MOLECULAR LIFE OF PLANTS

Sources: Lecture slides from HS19 provided via Moodle by the professors, own notes from lectures

PART RODRIGUEZ-VILLALON

EMBRYOGENESIS AND SEED DEVELOPMENT

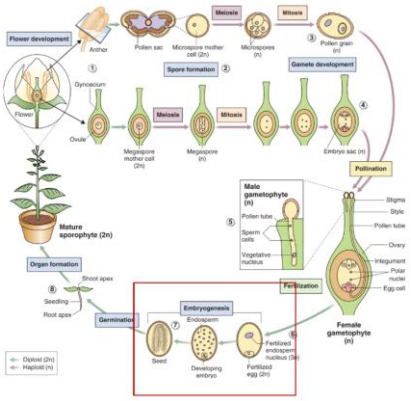
LEARNING GOALS

- Formation of the endosperm and the embryo
- Developmental stages of the embryo
- Apical-basal polarity and radial polarity
- Seed structure
- Germination
- Post-embryonic plant growth

THE CYCLE OF A FLOWERING PLANT

Three developmental stages of a seed plant life:

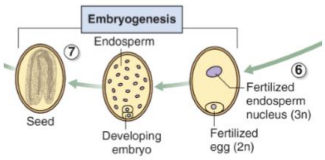
- Embryogenesis
  - Process by which a single cell is transformed into a multicellular entity having a characteristic organization
- Vegetative development
  - After germination, the seedling builds on its rudimentary form through the activity of the root and shoot apical meristems. Vegetative growth is indeterminate
- Reproductive development
  - Transition that involves the formation of specialized floral meristems that give rise to flowers

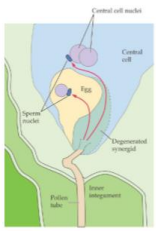


SEED DEVELOPMENT

Double fertilization: The pollen delivers two nuclei, one fertilizes the egg, giving rise to the diploid **embryo** and the other fuses with the two polar nuclei giving rise to the triploid **primary endosperm cell**.

After double fertilization, the **zygote** divides to produce the new embryo and the primary endosperm cell divides to form a unique nutritive tissue, the endosperm



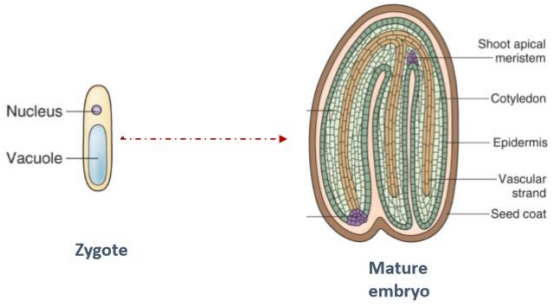


EMBRYOGENESIS

During embryogenesis, the basic architecture of the plant is established, including the elaboration of forms, the associated formation of functionally organized structures and the differentiation of cells to produce various tissues

A mature embryo consists of a radicle, an embryonic shoot apex including hypocotyl and an epicotyl

However complex the plant morphology eventually may become, in its essence it's a reiteration of a basic developmental program

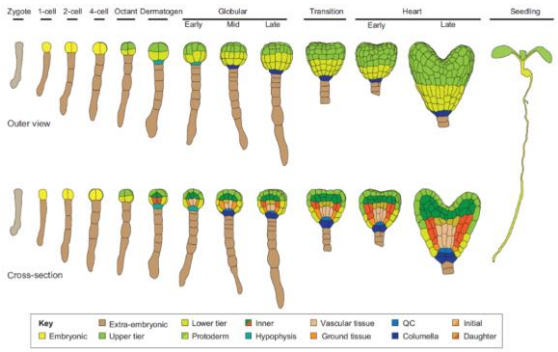


STUDY OF EMBRYOGENESIS

Confocal microscopy as well as genetic approaches allows the study of embryogenesis in Arabidopsis (Arabidopsis embryos are thinner than human hair). With confocal microscopy the structures can be studied layer by layer

DEVELOPMENTAL STAGES OF EMBRYOGENESIS

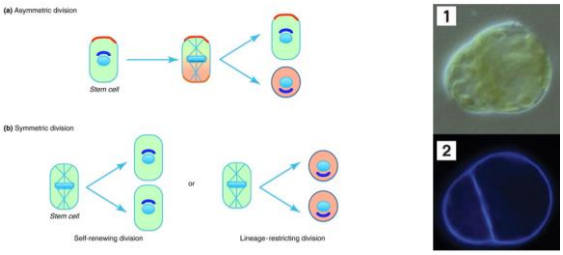
The globular stage is essential in embryogenesis



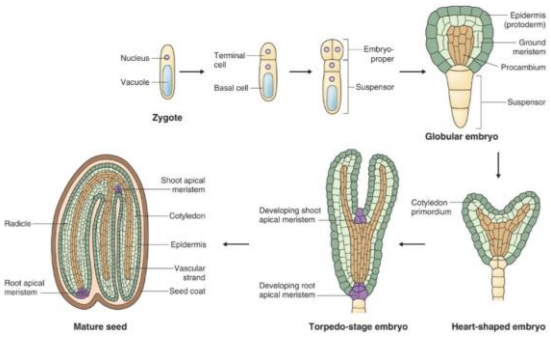
POLARITY

Asymmetric cell division: Mitotic event that generates 2 daughter cells of which one has a different cell size, structure, and fate from the original one

In plant development, asymmetrical divisions are essential to form new structures and to acquire new functions

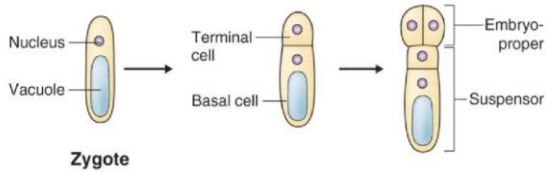


Apical-basal polarity: Polarity in which tissues and organs are arrayed in a stereotyped order along the axis that extends from the shoot apical meristem to the root apical meristem



ORIGINS OF POLARITY

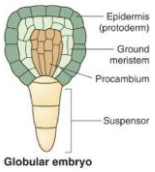
Zygotic stage: Polarized growth of this cell, followed by an asymmetric division gives rise to a small apical cell and an elongated basal cell. The apical cell will give rise to most of the embryo whereas the basal cell will ultimately form the extra-embryonic suspensor, which will attach the embryo to the vascular system of the plant. In some species, suspensor cells can synthesize auxin or gibberellins to support the development of the embryo. At the globular stage, the suspensor is not longer required and undergoes programmed cell death. If something goes wrong in the globular stage of embryogenesis it will affect the life of the plant



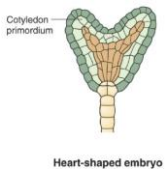
By the early globular stage, it can be distinguished:

- Apical region → cotyledons and shoot apical meristem
- Middle region → hypocotyl, root and apical domain of root meristem

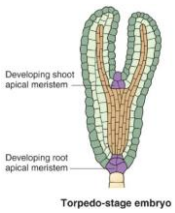
Globular stage: The apical cell undergoes a series of divisions to generate a globular embryo exhibiting radial symmetry, creating the outer layer termed protoderm, which will become the epidermis and the inner tissues, which will form the vascular and ground tissues. At about the same time, the suspensor asymmetrically divides to form the precursors of the quiescence center (QC) and the distal stem cells of the root meristem



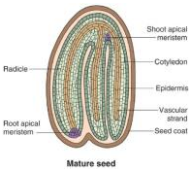
Heart stage: Rapid cell division in two regions on either side of the future shoot apical meristem form the cotyledon primordia, giving the embryo bilateral symmetry



Torpedo stage: Cell elongation throughout the embryonic axis and further development of cotyledons occurs



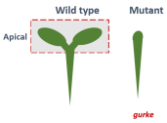
Mature stage: The embryo and seed lose water and become metabolically inactive as they enter in dormancy. Storage compounds accumulate in the cells at the mature stage



The specification of different cell identities during embryogenesis is tightly controlled by molecular signalling pathways and is often marked by the onset of specific gene expression patterns

MUTANTS WITH DISRUPTED APICAL-BASAL MORPHOLOGY

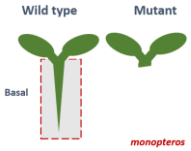
Gurke (GK): Cotyledons and apical meristem reduced or missing. It encodes an acetyl-CoA carboxylase. Very long chain fatty acids and sphingolipids required for embryogenesis



Fackel (FK): Malformed cotyledons, short hypocotyls and root. Sterol C-14 reductase

Gnom: Guanine nucleotide exchange factor involved in polar distribution of PIN proteins. Involved in the intracellular vesicle movement required to deliver PIN proteins to targeted sites

Monopteros (MP): Auxin response factor that regulates genes that guide auxin-dependant vascular development



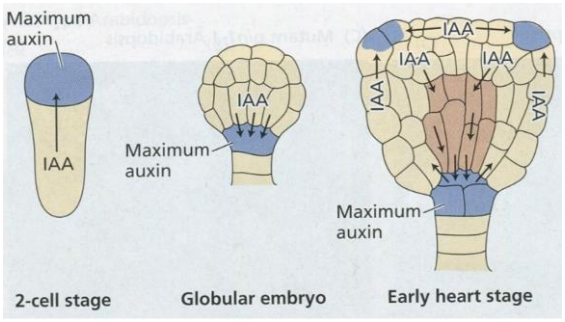
POSITION DEPENDANT SIGNALING

Important for exam!

Every cell has different information due to position

Position-dependant signals modulate the behaviour of cells (i.e. division pattern) depending on the position of these cells within the developing embryo

Auxin evokes a range of concentration-dependant responses. In the early embryo, auxin flows upwards into the globular embryo. Later (heart-stage) the auxin flow is reversed. Auxin transporters (PIN proteins) are asymmetrically distributed during early stages of embryogenesis. Different auxin availabilities in root and shoot regulate gene expression and contribute to the establishment of different developmental routes

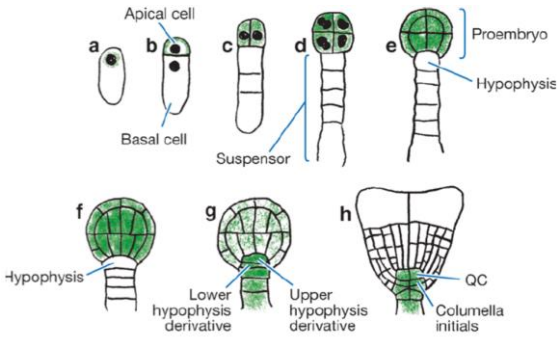


Blue areas denote cells with maximum auxin concentrations

ROOT APICAL MERISTEM (RAM) ESTABLISHMENT

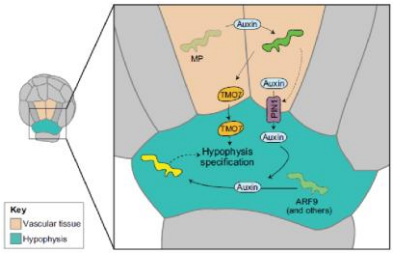
The RAM is a population of cells displaying stem cell features residing at the basal pole of the embryo that gives rise to root tissues. The uppermost cell of the hypophysis divides and forms four cells that will later form the quiescence center (QC) and precursors of the columella stem cells. The initial expression of QC-specific markers coincides with the basal shift of the auxin maximum

Green shading indicates the distribution of auxin

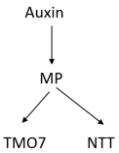


HYPOPHYSIS SPECIFICATION

The specification of the hypophysis is considered to be the initiation of the RAM. The specification of the hypophysis is a process highly regulated by auxin, which accumulates in this cell by the activity of PIN proteins. Auxin induces the expression of *MP* and Target of Monopteros 7 (*TMO7*), which is expressed in the vascular initials. *TMO7* protein acts as a mobile factor and gets transported to the hypophysis. Suppression of *TMO7* by RNA interference leads to abnormal hypophysis divisions and rootless phenotypes, similar to *mp*



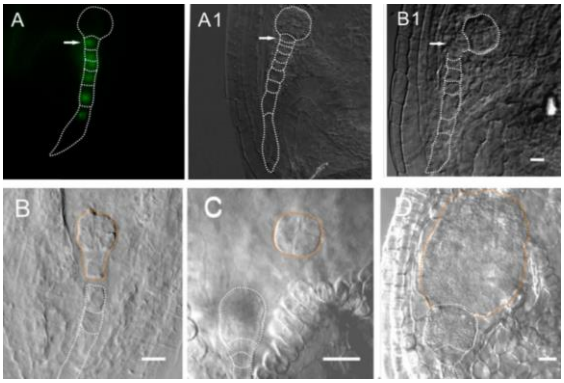
Auxin induces the expression of *MP* and its target No Transmitting Tract (*NTT*). *ntt* mutants exhibit defective post-embryonic root growth. *NTT* misexpression in other embryo or root tissues is sufficient to transform stem cell populations to a columella stem cell fate



SUSPENSOR CELLS HAVE EMBRYONIC POTENTIAL

The suspensor undergoes programmed cell death at the globular stage

After ablation of the first suspensor cell, the remaining suspensor cells can develop into a new embryo before heart-embryo stage. Once the embryo-suspensor connection is removed, suspensor at stages before globular embryo have embryogenic potential

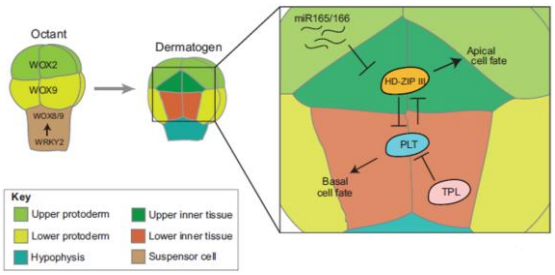


Once the first embryo-suspensor connection is removed, the first suspensor cell starts accumulating auxin and undergoes a regular pattern of cell divisions. The first suspensor cell becomes the hypophysis. Auxin comes from the maternal tissues

DETERMINATION OF ROOT AND SHOOT DOMAINS

The octant stage embryo can be anatomically divided in 2 domains: upper and lower tiers that will form aerial and root tissues

The Wuschel-related homeobox (*WOX*) family is differentially expressed in these domains. *WOX2* is expressed in the apical domain whereas *WOX9* is expressed in the basal domain. Plethora (*PTL*) gene family and the *HD-ZIP III* transcription factors display antagonistic roles in establishing apical and basal cell fate. *HD-ZIP III* genes are known to regulate formation of the SAM whereas *PLT* genes are known to determine basal cell fate. Ectopic expression of *PLT* genes in other regions induces these cells to acquire hypocotyl, root and shoot stem cell niches cell fate

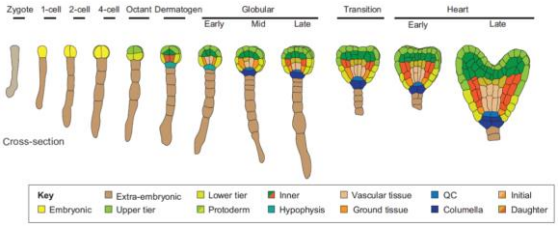


The genes are differently sensitive to auxin

RADIAL PATTERNING

Successive periclinal and anticlinal divisions will originate the distinct tissues:

- Protoderm → Epidermis
- Vascular primordium → pericycle and vascular stele
- Ground tissue → cortex and endodermis
- Hypophysis → quiescence center and root columella

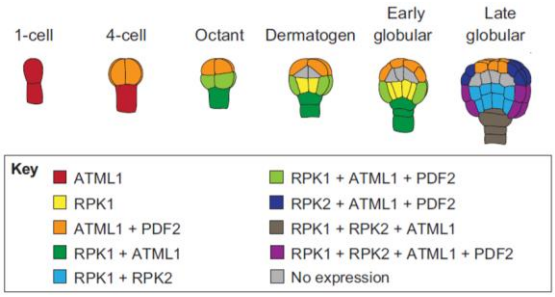


GENES INVOLVED IN THE ESTABLISHMENT OF TISSUE IDENTITIES

Geneses required for separation of the inner and outer layers:

- Arabidopsis thaliana meristem layer 1 (ATML1)
- Protodermal factor 2 (PDF2) transcription factors

The anatomical delineation between the outer layer and inner cells coincides with a change in gene expression pattern of ATML1



PDF2: ubiquitously expressed in 4-cell stage embryos, but its expression gets restricted to the outermost cell layers

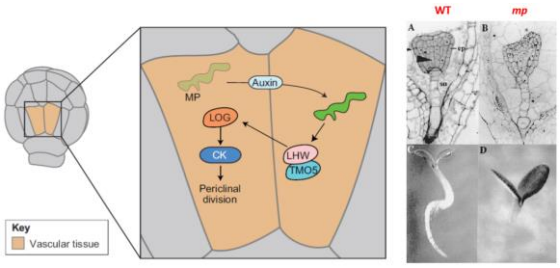
ATML1: sufficient to promote epidermal identity

***pdf2 atml1* double mutants**: lethal. Weak double mutant combinations lack epidermis and exhibit mesophyll cells at the surface

As soon as the outer cells start to express epidermis-specific genes, the inner cells are marked by the expression of other genes such as *Monopteros* or *PIN-formed 1*

VASCULAR DIVISION

All root vascular tissues are derived from four provascular initial cells in the early globular stage embryo that undergo several rounds of periclinal divisions. In future vascular cells, auxin promotes *Monopteros* and its target genes *TMO5* and *TMO5 Like1*. *mp* mutants display defects in the divisions that generate vascular tissues and they fail to establish an embryonic root. TMO5 forms a heterodimeric complex with the transcription factor *Lonesome Highway* and both promote the expression of the CK-biosynthetic enzyme Lonely Guy4 (LOG4). The TMO5/LHW-induced CK production is crucial for the activation of periclinal divisions and the establishment of vascular patterning

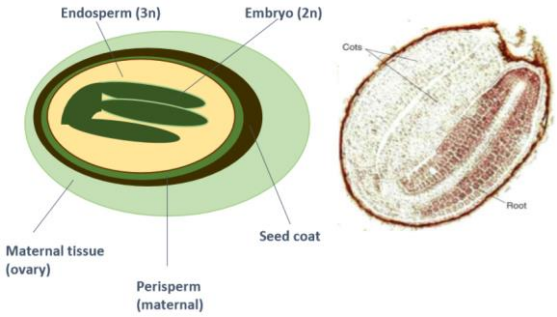


SEED STRUCTURE

The dispersal unit is composed of the seed, in some cases together with fruit and additional maternally derived tissues

The seed:

- Embryonic tissue (result of egg cell fertilization)
- Endosperm (storage tissue derived from the diploid central fertilization)
- Perisperm (maternal storage tissue derived from the ovary cells)
- Testa (maternal tissue derived from the ovary cells, forming the seed coat)
- Fruit (ovary derived tissue surrounding the seed to protect or aid dispersal)

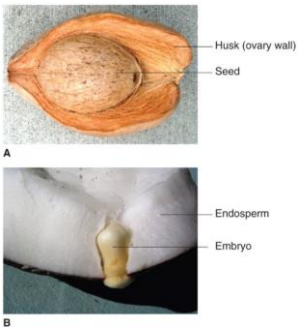


SEED COAT AND ENDOSPERM

Seed coats are made of layers of dead cells that protect the embryo. It is impregnated with phenolic compounds that protect the seed from attack by herbivores and pathogens and can impose dormancy by making the seed coat rigid or impermeable to water

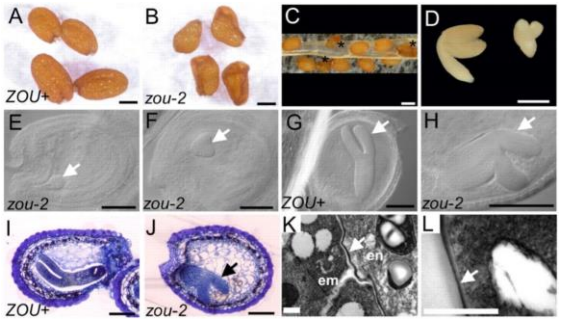
The endosperm contains stored food, which will be used to fuel growth of the seedling after germination. It either accumulates nutrients mainly in the form of starch (maize, wheat, rice,…) and serves as nutrient source for germination, or degenerates and provides its nutrients to the developing embryo (Arabidopsis)

A layer of living cells, the aleurone layer, surrounds the endosperm and produces enzymes that are required for nutrient mobilization from the endosperm during germination



INTERCELLULAR SIGNALS BETWEEN ENDOSPERM AND EMBRYO

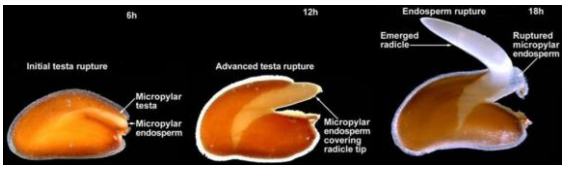
The growth of the seed coat, the endosperm and embryo must be stringently regulated to secure development of a viable seed. Endosperm and embryo develop autonomously during early seed development at early stages. However, the endosperm plays a crucial role in regulating embryo development at later stages and in controlling overall seed growth. *Zhoupi* (*ZOU*) is a transcription factor specifically expressed in the endosperm. *zou* embryos exhibit defects in cuticle formation and epidermal cell adhesion, demonstrating the non-cell autonomous activity of *ZOU* in embryo development. Moreover, *ZOU* controls endosperm breakdown



GERMINATION

Germination: Penetration of the seed coat by elongating embryonic root

The first step towards germination is hydration or imbibition, as the dry seed takes up water. This results in the activation of the living embryo, which starts to grow after germination

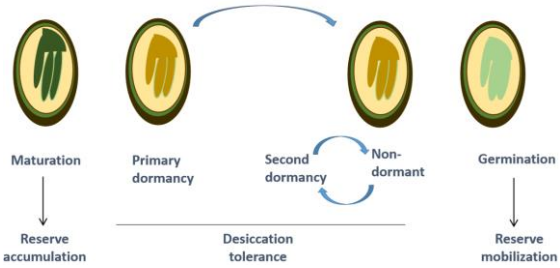


(6h: no germination. 12h and 18h: germination.)

