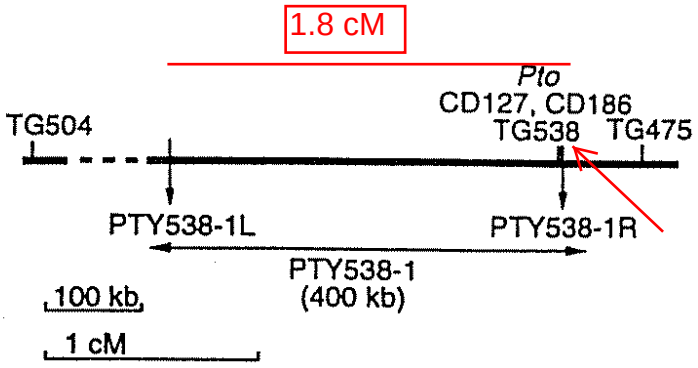
PLSC 731 -Plant Molecular Genetics
Second Half of Course
Higher Order Concepts

Map-based Cloning of Plant Genes

Phillip McClean

1. A genetic population of 251 F2 plants was screened with DNA probes, and the locus TG538 cosegregated with *Pto*.



From: Martin et al. 1993. Science 262:1432

2. A YAC library was screened with the TG538 probe, and the clone PTY538-1 was identified. Chromosome landing step

3. Primers that marked the end of the 400 kb clone were created, and the population was screened.

- PCR marker PTY538-1L was 1.8 cM from *Pto*
- PCR marker PTY538-1R cosegregated with *Pto*
- PCR marker PTY538-1R may be to the left of *Pto*; need to find a recombinant between this marker and TG538

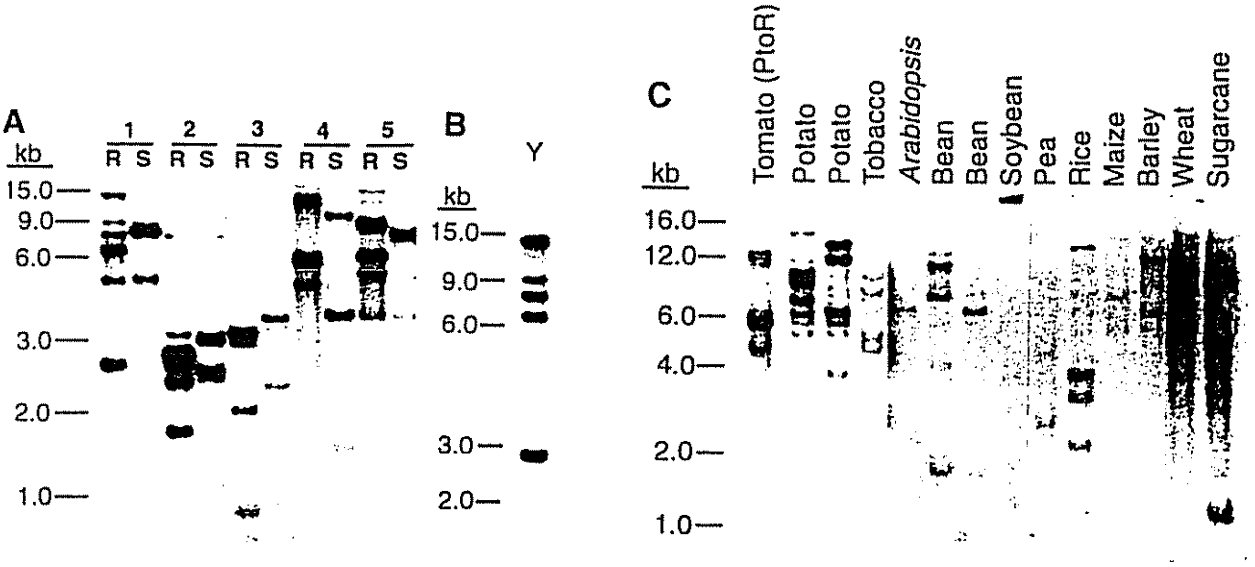
Is the Pto gene located on the clone?

4. 1300 plants from F2, F3 and cultivars were screened

- One plant with the *Pto* allele at the TG538 and the *pto* PTY538-1R allele was found. Thus, *Pto* must be located on the YAC clone PTY538-1.

5. A leaf cDNA library was screened with PTY538-1 DNA
   - 200 clones were found and 30 analyzed
   - 50 plants with recombination in the *Pto* region was screened
   - cDNA clone CD127 cosegregated with *Pto*.

6. Southern hybridization with CD127 detected many polymorphic fragments
   - **PANEL** A: The clone could be detecting exons or a multigene family
   - 14 other cross hybridizing clones were found that all represented six different classes of related genes
   - **PANEL B**: Analyses of PTY538-1 detected all (but one) of the genomic fragments that were detected with Southern hybridization.
   - It was concluded that PTY538-1 contained a multigene family.
   - **PANEL C:** Clone hybridized to multiple fragments in multiple species; clone detects a multigene family in other species.



7. Transformation vectors were created with CD127 (1.2 kb insert; vector pPTC5) and CD186 (2.4 kb insert; vector pPTC8).

- Two pPTC8 resistant plants were found.
- No pPTC5 resistant plants were found.

8. One resistant plant (genotype=*Pto*/*Pto*) was crossed to a susceptible cultivar (genotype=*pto*/*pto*).

- 9 plants contained the CD186 sequence and were resistant to the bacterial speck pathogen
- 13 without CD186 were susceptible to bacterial speck.

9. The clone CD186 was sequenced, and a 321 amino acid opening reading frame was found.

- The sequence of the open reading frame was similar to a serine-threonine protein kinase.

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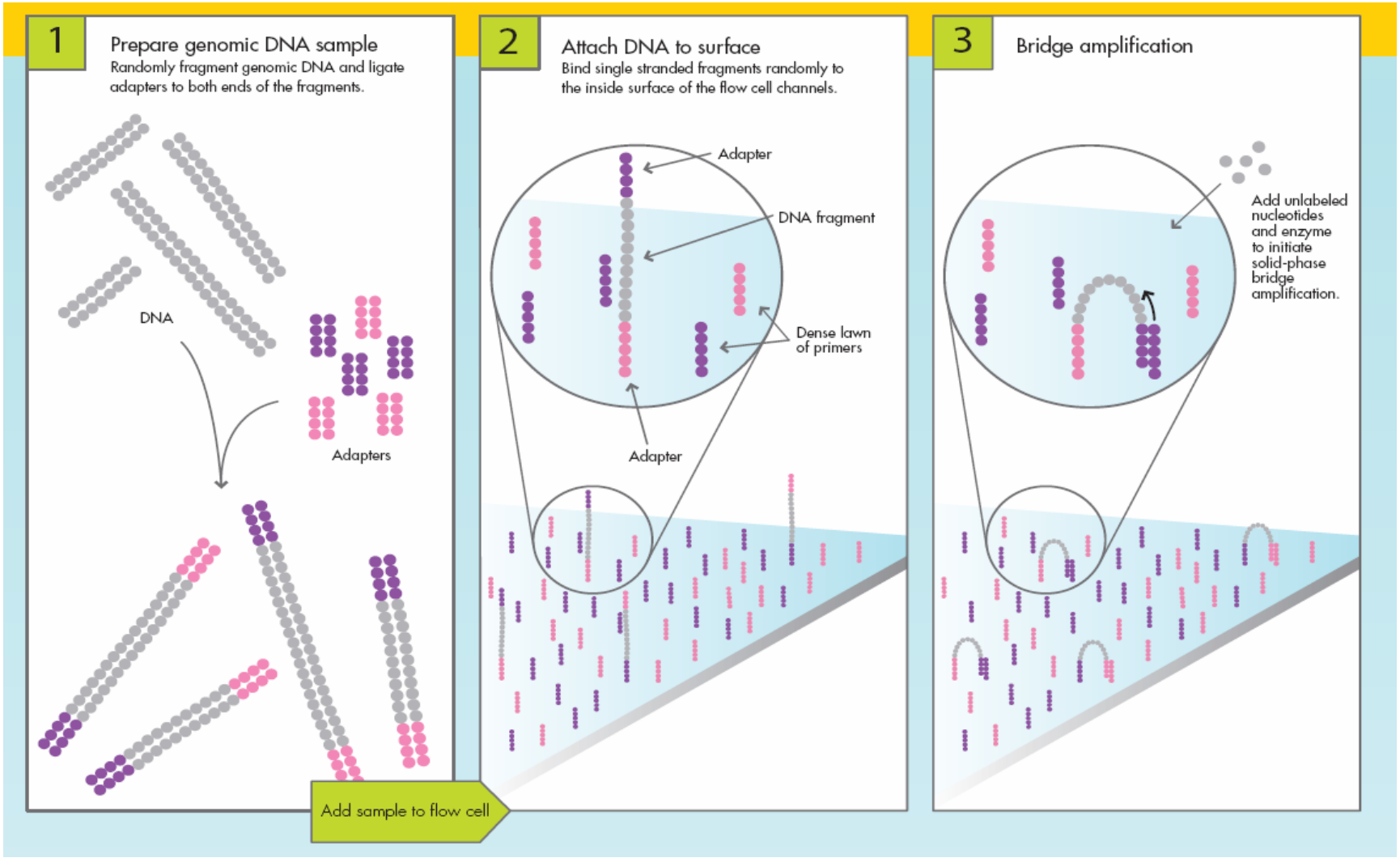
Genome Sequencing

Phillip McClean

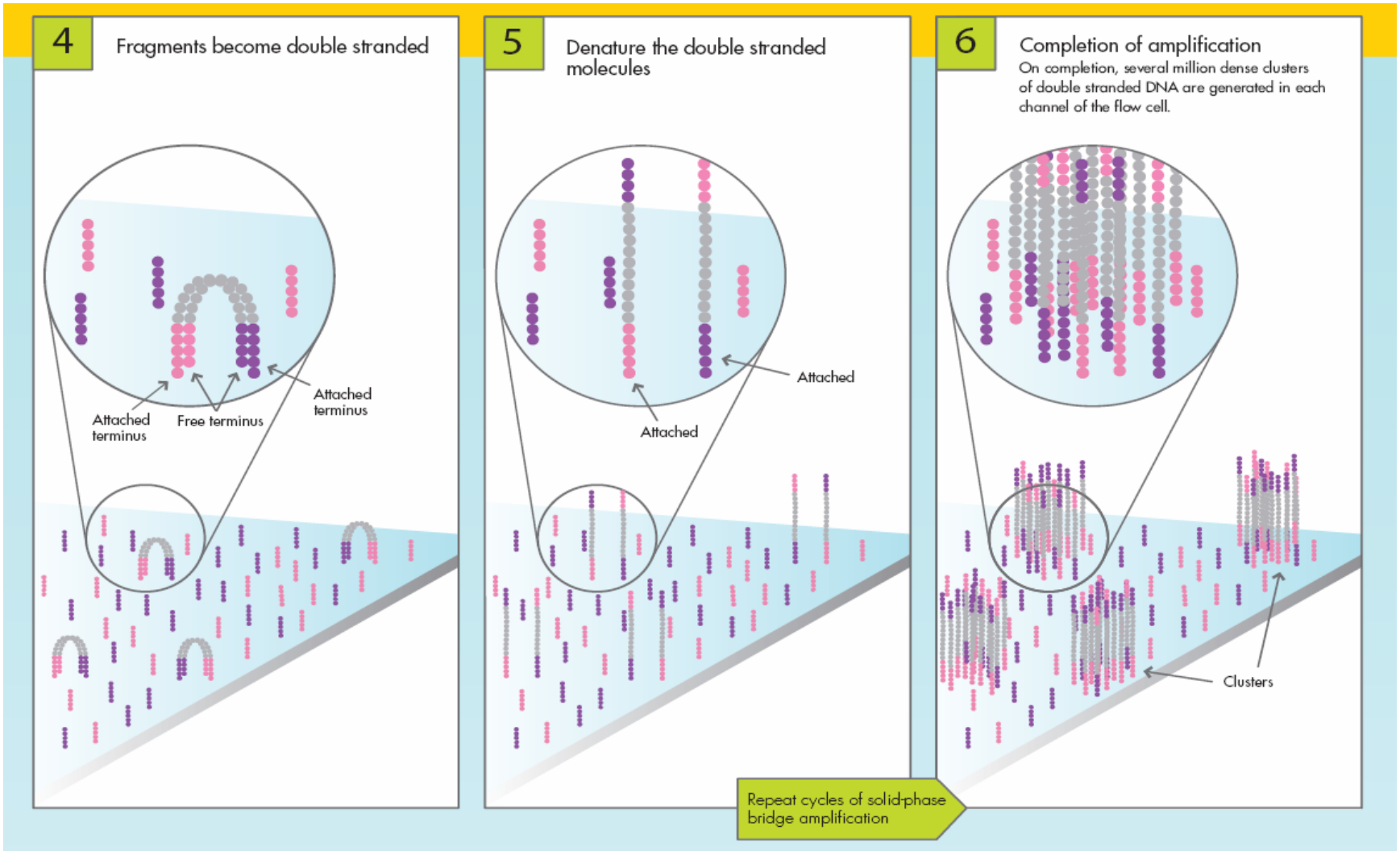
TODAY, Illumina is the MARKET LEADER in high throughput sequencing.

