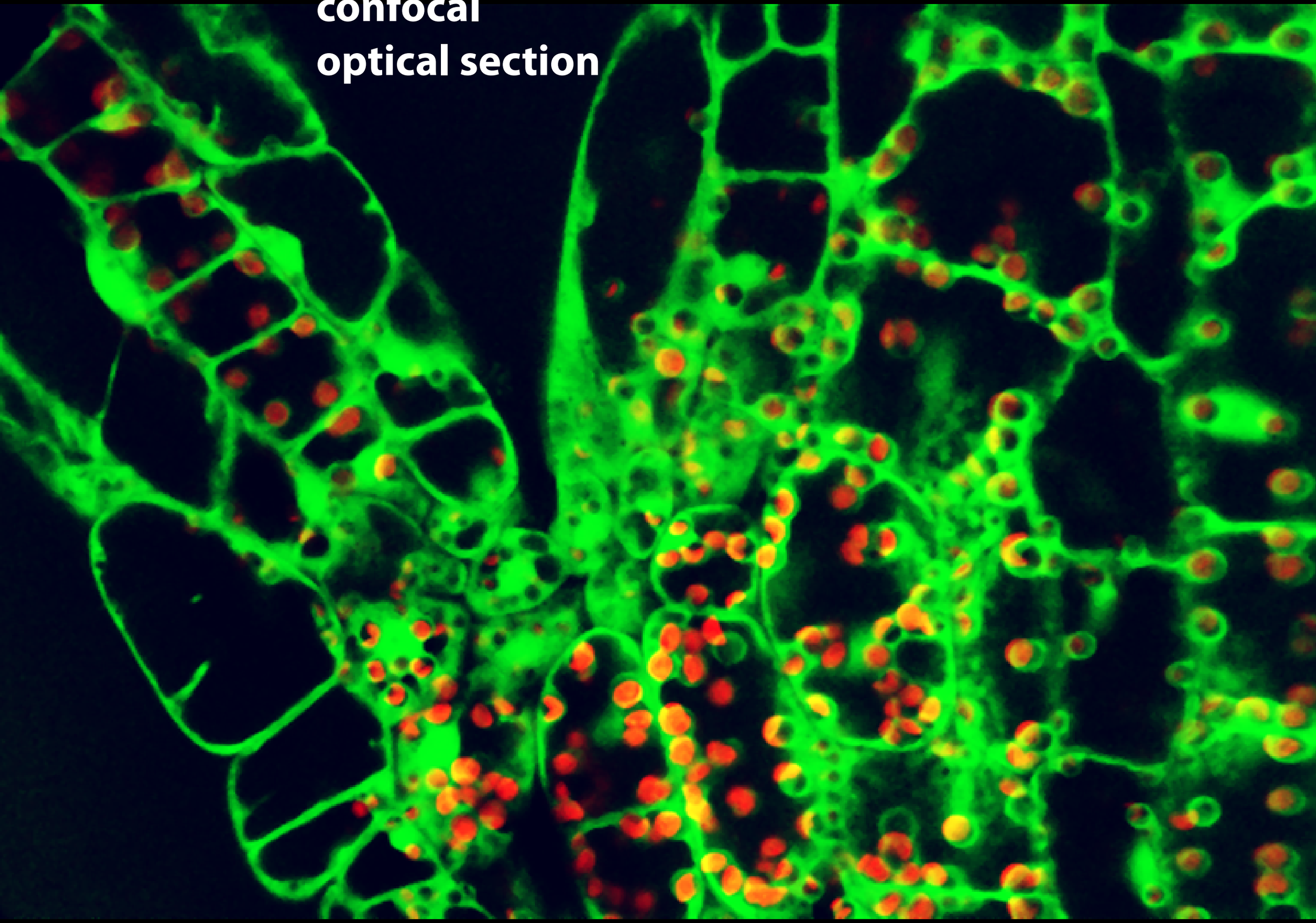


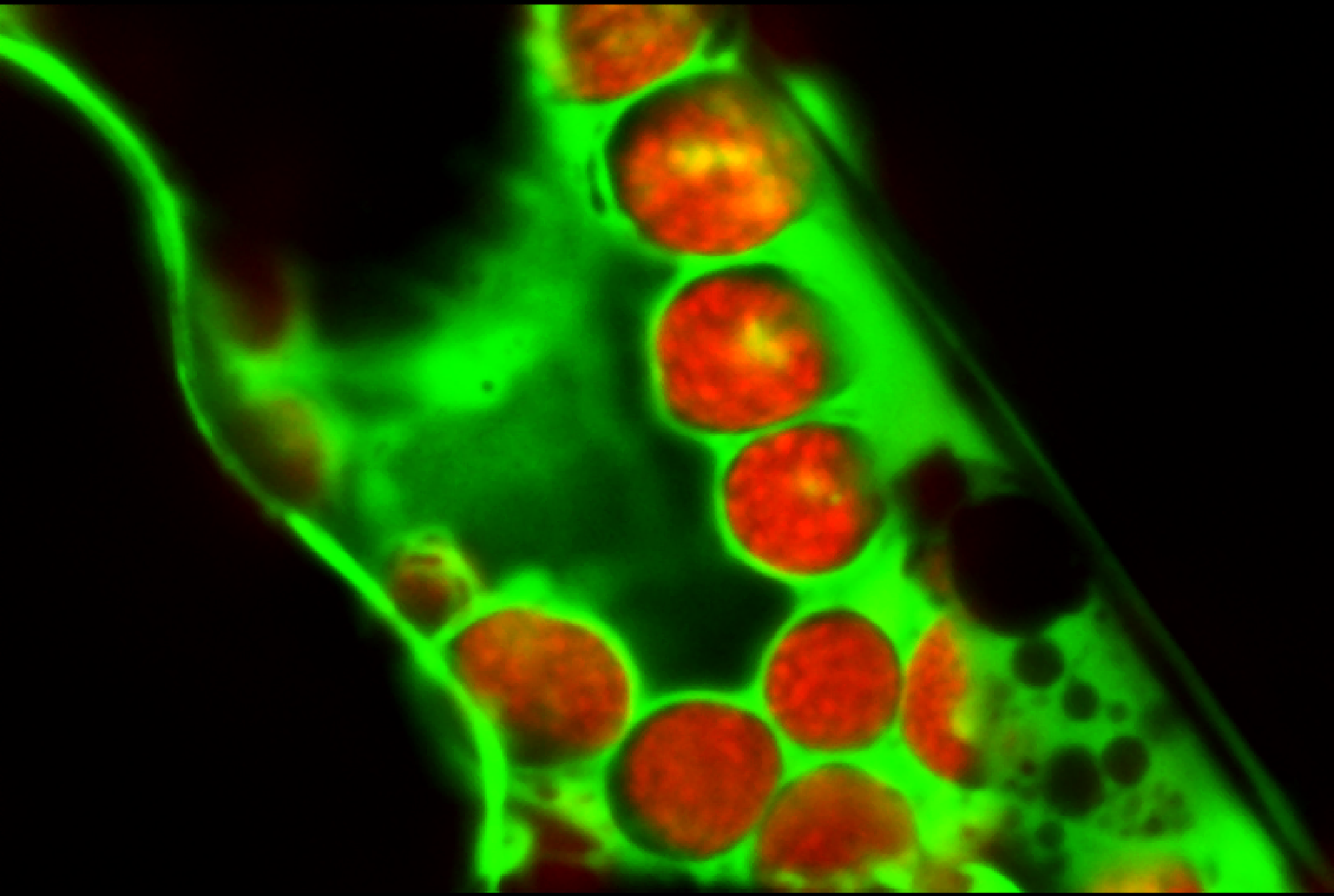