DORMANCY

Dormancy: Viable seeds that do not germinate under normally favourable conditions of water and oxygen availability

Seeds may undergo seasonal dormancy cycling if conditions are suboptimal, progressively gaining or losing dormancy until they germinate or die. In some cases, seed dormancy appears to be maintained by the seed coat, by the mechanical restraint of the embryo or by the impermeability of the seed coat to the uptake of water or gases



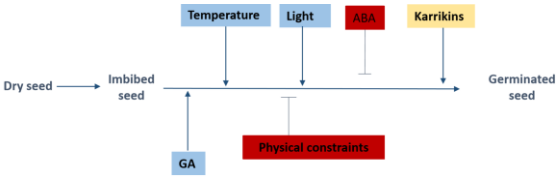
FACTORS REGULATING GERMINATION

Environmental signals such as temperature or light normally break dormancy. Most species have an optimal range of temperature for seed germination. Extreme temperatures (hot and cold) may be required to break dormancy. Light is an important trigger for germination via phytochromes. Light quality is important in preventing germination in shaded areas

Abscisic acid (ABA) is a potent repressor of germination, gibberellic acid (GA) promotes germination by counteracting the inhibitory effects of ABA

Many seeds exhibit seed-coat enhanced dormancy. Seeds imbibe but do not germinate. Other factors such as nitric oxide (NO), water or ammonium influence whether a seed will germinate or not

Karrikins (KAR) are a family of small organic compounds that are produced when plant material burns. When KAR are washed into the soil by rain, they stimulate the germination of certain plant species. These plants will rapidly grow, flower, produce new seeds and die after one year or two. Some plants that grow immediately after wildfires have evolved such that their seeds remain dormant in the soil until a fire generates KAR. A very specialized but successful strategy as fires release plant-bound nutrients and create an open habitat with minimal competition by other plants. In non fire-followers species such as A.thaliana, KAR influence seedling photomorphogenesis, causing smaller plants with larger leaves



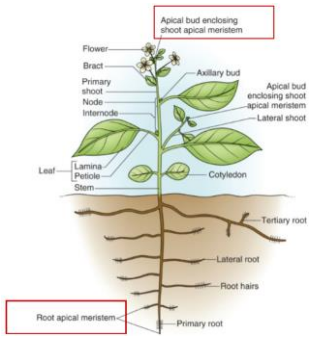
POST-EMBRYONIC GROWTH

Each plant organ is made up of:

- Dermal tissue system: epidermis, which covers and protects the internal organs. In the shoot, epidermis secretes a waterproof layer called cuticle, to slow water loss. In roots, cuticle is absent and epidermal cells form root hairs
- Ground tissue system: mainly formed by parenchyma cells, involved in the production and storage of carbohydrate and organic molecules
- Vascular tissue system: formed by the xylem, with tracheary elements that conduct water and the phloem, with sieve elements (phloem and companion cells) that transport photoassimilates

Plants differ from animals in possessing groups of cells that in principle can grow and divide indefinitely

Apical meristems, found at the tips of roots and growing points of shoots are formed of unspecialized, dividing meristematic cells that can produce new cells that differentiate into mature tissue



ROOT APICAL MERISTEM

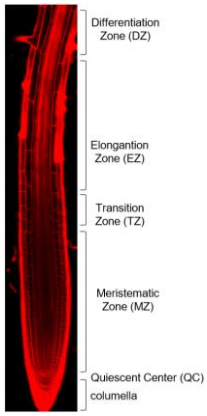
LEARNING GOALS

- Radial cell patterning of the root
- The root stem cell niche and its specification
- Ground tissue formation
- Vascular tissue formation
- Phloem and Xylem specification
- Root hair specification

THE ROOT SYSTEM

The root system of dicot species consists of a single primary root that often remains active throughout the plant's life cycle and can develop several lateral roots. In monocots, primary roots are embryonic, and crown-roots and lateral roots are formed during the post-embryonic growth of the plant

Longitudinal growth in roots is driven by cell division and subsequent cell expansion in the root tip. Cell division occurs in the root apical meristem (RAM) whereas expansion takes place in the elongation zone. When cells reached their final size, they enter the transition zone and acquire their final fate in the differentiation zone

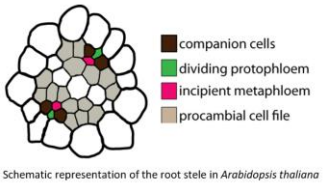


RADIAL PATTERNING IN THE POST-EMBRYONIC ROOT

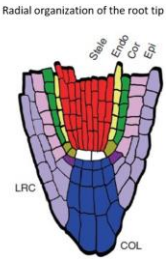
The radial symmetry of roots is already established during embryogenesis. At the distal tip of the root is the lateral root cap (LRC) and central columella (COL). The LRC protects growing root tip and it is required to enable gravitropism by perception of the gravity

The main body od the root consists of concentric tissue layers with different functional properties. From outside-in: Epidermis, Cortex, Endodermis, pericycle and the stele

The stele contains procambian cells that will form two specialized long-distant transport systems: the phloem and xylem



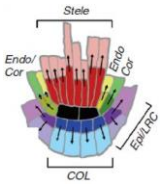
Schematic representation of the root stele in *Arabidopsis thaliana*



THE ROOT STEM CELL NICHE

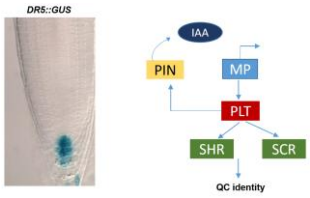
The initial cells divide to produce columella cells, the vascular tissue, the lateral root cap, epidermis, and the ground tissues (endodermis, cortex)

The quiescence cells have very low mitotic activity and they function as an organizing center. The initials are stem cells that can undergo regular division to extend the cells files of the root. The QC produces a signal that prevents the premature differentiation of the initials. The QC is required for the indeterminate root growth, in mutants which do not maintain QC, the root meristem is eventually exhausted, and cells cease to divide. QC cells acquire their fate most likely by positional information and not by cell lineage



SPECIFICATION OF THE QC

QC identity is specified early during hypophysis specification during embryogenesis. The specification of the QC is the result of an auxin concentration maximum. Auxin induces the expression of the *Plethora* (*PLT*) genes by the activity of the ARF factor *Monopteros* (*MP*). PLT proteins induce in turn the transcription of PIN proteins. PLT together with *Scarecrow* (*SCR*) and *Short-root* (*SHR*) are required to activate QC-specific promoters. In *plt1 plt2* and *scr* mutants, a functional QC is not maintained, and the root meristem collapses post-germination

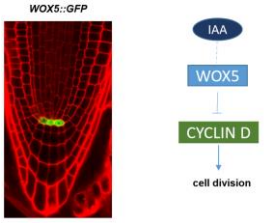


QC ACTIVITY AND REGULATION

WOX5 transcription factor is an important member of the Wuschel family of plant stem cell regulators that is specifically expressed in QC. WOX5 prevents the division of QC by inhibiting Cyclin D activity, a key regulator of the cell cycle

The auxin maximum in the QC restricts the expression domain of WOX5

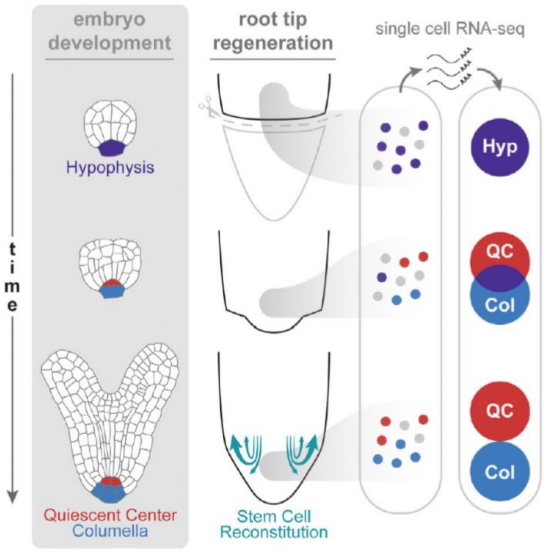
The QC has a more oxidizing environment that the adjacent, rapidly dividing cells. Auxin was suggested to control the redox status of the QC and slow down division by influencing the cell cycle



STEM CELL IDENTITIES DURING REGENERATION

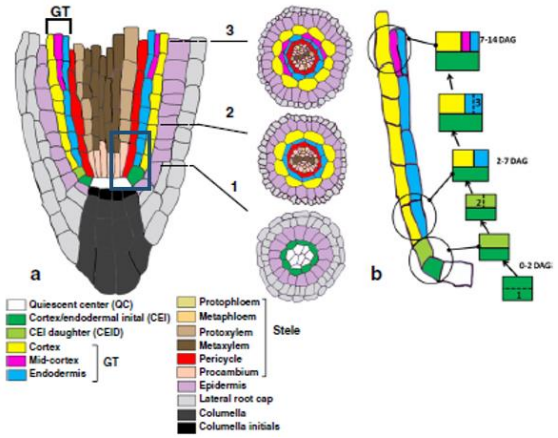
Positional information enables QC and columella regeneration already after one day. Stem cell like properties as the ones observed in QC are dispersed through the meristem, highlighting the great capacity of root cells for reprogramming

Stem cell formation in tip-cut roots is preceded by an embryonic-like developmental sequence: acquisition of hypophysis identity, followed by the establishment of cell populations with QC and columella identities



GROUND TISSUE FORMATION

The cortex/endodermis initials (CEI) first divide anticlinal to generate the CEI daughter cell (CEID) and regenerate themselves (during/soon after germination). CEID then undergoes an asymmetrical periclinal division, resulting in one cortex and one endodermis cell. In the transition zone, periclinal division of the endodermis generates the middle cortex

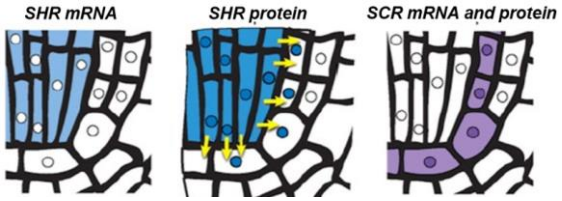


SCR is expressed mainly in cells that produce the endodermis and cortex cell layers by division. *scr* mutants exhibit only one ground tissue layer with both features of cortex and endodermis, suggesting that SCR regulates CEID division

SHR is mainly expressed in the stele. SHR protein moves to cells of the QC and neighbouring

precursor cells, where it accumulates in the nucleus and activates *SCR* expression. *shr* mutants contain also only one ground division with cortical identity

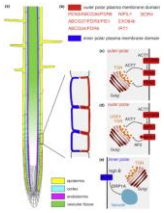
Thus, SCR controls CEID division and SHR controls cell division and cell fate



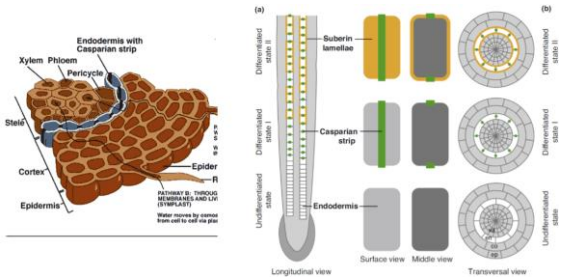
SCR is strongly downregulated in *shr* mutants. *scr* and *shr* mutants behave like *shr* mutants, indicating epistatic effects. *SHR* acts upstream to activate *SCR*

ENDODERMIS DIFFERENTIATION

To reach the central vasculature of the root, water and nutrients radially cross all external cell layers. The endodermis controls the nutrients transport from the soil to the stele and prevents their backflow to the stele. Inner and outer polarity can be observed in endodermal tissues and varies upon environmental conditions



In a first differentialtion step, lignin, which is a waterproof polymer, accumulates in a particular membrane domain named Casparian domain. In a second step, suberin accumulates. There is a crosstalk between the lignification and suberization processes

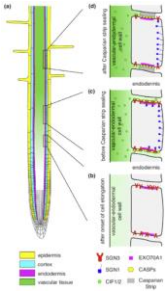


Schengen3 (SGN3): a LRRK-like receptor, accumulates in certain regions of apical and basal domains of the plasma membrane

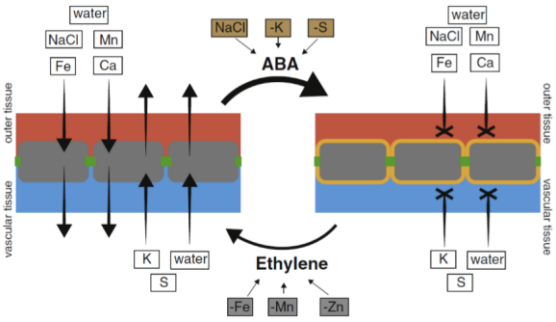
Schengen1 (SGN1): a peptide, translocated from the stele to the endodermis, where it binds SGN3

CASP1: a membrane protein, accumulates in the CASP domain and recruits lignin metabolic enzymes in the apoplast space

Suberin: accumulation occurs afterwards and its deposition is coordinated with lignin formation

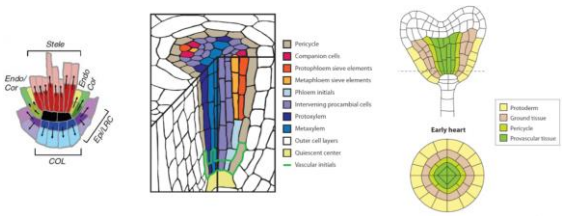


Salt, iron, manganese and calcium can pass through the endodermis to reach the stele as long as endodermal cells are not suberized. An excess of salinity increases ABA-mediated suberization of the endodermis. A deficiency of Fe, Zn or Mn represses the suberin accumulation via modulating ethylene levels



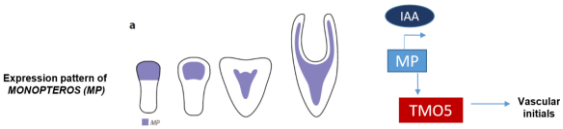
VASCULAR TISSUE FORMATION

The procambium is a vascular meristem containing stem cell populations that function to generate eventually fully differentiated vascular cells, xylem and phloem, during plant development. The vascular initials produce xylem and a phloem initial that periclinally divides gives rise to the phloem. The specification of vascular stem cells is already initiated at the embryogenesis stage, even if there is no xylem or phloem differentiation during embryogenesis



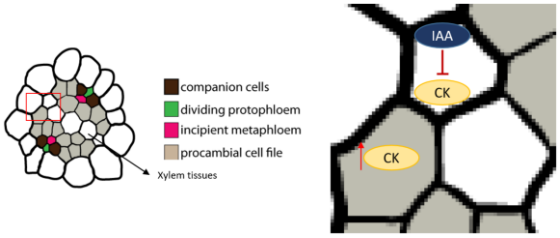
ESTABLISHMENT OF EMBRYONIC VASCULAR TISSUES

Auxin accumulates within developing provascular cells in the embryo. *MP* expression is upregulated where auxin accumulates. *TMO5*, a bHLH transcription factor, exhibits a similar expression pattern as *MP*. It is expressed in the four vascular initials and later it is restricted to xylem cells



PROCAMBIAL VS. PROTOXYLEM DEVELOPMENT

The procambial tissue, which remains undifferentiated, proliferates during root development. High auxin content in protoxylem cells inhibit cytokinin (CK) signalling in procambium cells, where CK levels are high and induce procambium cell fate. Two *AT-hook motive nuclear localized protein (AHL)* genes, *AHL3* and *AHL4* are required to form a procambium-xylem boundary and restrict the xylem axis to a single-cell layer



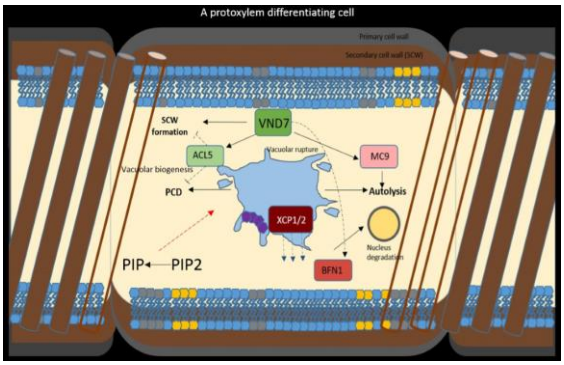
ESTABLISHMENT OF PROTOXYLEM TISSUES

TMO5 interacts with the transcription factor LHW (*Lonesome Highway*) in the xylem vascular initials. TMO5-LHW dimer triggers periclinal divisions in adjacent cells. However, TMO5-LHW dimer is not required to establish cell identity

DIFFERENTIATION OF PROTOXYLEM

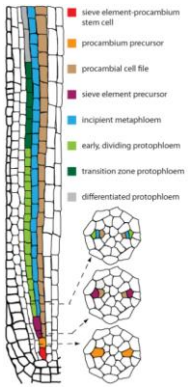
Cell wall reinforcement by cellulose and lignin deposition occurs concomitant with the expression of several PCD markers. Vacuolar compartments enlarge and the tonoplast breaks down. The release of hydrolytic enzymes promotes the degradation of all organelles

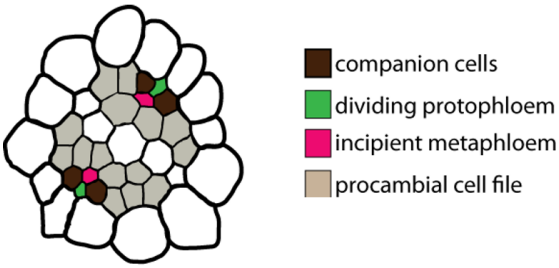
Vanadate7 (VND7): master regulator of protoxylem differentiation. It coordinates PCD, autolysis and secondary cell wall (SCW) formation. Ectopic expression of VND7 is sufficient to promote xylem identity and differentiation



SPECIFICATION OF PHLOEM LINAGE

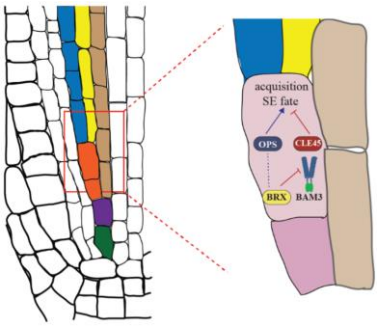
Unlike xylem tissues, phloem derives from a vascular initial that periclinally divides to form a procambium cell file and phloem tissues. Phloem tissues are formed by the sieve elements (proto- and metaphloem cells) and the companion cells



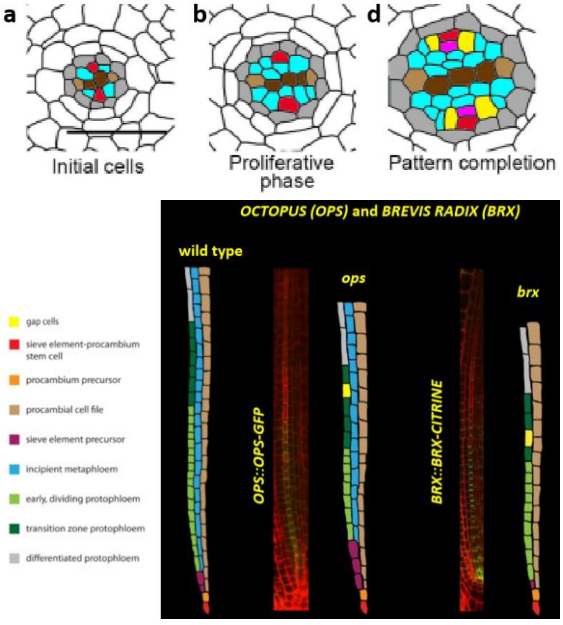


A small peptide called CLE45 and its receptor Barely any meristem3 (BAM3) are specifically expressed in the root phloem tissues. CLE45-BAM3

prevent phloem cell fate acquisition by maintaining the cells in a precursor developmental stage



Pear transcription factors are specifically expressed in the protophloem cells, but the protein moves to the surrounding cells to promote the periclinal divisions of these cells. Cell-to-cell communication is essential to establish the vascular pattern

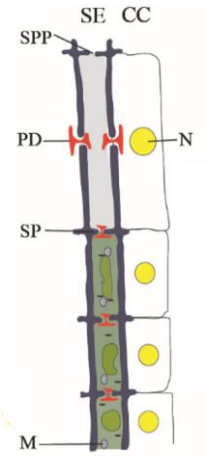


DIFFERENTIATION OF PROTOPHLOEM STRANDS

Protophloem differentiation takes place within the root meristem. Protophloem differentiation is an extremely complex process involving the degradation of mainly all organelles. At the end of this process, cells will become empty conducts to support the transport of photosynthetic products. Metabolic functions are provided by companion cells

NAC45/86-dependant exonuclease-domain protein1 and 4 are two transcription factors involved in nucleus degradation

Phloem and companion cells are interconnected to each other by plasmodesmata, which regulate the traffic of small RNA, proteins and other signalling compounds between CC and SE



SE: sieve element; CC: companion cell; SPP: sieve plate pore; PD: plasmodesmata; SP: sieve plate; N: nucleus; M: mitochondria.

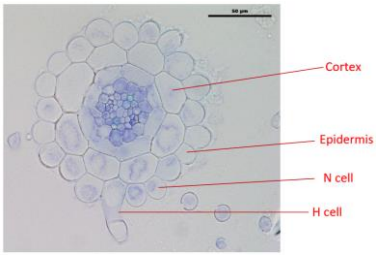
PROTOPHLOEM AND ROOT MERISTEMATIC ACTIVITY

Interruption of protophloem strand continuity has a big impact on root meristematic activity. It affects auxin unloading in the root meristem

In Arabidopsis mutants with a defective protophloem differentiation, root meristematic activity is affected and in turn, post-embryonic growth of the primary root is compromised. Higher accumulation of auxin in the maturation zone of the root leads to an increase in lateral root density

ROOT HAIR DEVELOPMENT

The Arabidopsis root epidermis is composed of two cell types: epidermal cells that are positioned in a cleft between two underlying cortical cells that will develop as hair (H) cells and the ones overlying one cortical cell that will be hairless (N). The fate of an epidermal cell is determined by whether or not a genetic pathway that inhibits hair cell differentiation is expressed

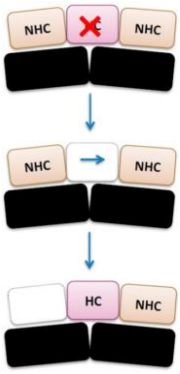


CELL FATE OF EPIDERMAL CELLS

If a root epidermal cell is ablated (X) and its neighbour fills the gap, this cell will take on the cell fate of its new position. Cells continuously respond to positional cues. The determinants of cell fate are most likely located in the cell walls between the epidermis and cortex

Scrambled (SCM): encodes a leucine-rich repeat receptor-like kinase and was isolated as a candidate for mediating the positional cue

The extracellular receptor domain of SCM on H cells may detect some unknown positional signals localized between cortical cells, and then an intracellular domain may transmit its signal into the epidermal cell to establish the appropriate cell fate

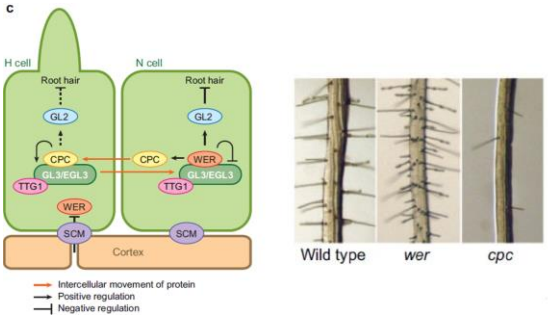


Werewolf (WER): a MYB transcriptional regulator that acts within the N cells to ensure they adopt the non-hair cell fate

Glabra2 (GL2): WER protein binds GL2 promotor and cells expressing GL2 differentiate into non-hair cells

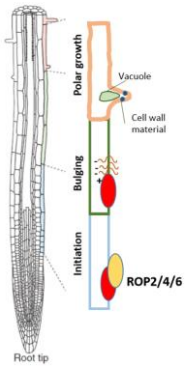
Caprice (CPC): a positive regulator of root-hair cell specification. CPC encodes a small protein with a MYB-like DNA binding domain but without a typical transcriptional activation domain

A positional cue from SCM represses *WER* expression in H cells. *WER* forms a complex with TTG1/GL3/EGL3 and promotes the expression of *GL2* and *CPC*. GL2 in N cells promotes non-hair cell fate. CPC moves to the H neighbouring cell and competes with WER to bind TTG1/GL3/EGL3 complex, resulting in a decrease of GL2 expression. Thus, a lack of GL2 expression allows the acquisition of root hair cell fate



DIFFERENTIATION OF ROOT HAIRS

The Root hair initiation Domain (RHID) is established when ROP proteins accumulate at one microdomain of the plasma membrane. During the bulging phase, ROP C-terminal interacts with the negatively charged lipids of the plasma membrane, amplifying the polar RHID. During polar growth, root hairs grow unidirectionally breaking the cell symmetry. The polar growth is mainly driven by the turgor pressure exerted by the vacuole and the deposition of cell wall material at the plasma membrane by vesicle trafficking



PART BOMBLIES

GENOMICS

LEARNING GOALS

- How we sequence genomes
- Different kinds of sequencing
- The whys and hows of plant genome size variation
- "omics approaches"
- Recombination rates
- Recombination rate plasticity
- How changes in genomes arise
- Outcomes of such changes
- Plant sex chromosomes and plant sex chromosome evolution

HISTORY OF GENOMES

1882: Discovery of chromatin by Walther Flemming

1888: Densely staining bodies that form during cell division termed "chromosome" by Heinrich Wilhelm Gottfried von Waldeyer-Hartz

1888: Theodor Boveri establishes individuality and continuity of chromosomes (constant number over generations)

1892: Germ Plasm Theory by August Weisman: germ cells, not somatic cells, pass on traits and hereditary material affects soma, not vice versa

1902: Theodor Boveri and Walter Sutton suggest chromosomes bear hereditary factors fitting Mendel's laws

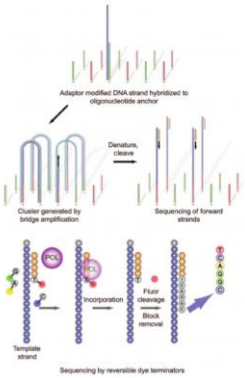
1920: Hans Winkler coined the term "genome" (GENe + chromosOME) → all the DNA in a cell

Genomics → quest to understand "all the DNA in a cell"

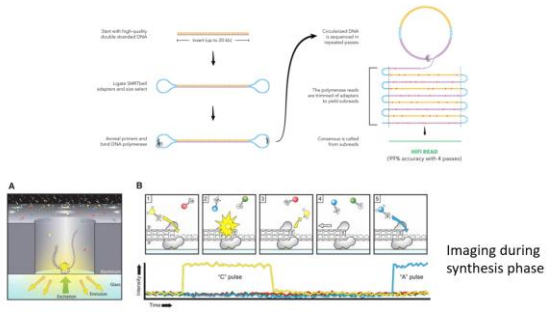
GENOME SEQUENCING

Basic concept: fragment DNA, sequence fragments, reassemble sequence

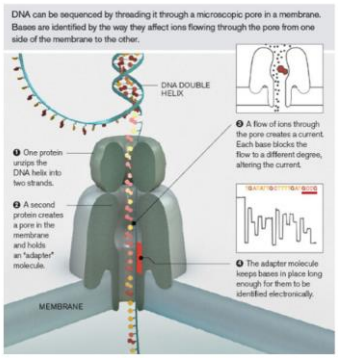
Ilumina sequencing (short read): versatile and cost effective, genome sequencing (best when there's a reference), transcriptome sequencing, ChIP, HiC, other genomic approaches



PacBio (long read): lower error rate (1%)



Single molecule approaches: long reads, high error rate (5-15%), scaffold for other technologies e.g. as part of a genome sequencing project, standalone technology

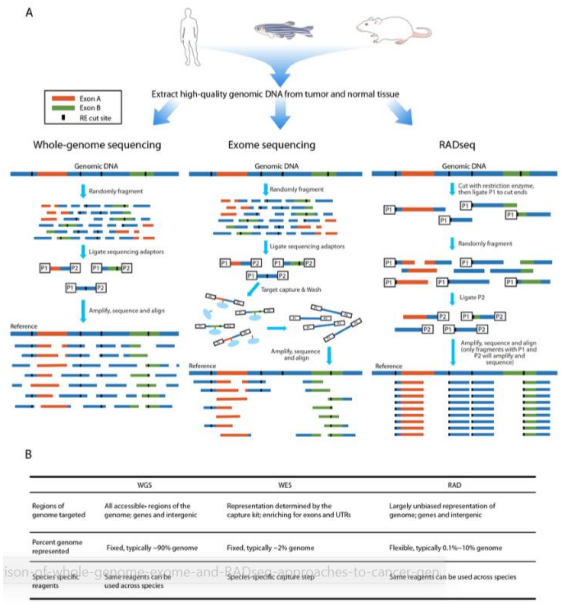


SORTS OF SEQUENCING

Exomeseq: moderate cost, bioinformatically simpler, good when you just care about genes or a subset of genes

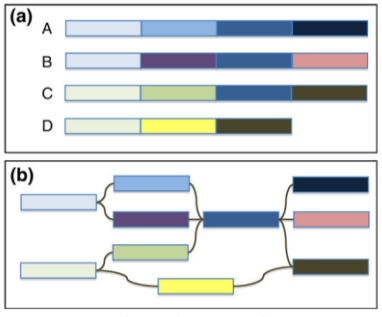
RADseq Advantages: Low cost, bioinformatically simpler, good for markers (mapping, biogeography, phylogenetics,…) → target for GC regions

Caution: dropout alleles



CHALLENGES IN PLANT GENOME SEQUENCING

- Large and highly repetitive genome
- Duplicate genes and history of polyploidy
- Large gene families and abundant pseudogenes
- Difficult to get high quality DNA from plant samples
- Issue of assembling genome-wide, repeat blocks



An example of a "gene graph"

From Schatz et al. Genome Biol. 2012

PLANT GENOMES SIZES

c-value: how much DNA a genome has

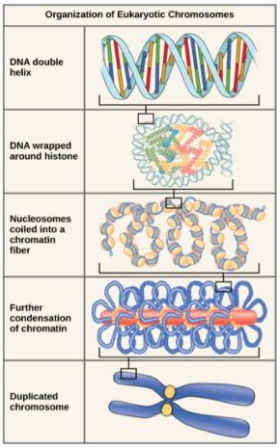
The c-value paradox: Complexity correlates with genome size, but it doesn't scale → a 50x larger genome doesn't make an organism 50x more complex

Sizes of plant genomes vary widely, due to repeat extensions and genome duplications

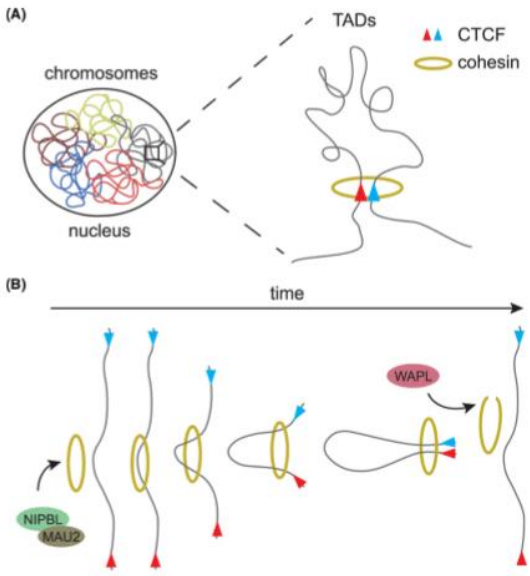
Hidalgo et al.: giant genomes evolved several times independently but seem to max out at 130-150 Gb. Why could that be?