Illumina Sequencing by Synthesis Technology

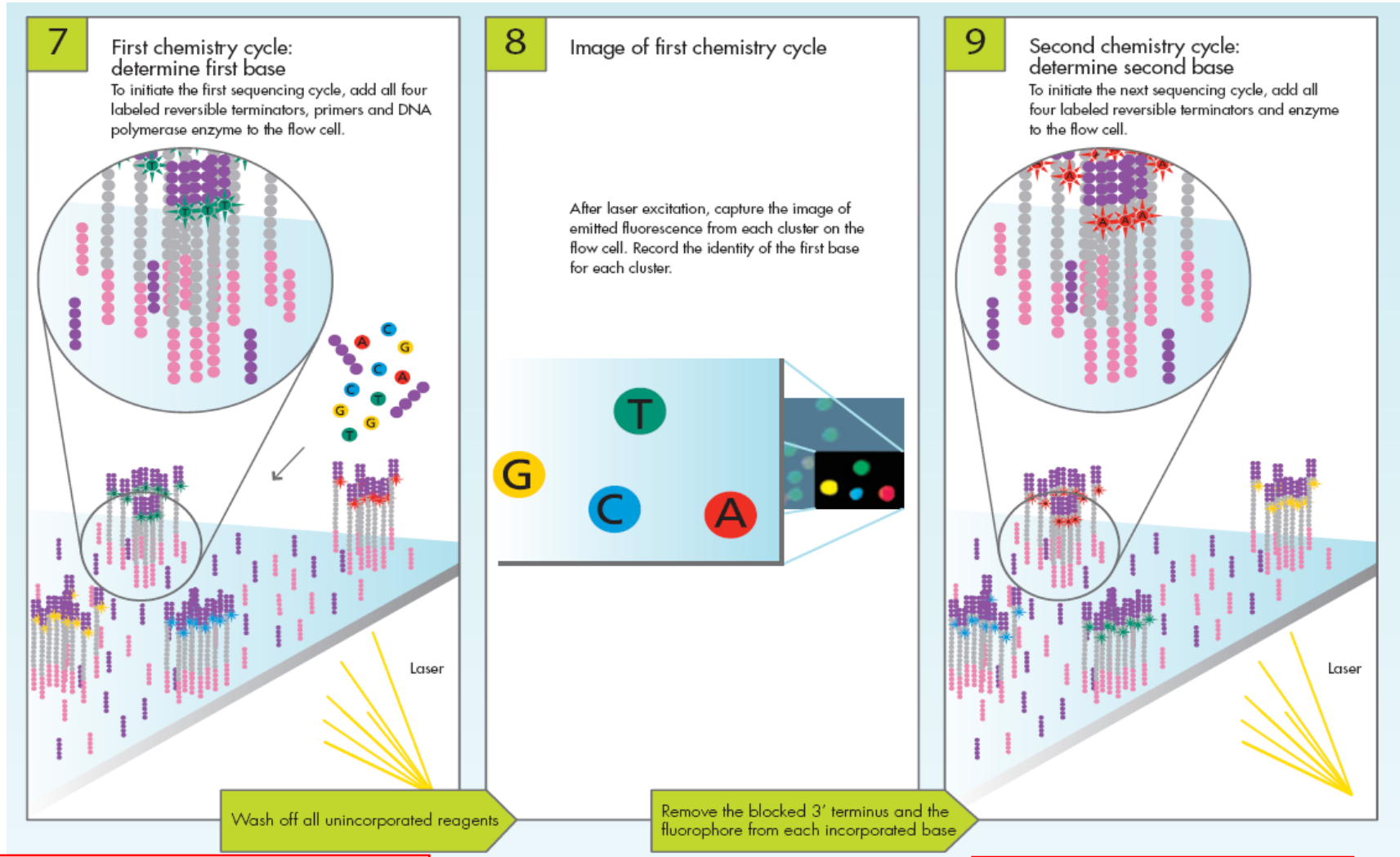


A SINGLE STRAND molecule is bound to the flow cell.

BRIDGE AMPLIFICATION:
***Steps 3-6**



EVERY MOLECULE IN THE CLUSTER IS AN IDENTICAL TEMPLATE FOR SEQUENCING!!



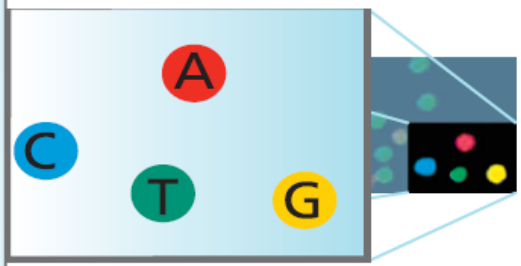
The first base is added to the template of each cluster with a blocker that prevents other bases from being added.

A PICTURE is taken of the flow cell; the color emitted determines the base added to the cluster. The blocker is removed.

The LAST TWO steps are repeated until the desired read length is reached.

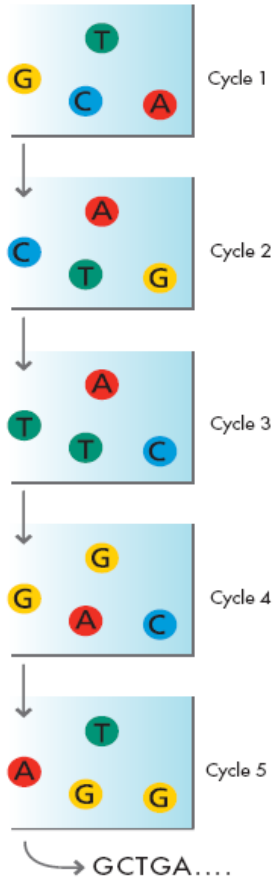
10 Image of second chemistry cycle is captured by the instrument

After laser excitation, collect the image data as before. Record the identity of the second base for each cluster.

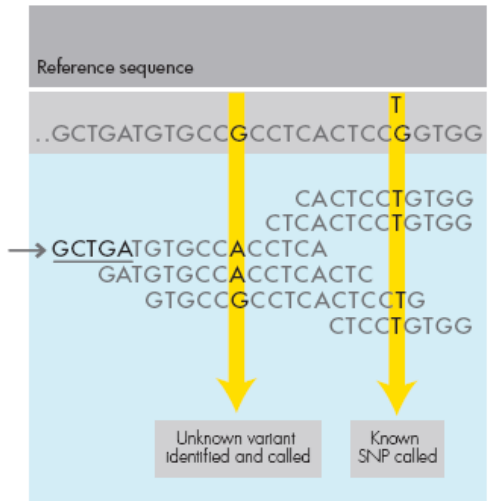


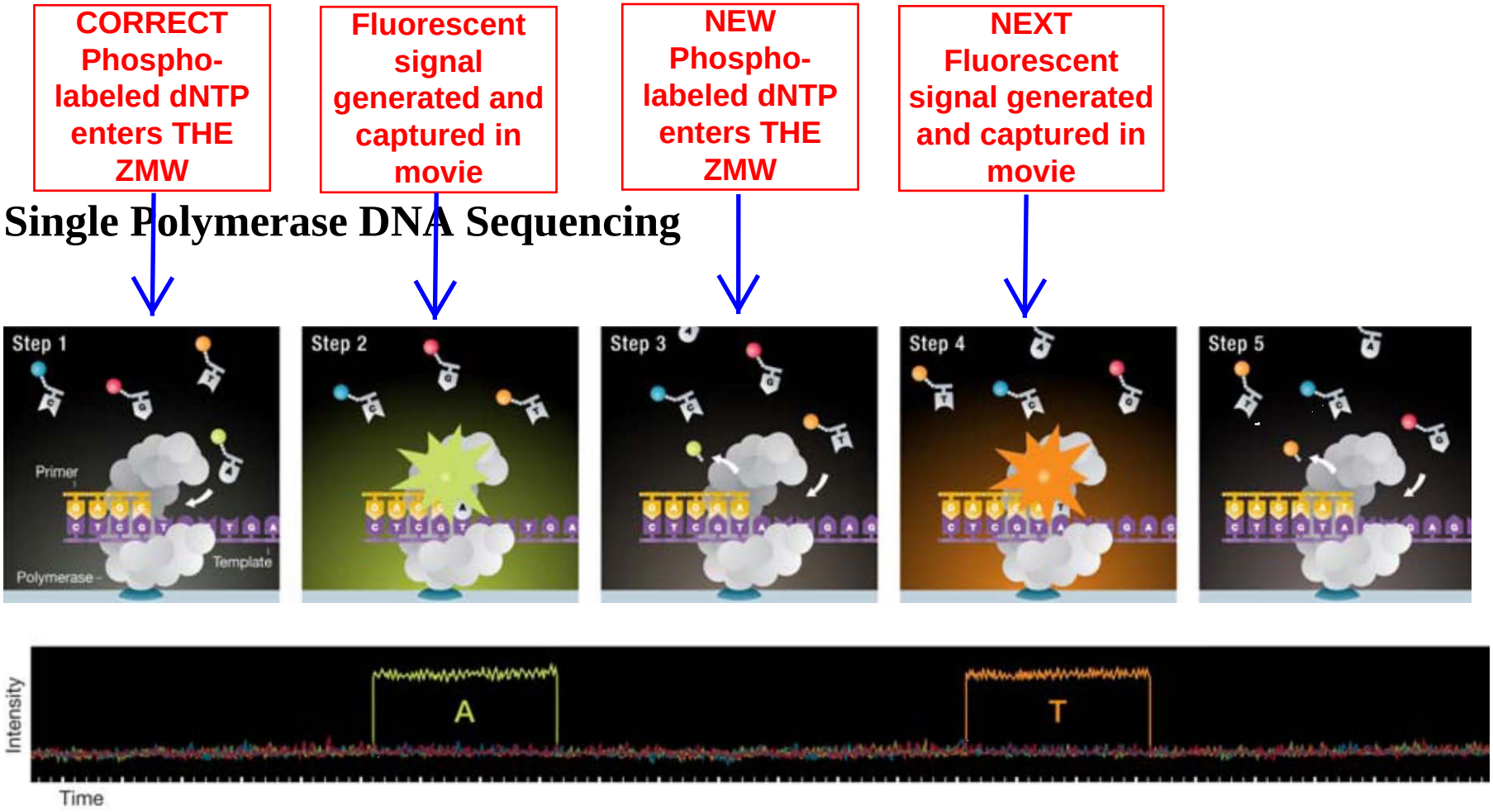
11 Sequence read over multiple chemistry cycles

Repeat cycles of sequencing to determine the sequence of bases in a given fragment a single base at a time.



12 Align the new data to a reference and identify sequence differences





Step 1: Fluorescent phospholinked labeled nucleotides are introduced into the ZMW.
Step 2: The base being incorporated is held in the detection volume for tens of milliseconds, producing a bright flash of light.
Step 3: The phosphate chain is cleaved, releasing the attached dye molecule.
Step 4-5: The process repeats.

Modern Long Read PacBio Sequencing Genome Sequencing Approach

1. Create 20kb insert libraries

2. Sequence with PacBio single molecule technology
   - Reads generally 10-15 kb in length

3. Add short read (150bp) paired end data to correct for inherent PacBio errors

4. Assembly reads into contigs
   - Contigs MUCH longer than with Sanger sequencing

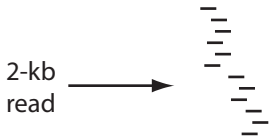
5. Scaffolds developed by long-range scaffolding methods
   - BioNano restriction enzyme mapping
   - Hi-C cross-linked DNA library sequencing
   - 10X linked read sequencing

6. Pseudochromosomes assembled based on homology of scaffolds to the markers located on a high-density genetic map

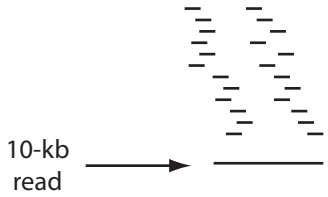
Scaffold Assembly

Building a Scaffold Using Paired-end Reads of Different Sized Sequences

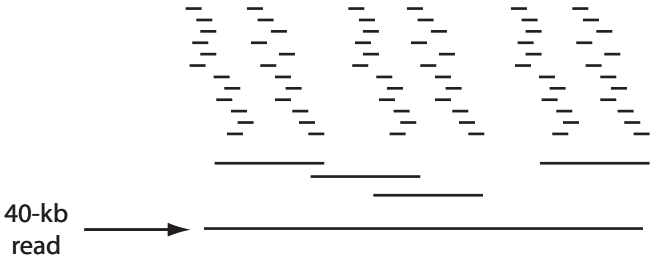
Step 1: Build a contig with overlapping 2-kb paired-end reads



Step 2: Link two contigs with 10-kb paired-end reads

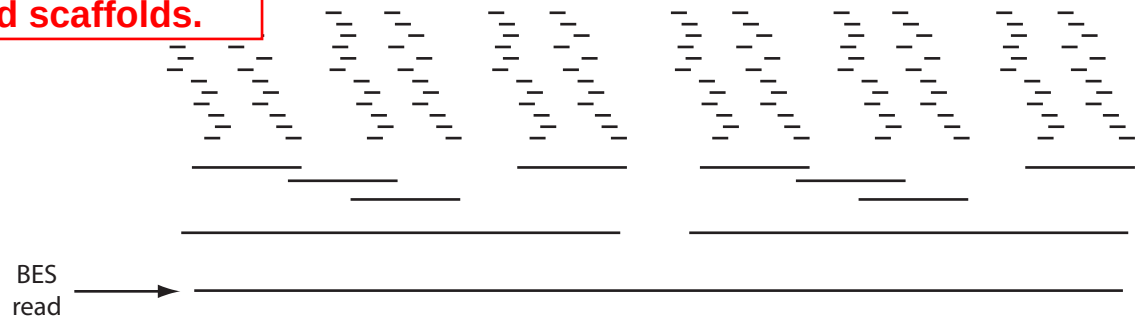


Step 3: Link three 10-kb contigs with 40-kb paired-end reads

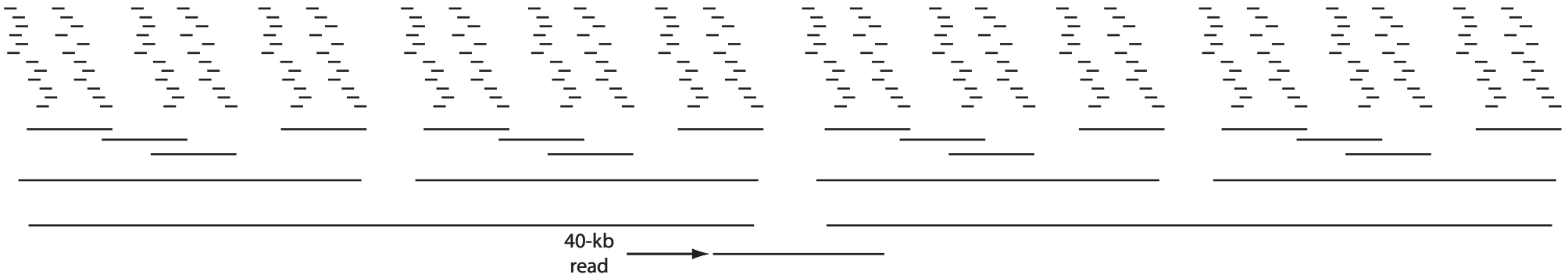


This ASSEMBLY approach is the TRADITIONAL method. Sequence data from different library sizes is used to contact data into assembled scaffolds.