NEC
Newport

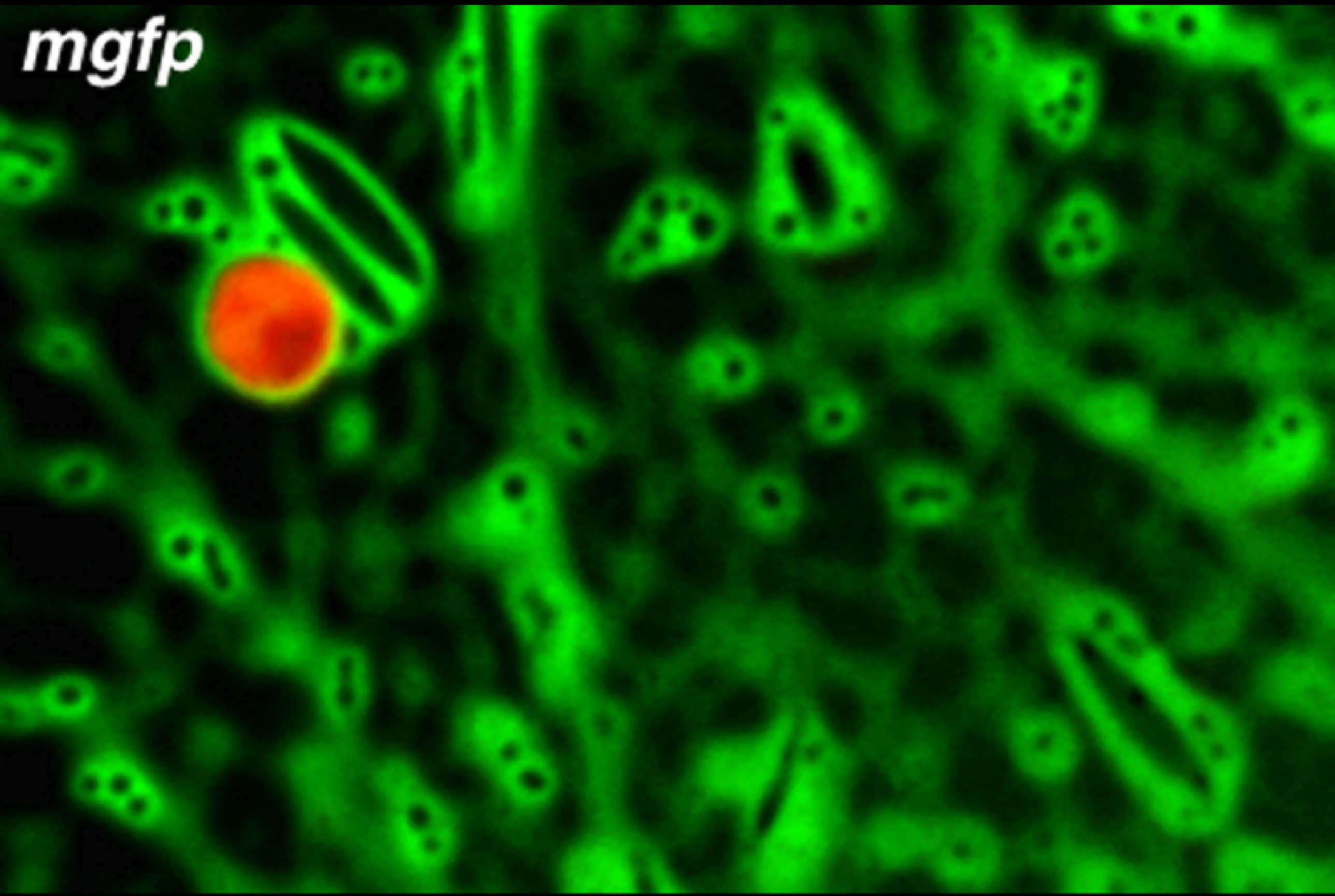
out-of-focus blur