- Biochemical/energy costs become limiting (DNA duplication, histone synthesis, transposon silencing, DNA repair)
- Geometric constraints → surface area to volume rate decreases → could affect membrane transport and gas exchange
- Timing (DNA replication needs time, growth could be slowed down too much)
- Repeat regions become more locked down (tighter packing to use less space) and may not sustain enough variation for evolution to progress

DNA PACKING



Plants have cohesin, condensin and chromatin loops



Genome architecture studied with HiC sequencing

3D genome architecture important for DNA packaging:

- gene regulation,
- co-regulation,
- limiting spreading of silencing marks
- chromosome pairing/segregation

WAYS OF UNDERSTANDING WHAT GENOMES DO

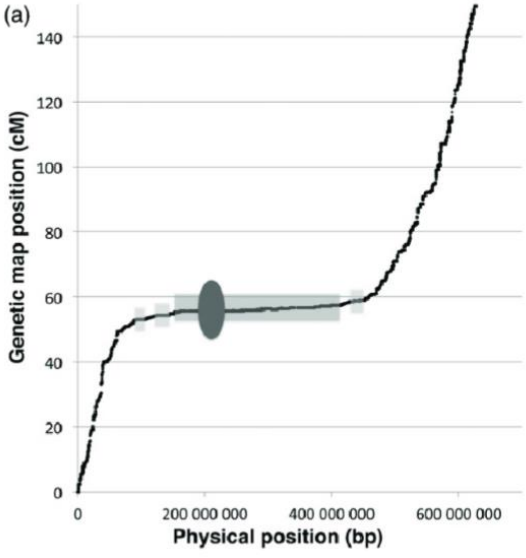
Transcriptomics: expression profiles of genome

ChIP seq.: gene regulation → binding of proteins to DNA (transcription factor targets, cohesin binding, histone positioning, replication,…)

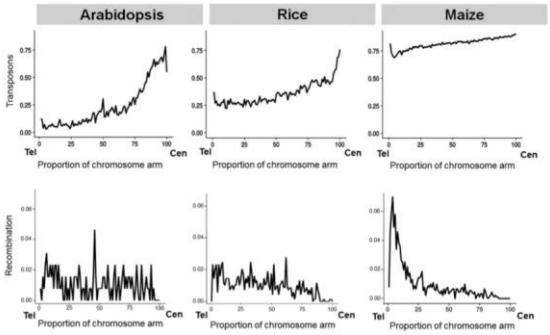
RECOMBINATION

- Gene shuffling
- Important for evolution/adaption
- Makes offspring similar but not identical to parents
- Mechanically important for chromosome segregation in meiosis I

Marey Maps: correlation of physical position and genetic map position

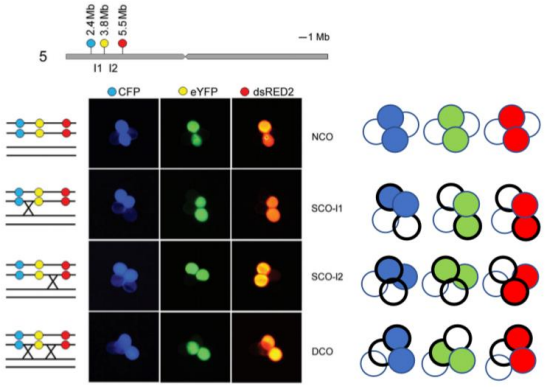


Recombination frequency and placement varies among species



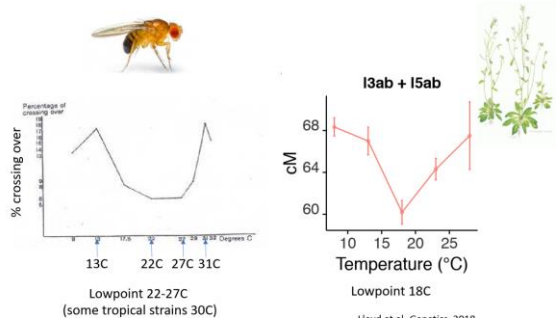
Measuring recombination:

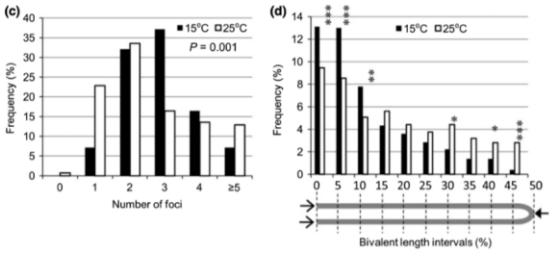
- Under the microscope → place transgenes all over the genome (different colors), cross to noe-carriers with quartet mutation, assay quartets for recombination outcomes



- Analyze pollen by flow cytometry

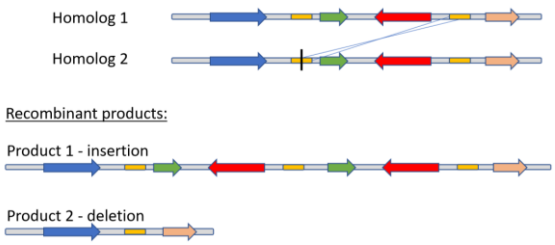
Recombination is plastic to environment (majorly in male meiosis, female meiosis is more stable) and crossover positions can also change with temperature



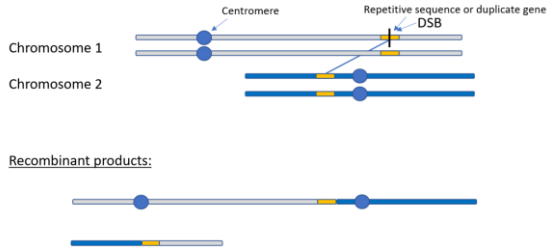


ERRORS IN RECOMBINATION

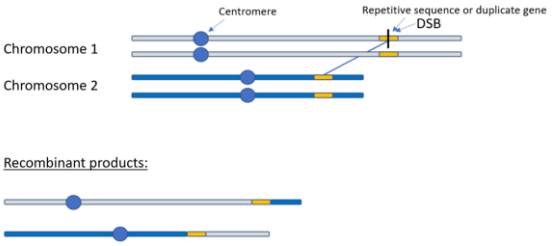
Unequal recombination: incorrect partner sequence used as a template due to repeats → expanding or reducing gene content as a result



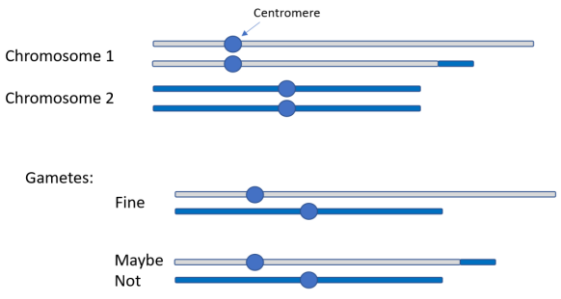
Illegitimate recombination on opposite sides of a centromere: incorrect partner sequence as template due to repeats (non-homologue as template) → dicentric or acentric fragments as a result (generally lost in meiosis, does not make it to progeny)

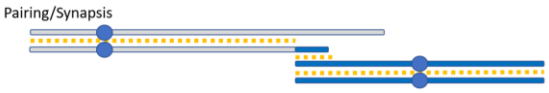


Illegitimate recombination on same side of centromere: incorrect partner sequence as template due to repeats (non-homologue as template) → results in translocation

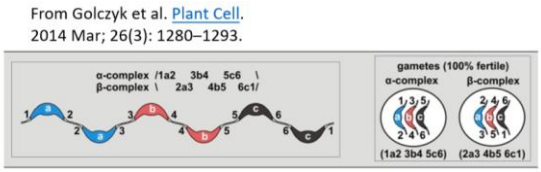


Translocation heterozygosity can (but only maybe) be a problem because essential genes could be lost or there could be problems in meiosis



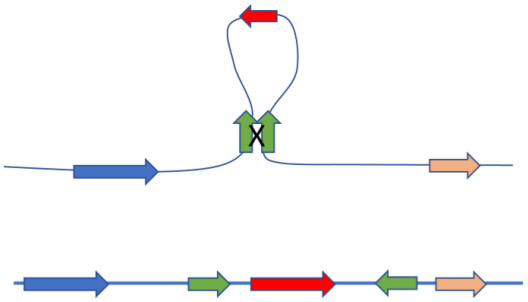


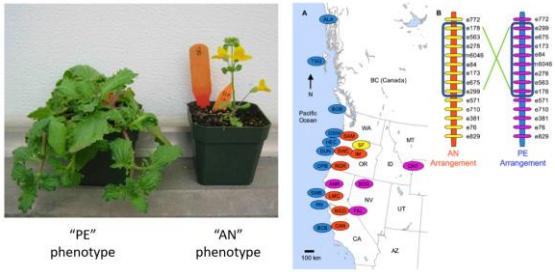
But it can work out totally fine



Inverted repeats: can cause inversions → inversion creates "supergene" because of no recombination in inversion ("co-adapted complex")

Can cause inversions…





SEX CHROMOSOMES IN PLANTS

Homomorphic sex chromosomes: heterozygous (XY) individuals are male (except strawberries)

Heteromorphic sex chromosomes: either "active Y" systems where Y is male and dominant (plant will be male no matter how many X chromosomes are present) or dosage of X chromosomes determines sex

A diversity of plant sex chromosome systems exists:

- Males, females and hermaphrodites exist, YY is lethal (Papaya)
- Two Y chromosomes are needed to be fertile, but sex is determined by X chromosome dosage (Rumex acetosa)

Sex chromosome evolution:

1. Male or female sterile mutation occurs, and recombination is suppressed. At this stage YY is still viable (e.g. Asparagus)
2. Chromosomes still homomorphic. Recombination suppression begins to spread, leading to degeneration and formation of small MSY. Gene loss causes YY to be lethal (e.g. Papaya)
3. Chromosomes heteromorphic. Transposons and duplications accumulate. Recombination suppression spreads to most of Y, further degeneration (e.g. Silene)
4. Severe degeneration and shrinkage of Y (e.g. mammals). Small proportion of the sex chromosomes is still recombining to allow X and Y to segregate against each other
5. Recombination suppression spreads to entire Y, Y may be lost and sex determination controlled by X to autosome ratio (e.g. Sorrel, Japanese hop)

DISCUSSION OF QUESTION THAT CAME UP

Why might selfers have a higher recombination rates?

The answer is not clear

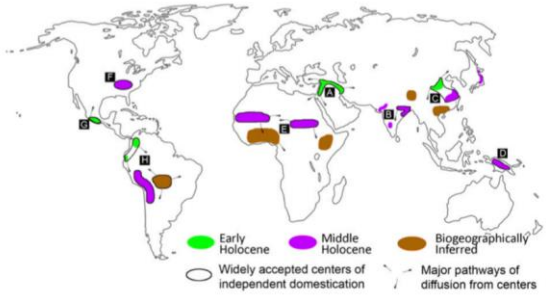
Might be evolutionary selection for higher recombination due to low effective recombination rate of selfers as they are highly homozygous → lines more likely to create recombinants might adapt faster → increased recombination rate increases the likelihood of a recombination event falling in a heterozygous block

DOMESTICATION

LEARNING GOALS

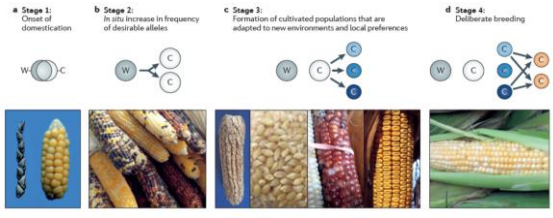
- Where did crops come from and when?
- What are the "phases" of domestication/cultivation?
- What are the pros and cons of genetic diversity in crops?
- Some of the good and bad that can come from breeding with wild species
- Biotechnology – some tools of what some hope to be another green revolution
- Make sure you understand CRISPR/Cas9 – it is revolutionizing basic and applied research!

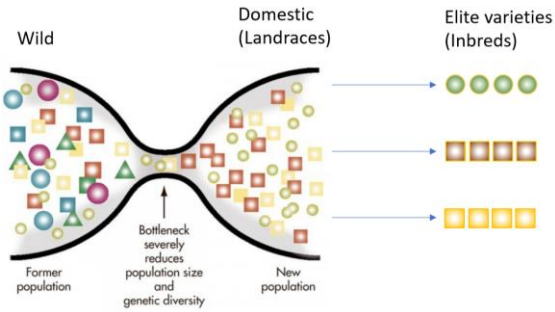
ORIGIN OF CROPS



PHASES OF DOMESTICATION

1. Wild plant brought into casual cultivation
2. Selection of desirable traits (locally) leads to LAND RACES (9000 years ago)
3. Some land races used to make predictable ELITE VARIETIES (60 years ago)
4. Intensive breeding for new traits, crop wild relatives (last 50 years)





Example Maize

How to know what the earliest traits were:

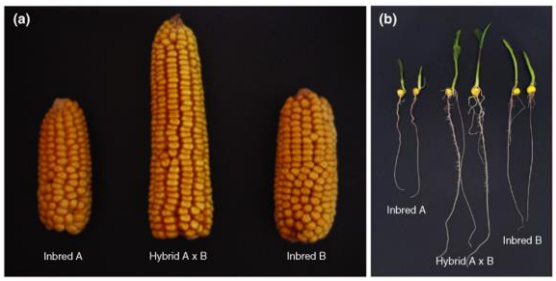
- Archaeology
- Traits that differentiate all maize from teosinte
- Genome analysis → genetic mapping, QTL mapping, GWAS, WGS

Selection of desirable traits:

- Reduced shattering
- Larger ears
- Less bushy plants
- More emphasis on reproductive parts

Improvement of land races:

- Bred for particular traits, especially yield
- Crossing of lines to make up for low fitness coming from low diversity
- (Only F1s are sold)



The green revolution:

- Development of high-yielding, especially dwarf-varieties and hybrids of elite lines
- Monoculture
- Chemical fertilizers and agro-chemicals
- Irrigation
- Mechanization

DIVERSITY

Monocultures also have their downsides → bring back traits from wild relatives

Wild relatives can bring adaptive traits but also unwanted traits

Example banana

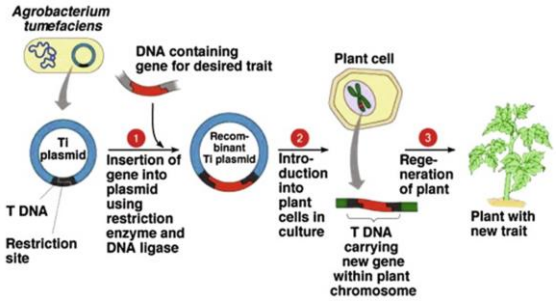
Problems with Banana Streak Virus (BSV)

Crossing of wild relative BSV resistant bananas with unresistant bananas results in 50% infected offspring (could transmit the virus)

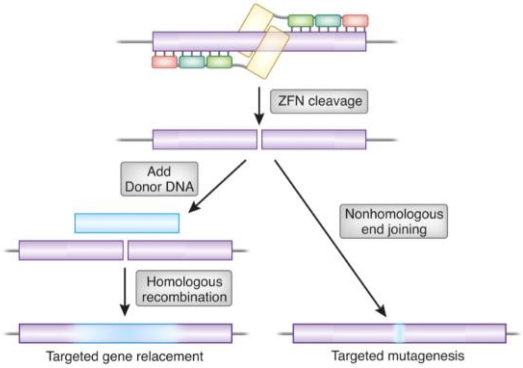
Found out that the resistant strain was heterozygous for a gene causing BSV disease probably by disrupting the genes (one allele can make the active virus, the other one can't)

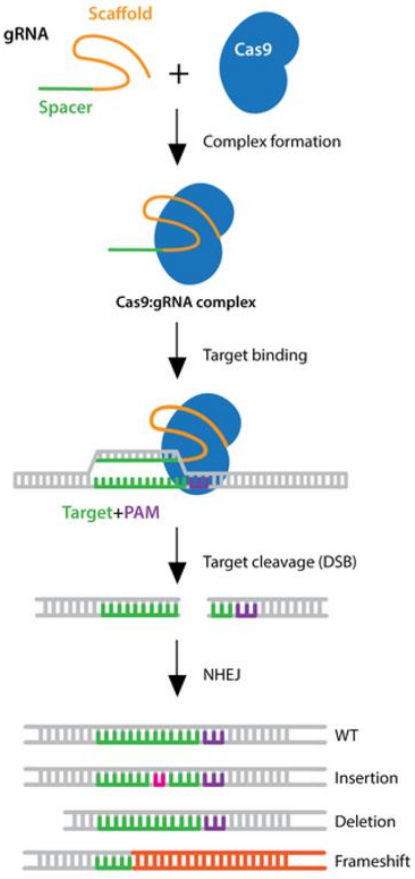
HOW TO MAKE TRANSGENIC PLANTS

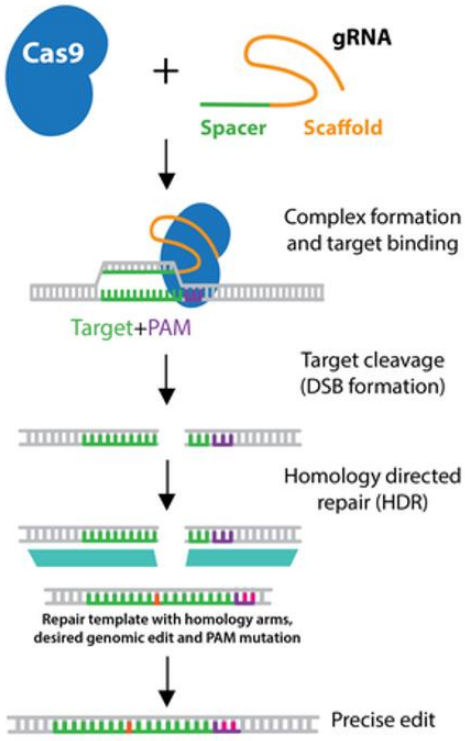
Untargeted: insteriton of T DNA



Targeted: proteins have specific recognition sites, proteins cleave DNA, if DNA is added it can be used to replace the cut sequence







Additional modifications possible with CRISPR/Cas9:

- Base editing
- Gene activation/repression
- Epigenetic modification

EXAMPLES OF MODIFIED CROPS

FlavrSavr tomato: less prone to rotting, but lower yields

Bt Corn: proteins make pores in insect gut and kills it

Papaya: most of Hawaii's papaya are resistant to ringspot virus after introduction of resistant transgenic line

Zucchini: some resistant to three major squash viruses

Golden rice: transgenes express enzymes for beta-carotene biosynthesis

Cassava: transgenic line created to lower levels of cyanogen glucosides

Canola: improve oil content and herbicide resistance

RoundUp ready soybean: herbicide resistant

Banana: production of human vaccines against Hepatitis B

POLYPLOIDY

LEARNING GOALS

- Understand the differences between different kinds of polyploids (allo- vs. auto-, odd vs. even), their origins and the challenges the face
- Understand how to detect ancient vs. new WGD events
- Understand what goes wrong with triploids
- Understand how multivalent pairing is prevented in auto- vs. allopolyploids
- Understand chromosome and allele segregation in different types of polyploid
- Understand duplicate gene resolution (in allopolyploids) – how, why, what might prevent/promote it, different modes
- Understand the potential for diploid-tetraploid gene flow
- Speculate about the link to cold

EXAMPLES OF POLYPLOIDS

"X" vs. "N": "2N" refers to diploid stage of haplo-diploid life cycle → "X" is used instead of "N" to denote genome copies

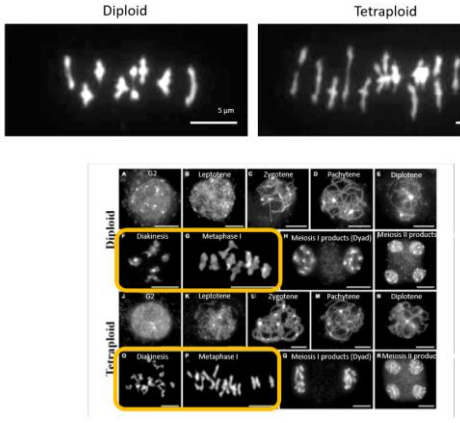
- Sugar cane: 12X
- Coffee: 4X
- Potato: 4X
- Strawberry: 8X

Polyploidy also occurs in animals and fungi

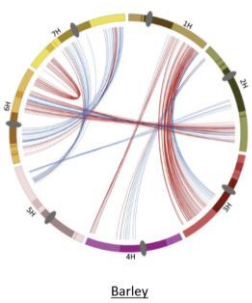
Correlation of polyploid occurrence and climate: more polyploids in extreme north, south and altitude (cold places) → polyploids might be more fertile and therefore survive better in cold regions

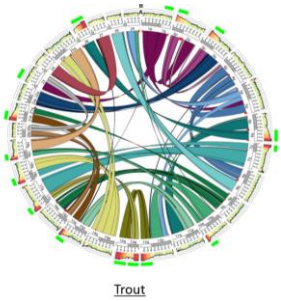
IDENTIFYING RECENT AND ANCIENT POLYPLOIDS

Recent polyploids: easy to identify → most obvious in diakinesis and metaphase I (yellow rectangles)



Ancient polyploids: look for big junks of chromosomes where genes are in the right order, but junks are spread on different chromosomes → most likely from ancient genome duplications


Barley


Trout

TYPES OF POLYPLOIDS

Allopolyploids:

- Have a hybrid origin
- Have subgenomes (Homologs and Homeologs)
- Chromosome pairing preferences
- Often selfers, some outcrossers