Step 4: Link two 40-kb contigs with 100-kb BAC end sequences (BES)



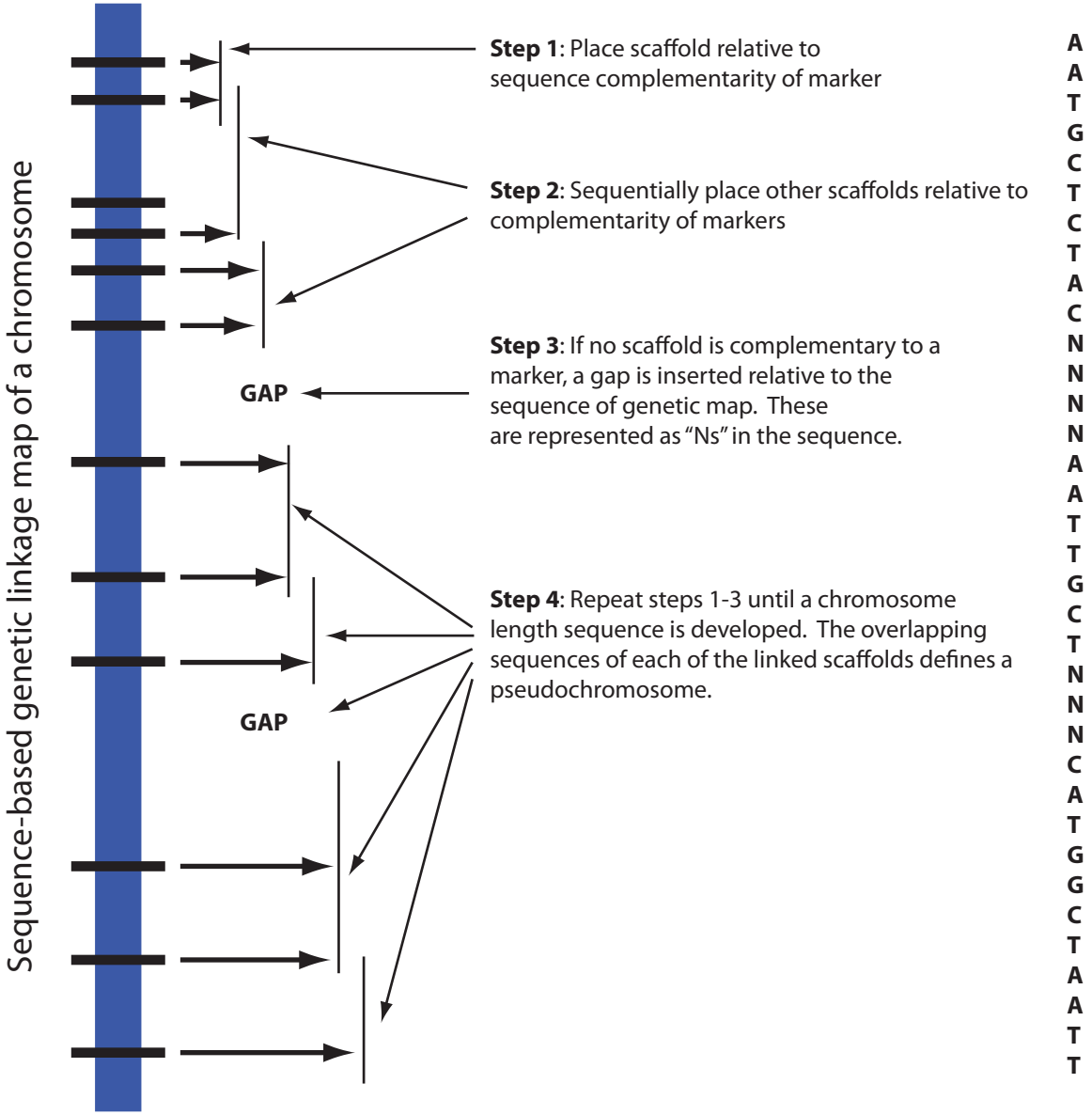
Step 5: Here link two100-kb BAC sized contigs with a 40-kb paired-end read; other sized reads can also be used for this linking



Step 6: Continue linking larger blocks of sequences until the block can not be linked with another block. This block is defined as a scaffold.

Genome Assembly

Linking Scaffolds to a Dense Genetic Map



This figure represents assembling PSEUDOCHROMOSOMES by linking scaffolds using marker locations. The sequence of the markers provides a accurate data for the organization of the scaffolds. REMEMBER that genetic data is still the most useful data for assembly. It is directly related to recombination events.

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Plant Genome Evolution

Phillip McClean

Why Should We Study Plant Genome Evolution???

Genomic Synteny

- Large sequence **BLOCKS** Shared in the Same Order between species
  - Same **GENES MAY** control the Same PHENOTYPES
- Warm season legumes have shared gene **MACROSYNTENY**
  - Lonardi et al. (2019) *The Plant Journal*, *98*:767.

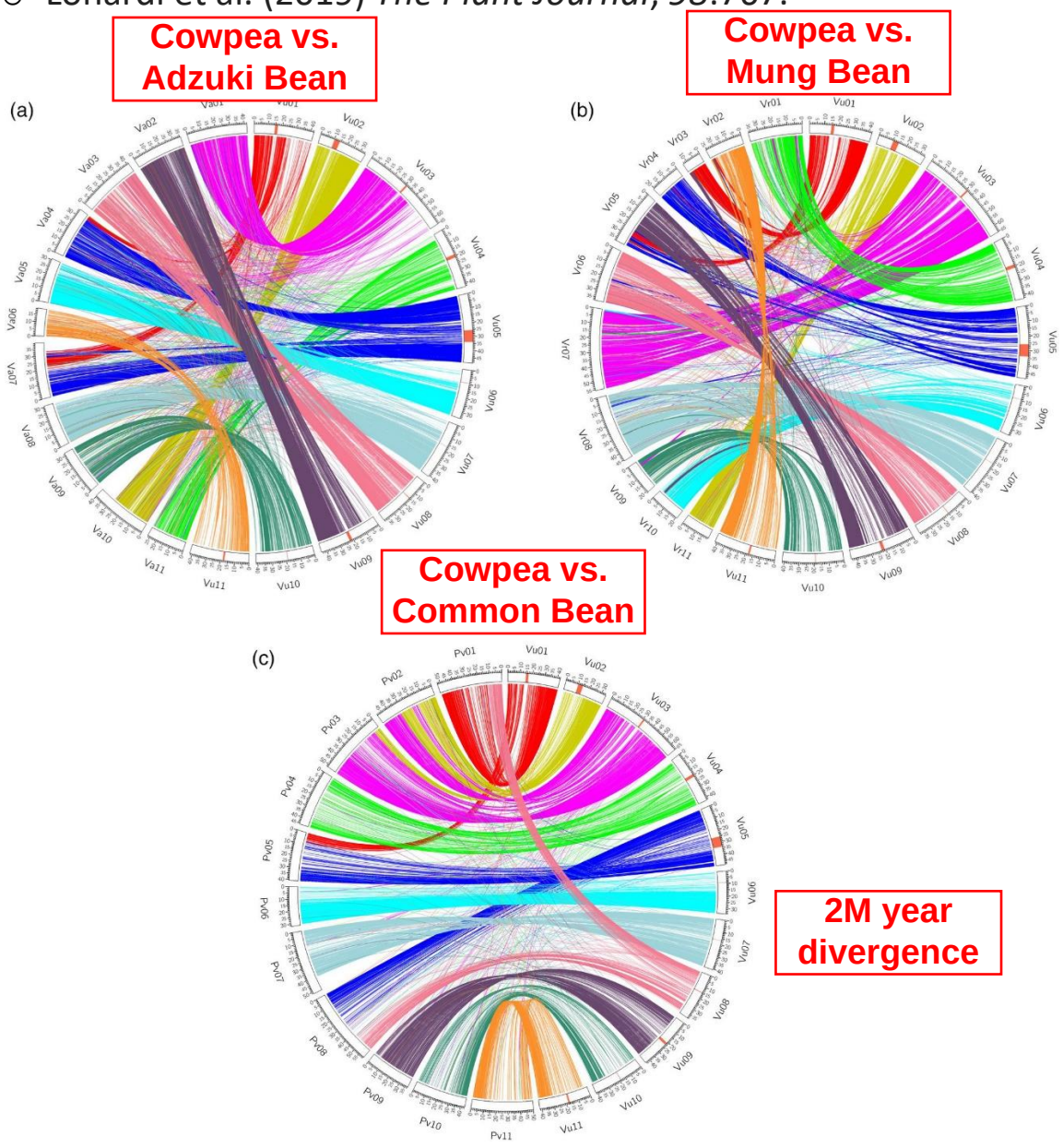


Figure 3. Synteny view between cowpea (**Vu**; *Vigna unguiculata*) and other closely related diploid species. These include: (a) adzuki bean (**Va***; Vigna angularis*); (b) mung bean (**Vr**; *Vigna radiata*); and (c) common bean (**Pv**; *Phaseolus vulgaris*) using the revised cowpea chromosome numbering system.