Arabidopsis seedling apex

confocal
optical section

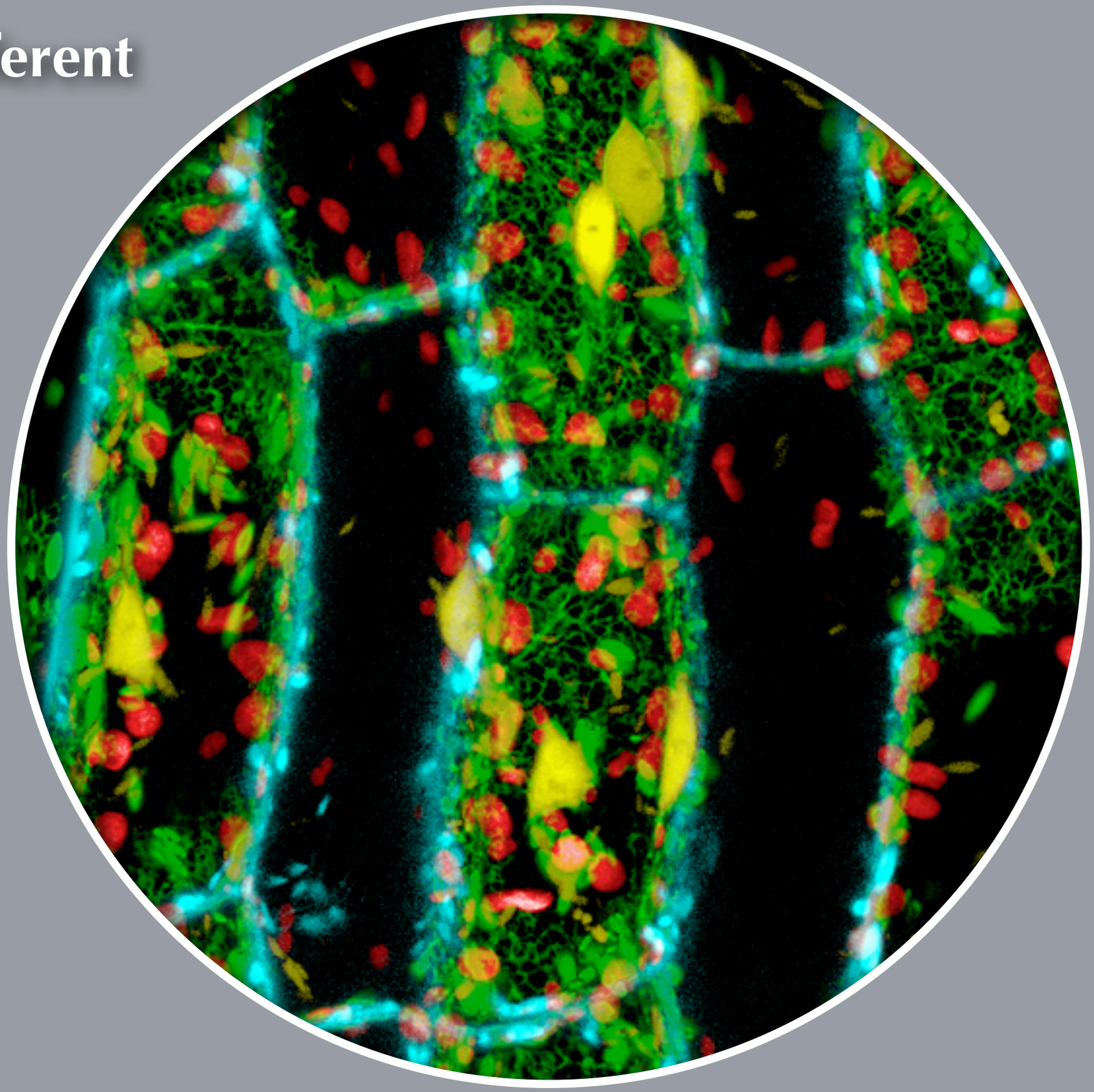