Examples: Wheat, cotton, maize, strawberries, coffee

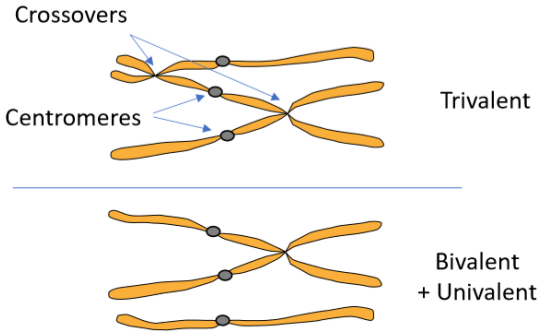
Autopolyploids:

- Have a within species origin
- Single genome (only Homologs)
- No chromosome pairing preferences
- All known ones are outcrossers

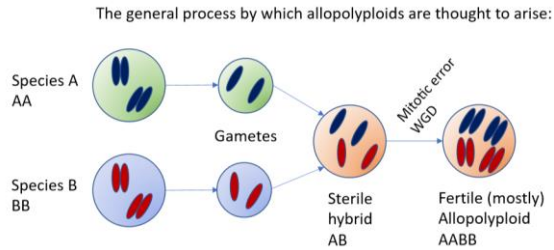
Examples: Potato, blueberries, cranberries, alfalfa, Arabidopsis arenosa

Even polyploids: even number of chromosomes

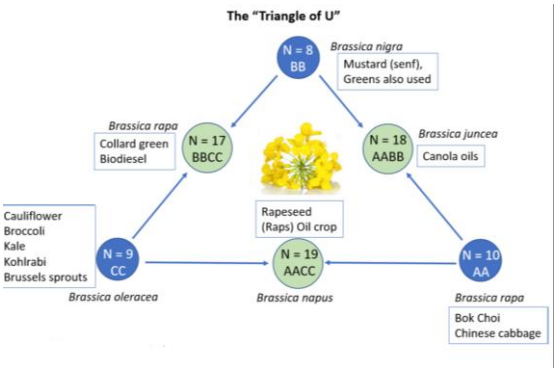
Uneven polyploids: uneven number of chromosomes → in general infertile (seedless), though in rare cases triploids can have some fertility → give rise to aneuploid swarms → over time resolve to 2X and 4X (triploid bridge)

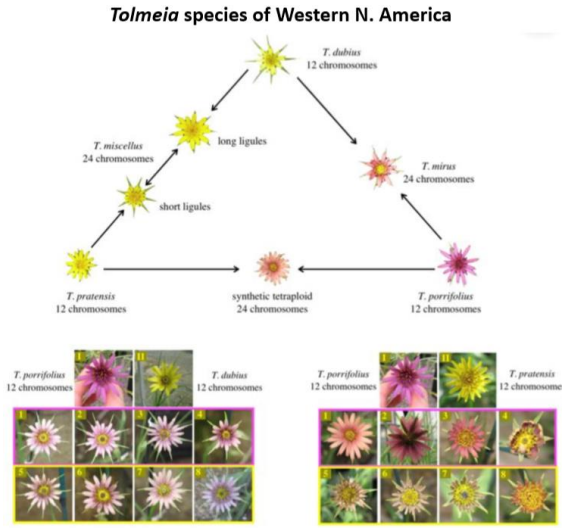


ALLOPOLYPLOIDS



Examples from nature:



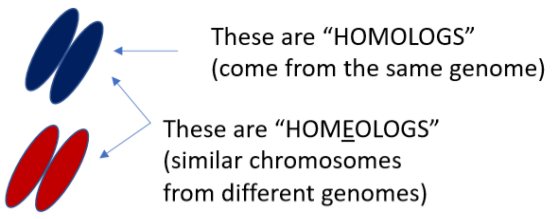


HOMOLOGS VS. HOMEOLOGS

Homologs: Chromosome set origins from same genome

Homeologs: Similar chromosomes that origin from different genomes

The red set is one subgenome, the blue set another



CHALLENGES ALLOPLOIDS FACE

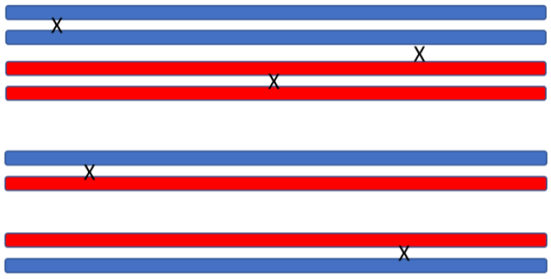
No problem if homologs pair, but homeologs don't

Progeny:

If there are multivalent pairings it can be hard for spindle to segregate (uneven segregation or broken chromosomes are unwanted)

Progeny have dosage imbalance which may or may not be a problem

Differences that allow partner preferences are destroyed → higher risk of multivalent formation in progeny

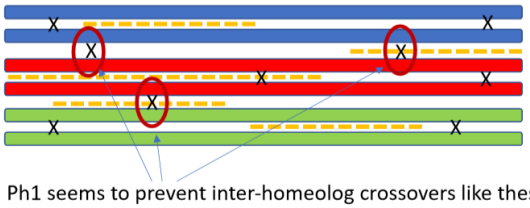


Progeny:

PREFERENTIAL PAIRING OF ALLOPLOIDS

Genetic basis of homeolog pairing preferences Ph1 (example in wheat)

- Subgenomes do not pair
- Genetic locus that causes it – deletion allows homeologs to pair



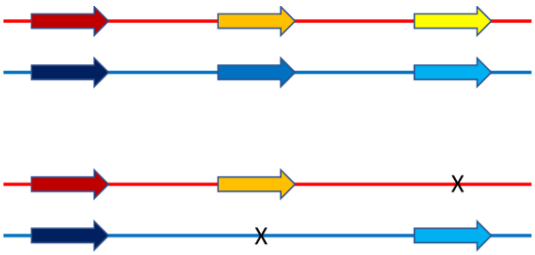
Ph1 seems to prevent inter-homeolog crossovers like these

So in the end you are left with only interhomolog crossovers (=pairing preferences)

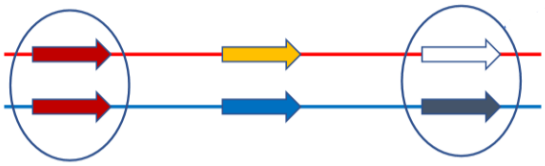
GENE EXPRESSION DIVERSITY GENERATED BY ALLOPLOIDY

- Alloploidy triggers a cascade of epigenetic and genetic changes (transposon activation, gene expression changes, rearrangements, insertions, deletions,…)
- Differences in genome expression dependant on parent of origin → sub-genome dominance
- Subgenomes decay over time and become nonredundant

Gene loss: lost by deletions, silencing, etc.



Non-redundancy: sub-functionalization (they each take over a subset of ancestral functions or expressions) or neo-functionalization (one copy takes on a new function)



Duplicates of one subgenome tend to be more or less retained

The Gene Balance Hypothesis: genes are retained to maintain the balance of protein complexes → predicts that dosage sensitive genes should be over-retained after WGD but under-retained after smaller scale local duplications

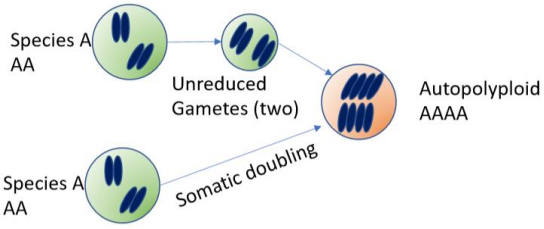
Over-retained after WGD:

- Kinases
- Transcription factors
- Genes highly connected in gene networks

Under-retained after WGD:

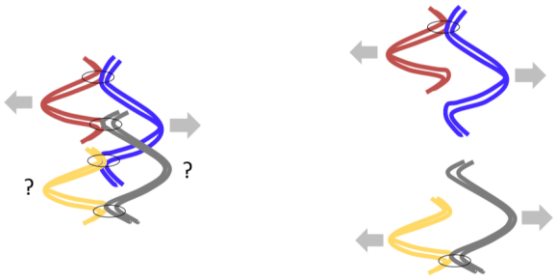
- DNA repair
- Homologous recombination
- Structural proteins
- Proteins prone to dominant-negative effects

AUTOPOLYPLOIDS

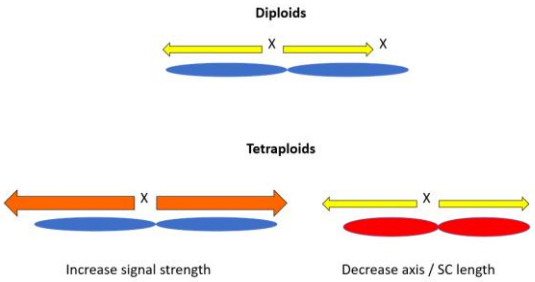


CHALLENGES AUTOPLOYPLOIDS FACE

Different possibilities to pair up → crossover reduction as a solution to achieve meiotic stability, though pairing among homologs remains random

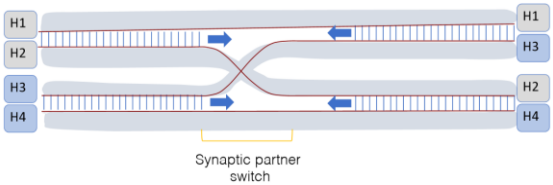


Hypothesis to reduce crossovers: Crossover Interference

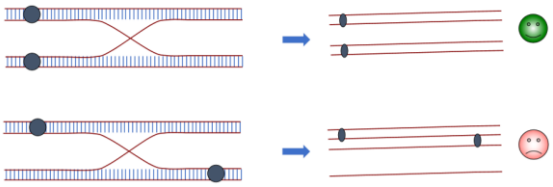


PARTNER SWATCHES AMONG CHROMOSOMES

Only occurs in polyploids



Depending on where the crossovers are it can be a problem

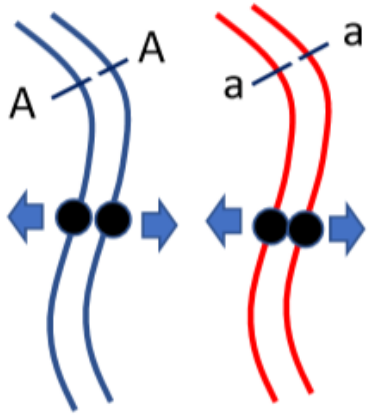


→ meiotic stability in autopolyploids results from:

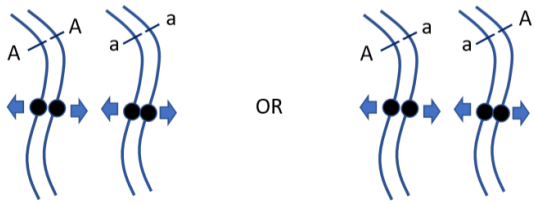
- Reducing the number of multichromosome associations that form
- Making those that do form more likely to be in the safe configuration

GENETICS IN ALLO- VS. AUTOPOLYPLOIDS

Allopolyploids: all gametes are Aa



Autopolyploids: gametes can be AA, Aa or aa



Distinguishing allo- and autopolyploids genetically:

	Autopolyploids	Allopolyploids
Parent	AABB	AABB (each on one homeolog)
Gametes	AA 1/6, AB 4/6, BB 1/6	AB 100%

BUT!! Only sometimes:

	Autopolyploids	Allopolyploids
Parent	AAAB	AAAB (each on one homeolog)
Gametes	AA ½, AB ½	AA ½, AB ½

WEIRD GENETICS

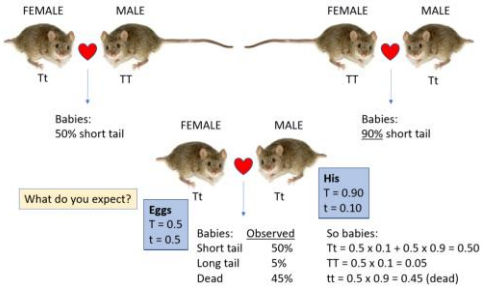
LEARNING GOALS

- Understand how genes can cause gamete killing type drive – work through the examples
- Understand why that systems require multiple genes and why recombination suppression is important
- Understand how "true meiotic drivers" work (the chromosome knobs) – how to cheat female meiosis
- Understand why recombination is important (and where) for female meiotic drive
- Understand what B chromosomes are and why they get maintained despite being usually costly

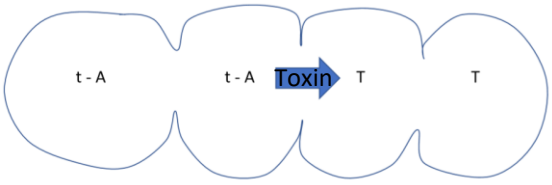
THE TRULY SELFISH GENES

Selfish genes: cheat the Mendelian system

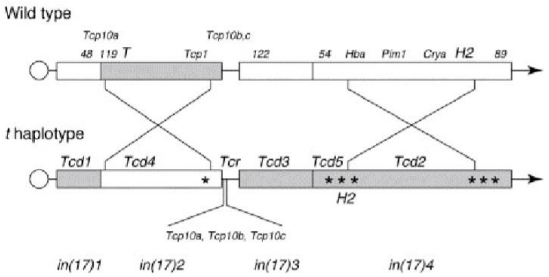
Example short tailed mouse



t is a gamete killer → t makes a poison that makes sperm immotile, but t has the "antidote" on the same chromosome. So, t sperms are immune, T sperms are not

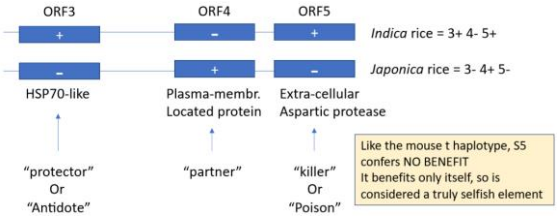


Genetic locus that causes it is called "t haplotype". It has five, genes, TCDs, that cause the effect, and the responder, TCR, that gives resistance. These genes are locked together by inversions. In competition t sperm underperforms relative to T sperms



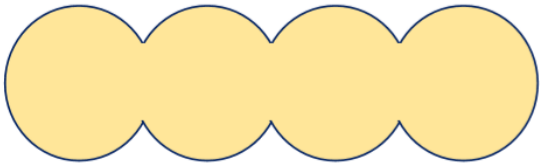
Example S5 system in rice

S5-caused gamete absorption occurs in crosses between rice subspecies → genes don't benefit the host

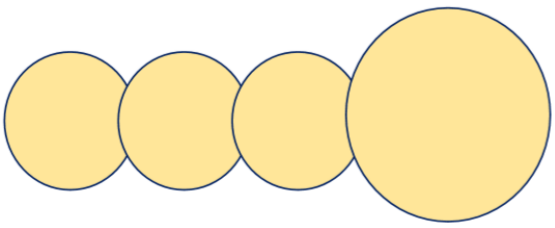


MEIOTIC DRIVE – DIFFERENCES IN MALE AND FEMALE MEIOSIS

Male meiosis product: A syncytium, all four become gametes → male meiotic drive often involves killing competing gametes

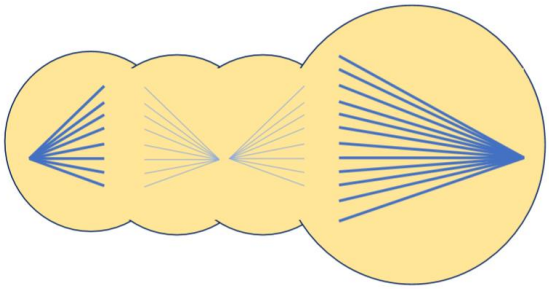


Female meiosis product: often NOT a syncytium, only one becomes the egg, others are polar bodies → female segregation distortion often involves getting into the egg (true meiotic drive)



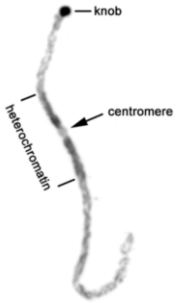
HOW TO CHEAT THE FAIRNESS OF FEMALE MEIOSIS AND GET TO THE EGG

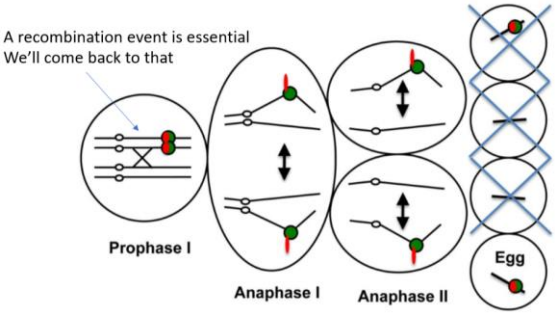
How do selfish genes know, which will become the egg? → The spindles that go to the outer meiosis I products (one will be the egg) are bigger → have a stronger pull

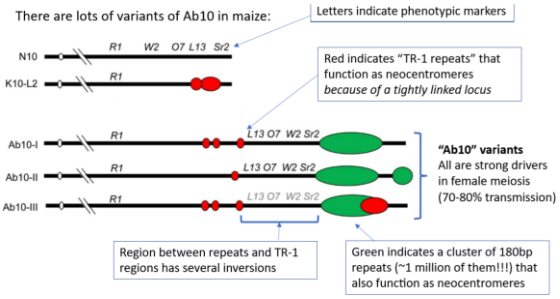


KNOBS ON CHROMOSOMES (MEIOTIC DRIVER)

Unusual chromosomes have "knobs" → these chromosomes are often ahead of other chromosomes on meiosis II spindles. The "knobs" are in fact **neocentromeres** → additional centromere for spindle attachment → extra attachment makes them preferentially move to the stronger spindle pole

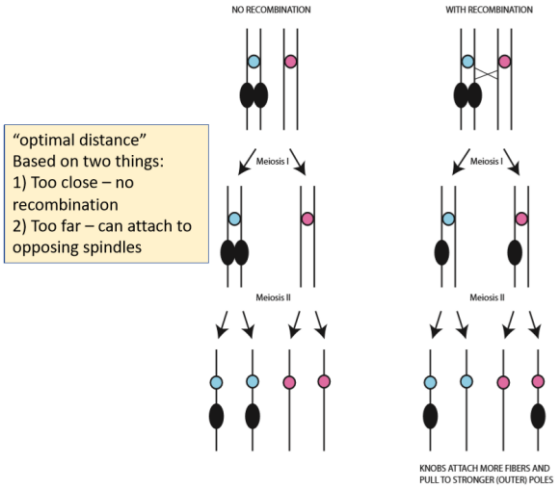






In the knob system recombination is wanted → essential to be effective

Knobs are in optimal distance from centromere

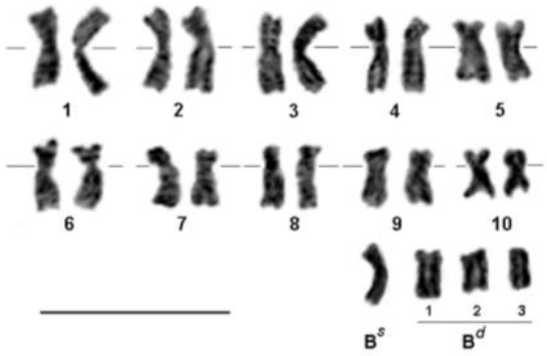


Why are these knobs not everywhere?

- It drags in male meiosis → sperms with abnormal chromosomes are less fit
- B chromosomes are also common an cause a knob loss
- Knobs replicate late and can cause mitotic abnormalities
- Suppressors can evolve on other chromosomes

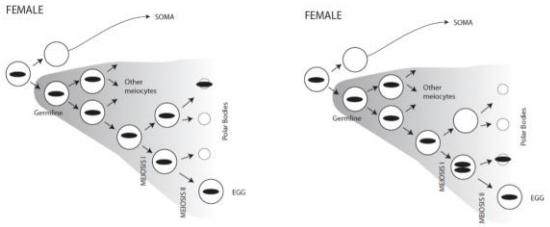
B CHROMOSOMES (MEIOTIC DRIVER)

- Highly heterochromatic
- Usually silent
- Usually full of repeats
- Few or no genes
- Sometimes costly
- Sometimes beneficial
- Usually decrease fertility but:
    - Rye, Lolium, maple: increase survival at high density only
    - Pearl millet: good at low density, bad at high density
    - Chives: increase drought tolerance, but decrease fitness otherwise
    - B chromosomes are worse in odd numbers than even



B chromosomes have big centromeres, so they drive by the same mechanism as knobs

Drive in meiosis II (left) is more common, but drive in meiosis I (right) also works

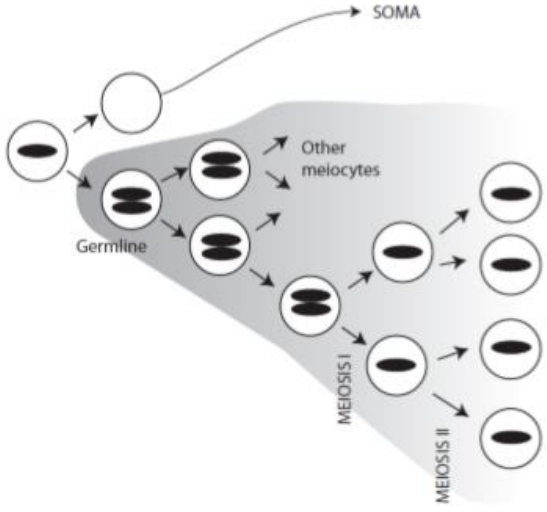


PRE-MEIOTIC DRIVE IN B CHROMOSOMES

Example Crepis capillaris

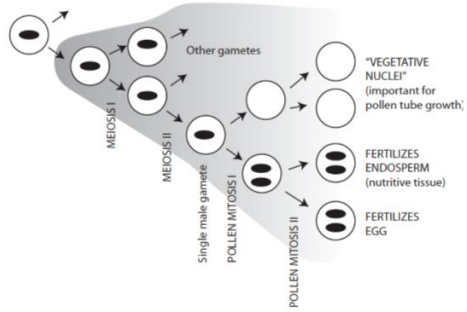
Rare in plants as they don't have a true germline like animals do

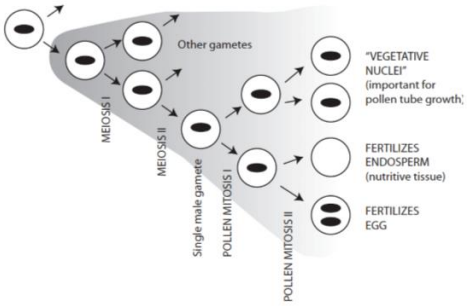
Early in reproductive development, B chromosomes can decondense and become active. There is a non-disjunction to give 2B and 0B cells, where 2B cells are at growth disadvantage, but the 2B cells poison the 0B cells so that they take over the reproductive tissue and get into all gametes



B CHROMOSOMES CAN DRIVE MALE GAMETOPHYTE DEVELOPMENT

Most plants do a pollen mitosis I drive, some do a pollen mitosis II drive, which is not so adventitious





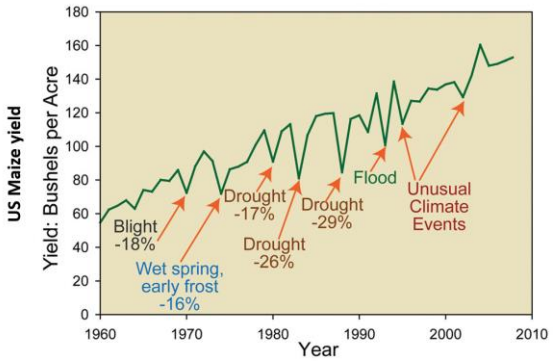
PLANTS AND ENVIRONMENT

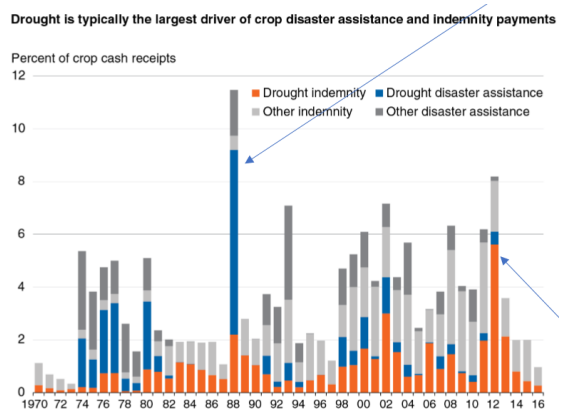
LEARNING GOALS

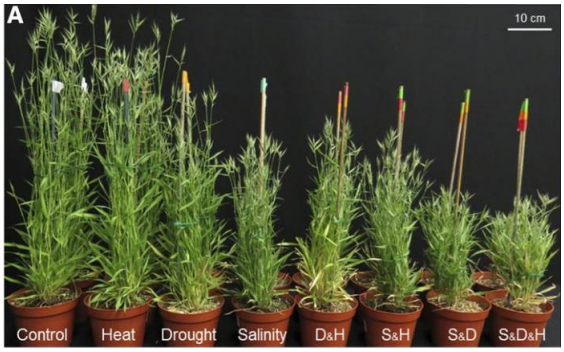
- Plant stress and why it matters
- Mechanisms of plant signalling
  - ROS
  - Calcium
  - How stress is signalled in different cell structures
- Drought stress
  - How osmotic stress is sensed
  - Osmolytes and their role in turgor and cell protection
  - ABA – the plant stress hormone
  - Balancing osmotic stress and growth
- Salinity stress
  - How it is sensed, how it signals
  - ScaBP/SnRK interactions in plant stress
- Temperature sensing
  - Heat and cold responses and how they are integrated into a coordinated output