Viridiplantae Phylogeny
Nature (2019) 574:679

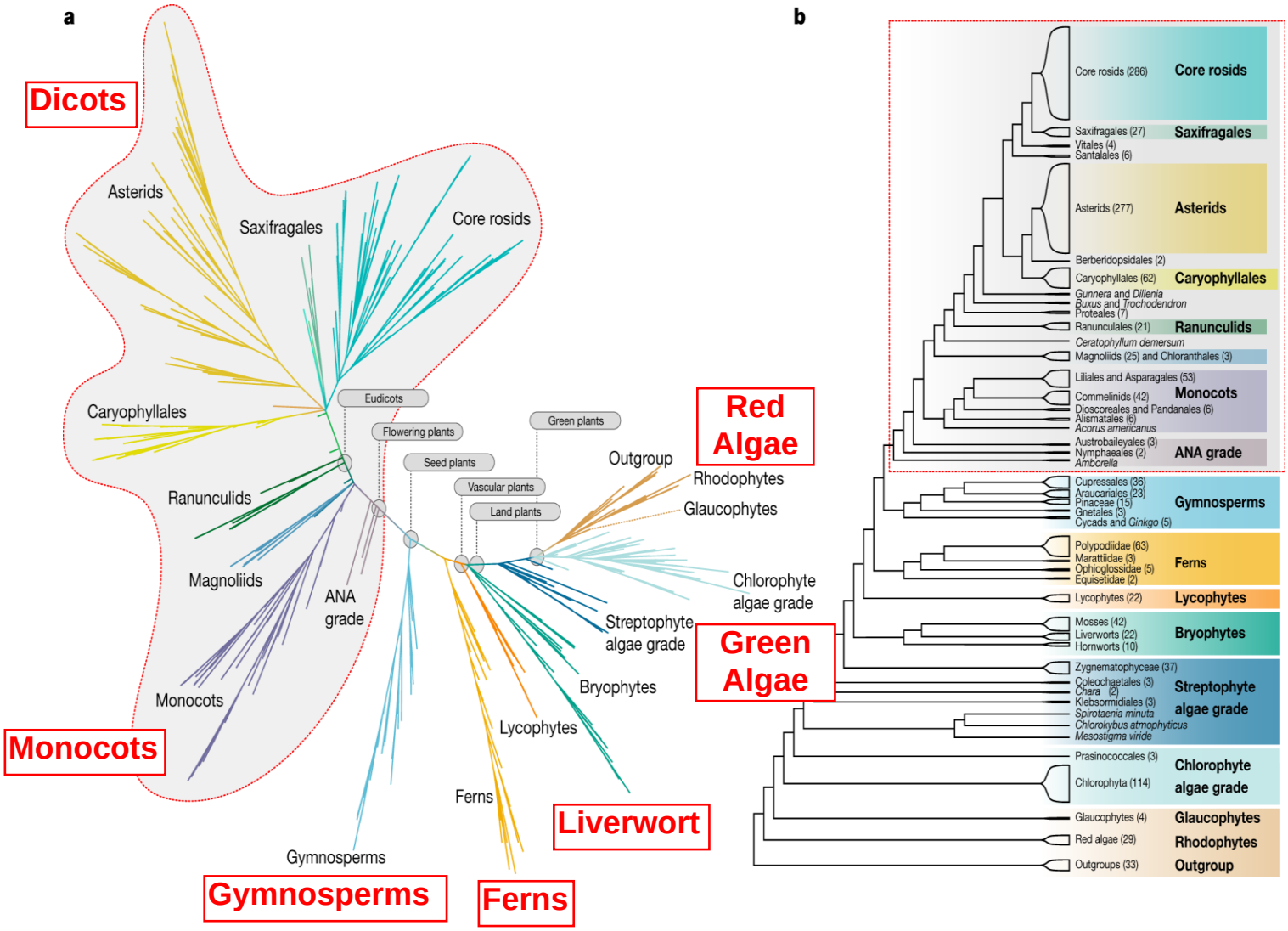


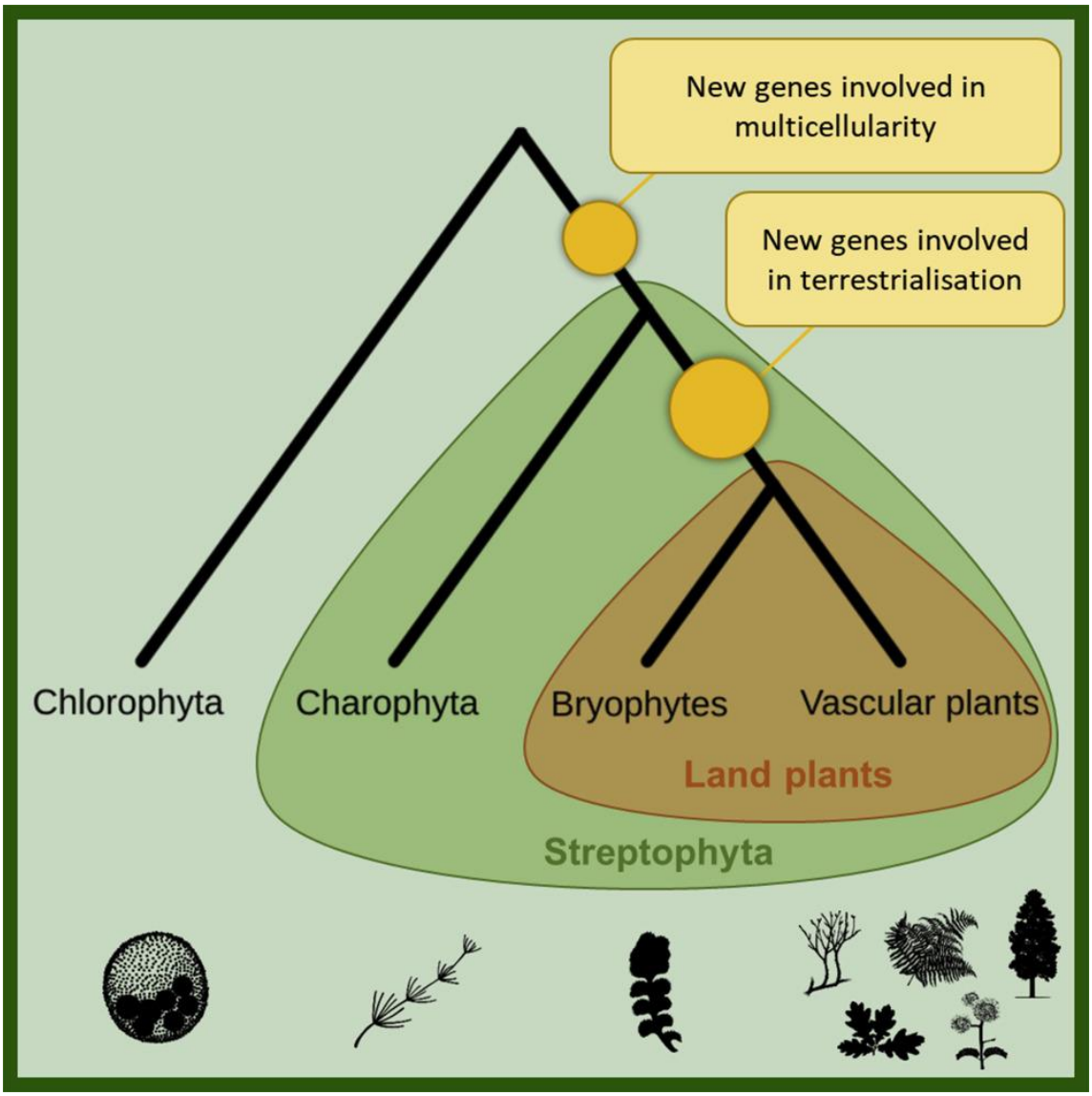
Fig. 2: Phylogenetic inferences of major clades. Phylogenetic inferences were based on ASTRAL analysis of 410 single-copy nuclear gene families extracted from genome and transcriptome data from 1,153 species, including 1,090 green plant (Viridiplantae) species (Supplementary Table 1). **a**, Phylogram showing internal branch lengths proportional to coalescent units ($2N_e$ generations) between branching events, as estimated by ASTRAL-II15 v.5.0.3. **b**, Relationships among major clades with red box outlining flowering plant clade. Species numbers are shown for each lineage. Most inferred relationships were robust across data types and analyses (Supplementary Figs. 1–3) with some exceptions (Supplementary Fig. 6). Data and analysis scripts are available at https://doi.org/10.5281/zenodo.3255100.

Plant Genome Evolution

How have plants evolved over time to express their extensive biological, cellular, and molecular diversity?

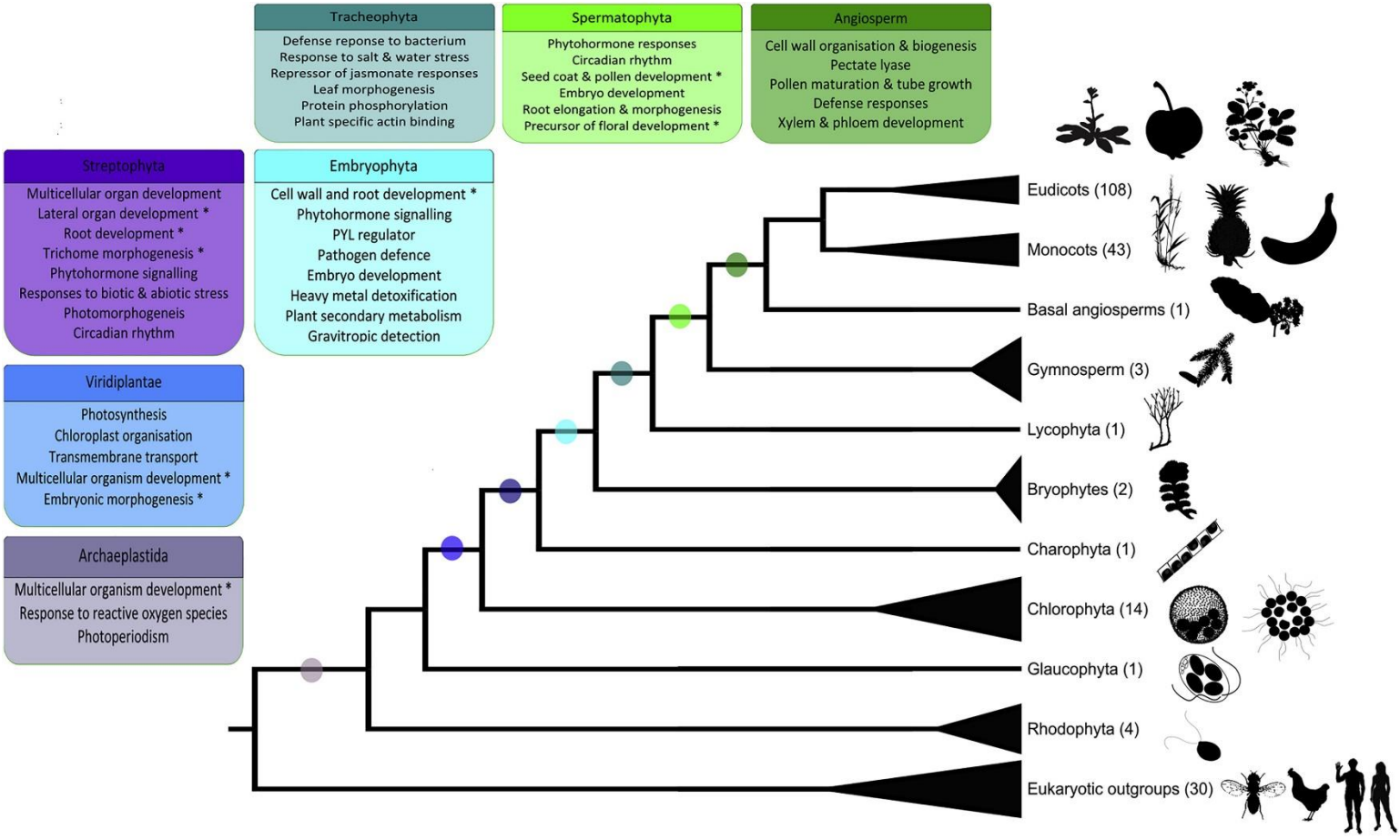
From: ***Bowles et al (2020) Current Biology 30:530***

- **Compared gene sets from 208 sequenced genomes across the photosynthetic organisms**
  - Viridiplantae = photosynthetic organisms
    - 500,000 species
      - Chlorophyta – Green algae
      - Streptophta – other algae and land plants
  - Two functions added over time BEFORE the appearance of land plants
    - Multicellularity
    - Terrestrailization



Viridiplantae Added Complexity During Evolution

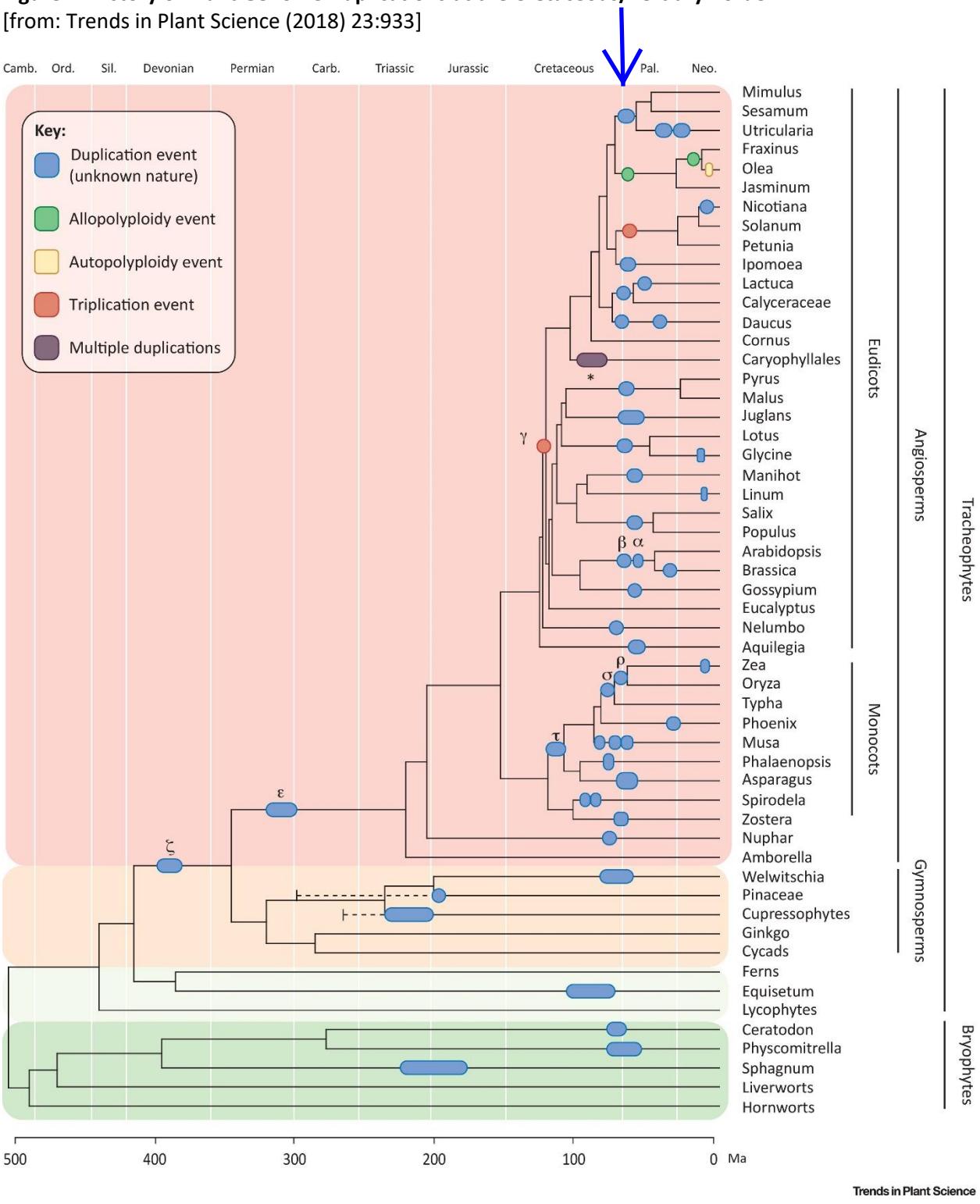
From: ***Bowles et al (2020) Current Biology 30:530***