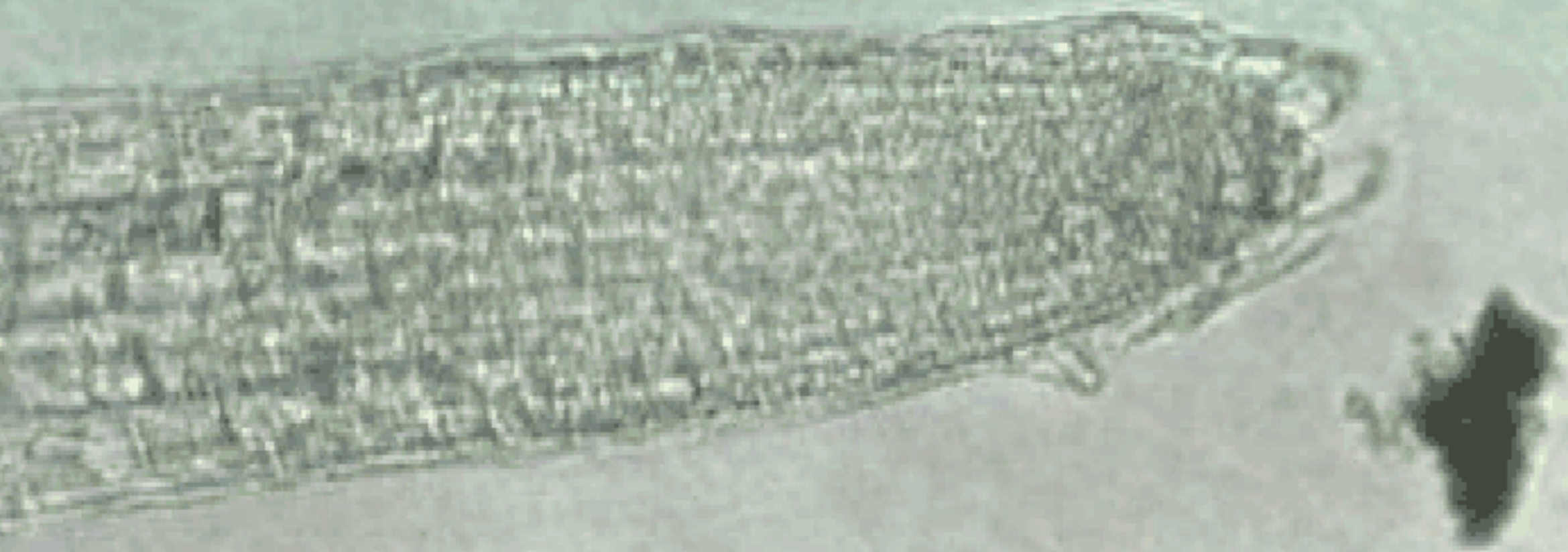

mgfp