STRESS IS A BIG DEAL

Temperature, drought, flooding, and salinity are some of the biggest abiotic threats to plant health and human food supply. A combination of stresses is even worse than one stress alone







DEFINITION OF TERMS

Productivity: growth and production of the parts we care about (grain, tubers, leaves, fruit,…)

Fitness: reproductive potential in the Darwinian sense

Elastic response: responses are reversible shifts in biology that can return to baseline when the stress is removed

Plastic response: responses that are irreversible structural or physiological changes

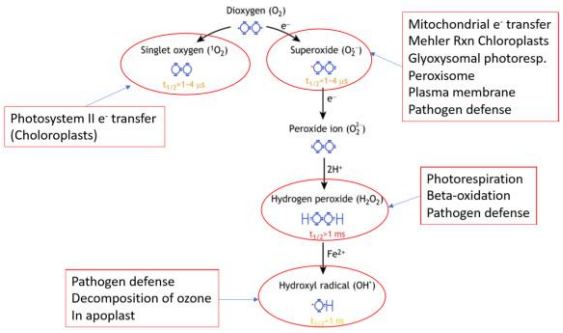
System failure: all ability to respond exceeded → usually death

REACTIVE OXYGEN SPECIES (ROS)

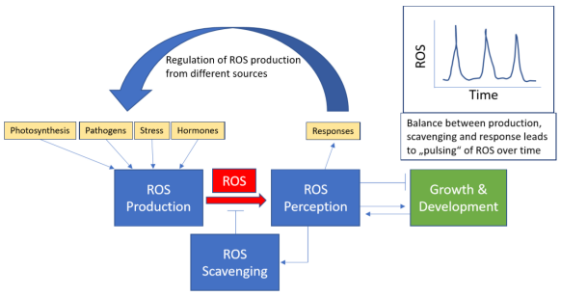
Triggers:

- Ozone
- Drought
- Pathogens
- Heat, cold
- Heavy metals
- Root nodulation
- Salinity
- Anoxia
- High light
- Wounding
- Herbicides
- Senescence

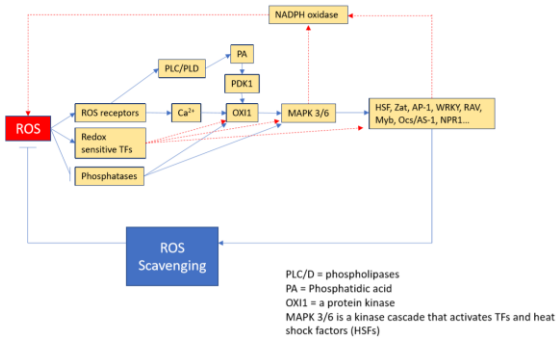
ROS have several sources



ROS are continuously produced by aerobic metabolism, but during stress ROS production can increase and/or scavenging is decreased, so ROS accumulate

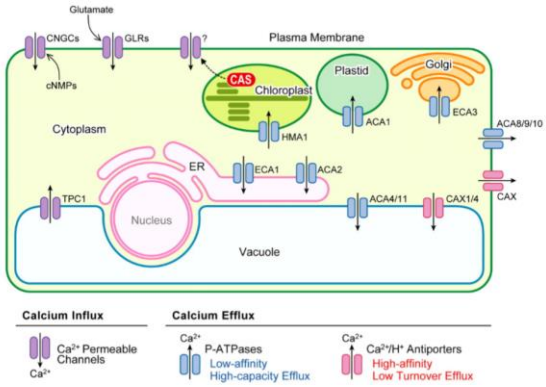


Pathway of ROS sensing (don't memorize all proteins involved)

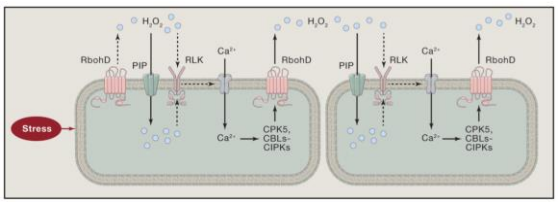


CALCIUM

Calcium is an important signalling factor but what makes it specific?

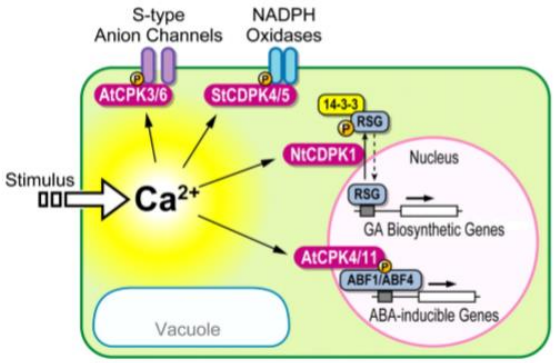


Calcium influx is common to most abiotic and biotic stresses. It activates CPKs (Calcium-dependant protein kinases) and CBL/CIPK proteins. These phosphorylate and activate RbohD. RbohD generates H_2O_2 that diffuses through the cell wall to neighbouring cells. There it induces calcium through receptor-like kinases (RLKs) or enters cells through PIP. This cross-activity generates calcium and ROS waves in tissues that can travel up to 1000µm/sec.



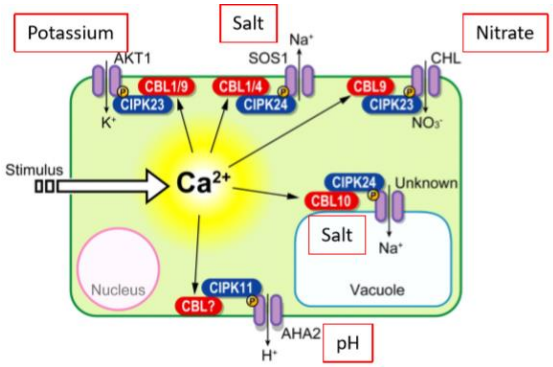
TRANSLATING CALCIUM INFLUX INTO SIGNALS

Calcium-mediated CDPK signalling: Calcium binding to the EF-hand domain of CDFKs relieves autoinhibition, then they autophosphorylate to enhance their own activity



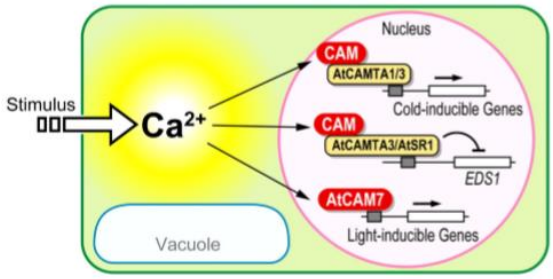
(Don't memorize all proteins)

Calcium-mediated Calcineurin-like signalling: Calcium binding to the EF-hand domain of CBLs interact with CIPKs and activate them. Specificity may be conferred by combinations



(Don't memorize all proteins)

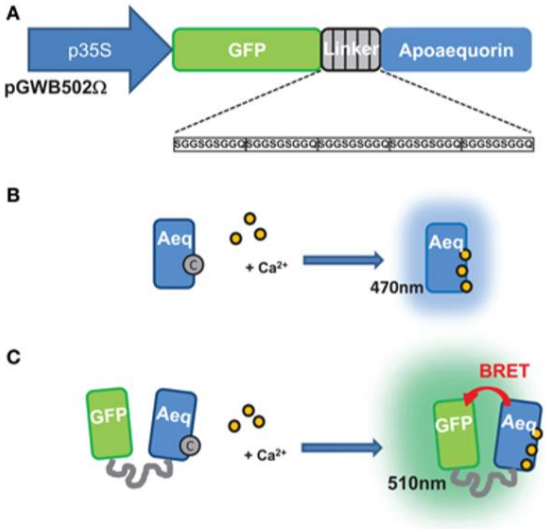
Calmodulin-mediated transcription factors: Calcium binding causes conformational changes in calmodulins, that then interact with CAMTAs to regulate transcription, or in rare cases (CAM7) even do so on their own



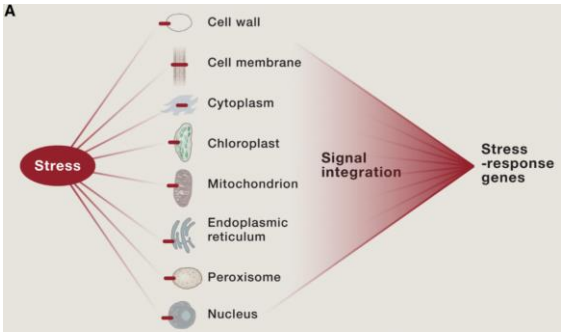
(Don't memorize all proteins)

IMAGING CALCIUM SIGNALS IN PLANTS

Apoaeqorin is a bioluminescent protein from jellyfish that emits light when calcium is present. Apoaeqorin luminescence can be detected with fluorescence microscopy, even with live imaging, but signal is faint. Adding GFP enhances the signal via BRET (**B**ioluminescence **R**esonance **E**nergy **T**ransfer). This construct is called G5A



STRESS SENSING IN DIFFERENT PARTS OF THE CELL



E.g. chloroplasts are prone to light stress and the ER is prone to heat stress. When only chloroplasts send a stress signal, the plant knows that it is light stress

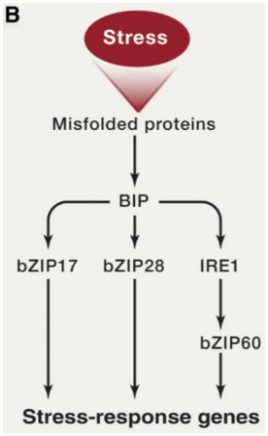
STRESS SENSING IN THE ER

Many proteins are folded/processed in the ER. Stress can affect protein folding or slow degradation of unfolded or misfolded proteins. This can be sensed by the ER and initiate a so-called Unfolded Protein Response (UPR).

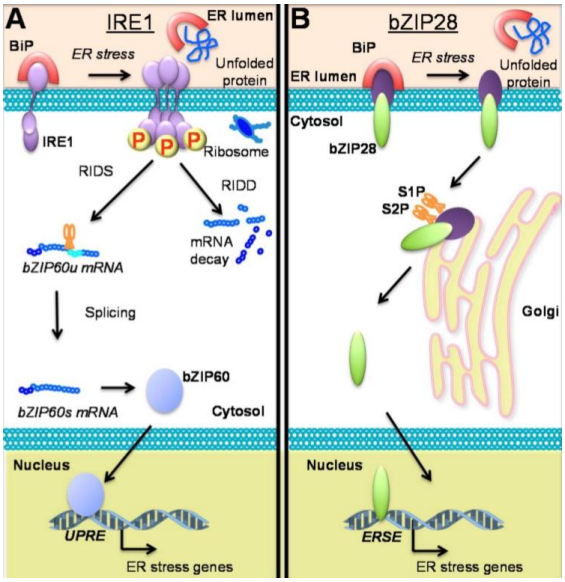
UPR involves: upregulation of chaperones and other proteins that aid folding, slowing of translation → help to restore balance of folding demand and capacity

The best know stress sensing mechanism depends on an ER-membrane localized chaperone called

BIP. One protein that interacts with BIP is bZIP28 – a basic leucine zipper protein. As unfolded or misfolded proteins accumulate during osmotic stress, they interact with BIP and displace bZIP28. bZIP28 is then transported to the Golgi, where it is cleaved. The cytosolic portion relocates to the nucleus where it activates stress responses. bZIP17 is activated in a similar manner but by salt. IRE1 is another protein that resides in the ER. When it binds unfolded proteins, it activates, splices the bZIP60 transcript, which generates an alternate version that returns to the nucleus to activate UPR.



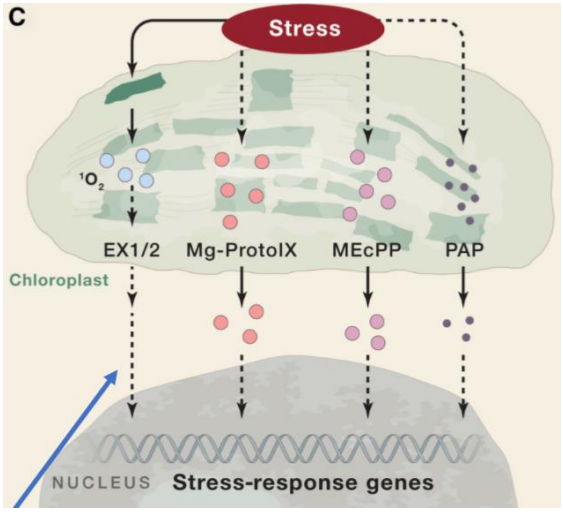
BIP blocks self-activation. When BIP is removed, IRE1 oligomerizes and autophosphorylates its cytosolic kinase domain. Phosphorylation activates IRE1´s ribonuclease domain. It splices specific targets (like bZIP60). (RIDS: Regulated IRE1-dependant splicing) IRE1 can also bulk mRNA degradation (RIDD: Regulated IRE1-dependant decay).



STRESS SENSING IN THE CHLOROPLAST (AND MITOCHONDRIA)

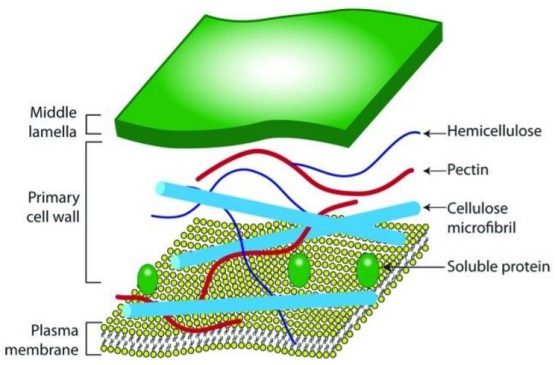
Stress, especially high light, causes the chloroplast to make too much ROS. Accumulation of singlet oxygen is (somehow) sensed by thylakoid-localized receptors EX1 and EX2, which (somehow) signal the nucleus (retrograde signal) to initiate a stress response. Stress also increases the levels of plastid metabolites MEcPP and PAP, which directly function as retrograde signals to the nucleus

In mitochondria, dysfunction also causes ROS accumulation, which in that case triggers the production of ABA stress hormone



STRESS SENSING IN THE CELL WALL

Accumulation of ROS during stress causes cell wall stiffening and remodelling. In yeast, there are sensors that detect cell wall stress and turgor pressure. They activate Rho GTPases, protein kinases and MAPK signalling. In plants there also seem to be receptors like this, but they are not well understood



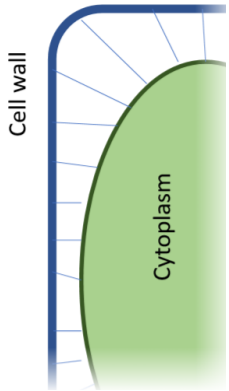
DROUGHT STRESS

Dehydration activates two types of response

- Avoidance – Balance water uptake and loss
  - Increase uptake and/or reduce loss
  - Increase osmolyte production
  - Slow shoot growth
  - Suberin (hydrophobic) biosynthesis
  - Stomatal closure
- Tolerance – Protect against cellular damage
  - Detoxification of ROS
  - Increase concentrations of protective compounds and proteins
  - Activation of response genes

SENSING DROUGHT STRESS – CELL MEMBRANE DISTORTION

The plasma membrane is attached to the cell wall via Hechtian Strands. When the plasma shrinks or expands these strands can exert tension on the membrane



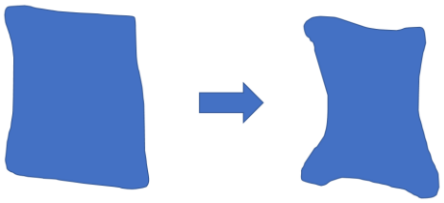
Membrane tension can cause opening of plasma membrane localized mechanosensitive calcium channels. In plants there are known examples: MSL, CSC1 and OSCA1

OSCA1 is part of a 15-member family. Another member, CSC1, also generates calcium transients in response to osmotic stress

osca1 mutants have weak calcium responses to osmotic stress and are very drought sensitive

SENSING DROUGHT STRESS – CELL WALL DISTORTION

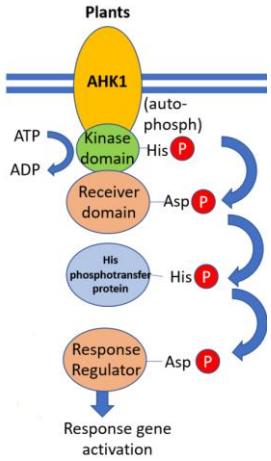
When cells shrink due to dehydration, cell walls can buckle inwards. This can activate cell wall monitoring receptor-like kinases (RLKs). Not much is known about these or how they signal



SENSING DROUGHT STRESS – TWO COMPONENT SYSTEMS

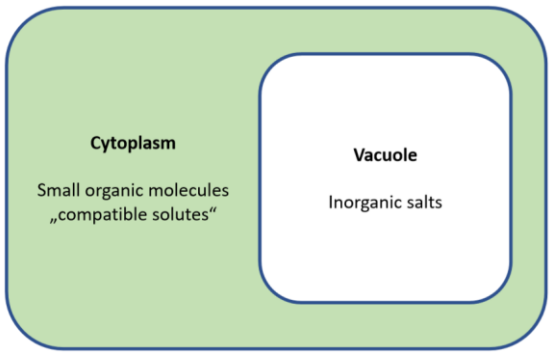
Increased AHK1 expression results in:

- Increased NCED3 (ABA synthesis)
- Stomatal closure
- Other avoidance responses



STRESS AVOIDANCE -ALTER OSMOLYTE CONCENTRATION

Alter osmolyte concentration is important to maintain turgor pressure and water for cellular processes



Example of compatible solute Proline

Osmotic stress causes accumulation of Proline

Proline has multiple factors:

- Increases water uptake into cell (safely)
- Stabilizes proteins and cell structures (works a bit like a chaperone → protects hydration shells of proteins)
- Stabilizes membranes
- Buffers pH
- Buffers Redox fluctuations
- Free radical scavenger (acts as antioxidant)
- Via protein partners it can induce gene transcriptions (many stress-response genes have Proline-response elements in their promoters)

Proline accumulation is reversible → elastic response

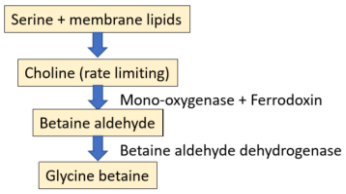
Functionally similar are polyhydric alcohols Mannitol and Pinnitol

Example of compatible solute Glycine betaine

Osmotic stress causes accumulation of Glycine betaine in halophytic and drought tolerant plants (maize, spinach, sugar beet, barley)

It accumulates in chloroplasts where it protects thylakoid membranes

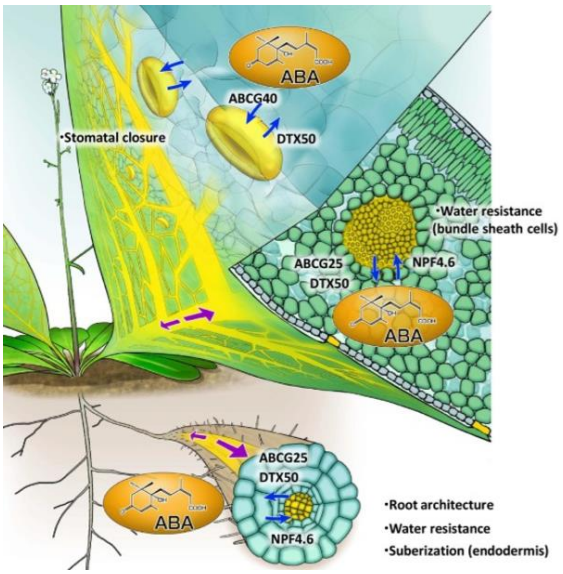
Glycine betaine production:



Glycine betaine is irreversible → plastic response → once the plant experienced drought stress it stays drought tolerant

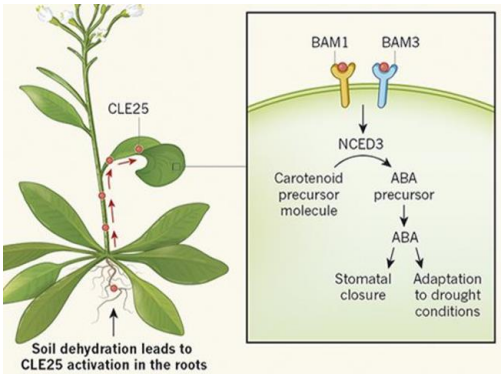
INDUCTION OF THE PLANT STRESS HORMONE ABA

Abscisic acid (ABA) is produced as a response to a hydraulic signal (loss of xylem water pressure). ABA is rapidly synthesized in the vasculature, roots and stomata and redistributed throughout the plant. Transporters that bring it in and out of the vasculature are known. ABA leads to osmotic stress response activation, stomatal closure, etc.

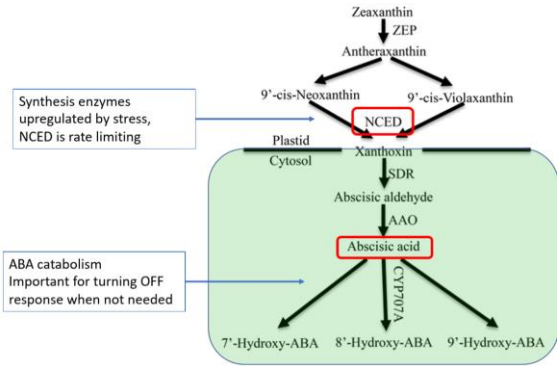


(Don't memorize all protein names)

ABA synthesis can also be induced independent of a hydraulic signal



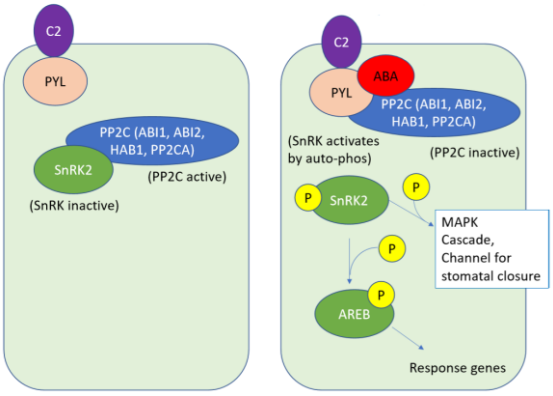
SYNTHESIS OF ABA



(Don't memorize the pathway)

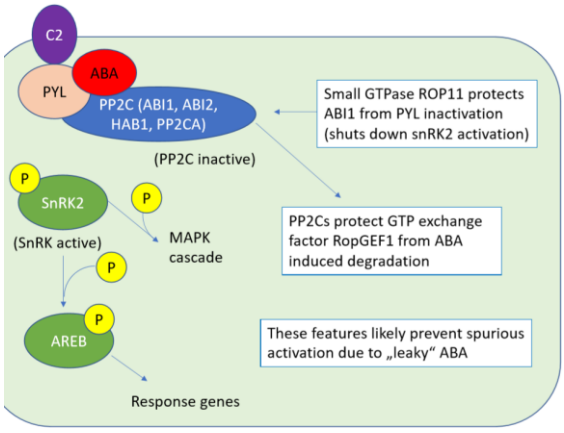
SENSING AND TRANSDUCTION OF THE ABA SIGNAL

Binding of ABA by PYL is enhanced by PP2C proteins – these are considered co-receptors



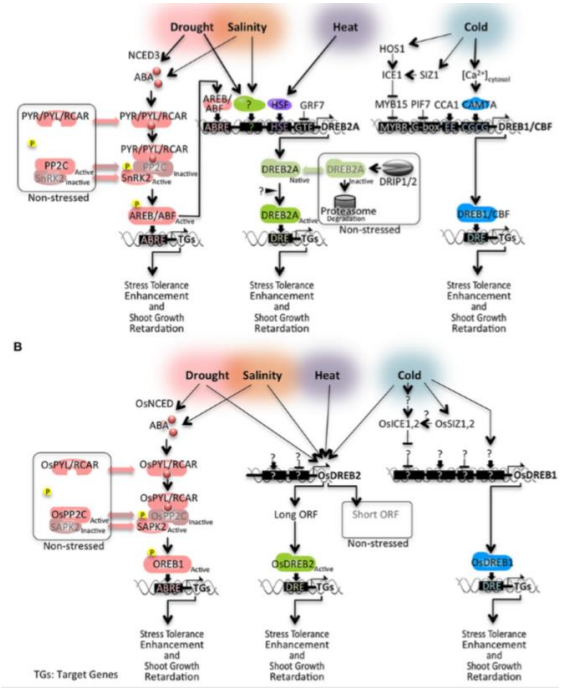
(Don't memorize all proteins, only general concept (self-phosphorylation) is important)

There is a safety mechanism built in the pathway



The stress signal can also be transduced independent of ABA

DREB2 is a transcription factor that contributes to stress tolerance independently of ABA