Consistent with other research

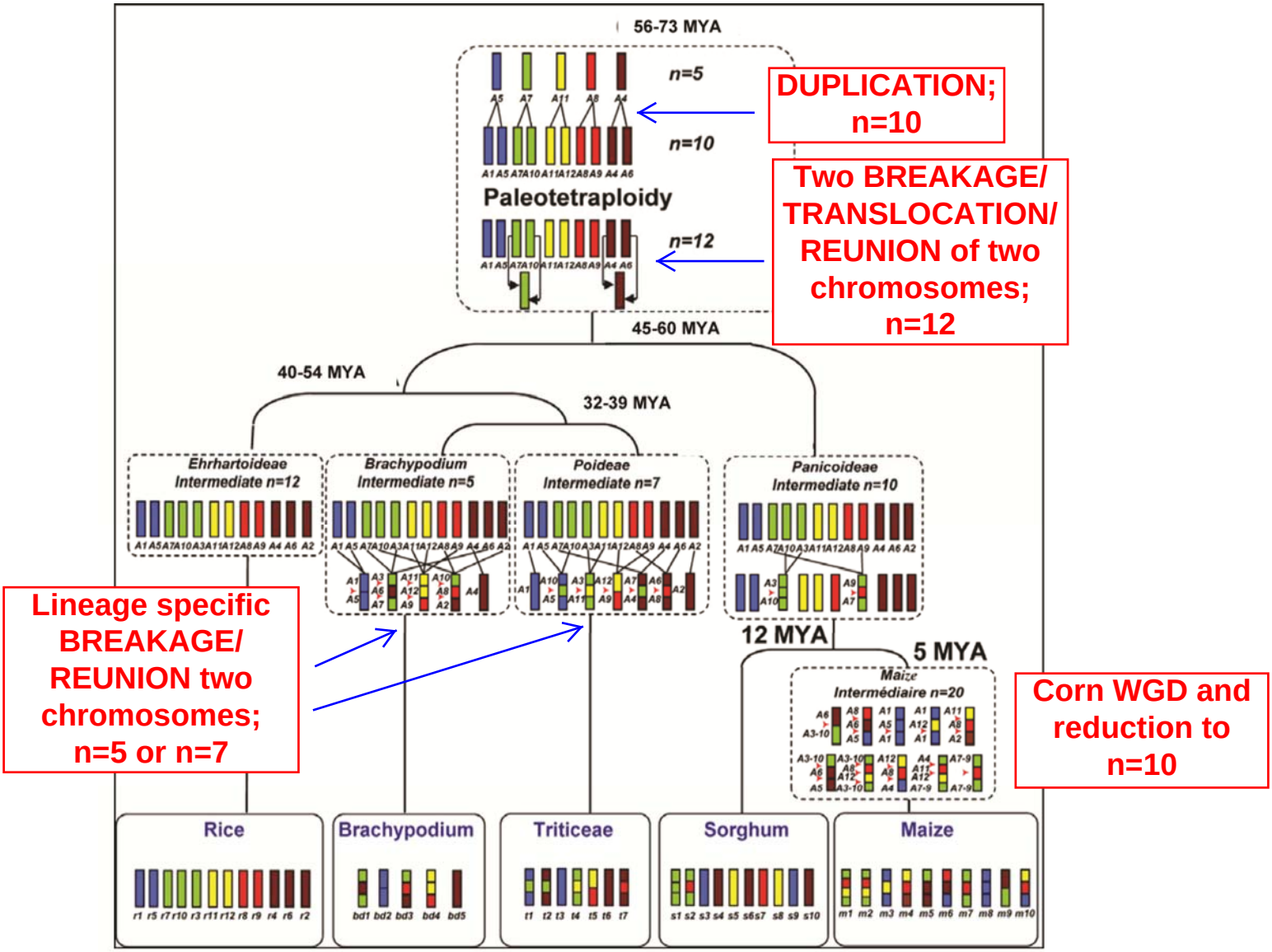
- **Evolved basic functions of plants included**
  - Embryogenesis
  - Plant hormones
  - Symbiotic interactions with:
  - Arbuscular mycorrhizae
  - Rhizobacteria

Figure 7. History of Plant Genome Duplications at the Cretaceous/Tertiary Border
[from: Trends in Plant Science (2018) 23:933]



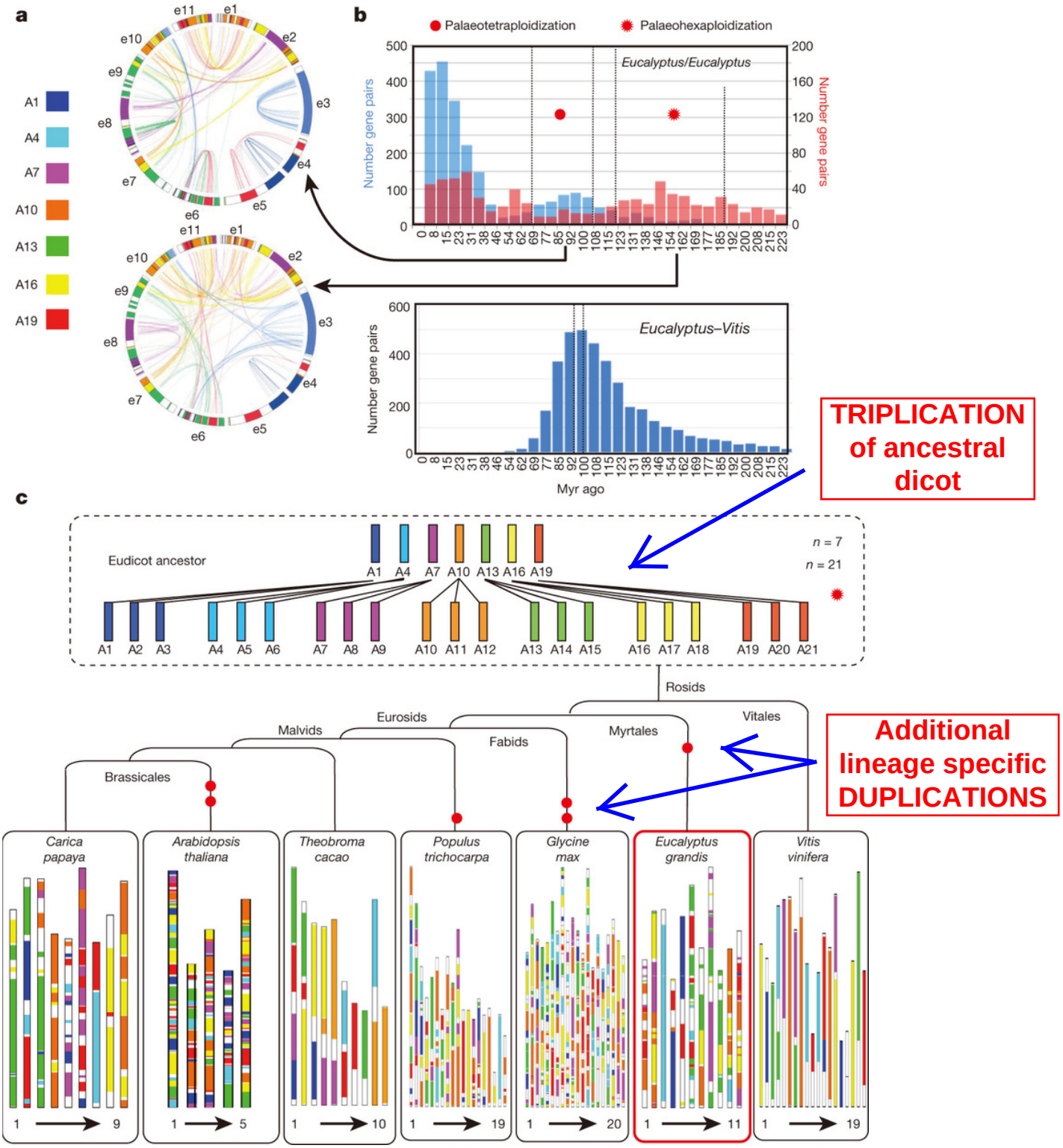
Journal Article Figure 13. Figure 1. The Distribution of Known Whole-Genome Duplication (WGD) Events within the Plant Kingdom. Most events are shown from Van de Peer *et al.* [91] but have been updated. The length of each bar along the branch indicates the current estimate for its age. Duplication events of unknown origin are shown in navy blue, triplications in red, known autopolyploidy events in yellow, and allopolyploidy events in green. The white bar associated with Caryophyllales represents 26 independent WGD events, some of which are autopolyploidy and some allopolyploidy. Named duplication events are shown alongside their Greek letter. Abbreviations: Camb., Cambrian; Carb., Carboniferous; Ord., Ordovician; Neo., Neogene; Pal., Paleozoic; Sil., Silurian.

Figure 6. A unified model of grass genome evolution. (from: Vogel et al. 2010. Nature 463:763.)



Supplementary Figure 18. Grass chromosome evolution model. The monocot chromosomes (r1-r12 for rice, t1-t7 for Triticeae, bd1-bd5 for *Brachypodium,* s1-s10 for sorghum, and m1-m10 for maize) are represented with a five colour code to illustrate the evolution of segments from a common ancestor with five proto-chromosomes and a n=12 intermediate as described in [62], and are named according to the rice nomenclature. The events that have shaped the structure of the 5 different grass genomes including the 7 *Brachypodium* chromosome nested insertion events during their evolution from the common ancestor are indicated as whole genome duplication, ancestral chromosome translocations and fusions, and lineage- specific nested chromosome insertions.

Evolution of Eudicot Genomes (from: Nature 510:356)



a, Paralogous gene pairs in *Eucalyptus* for the identified palaeohexaploidization (bottom) and palaeotetraploidization (top) events. Each line represents a duplicated gene, and colours reflect origin from the seven ancestral chromosomes (A1, A4, A7, A10, A13, A16, A19). b, Number of synonymous substitutions per synonymous site (K_s) distributions of *Eucalyptus* paralogues (top) and *Eucalyptus*–*Vitis*orthologues (bottom). Blue bars (top) indicate K_s values for 378 gene pairs from the palaeotetraploidization WGD event (red dot), and red bars show K_s values for 274 gene pairs of the palaeohexaploidization event (red star). c, Evolutionary scenario of genome rearrangements from the Eudicot ancestor to *Eucalyptus* and other sequenced plant genomes; palaeohistory modified from ref. 49.

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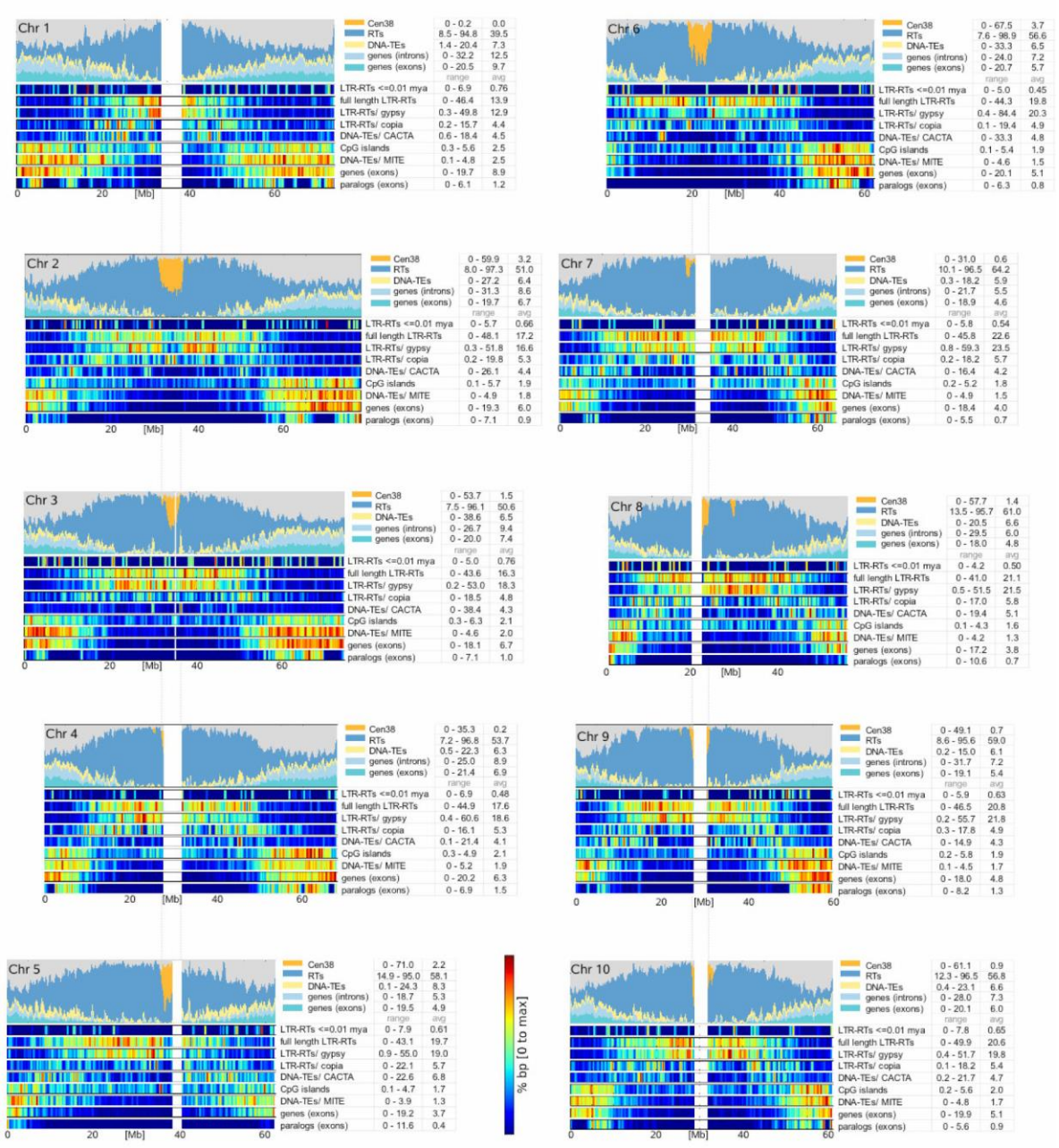
Plant Transposable Elements

Phillip McClean

Distributions of Transposable Elements in Sorghum

Paterson et al. 2010. Nature 457:551 (see supplement)

- In sorghum, ***Gypsy*** retrotransposons are clustered in the central heterochromatic region
- ***Copia*** retrotransposons and **DNA elements** distributed across the chromosomes
- Expressed genes found at the ends of chromosomes
    - Sorghum example



Examples of Transposable Element Insertional Mutants

- **Pea R shrunken seed locus**
  - Ac/Ds type transposon
    - **Starch branching enzyme**
- **Maize waxy gne**
  - Ac/Ds DNA transposon
    - **Starch granule-bound glucosyl transferase**
- **Rice glutinous kernel**
  - LTR retrotransposon
    - **Granular bound starch synthase**
- **Sorghum color gene Y**
  - CACTA DNA transposon
    - **MYB transcription factor**
- **Arabidopsis FAR1/FHY3 regulated phytochrome A response**
  - MULE DNA transposon
    - ***FAR1/FHY3* transcription factors**

Promoter Transposable Element Effects

- **Rice blast disease resistance**
  - LTR retrotransposon
    - **Element present = resistance; Element missing = susceptibility**
- **Grape branching**
  - hAT DNA transposon
    - **Activated enhancer = greater inflorescence branching**
- **Orange slice color**
  - *Copia*-like retrotransposon
    - **Cold-induced expression = darker slice color**