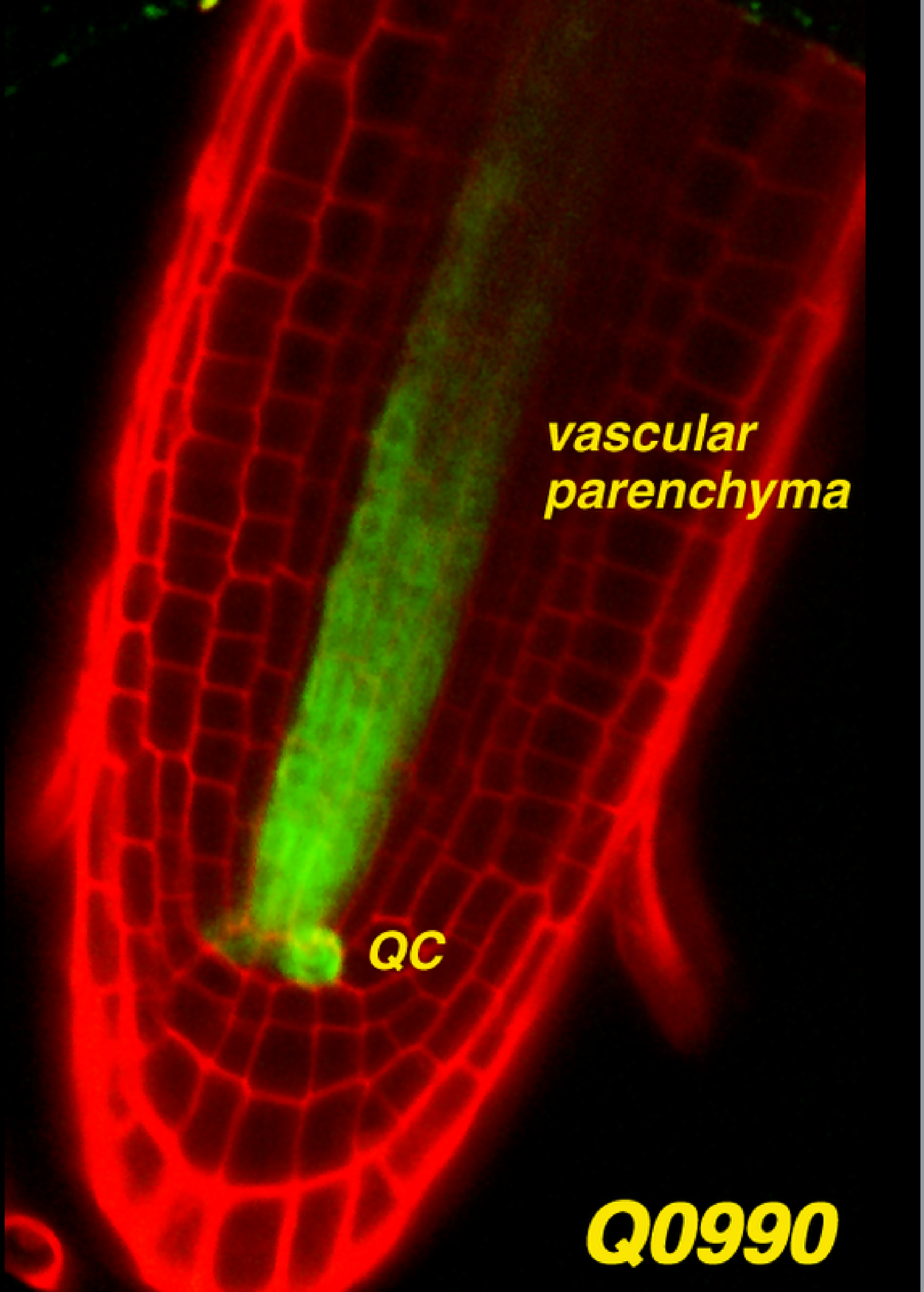
Multi-spectral imaging with different GFP variants