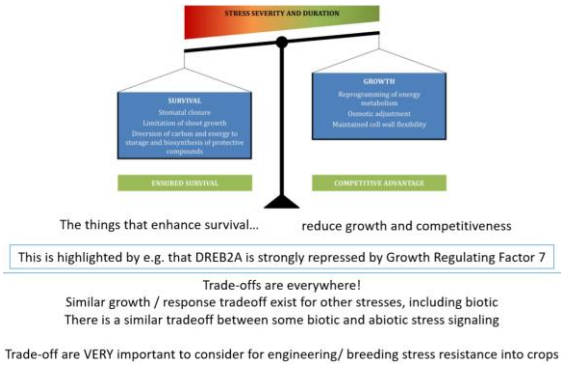
(Don't memorize all proteins)

STRESS RESPONSE

Osmotic stress leads to induction of different sorts of genes:

- Functional genes
  - Chaperones, LEA proteins (hydrophilins)
  - Osmolyte biosynthesis enzymes
  - Aquaporins
  - Transporters
  - Detoxifying enzymes
  - Cutin biosynthesis (cutin also feeds back to ABA)
- Regulatory genes
  - Transcription factors and other proteins that activate further signaling

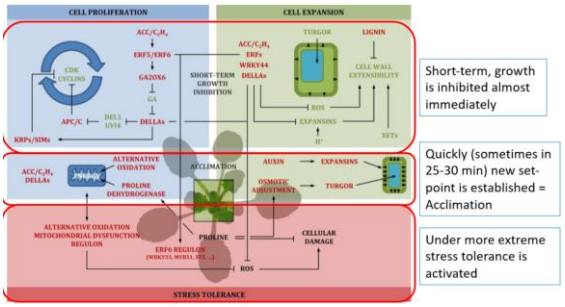
BALANCE BETWEEN GROWTH AND RESPONSE



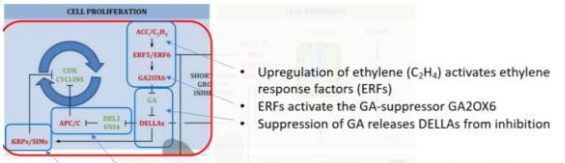
This is highlighted by e.g. that DREB2A is strongly repressed by Growth Regulating Factor 7

Trade-offs are everywhere!
Similar growth / response tradeoff exist for other stresses, including biotic
There is a similar tradeoff between some biotic and abiotic stress signaling

Trade-off are VERY important to consider for engineering/ breeding stress resistance into crops



Drought responses suppress the cell cycle

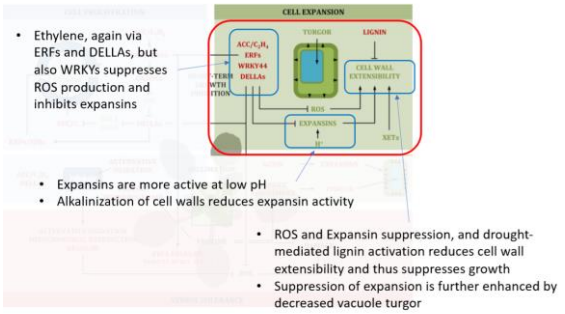


- Upregulation of ethylene (C_2H_4) activates ethylene response factors (ERFs)
- ERFs activate the GA-suppressor GA2OX6
- Suppression of GA releases DELLAs from inhibition
- DELLAs supress transcription of APC/C inhibitors DEL1 and UVI4. APC/C upregulation suppresses cell proliferation by degrading CDKs, Cyclins.
- DELLAs also upregulate transcription of KRPs/SIMs, which also inhibit cell proliferation

ABA's role is a bit odd in all this – it can directly inhibit growth, but it can also supress ethylene production

Red text = upregulated by stress, Green text = downregulated by stress

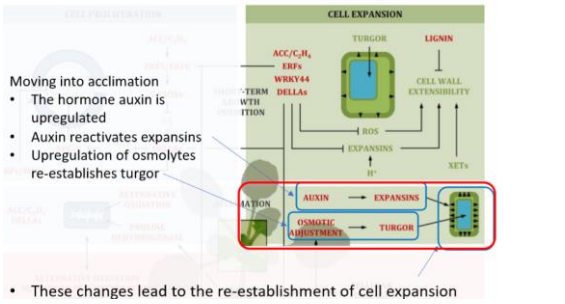
Drought responses suppress cell expansion



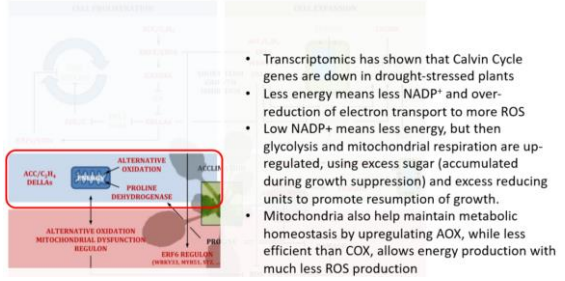
- Ethylene, again via ERFs and DELLAs, but also WRKYs suppresses ROS production and inhibits expansins
- Expansins are more active at low pH
- Alkalinization of cell walls reduces expansin activity
- ROS and Expansin suppression, and drought-mediated lignin activation reduces cell wall extensibility and thus suppresses growth
- Suppression of expansion is further enhanced by decreased vacuole turgor

Red text = upregulated by stress, Green text = downregulated by stress

Suppression of cell expansion is alleviated during acclimation



Energy metabolism is reprogrammed during drought in growing leaves



- Transcriptomics has shown that Calvin Cycle genes are down in drought-stressed plants
- Less energy means less NADP⁺ and over-reduction of electron transport to more ROS
- Low NADP+ means less energy, but then glycolysis and mitochondrial respiration are up-regulated, using excess sugar (accumulated during growth suppression) and excess reducing units to promote resumption of growth.
- Mitochondria also help maintain metabolic homeostasis by upregulating AOX, while less efficient than COX, allows energy production with much less ROS production

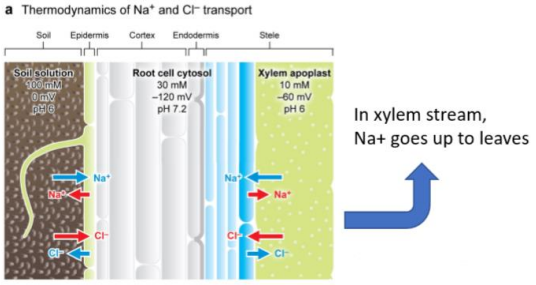
SALT STRESS

Salinization can be caused naturally by insufficient rainfall, but a major cause is agriculture and irrigation in arid regions. Salinity can cause crop yields to drop or in extreme cases make land unusable

- Shallow-rooted crops don't pull up groundwater like deep rooted trees or native perennials. Water table can rise, drawing salt from lower layers nearer the surface
- Irrigation – as plants use irrigation water, or the extra evaporates, salt is left behind

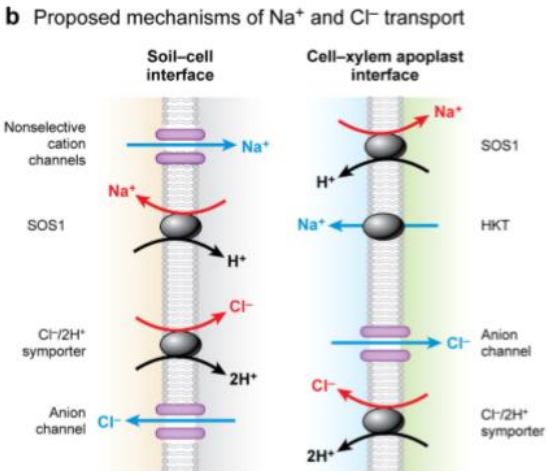
Plants vary in their salinity tolerance

SODIUM UPTAKE IN ROOTS



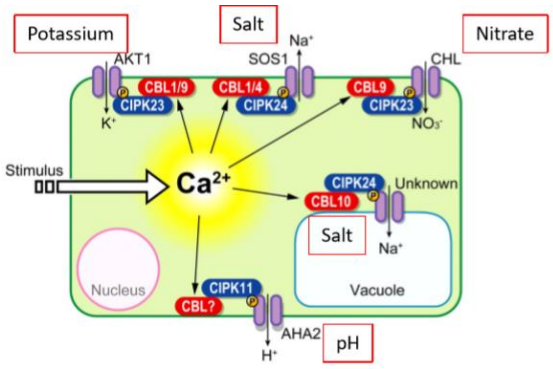
Blue arrows: passive transport

Red arrows: active transport

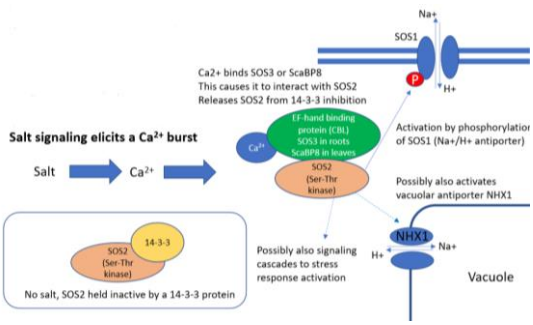


SENSING AND SIGNALING SALT STRESS

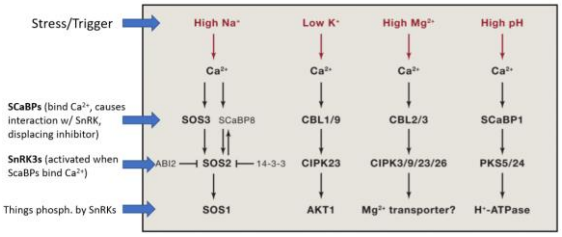
Calcium-mediated Calcineurin-like signalling: Calcium binding to the EF-hand domain of CBLs interact with CIPKs and activate them. Specificity may be conferred by combinations



(Don't memorize all proteins)



SOS2 is a member of a large kinase family called SnRks (SNF1-related protein kinases). All SOS2-like SnRK3s interact with SOS3-like calcium binding proteins (ScaBPs)



TEMPERATURE

Temperature stress causes:

- Membrane hyperfluidity (heat) or rigidity (cold)
- Membrane disruption
- Disruption of cytoskeleton
- Protein misfolding, aggregation, over-/under-stability
- Metabolic imbalances
- Generation of harmful levels of ROS

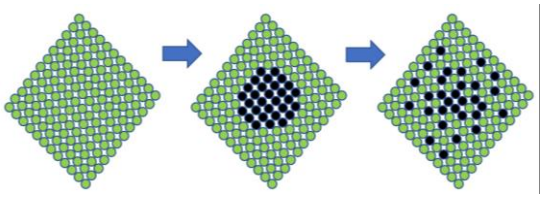
PERCEPTION VS. SENSING

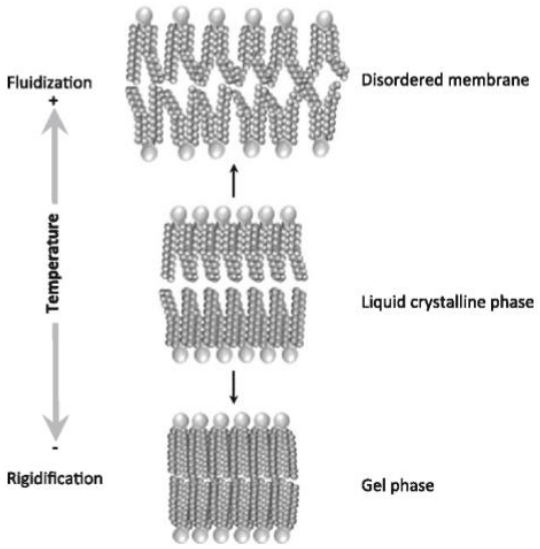
Temperature affects the thermodynamic properties of essentially all proteins and structures. Thus, in principle almost any protein or structure can **perceive** temperature. So, what does it mean to say something is a **sensor**? To consider something a **sensor**, it has to not only **perceive** a stress, it has to trigger a **response.**

EVIDENCE FOR POSSIBLE TEMPERATURE SENSORS IN PLANTS

Membrane fluidity

Measured in FRAP (**F**luorescence **r**ecovery **a**fter **p**hotobleaching) experiments





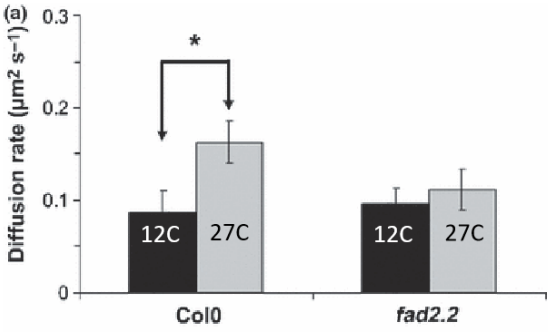
In moss primary thermosensors are heat-responsive calcium channels in plasma membrane

Membranes can be treated with chemicals:

- BA (Benzyl alcohol) → fluidizer
- DMSO (dimethylsulfoxide) → rigidifier

A study showed that A. thaliana actively compensates the temperature effect on membrane fluidity by dynamically altering fatty acid saturation

A fad2.2 (fatty acid desaturase) mutant has reduced responsiveness to temperature



Conformational changes (unknown if it happens in plants)

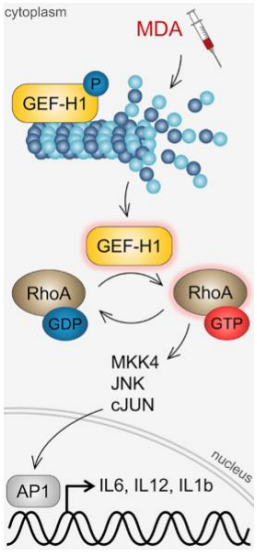
In bacteria: conformation changes can be induced in proteins (Unknown if this also happens in plants!) → e.g. DesK protein → phosphatase at 37°C, kinase at 25°C

Microtubule/microfilament stability

Microtubules and microfilaments dissemble at high and low temperatures

In Brassica, taxol and jaspakinolide (stabilizers of microtubules and microfilaments) inhibit activation in cold of a cold-responsive gene, while the effect of cold was mimicked at normal temperatures by oryzalin, colchicine and latrunculin B (chemicals that destabilize microtubules and microfilaments). Heat response activation is similarly prevented in high temperature by microtubule stabilizers and promoted at lower temperature by destabilizers. Similar trends were seen with calcium influx in Medicago

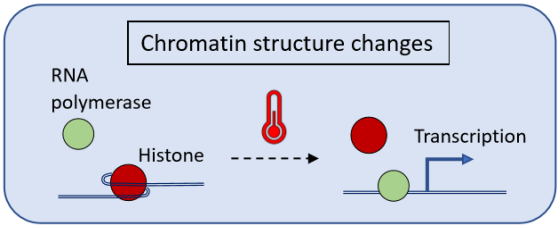
Maybe it is sensed similar as in animals:



(Don't memorize proteins, only concept is important)

Chromatin structure changes

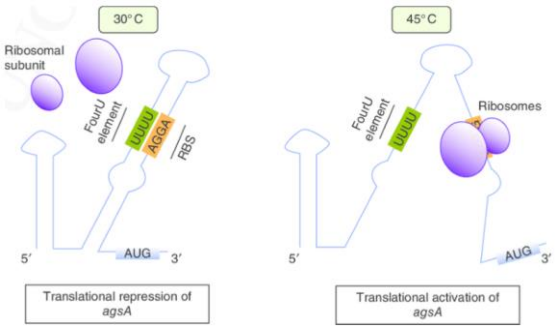
In A.thaliana exposed to increased temperature, a histone variant, H2A.Z is rapidly displaced from promoters to allow transcription. Though this does not occur in vitro, suggesting it is an indirect response. Eviction requires HSFA1a (activated by heat). More work is required to link intrinsic chromatin structure changes to temperature sensing



mRNA 2nd structure – translation

Temperature sensitive RNA switch in bacteria

It has not been directly demonstrated in plants, but there are hints that it may work in plants too. mRNAs associated with stress responses have high free energy and/or longer loop lengths → these features are temperature sensitive and thus useful for environmental sensing

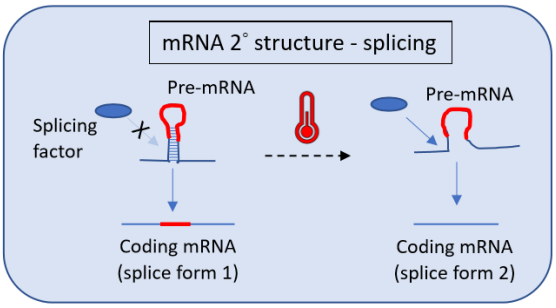


Alternative splicing

Two examples of alternative splicing leading to temperature-influenced outcomes in plants:

- Flowering locus M (FLM)
  - Gene encodes a protein that promotes flowering
  - Comes in several isoforms → ratio of FLM-δ and FLM-β changes with temperature

- FLM-β contributes to thermosensitive flowering, why it is alternatively spliced at different temperatures is **not** clear
- Late elongated hypocotyl (LHY)
  - Gene encodes a protein that is part of the circadian clock and regulates flowering
  - Comes in several isoforms
  - Decreased temperature leads to increased retention of intron 1 and degradation via NMD
  - May be due to structural features of the mRNA



Protein structure/activity

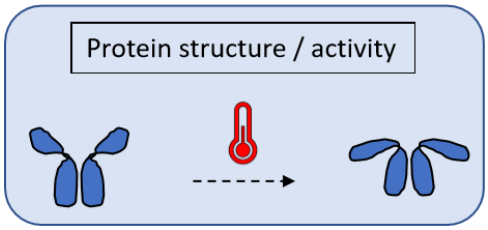
Temperature affects structure of most proteins. Effects of enzymatic reactions can alter metabolism and lead to ROS accumulation. Also, protein misfolding can activate the UPR.

Warm: Fatty acid desaturase 8 (FAD8)

- Has a C-terminal region that destabilizes the protein at 27°C relative to 22°C
- Leads to lower desaturated fatty acid content at higher temperature (compensating for the greater membrane fluidity due to temperature effects alone)

Cold: HvCBF2

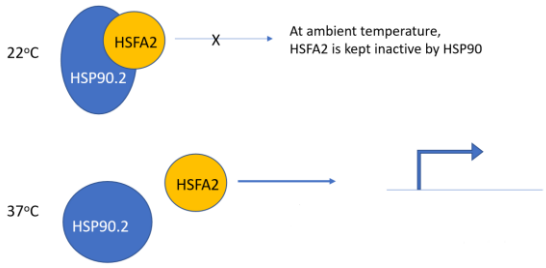
- Is a cold response gene activator related to DREB1
- Has no activity at 30°C, but becomes progressively more active as temperatures drop, presumably due to a conformational change



Protein interactions

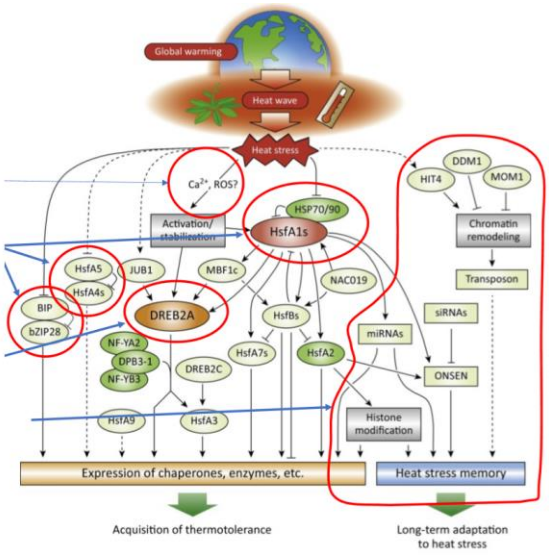
At warmer temperatures, HSP90 has a conformational change, dissociates from HSFA2 which then activates heat responses

At cool temperatures, HSR can be induced by Geldanamycin or Radicicol (HSP90 inactivators)



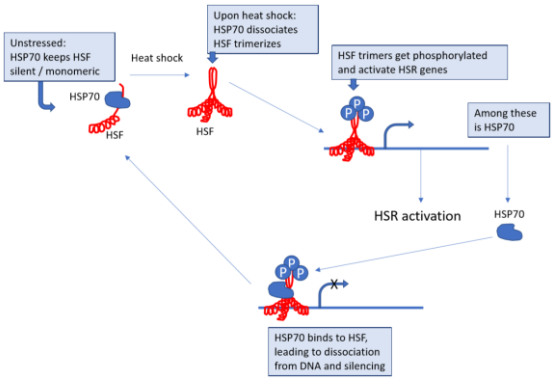
RESPONSES TO HEAT

1. A calcium/ROS burst
2. Proteins bound by repressors (in this case TFs) that are released by stress
3. DREB2 (TF)
4. Chromatin remodelling as epigenetic memory



(Don't memorize all proteins)

HEAT SHOCK FACTOR CYCLE



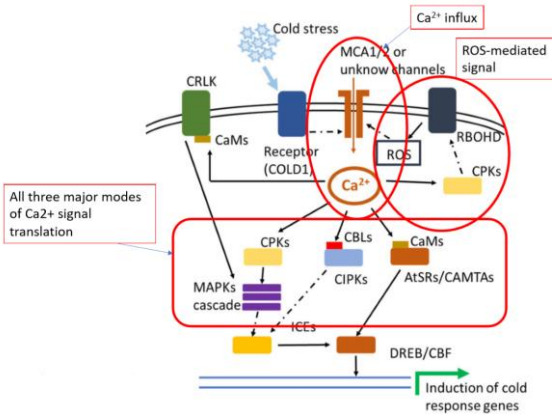
HEAT SHOCK RESPONSE

One of the strongest responses is upregulation (by heat-activated HSFs) of Heat Shock Proteins (HSPs). These have several functions – e.g. can oligomerize and insert into membranes to stabilize them. Also act as chaperones to assist in protein folding. Expressing HSPs is costly – which is why they are inducible. Transgenic overexpression of HSFs can lead to increased thermotolerance, but even more effective is prior heat exposure

Activation of the HSR primes plants for greater resistance to later heat shocks → Acclimation Priming

The greatest protection is provided by gradual temperature increase, but quick shock also works

RESPONSES TO COLD

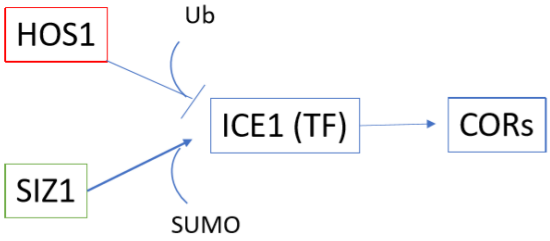


(Don't memorize all proteins)

CHECKS AND BALANCES IN THE COLD RESPONSE

HOS1: a ubiquitin ligase, ubiquitylates ICE1 and targets it for degradation

SIZ1: a sumo ligase, Polysumolates ICE1 and protects it from HOS1



COR GENES

Overexpression of COR genes is linked to cold tolerance

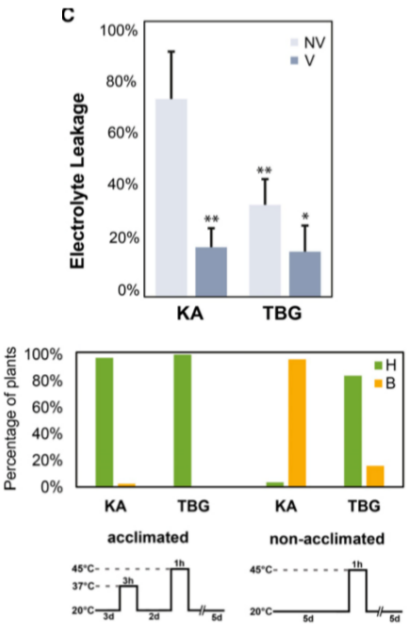
Example Mountain vs. railway A.arenosa

Mountain: temperature quite stable

Railway: fluctuating temperature → natural priming

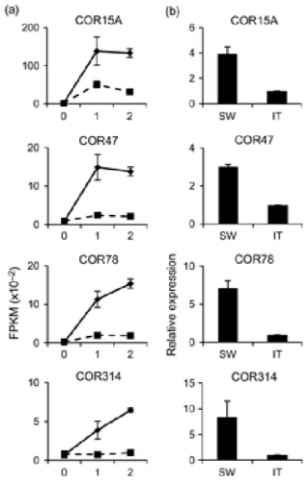
Cold: transcriptome study showed that railway (TBG) constitutively expresses COR genes, railway has better freezing tolerance

Heat: Transcriptome study showed that railway (TBG) constitutively expresses HSF1 and HSP101, mountain (KA) acclimates just fine, railway has better basal tolerance



Example A.thaliana CBF2 mutant

The IT (Italy) has an inactive CBF2 and has reduced COR gene expression compared to SW (Sweden). The CBF2 mutant has a reduced cold tolerance



Example rice

KMXBG is a cold tolerant rice, Towada is not. The responsible gene has been isolated. It is a receptor-like kinase that interacts with ATP-synthase. Upregulated CTB4a correlates with higher ATP and improved yield in cold stress (mechanism still unclear)

HEAT AND COLD ACCLIMATION

A.thaliana plants can survive to -5°C without priming, but with priming they can survive to -13°C