How TEs Can Drive Evolution of Function

- **Vicient and Casacuberta. 2017. Annals of Botany 120:195.**

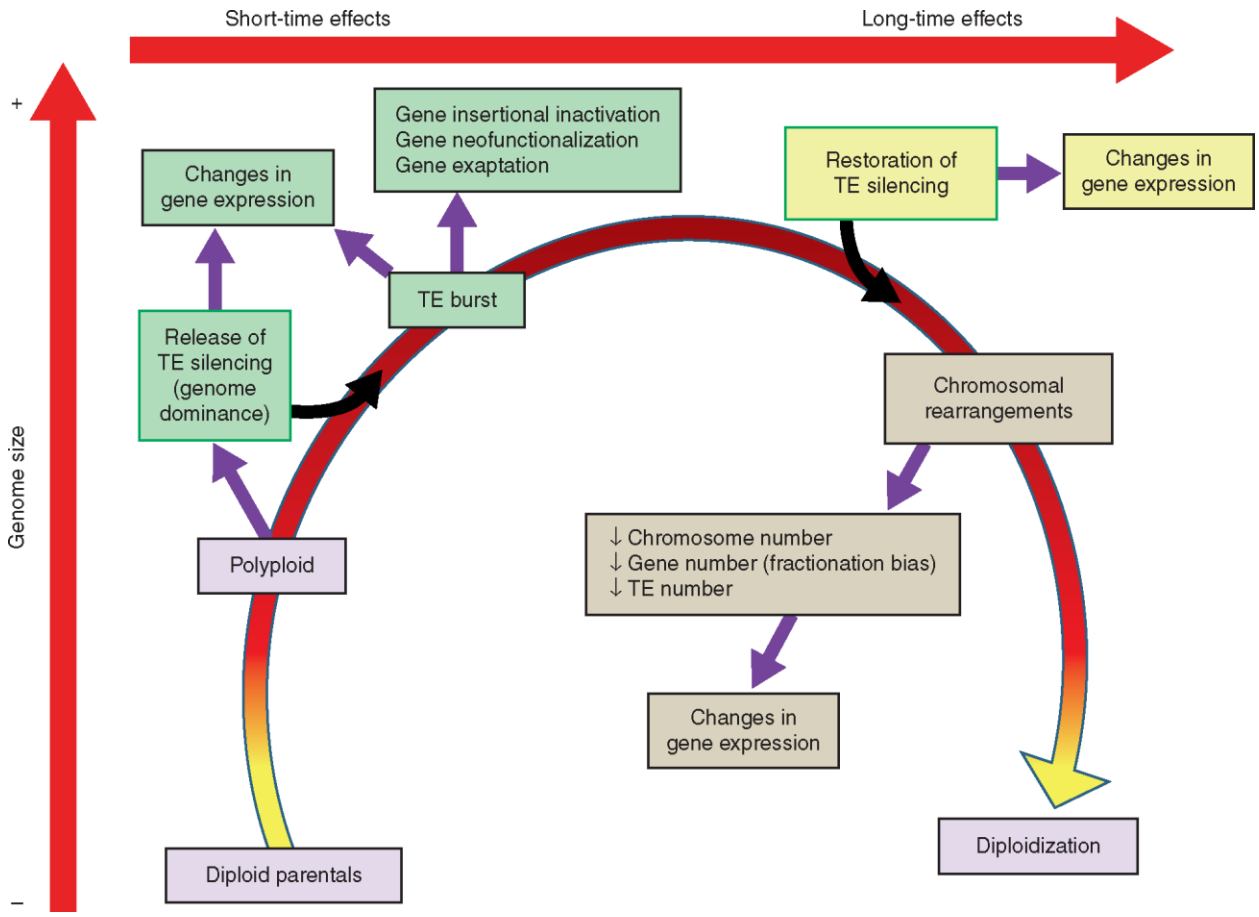


Figure 1. The close connections of polyploidization and TE dynamics. **POLYPLOIDIZATION** is accompanied by a **release of TE silencing**, which may be different for parentally or maternally inherited TEs. This release, in addition to **ACTIVATING TE MOBILIZATION**, may **induce changes in the regulation of genes located near TEs**. The **BURST OF TEs** will produce **new TE insertions that can modify the coding capacity of genes or their regulation**. The **RELEASE OF TE SILENCING** is **reversed after few generations**, and **TE SEQUENCES AGAIN BECOME** the **target of epigenetic silencing mechanisms**. The **SILENCING OF TEs**, including the new insertions resulting from the TE burst, will **influence the expression of genes located nearby**. This may result in **CHANGES OF GENE EXPRESSION** with respect to the **early phases of polyploidy** but also with respect to the diploid parents. **TEs WILL ALSO BE IMPORTANT FOR THE DIPLOIDIZATION** of the polyploid genome, as the different **TE copies may provide sequence homology for recombination**, leading to deletions and chromosome rearrangements.

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Plant Transcription, Transcription Factors and Chromatin Remodeling

Phillip McClean

Transcription Factors: General Terms and Concepts

Promoter

- Difficult to define
- General definition
    - ***All the DNA sequences containing binding sites for RNA polymerase and the transcription factors necessary for normal transcription***

Transcription Factor

- ***Any protein other than RNA polymerase that is required for transcription***

Functions of Transcription Factors

- Bind to RNA Polymerase
- Bind another transcription factor
- Bind to cis-acting DNA sequences

Basal Transcription Apparatus

- RNA polymerase + General transcription factors
- Both needed to initiate transcription
    - ***These steps are the minimum requirement for transcription***

Upstream Transcription Factors

- Ubiquitous factors that increase the efficiency of transcription initiation
    - ***Set of factors necessary to for expression of each gene***

Inducible Transcription Factors

- Act in the same manner as an upstream factor
    - **BUT**
        - ***Their synthesis is regulated in a temporal or spatial manner***

MADS Box Binding Example

- **Smaczniak et al. 2012. Development 139, 3081-3098 (2012)**

MADS box genes

- **Key regulatory of growth**

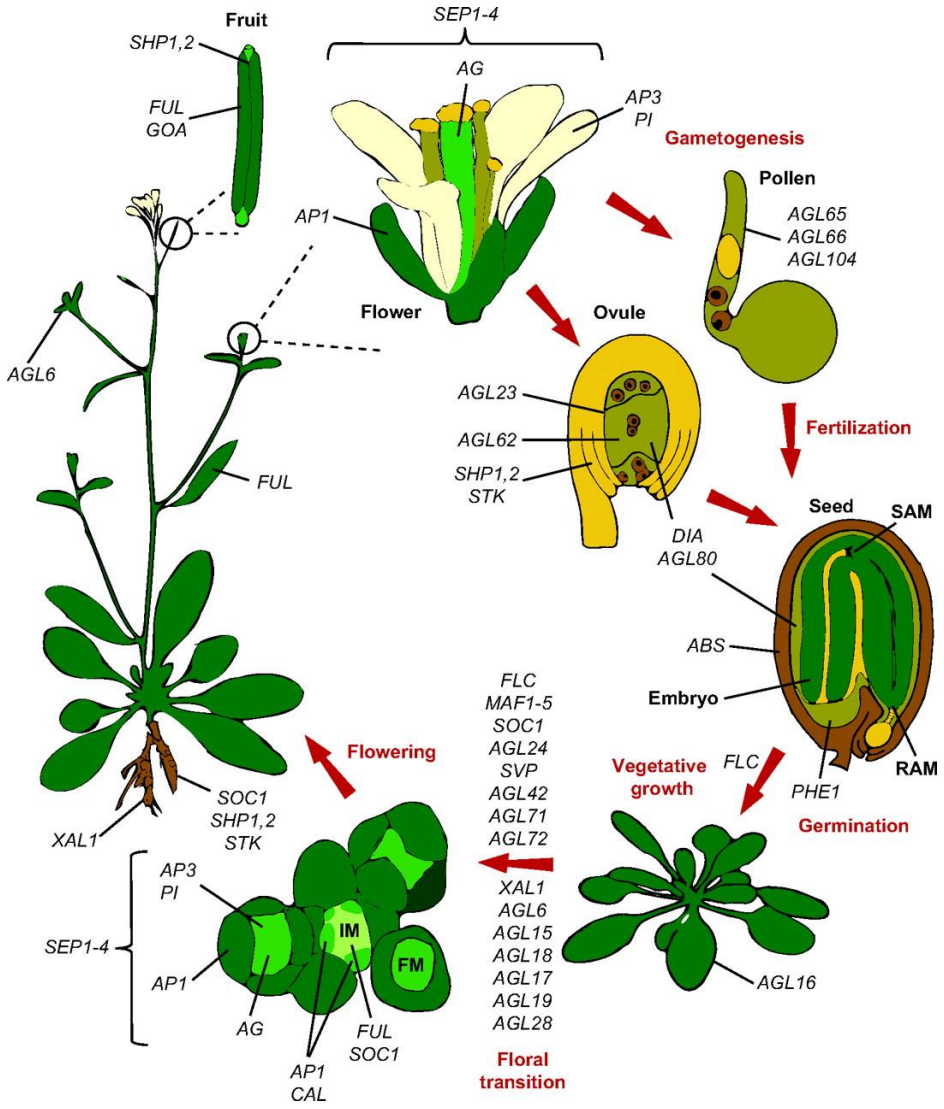


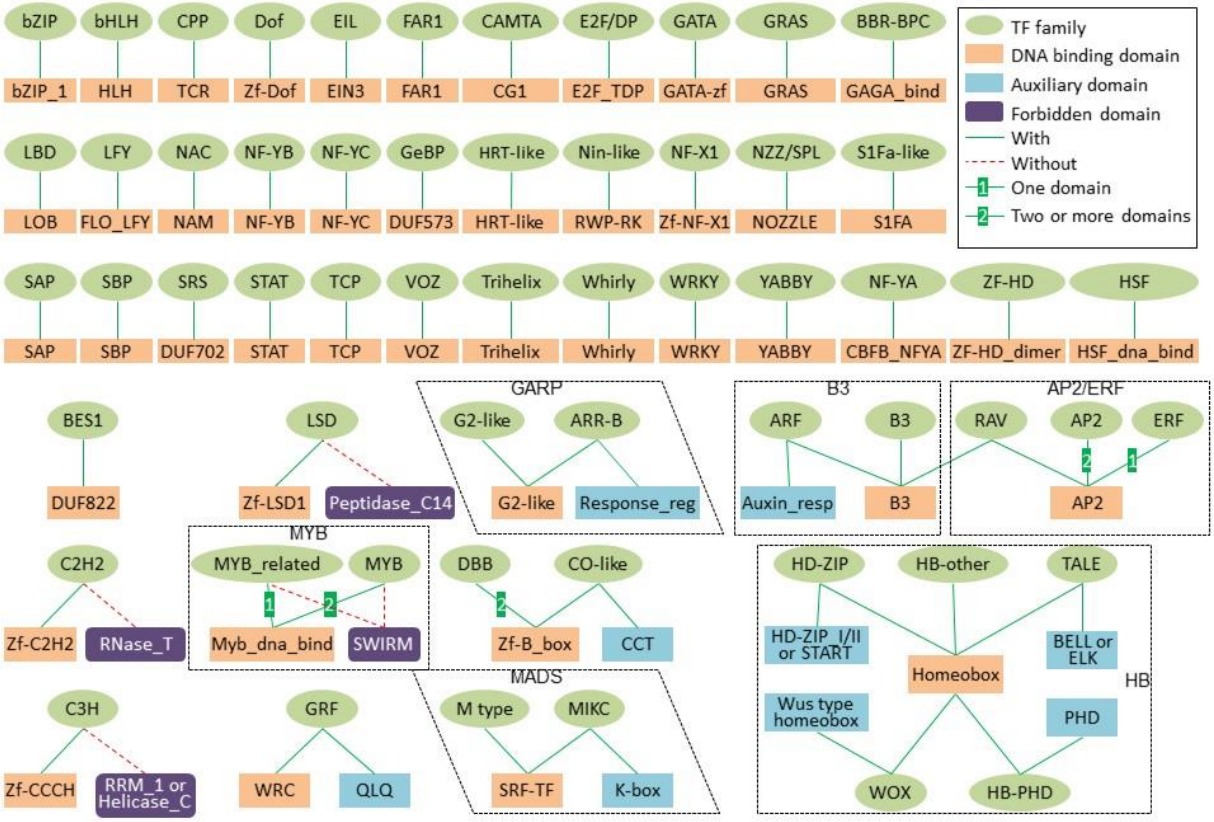
Fig. 2. Functions of MADS-box genes throughout the life cycle of *Arabidopsis thaliana*. *Arabidopsis* progresses through several major phase changes during its life cycle and MADS box genes play distinct roles in the various developmental phases and transitions. **Reproductive development** starts with the generation of male and female haploid gametes (gametogenesis) and, after double fertilization, this results in a developmentally arrested embryo that possesses a root apical meristem (RAM) and a shoot apical meristem (SAM), enclosed within a seed. Under favorable conditions, **seeds germinate** and young plants go through the **vegetative phase** of development in which leaves are formed and plants gain size and mass. Finally, the plant is ready to flower and the **floral transition stage** results in the conversion of vegetative meristems into inflorescence meristems (IMs) and floral meristems (FMs) that produce floral organs. Subsequently, **gametes are formed** within the inner flower organs, thus completing the cycle. The **MADS box genes that are involved in each of the various stages of development are indicated.**

Plant Transcription Factor Database: Plant TFDB

http://planttfdb.cbi.pku.edu.cn/index.php

- How transcription factors are defined
  - ***Some*** Pfam domains have ***DNA binding functions***
  - The DNA domain they bind to is the ***cis-acting element***
    - **Proteins with Pfam-defined DNA binding domains are considered *TRANSCRIPTION FACTORS***
- **Family assignment rules:**

  http://planttfdb.cbi.pku.edu.cn/help_famschema.php)