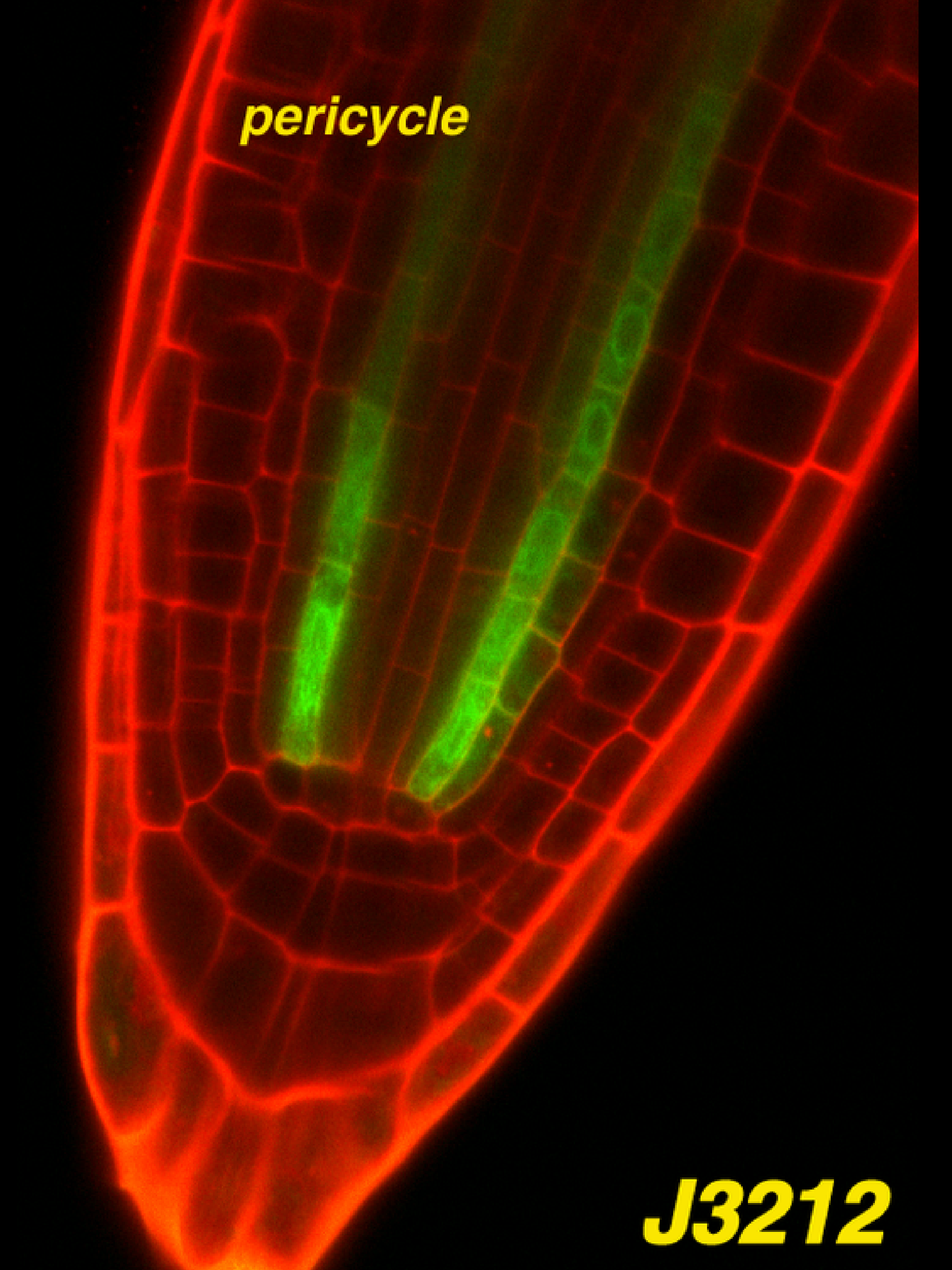
Extensin-CFP
GFP-ER
Histone2b-YFP
Chlorophyll