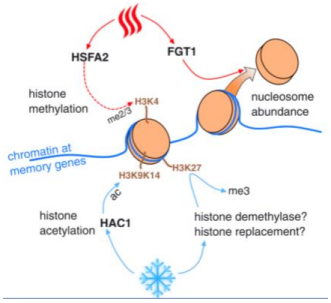
Priming involves epigenetic memory

Cold:

- HAC1: required for histone acetylation and for the cold priming response
- HDA6: histone deacetylase required for freezing tolerance
- HDAC: de-acetylates histones 3 and 4, required for freezing tolerance and Cor and DREB1 activation

Heat:

- HSFA2: required for heat priming. HSFA2-dependant heat memory genes include sHSPs, APX2 (ROS detox), these show enhanced induction on second HS that is HSFA2-dependant. Heat memory loci are marked with H3K4me2/me3, which mark genes that have strong reinduction
- FGT1: required for sustained expression of memory genes. Interacts with known chromatin remodellers
- SPLs: TFs that normally silence heat memory genes. Silenced during memory phase by miR156 (microRNA induced by heat shock)



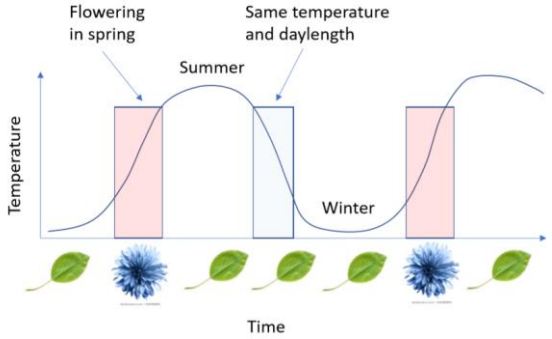
Priming for cold and heat can also protect against other stresses

Primary stress	Secondary stress	Species
Heat	Cadmium	*Oryza sativa* (rice)
Heat	Salinity	*Hordeum vulgare* (barley), *Brassica campestris*
Cold	Heat	*Vitis vinifera* (grape) *Hordeum vulgare* (barley), *Solanum lycopersicum* (tomato)
Cold	Cu & Cd	*Pisum sativum* (pea)
Cold	Freezing	*Arabidopsis thaliana*
Heat	Chilling	*Solanum lycopersicum* (tomato)
Cold	Drought	*Phaseolus vulgaris* (bean)

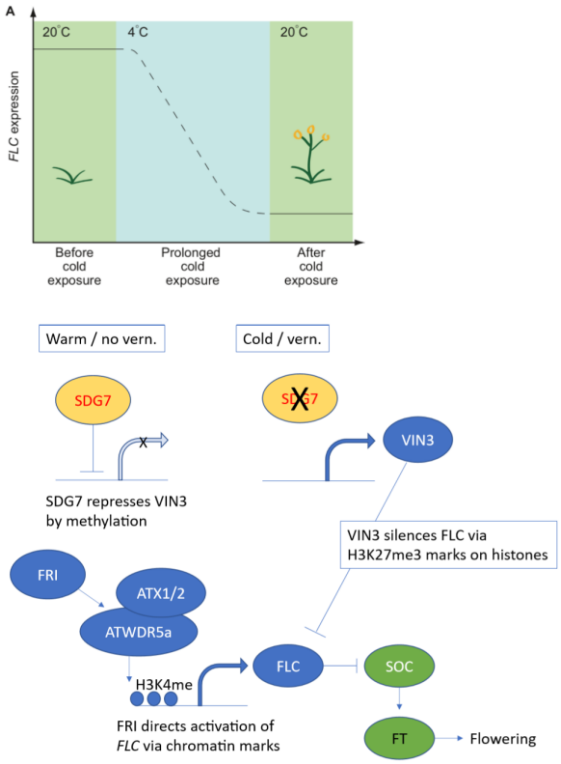
VERNALIZATION

Vernalization: remember longer-term cold

Day length and temperature are major factors

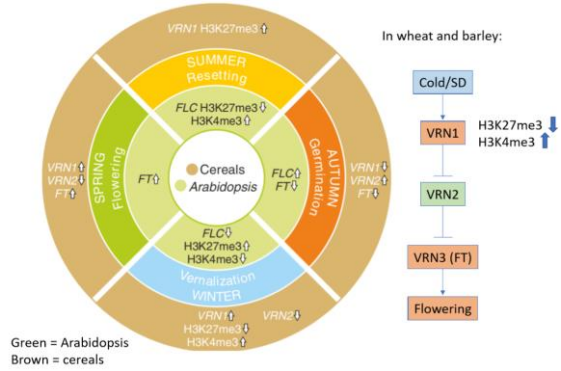


Cold leads to the slow downregulation of a floral repressor, FLC

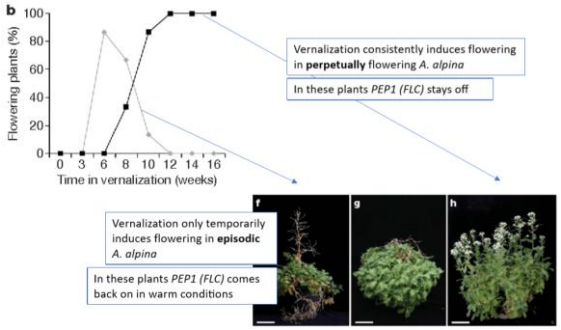


FLC is progressively silenced by the PRC during cold, with help of a long noncoding antisense RNA

Cereals also respond to vernalization, but the mechanism is different



VERNALIZATION IN PERENNIALS



There can be variation in the extent of vernalization need and this can correlate with habitat

PART ZEEMAN

MOBILISATION OF SEED RESERVES

LEARNING GOALS

- Events during seed germination
- Seed storage compounds
  - Carbon, nitrogen, sulphur, phosphate, ions
- Starch and its mobilisation
  - Hydrolysis of starch in the cereal endosperm
- Protein and phytate mobilisation
  - Seed storage proteins and phytate inclusions

DIFFERENT METHODS OF PLANT PROPAGATION

Seeds: grow without help of parental plant

Bulbs, tubers, runners and rhizomes: vegetative reproduction

SEEDS

Major advantages of seeds:

- Sexual reproduction
- Longevity
- Distribution

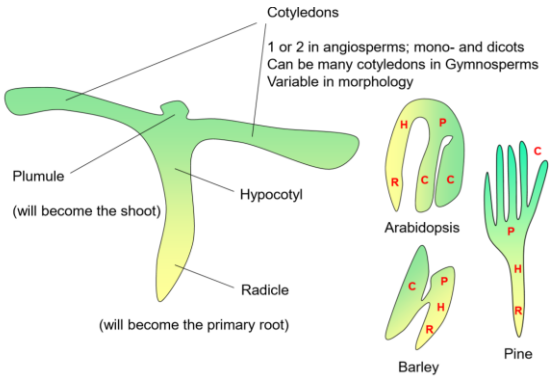
Seed plants include:

- Gymnosperms: probably not a monophyletic group, but often classified as such (800 species)
  - Conifers, cycads, gnetae, ginkgo
- Angiosperms: probably monophyletic, most abundant plants (> 0.5 mio. species)
  - Magnoliophyte, flowering plants, mono- and dicots

Big seeds: long to develop, don't disperse well, but grow and survive well

Small seeds: well dispersible, plant can produce many, might not survive so well

THE EMBRYO



GERMINATION

Germination: When micropylar endosperm and seed coat are disrupted

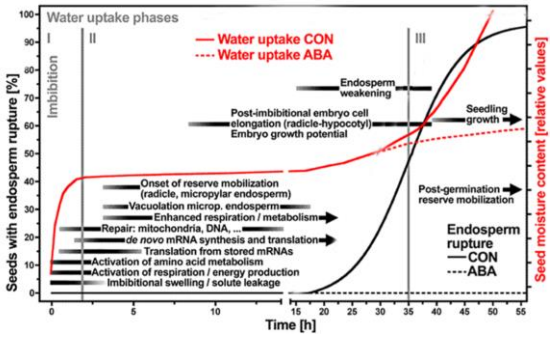
IMBIBITION

Uptake of water through hydration of carbohydrate matrices e.g. cell walls and storage carbohydrates

Dependant on:

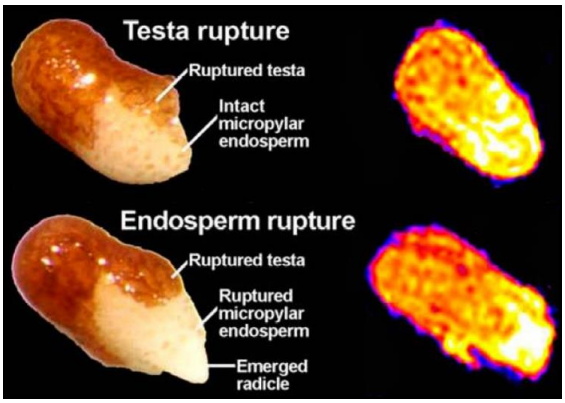
- Water potential
- Water conductance through soil/to seed
- Permeability of the seed coat

Radical grows at the micropyle, where the pollen entered the egg



The energy is not used to start germination, but to establish the seedling

Example: water contents of germinating tobacco seeds



(White: lots of water, red: not much water)

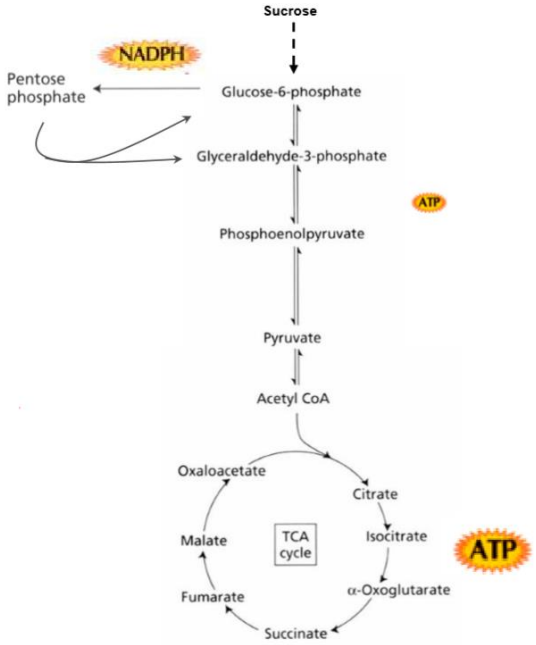
ONSET OF RESPIRATION

Uptake of O_2, production of CO_2, consumption of seed sugars

Production of ATP via glycolysis, conversion of glucose-6-phosphate to pyruvate (fast, oxygen-independent)

Production of ATP via tricarboxylic acid cycle (Krebs cycle), transport of pyruvate into the mitochondrion, its conversion to acetyl-CoA, and subsequent oxidation to produce ATP (slow, oxygen-dependent, otherwise fermentation occurs)

Production of NADPH via oxidative pentose phosphate pathway to create reducing power, conversion of glucose-6-phosphate into pentose-phosphate (release of CO_2, pentose recycled into hexose-phosphate and triose-phosphates)



ONSET OF GENETIC ACTIVITY

- Protein synthesis from existing mRNA
- DNA repair
- RNA synthesis
- DNA synthesis and cell division

→ All processes dependent on respiratory ATP and NADPH production

SEED CONTENTS

Carbon: starch (glucose polymer), sugars (sucrose), raffinose family oligosaccharides, lipids (triacylglycerol), fructans, cell walls

Nitrogen: storage proteins, albumins (readily extracted in water), globulins (extracted with salt solutions), proalmins (extracted with alcohol), glutelins (acid- or alkali- soluble)

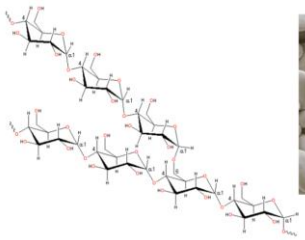
Phosphate and mineral nutrients: phytate, polyphosphate

STARCH

Starch: comprised of glucose polymers which take the form of insoluble granules

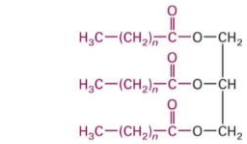
Amylopectin: major polymer, has up to 500'000 glucose residues per molecule. These are linked with α-1,6-bonds (branch points)

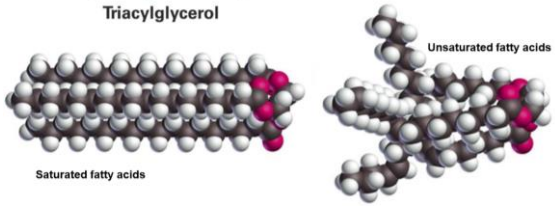
Amylose: minor polymer is linear with a few thousand residues



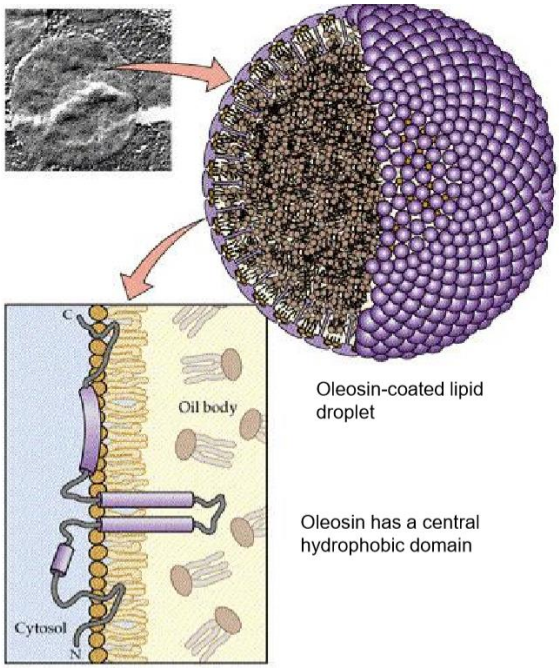
LIPIDS

Storage lipids are triacyglycerols, usually containing unsaturated fatty acid chains of 16 – 18 carbon atoms in length with 1 or 2 C=C double bonds



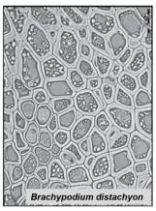


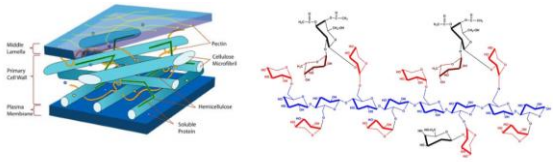
Lipids accumulate in oil bodies which are surrounded by a phospholipid monolayer studded with proteins (e.g. oleosin) which prevent oil body fusion



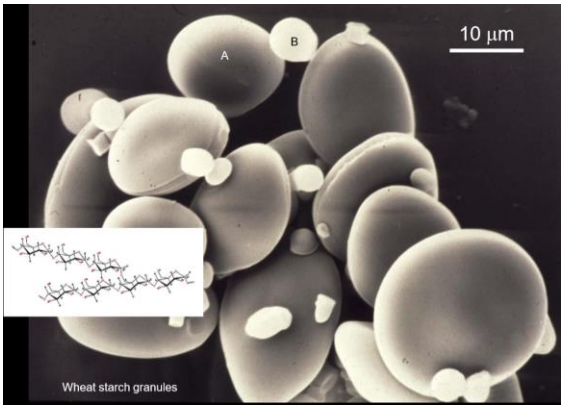
CELL WALLS

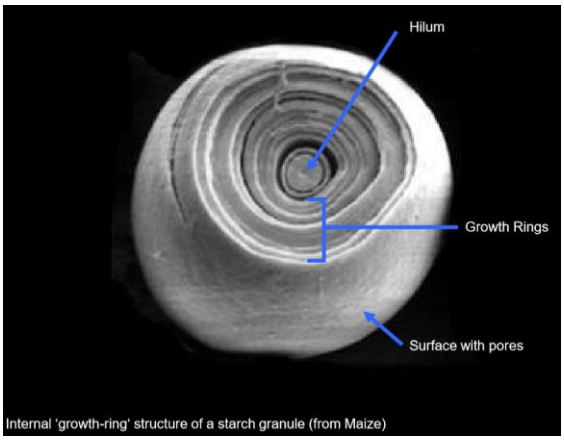
In many seeds cell walls serve as a storage carbon reserve in addition to or instead of being structural feature. Cell walls of the maternal seed coat tissues are rich in cellulose and can be lignified. Cell walls of the endosperm are rich in hemicelluloses and pectins



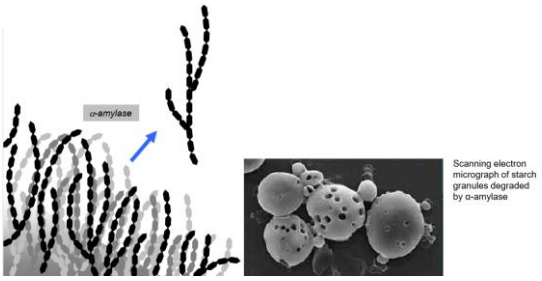


STARCH BREAKDOWN





Enzymes can get through pores into the center to degrade starch (unusual)



α-amylase: endoamylase → acts in starch structure

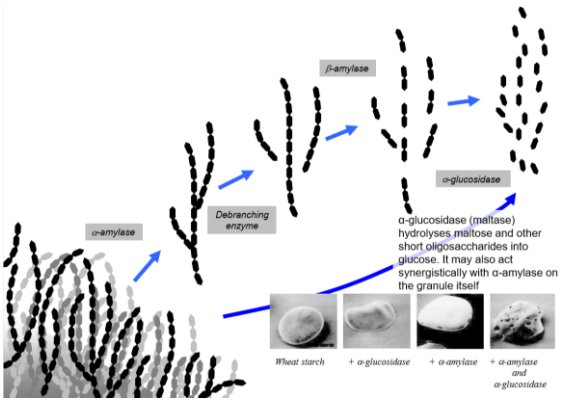
Products of α-amylotic degradation of amylopectin:

- Maltose
- Maltotriose
- Maltotetraose
- Maltopentaose
- α-maltosyl-maltohexaose (branched)

Debranching enzymes fall into two classes: Isoamylase and limit dextrinase, both hydrolyse α-1,6-bonds

β-Amylase: processive enzyme which cuts sequential maltose units from a linear chain

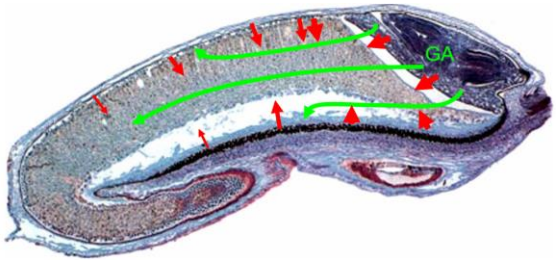
α-glucosidase (maltase): hydrolyses maltose and other short oligosaccharides into glucose. It may also act synergistically with α-amylase on the granule itself



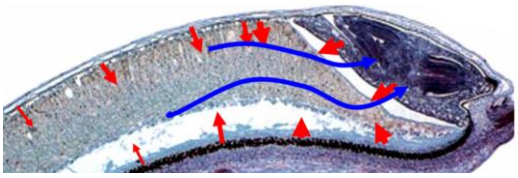
It's a network of pathways and not a linear pathway

GIBBERELLIC ACID (GA)

Gibberellic acid (GA): produced by embryo and scutellum, diffuses to the aleurone layer, promotes aleurone and scutellar production and secretion of enzymes (α-amylase, limit dextrinase, α-glucosidase, cell-wall degrading enzymes, proteases)



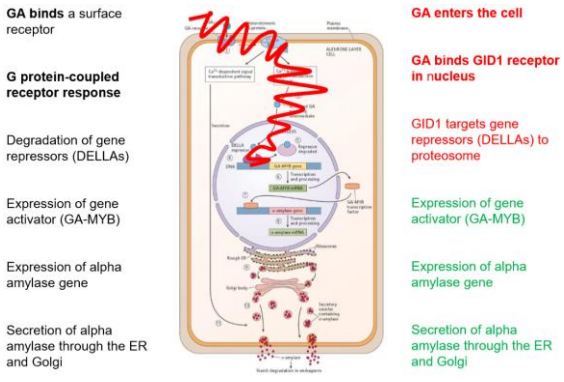
1. Cell wall degradation, starch degradation and storage protein degradation begin
2. Pre-formed β-amylase activated by proteolysis
3. Sugars and amino acids taken up by the scutellum



Imbibed, non-dormant seeds will not mobilise their reserves if embryo is removed

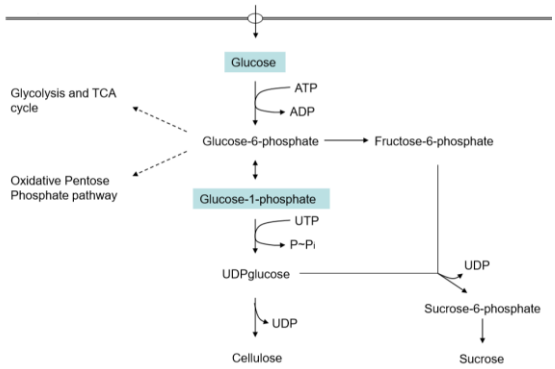
Embryo-deficient seeds will mobilise their reserves if treated with GA

MODEL FOR GA INDUCTION OF A-AMYLASE BY THE ALEURONE LAYER CELLS

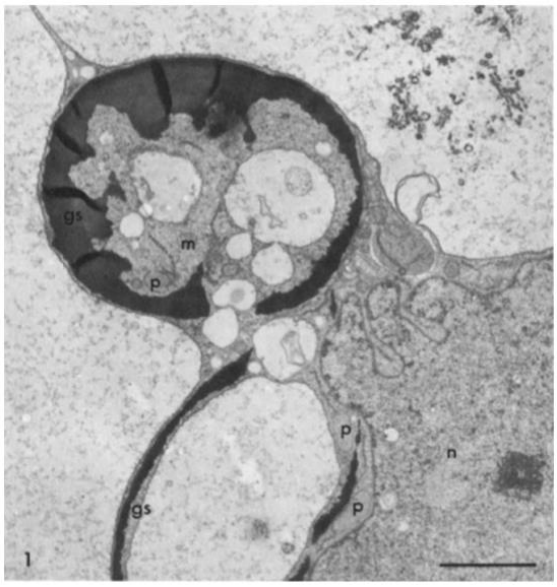


(Red is incorrect)

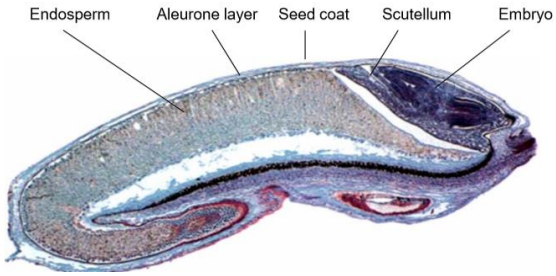
ENTRY OF STARCH BREAKDOWN PRODUCTS INTO METABOLISM



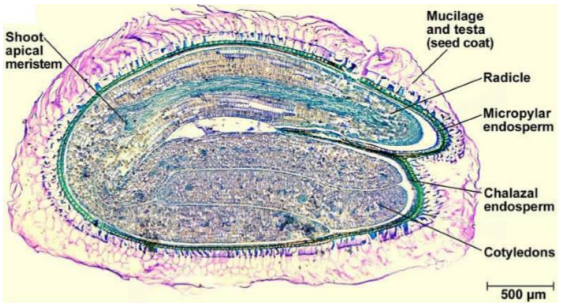
Starch degradation in the cotyledons of *Vici faba*. The amyloplasts in which the starch was synthesized degenerates and starch granules are degraded in the cytosol of the cotyledon cells, later studies implicated autophagy in this process



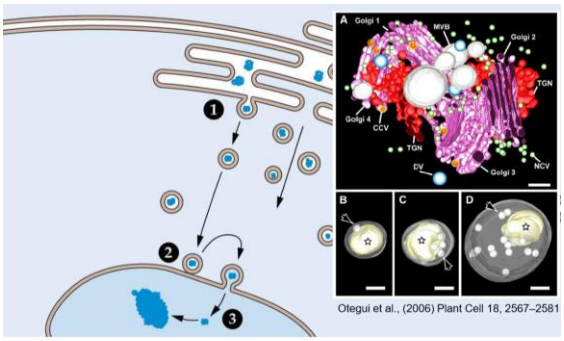
Barley seed (monocot): Endosperm is dominant tissue, cotyledon is modified to form scutellum, most storage reserves in the form of endosperm starch and proteins, aleurone layer and scutellum contain sugars, phytate and protein



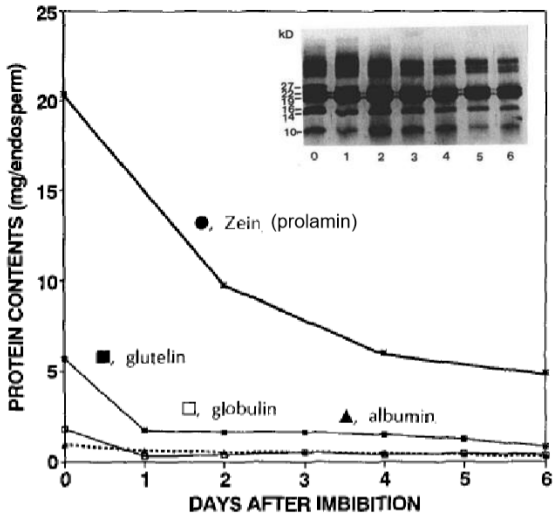
Cress seed (dicot): Endosperm is a single cell layer, cotyledons contain the storage reserves (lipids, proteins, phytate, sugars)



SEED STORAGE PROTEIN DEGRADATION



1. ER-synthesised storage proteins are packaged into Golgi vesicles for translocation to the vacuole
2. Vesicles dock and fuse with the tonoplast
3. Storage proteins aggregate within the vacuole

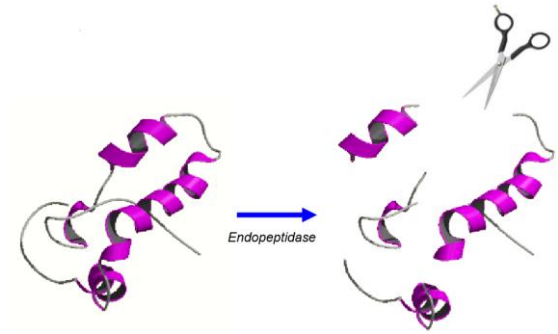


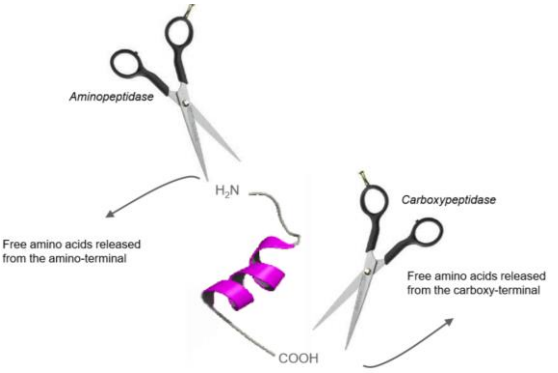
Change in protein contents in maize endosperm tissue.