TF Family Examples

- **bHLH Family** (2nd family, first green family row)
  - Has the HLH (Pf00010) domain
- **NAC Family** (3rd family, second green family row)
  - Has the NAM (Pf02365) domain
- **ARF Family** (5th family, fourth green family row)
  - Has the Auxin_resp auxillary domain (Pf06507) **AND** the B3 (Pf02362) domain

Cis-acting Elements Vary Among Gene Family Members

- **Example: *rbcS*: small subunit of RUBISCO**
- **Manzara et al. 1991. The Plant Cell 3:1305**

Conserved cis-acting element

Table 1. Conserved Sequence Motifs in *rbcS* Promoters

Number/ Symbol	Motif	Sequence	Binding Factor	Putative Function	References
	L box	AATTAACCAA		unknown	Ueda et al. (1989)
1		pyrimidine rich		unknown	
2	I box	GGATGAGATAAGATTA (C C)	GA-1	unknown	Ueda et al. (1989)
2	I box	GATAAG	GA-1	"enhancer"	Donald and Cashmore (1990)
2	GATA	GATGAGATA (T)	ASF-2 [a]	leaf-specific	Lam and Chua (1989)
3	G box	CACGTGGC (T)	GBF	"enhancer"	Donald and Cashmore (1990)
5		TTAAATAGAGGGCGTAA		unknown	
8	Box II	GA A A A TTGTGPuTAATPuT	GT-1	light regulation	Lam and Chua (1990)
9		A C TTTGTAATGTCAA		unknown	
10		A GAGCCACA		unknown	
12	CAAT box	T ATCCAAC		unknown	
13		GGTTAC		unknown	
15		AGATGAGG	LRF-1 (?)	light regulation	Buzby et al. (1990)
16		T A A TTTGTGTCCGTTAGATG		unknown	
18	"LRE"	CCTTATCAT		unknown	Grob and Stuber (1987)
19	TATA box	C T TTATATAAA	TFIID [a]	transcription initiation	Gasch et al. (1990)
	A-T rich	(varies)		unknown	
	A-T rich	AATATTTTTATT	AT-1	unknown	Datta and Cashmore (1989)
	A-T rich	AAATAGATAAATAAAAACATT	3AF-1 [a]	"enhancer"	Lam et al. (1990)

[a] These genes have been cloned.

Family member specific cis-acting elements

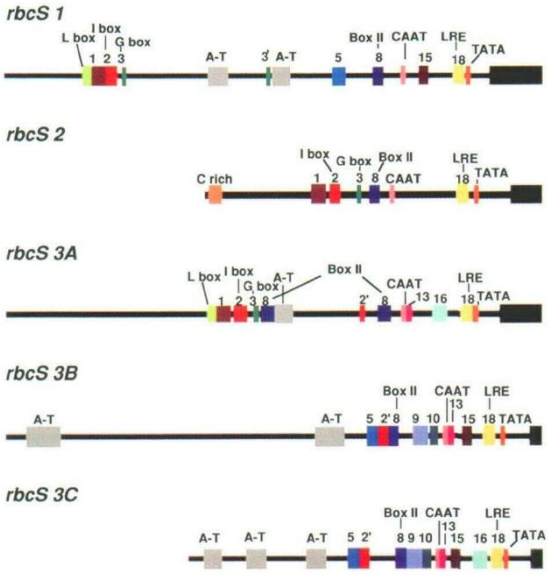


Figure 1. Conserved DNA Sequence Motifs in the 5′ Upstream Regions of the Tomato *rbcS* Genes.

Heavy black lines represent the 5′ upstream region of each gene. Conserved sequences are designated by colored boxes (for key, see Table 1). Solid black boxes at the 3′ end (right) of each upstream region represent the 5′ untranslated region of each gene. The numerical designations for conserved sequences are from Manzara and Gruissem (1988). L-box, I-box, and G-box are the terminology of Ueda et al. (1989), box II is the terminology of Fluhr et al. (1986), and LRE is the terminology of Grob and Stüber (1987).

Transcription Factors Bind to Different Domains of a Promoter in Different Tissues

- **The Plant Cell (1991) 3:1305**
- White ovals = cis-element bound by proteins (TFs)
  - **Binding varies by developmental stage**

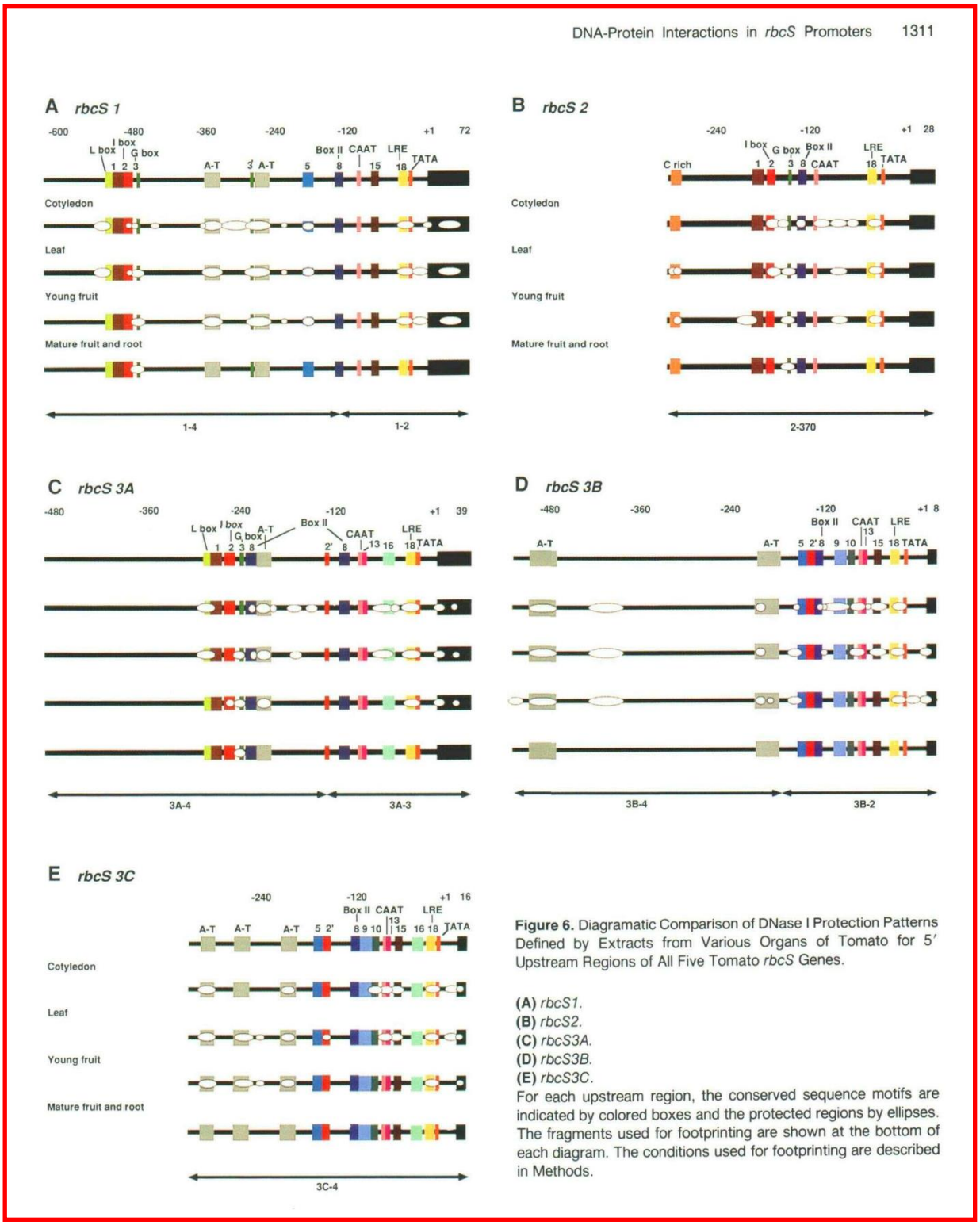


Figure 6. Diagramatic Comparison of DNase I Protection Patterns Defined by Extracts from Various Organs of Tomato for 5′ Upstream Regions of All Five Tomato *rbcS* Genes.

(A) *rbcS1.*
(B) *rbcS2.*
(C) *rbcS3A.*
(D) *rbcS3B.*
(E) *rbcS3C.*
For each upstream region, the conserved sequence motifs are indicated by colored boxes and the protected regions by ellipses. The fragments used for footprinting are shown at the bottom of each diagram. The conditions used for footprinting are described in Methods.

Remodeling Process

- **Remodeling is:**
  - **Alterations in chromatin structure that *activates* or *deactivate* gene expression**
  - **Involves transcription factors that actively recruit remodeling complexes**
- May be coupled to DNA replication
- Involves two steps

1. **Histone modification**
   - Specific lysine residues are modified by
     - **Acetylation** [by histone acetylases (HATs)]
       - ***Loosens structure***
       - **Transcription apparatus has access to promoter**
     - **Methylation** (by methylases)
       - ***Tightens structure***
       - **Transcription apparatus has access blocked to promoter**
     - **Ubiquitination** mediated protein degradation
       - Ubiquitin
         - Small protein that is attached to tail of histone protein
       - ***Often marks that protein for degradation***

2. **Recruitment of remodeling complexes**
   - **Swi/Snf family**
     - Contains helicases that **twist DNA on the nucleosomes**
       - **DNA slides on the histones**
         - DNA is more accessible to the transcription factors
       - **Complexes with other proteins to repress a transcriptional unit**

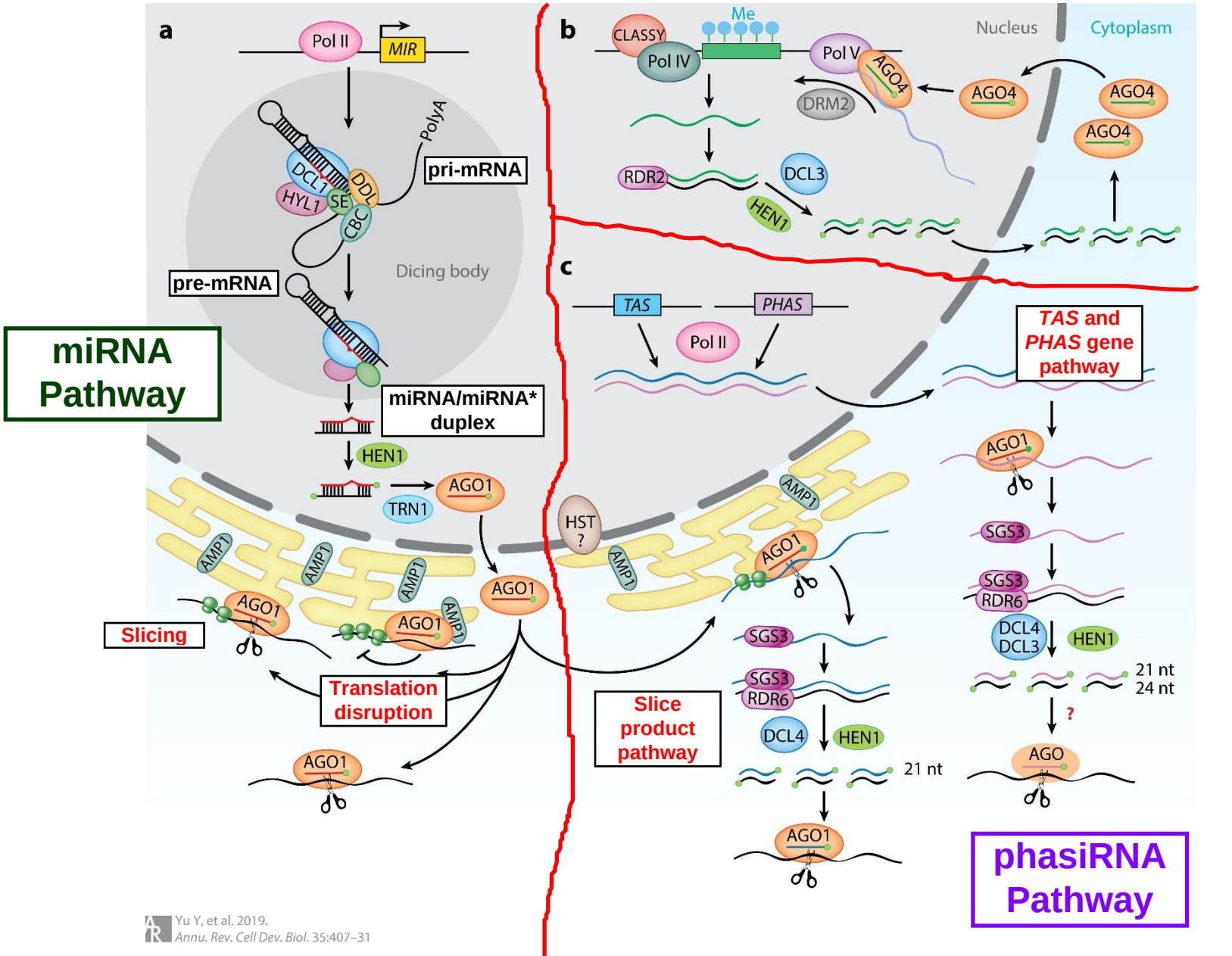
PLSC 731 -Plant Molecular Genetics
Second Half of Course
Higher Order Concepts

Plant Non-Coding RNAs

Phillip McClean

Synthesis and Evolution of Plant Small RNAs

- Yu et al. 2019. Ann Rev Cell Develop Biol 35:407



Yu Y, et al. 2019.
Annu. Rev. Cell Dev. Biol. 35:407–31

Figure 1 Biogenesis and modes of action of plant small RNAs. **(a)** A **MIR gene is transcribed** into a pri-miRNA, which is sequentially **processed** first **into** a pre-miRNA and then into a **miRNA/miRNA* duplex**. The **duplex** is **methylated** by HEN1, and the miRNA strand is **loaded into AGO1** in the nucleus. The **miRNA-AGO1** complex is **transported** to the **cytoplasm** and **regulates target gene** expression through **transcript cleavage and/or translation repression**. **(b)** Pol IV generates **single-stranded siRNA precursors**, which are **converted into dsRNAs** and **processed** into **24-nt siRNA duplexes**. **Methylated** siRNAs are **loaded into AGO4** in the **cytoplasm** and are **transported** to the nucleus, followed by the recruitment of these **siRNA-AGO4 complexes to Pol V transcripts**. The subsequent **recruitment of DRM2 catalyzes DNA methylation at RdDM target loci**. **(c)** **TAS or PHAS loci** are **transcribed** into **single-stranded RNAs** that are **targeted** by an **miRNA-AGO1/7 complex**. The 5′ or 3′ **cleavage fragment** is **protected** by SGS3 and **converted** into **dsRNA** by RDR6. DCL proteins **process** these dsRNAs into **21- or 24-nt phasiRNAs**. The 21-nt tasiRNAs, which are phasiRNAs from TAS loci, are primarily **loaded** into **AGO1** and guide **transcript cleavage** of their targets.