Indeterminate growth of the Arabidopsis root meristem

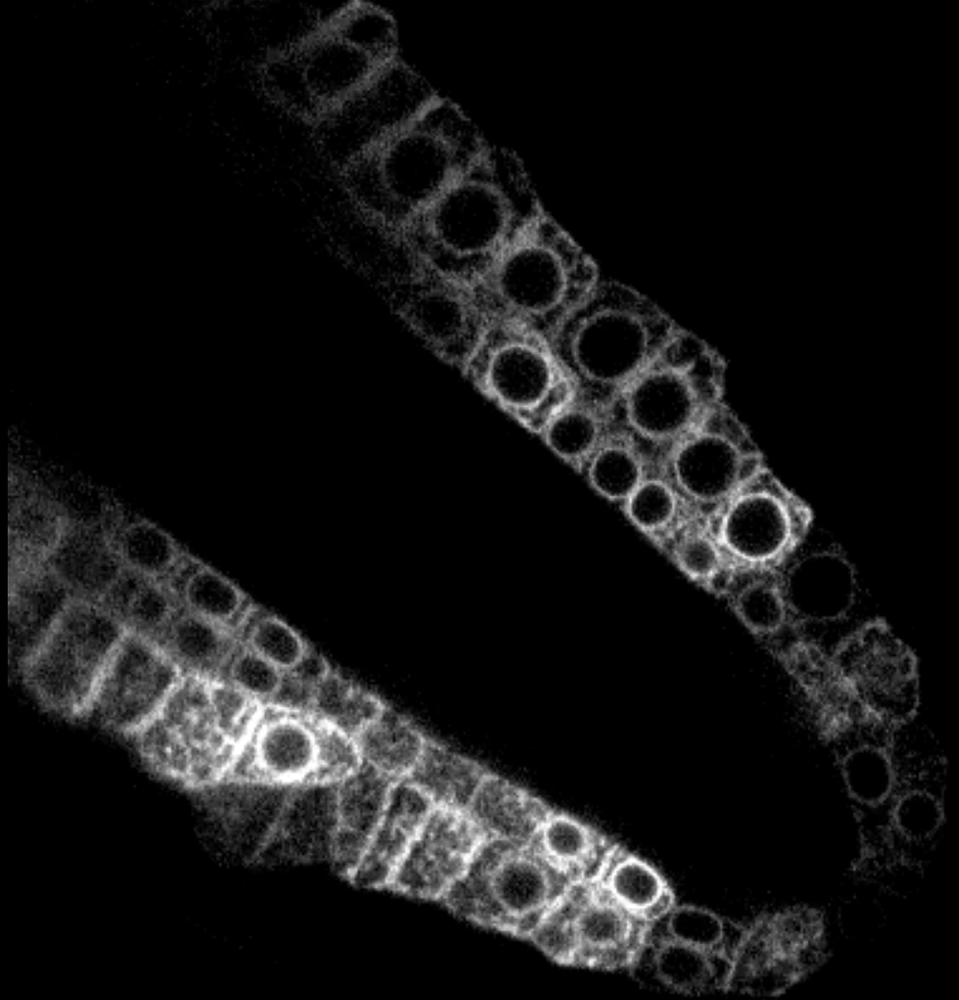



vascular
parenchyma
QC
Q0990