Abbreviations: **AGO**, ARGONAUTE; **AMP1**, ALTERED MERISTEM PROGRAM 1; **CBC**, CAP-BINDING COMPLEX; **DCL**, DICER-LIKE; **DDL**, DAWDLE; **DRM2**, DOMAINS REARRANGED METHYLASE 2; **dsRNA**, double-stranded RNA; **HEN1**, HUA ENHANCER 1; **HST**, HASTY; **HYL1**, HYPONASTIC LEAVES 1; **Me**, methylated; **phasiRNA**, phased siRNA; **Pol**, RNA polymerase; **pre-miRNA**, precursor miRNA; **pri-miRNA**, primary miRNA; **RdDM**, RNA-directed DNA methylation; **RDR2/6**, RNA-DEPENDENT RNA POLYMERASE 2/6; **SE**, SERRATE; **SGS3**, SUPPRESSOR OF GENE SILENCING 3; **siRNA**, small interfering RNA; **tasiRNA**, trans-acting siRNA; **TRN1**, TRANSPORTIN 1.

Plant Biological Processes Controlled by miRNAs

- **Yu et al. 2019. Plant Noncoding RNAs: Hidden Players in Development and Stress Responses. Ann Rev Cell Develop Biol 35:407**

miRNAs involved in ALL stages of plant growth, development, and abiotic/ biotic stress response!!!

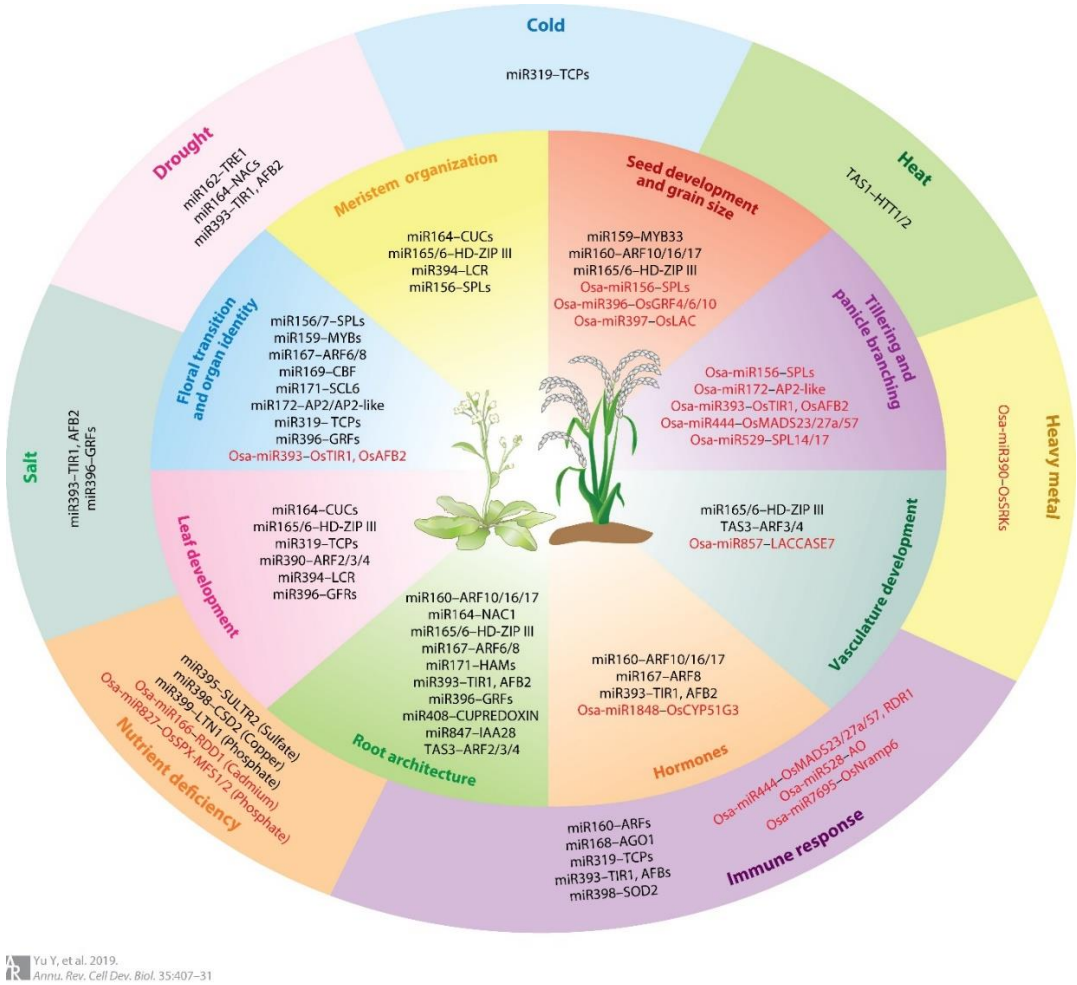


Figure 2 Functions of miRNAs in plant development and stress responses and an overview of the current understanding of miRNA-mediated regulation during development (inner circle) and responses to biotic and abiotic stresses (outer circle) in Arabidopsis and rice. Red font indicates miRNA-target modules that act in rice, but not in Arabidopsis.

Plant miRNA Stress Regulatory Pathway

- **Khraiwesh et al. 2012 Biochimica et Biophysica Acta 1819:137**

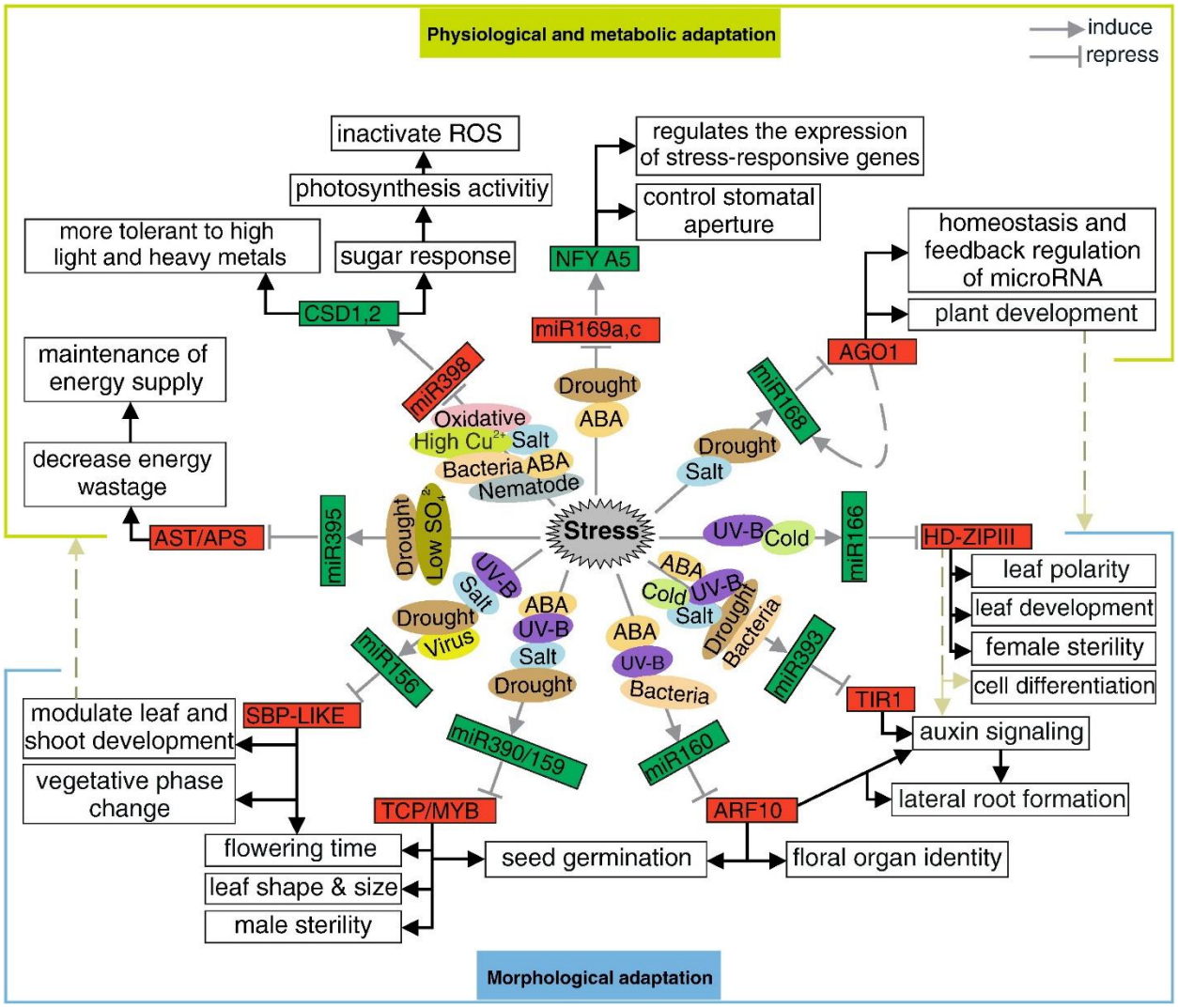


Fig. 3. Regulatory network of stress-responsive miRNAs in *Arabidopsis*. A network is proposed that describes the molecular mechanisms underlying the response of *Arabidopsis* plants to different biotic and abiotic stresses. The network is based on the changes in expression profiles of miRNA and subsequent target transcripts in plants under stress. Green boxes: upregulated RNAs; red boxes: downregulated RNAs.

Many signals activate miRNAs to regulate plant growth and development!!!

Functions of Plant lncRNAs

- **Table from: Rai et al 2019. Genomics 115:997.**

Table 1. List of plant long noncoding RNAs identified on the basis of their biological function and mode of action.

SPECIES	Name	Mechanism of action	Biological function	References
Arabidopsis thaliana	IPS1	Target mimicry: interacts with an miRNA, ath-mir399	Regulates **phosphate homeostasis**	[22]
	COLDAIR	**Epigenetic silencing**: modification of histones	Regulates **flowering**	[27]
	COOLAIR	**Epigenetic silencing**: Promoter interference	Regulates **flowering**	[88]
	HID1	Stage specific: Associates with chromatin and **represses transcription** of phytohormone interaction factor 3 (PIF3)	Regulates **photomorphogenesis**	[103]
	asHSFB2a	**Hijacks** nuclear Antisense transcription regulators	Regulates the **gametophytic and vegetative development**	[111]
	ASCO-lncRNA	Regulators of **alternate splicing**	Regulates the **development of lateral roots**	[5]
	APOLO	Causes the **formation of Chromatin loop** and regulates the expression of its neighboring genes	Regulated **plant development** through auxin signaling	[2]
	ELENA1	**Increases the expression** of genes involved in plant immunity, such as PR1 and PR2, B 1,3glucanase and salicylic acid genes	Upregulated in **disease** caused by Pseudomonas syringae	[61]
	Drought induced RNA (DRIR)	**Regulates** stress responses such as stomata closure	Upregulated in **drought and salt stress**	[72]
	CDF5 LONG NONCODING RNA (FLORE)	**Natural anti-sense transcript** of CDF5, upregulates flowering	**Photomorphogenesis**	[26]
Glycine max (soybean)	GmENOD40	Involved in the development of nodules	Regulates the **re-localization of proteins** from nucleus to cytoplasm	[117]
Hordeum vulgare (Barley)	HvCesA6 lnc-NAT	Acts as a **precursor for siRNA**	Involved in the **synthesis of cell wall**	[25]
Medicago truncatula	MtENOD40	Involved in the development of nodules	Regulates the re-localization of proteins	[83]
Oryza sativa (Rice)	OsPI1	Unknown	Regulates **phosphate homeostasis**	[106]
	OsENOD40	Involved in the development of nodules	Unknown	[42]
	LDMAR (P/TMS12-1)	Acts by causing **promoter methylation**	**Male fertility** regulation	[17]
	Cis-NATPHO1;2	Acts by **enhancing translation** of PHOSPHATE1;2	Regulates **phosphate homeostasis**	[30]
	lncRNAs: 2224 lincRNAs: 1624 lncNATs: 600	Tissue and stage specific: Anthers, pistils, seeds five days after pollination, and shoots 14 days after germination	Regulates **sexual reproduction**	[128]
Petunia hybrid	SHO lnc-NAT	**Degrades dsRNA**	Regulates the **synthesis of local cytokines**	[138]
Populus trichocarpa	incRNA20 lincRNA2752	**target mimic** of ptc-miR476 target mimic of ptc-miR169	Upregulated in **abiotic stress** (drought)	[80]
Solanum lycopersicum	TPS11	Unknown	Regulates **phosphate homeostasis**	[53]
	Slylnc0195 slylnc1077	**target mimic** of miR166 target mimic of miR399	Upregulated in infections caused by tomato yellow leaf curl virus	[97,101]