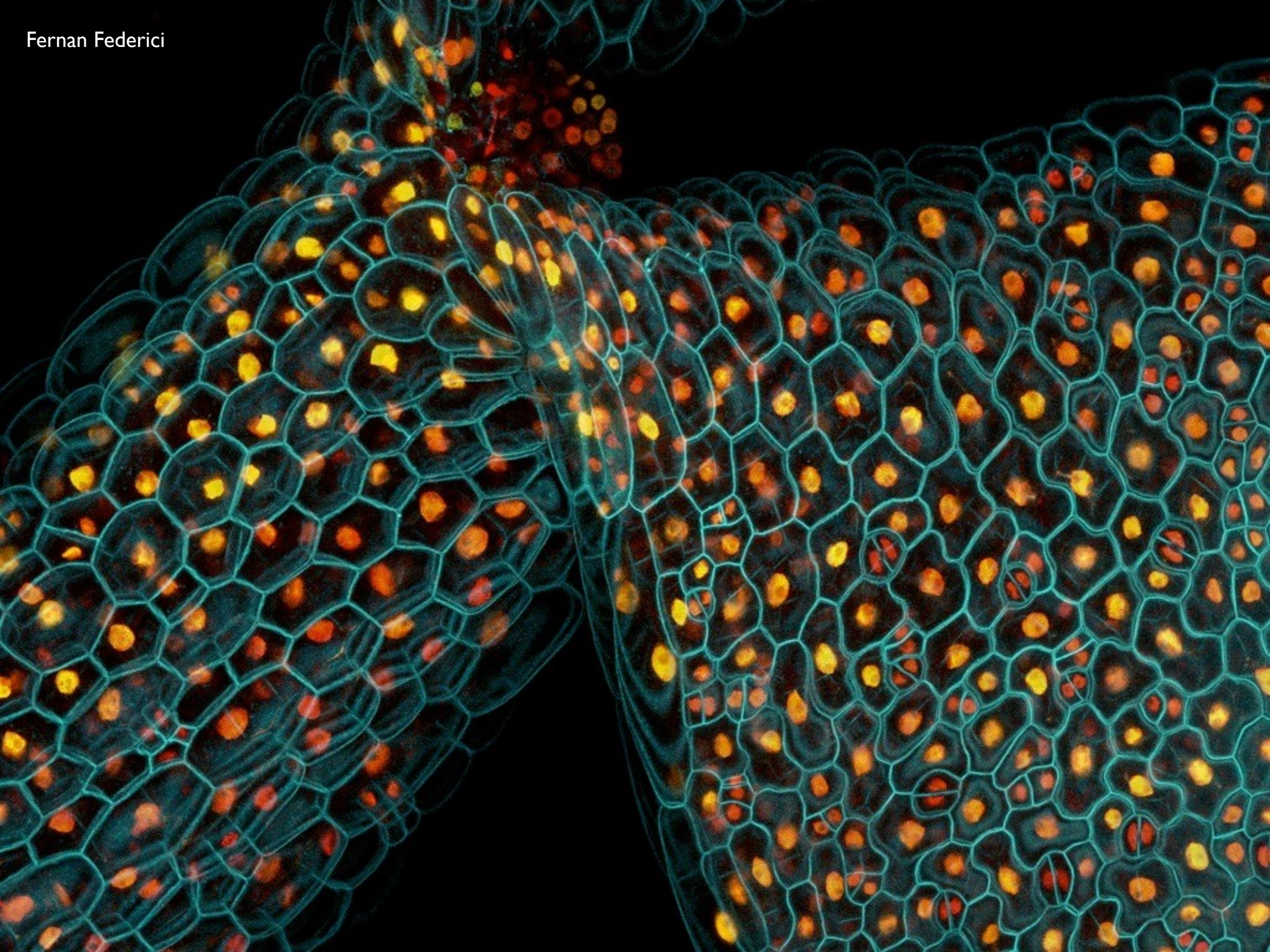

pericycle
J3212

endodermis
cortex

J0571



Fernan Federici



Credit: Dr. David Dobnik