PROTEOLYSIS

There are three classes of proteases involved in storage protein mobilisation:

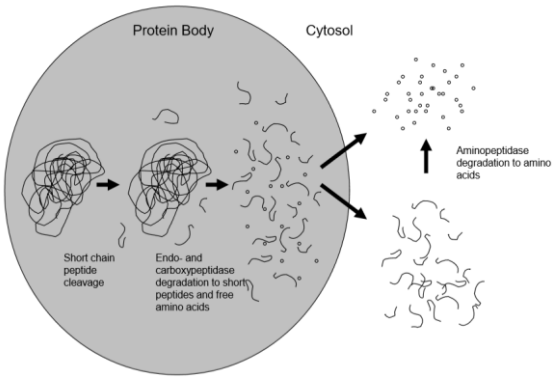
- Endopeptidases
- Aminopeptidases
- Carboxypeptidases



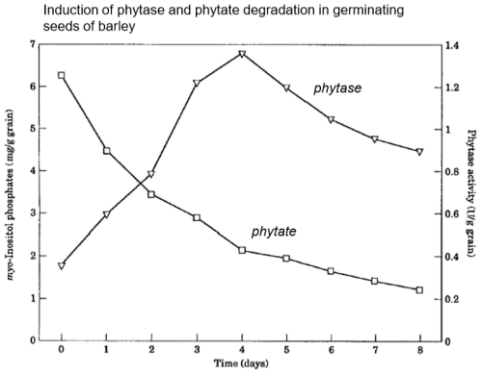


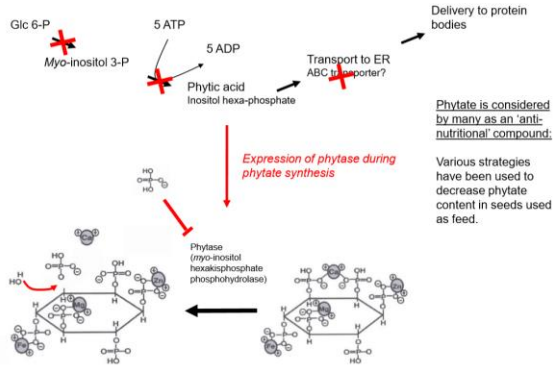
Proteases belong to C1 and C13 cysteine proteases, S8 and S10 serine proteases, A1 aspartic proteases, leucine aminopeptidases, as-yet unidentified metalloproteases

Possible way of storage protein degradation



PHYTATE METABOLISM





TAKE HOME MESSAGES

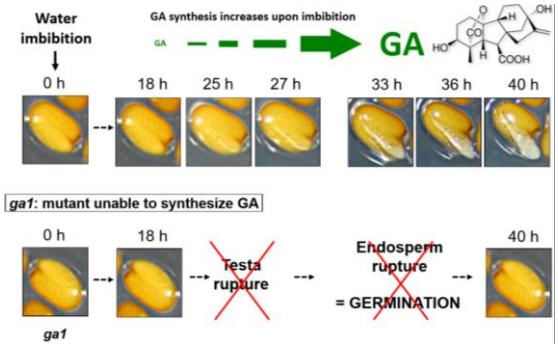
- Most seeds store carbon as sugars and as starch or lipids. Sugars fuel initial metabolic events upon imbibition and germination. Degradation of starch and lipid reserves serves mainly to support post-germinative growth
- Starch is readily converted into hexose-phosphates for entry into metabolism through hydrolysis in the non-living cereal endosperm, but the pathway may differ in the cotyledons of legume seeds
- Lipids are mobilised by lipolysis, followed by β-oxidation to yield acetyl CoA. A quarter of the carbon is lost as CO_2 as the glyoxylate cycle creates C4 acids which are decarboxylated to feed into gluconeogenesis
- Proteins are degraded by a combination of endo- and exo-acting peptidases, liberating free amino acids for transport and incorporation into new proteins
- Phytate is degraded by dephosphorylation of the *myo*-inositol backbone, simultaneously releasing P_i and chelated cations

GERMINATION AND ENVIRONMENTAL PERCEPTION

LEARNING GOALS

- Roles of GA and ABA controlling germination
- Dormancy
- Role of light in germination
- Light sensing via photoreceptors, influence on growth and development

ROLE OF GA IN GERMINATION



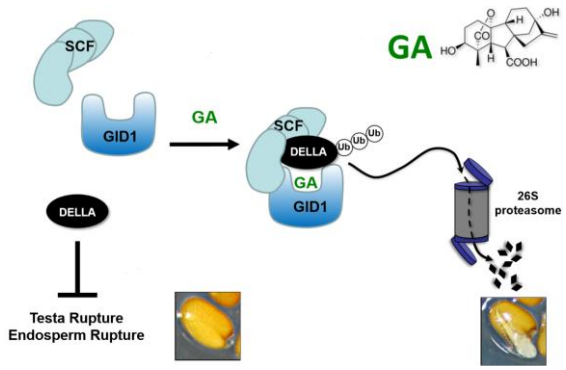
Without GA there are not even early stages of germination

SCF complex: Skp1, Cullin, F-box protein complex. Targets proteins to the 26S proteasome

GID1 receptor: Gibberellin Insensitive Dwarf 1. GA receptor protein

DELLA factors: modulate the activity of transcription factors

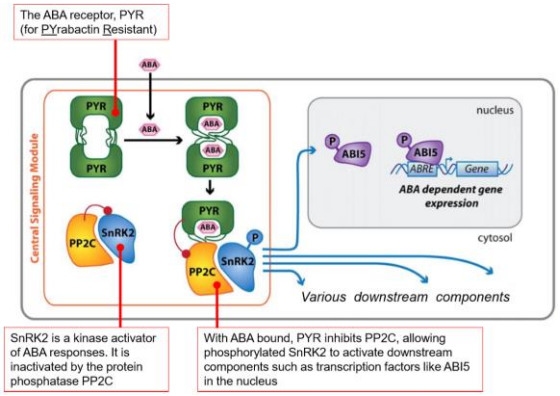
Model for GA action: GID receptors bring a SCF complex to the DELLA upon GA binding



ROLE OF ABA IN GERMINATION

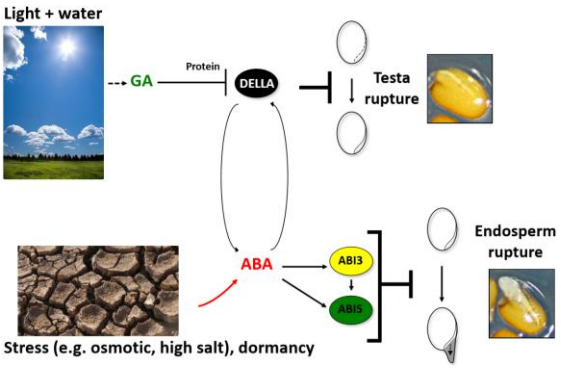
ABA signals osmotic stress and blocks endosperm rupture and cotyledon greening. ABA doesn't block testa rupture

EARLY EVENTS



ABA levels decline after imbibition

CONTROL OF SEED GERMINATION



SEED DORMANCY

Physical dormancy: waterproof seed coat that prevents imbibition, seed pod that requires burning before it will open

Physiological dormancy: A developmental arrest that occurs despite favourable conditions and cues for germination. Dormancy needs to be broken → time, cold period (stratification), light

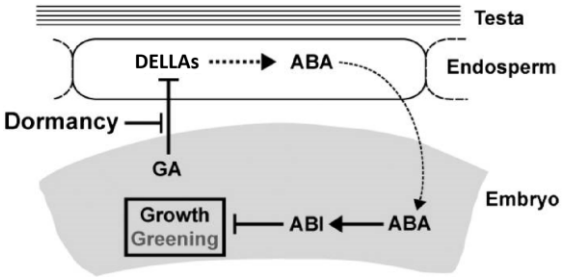
Morphological dormancy: very small embryo in a big seed (grows inside the seed prior to germination) e.g. coconut

ROLE OF TIME IN GERMINATION

Example: Arabidopsis

GA does not stimulate germination in dormant seeds

The seed coat is essential to maintain dormancy → dormant seeds with coat removed will germinate but dormant seeds with coat removed embedded on the removed coats don't germinate



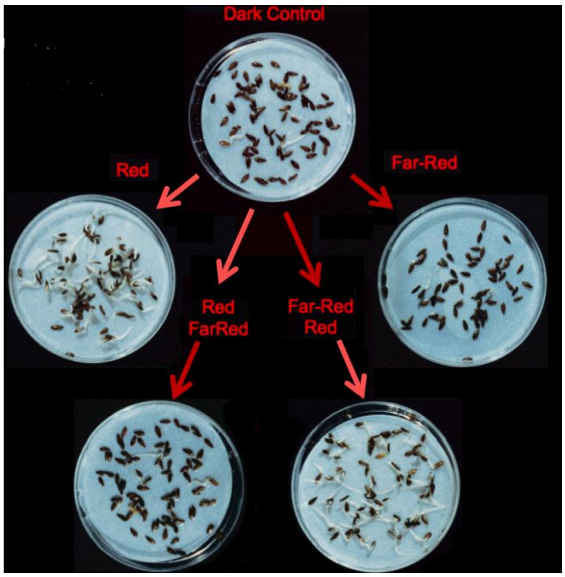
ROLE OF LIGHT IN GERMINATION

Example: Lettuce

Red light stimulates germination

Far-red light blocks germination

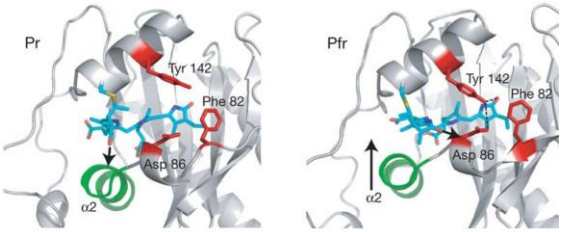
Experiments show that light-treatment is reversible → light seeds were exposed to last is important

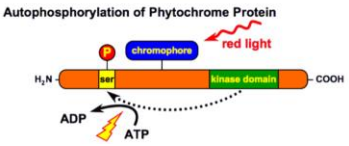


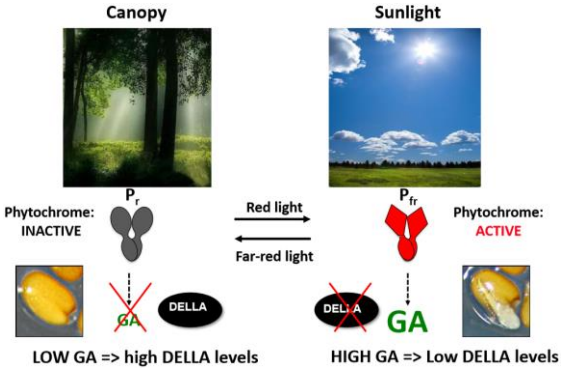
Possible explanations:

- 2 pigments which are antagonistic
- 1 pigment which is interchangeable

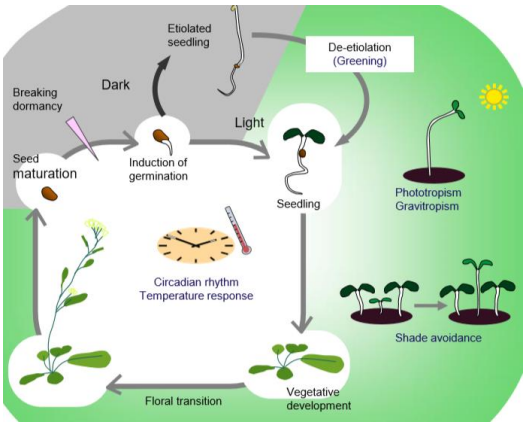
The latter is the case



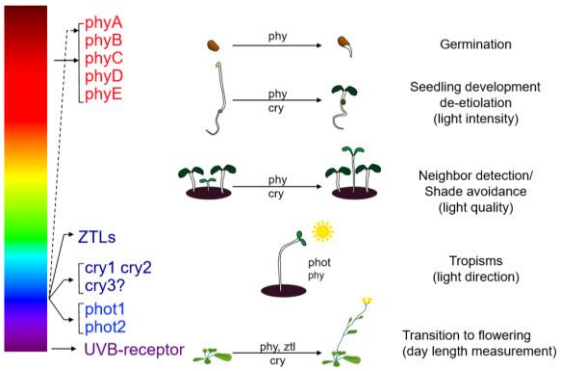




ROLE OF LIGHT IN LIFE CYCLE OF PLANT



ARABIDOPSIS PHOTORECEPTORS



Skotomorphogenesis: Development in the dark → etiolated seedlings, long hypocotyl, apical hook

closed, small and folded cotyledons, no expression of photosynthetic genes, no greening, short roots

Photomorphogenesis: Development in the light → short hypocotyl, apical hook open, expanded cotyledons, expression of photosynthetic genes, chlorophyll biosynthesis, longer roots

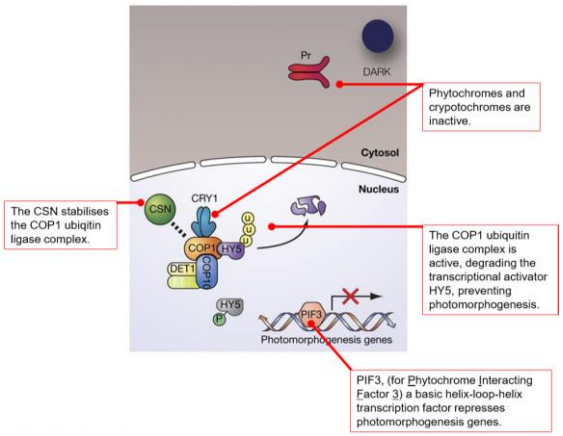
hy1 and *hy2*: lacks heme oxygenase and phytochromobilin synthase (chromophore for phytochromes), respectively. They cannot make phytochromobilin

hy3: lacks phytochrome B

hy4: lacks cryptochrome 1

hy5: lacks a bZIP transcription factor that is an activator of photomorphogenesis genes

hy8: lacks phytochrome A



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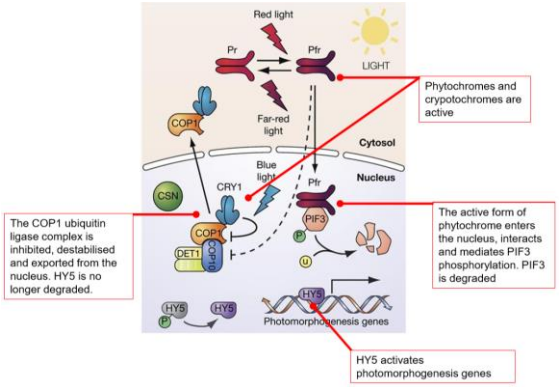
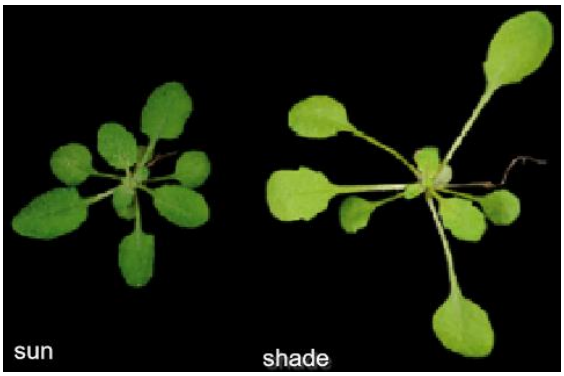
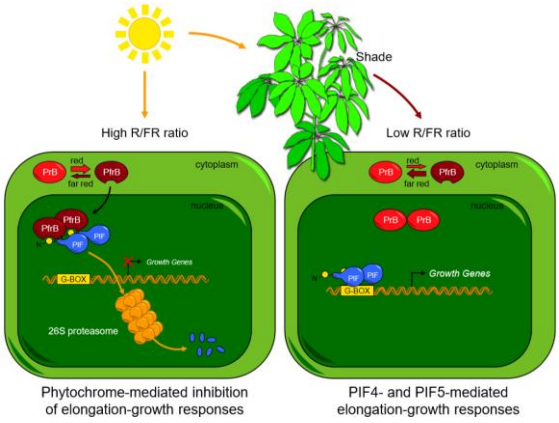


SHADE

Plants in shade grow differently than in light although they grow under the same light intensity. In shade the amount of far red light is higher

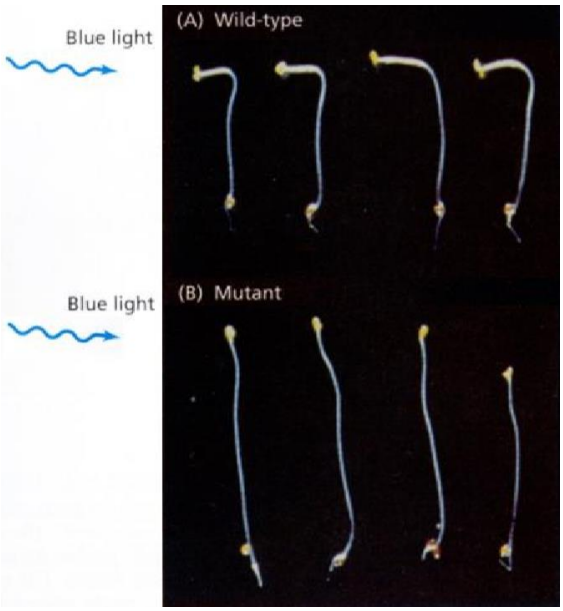


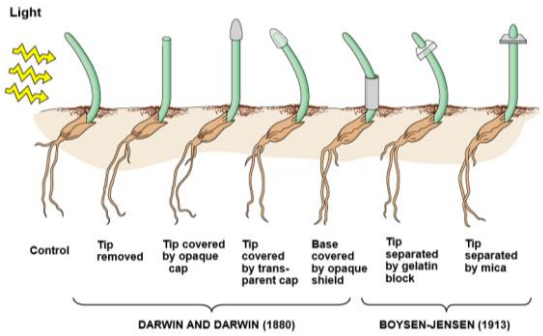


phyB: displays a constitutive shade avoidance response

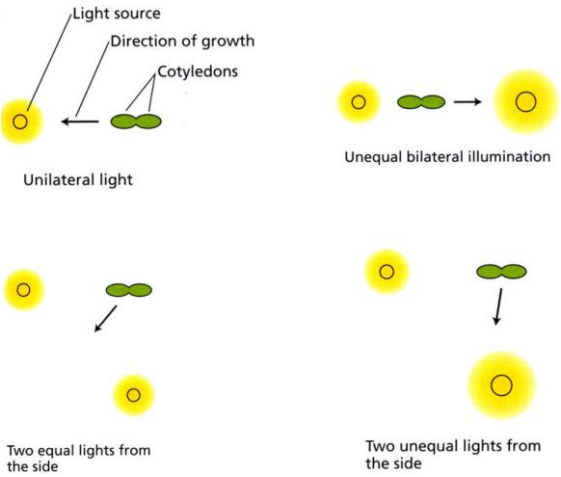
PHOTOTROPISM

Phototropism: Blue-light response resulting from asymmetric growth → bending

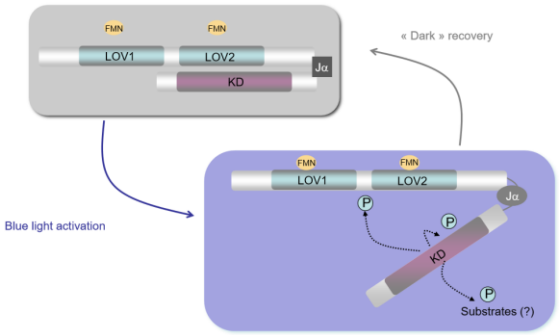




Plants grow towards most intense light source



Phot1 and phot2 are plasma membrane associated photoreceptors



TAKE HOME MESSAGES

- Light affects many aspects of growth
- Intensity and quality are sensed
- Blue, red, far-red and UV light is sensed by a set of complementary light sensory proteins
- Information is used for developmental decisions (germination, flowering)
- Information modulates growth (shade avoidance, phototropism)

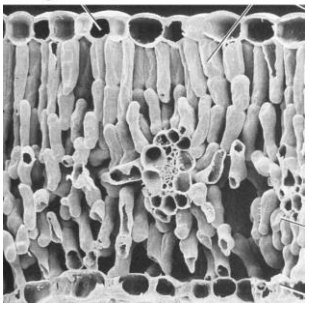
CENTRAL METABOLISM I

LEARNING GOALS

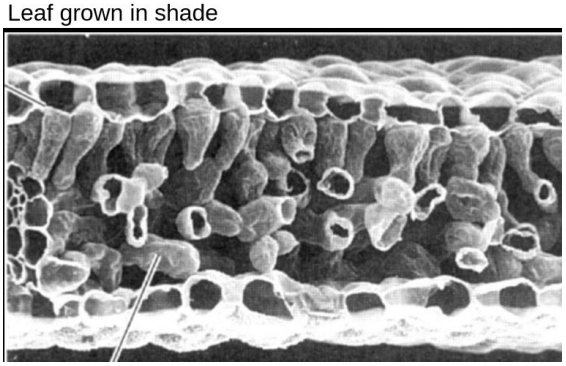
- Acclimation responses to different light environments
- Carbon assimilation via the Calvin-Benson Cycle
- Primary products of photosynthesis
- Control of partitioning

LEAF ANATOMY IN DIFFERENT LIGHT ENVIRONMENTS

Leaf grown in sun



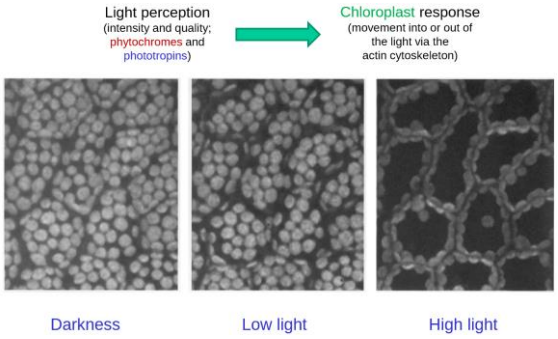
Leaf grown in shade



Column-like palisade cells facilitate the penetration of light into abaxial cell layers → more equal distribution of light within photosynthetic tissue

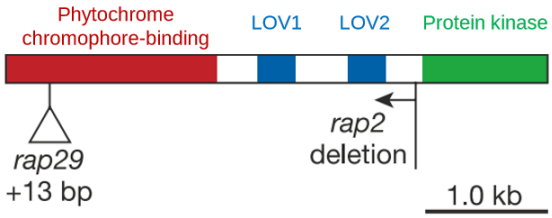
CHLOROPLAST DISTRIBUTION IN PALISADE MESOPHYLL

High light: Chloroplasts move to the side walls, where they shade each other, avoiding damage caused by the absorption of excess light



Chloroplast movements:

- Chloroplasts move into weak light and away from high light
- A blue light response in angiosperms
- A blue and red light response in ferns

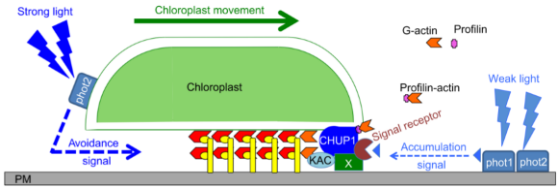


(Unique in ferns)

phot2: blue light receptor. Localizes in plasma membrane (PM) for accumulation response and to chloroplast envelope for avoidance response

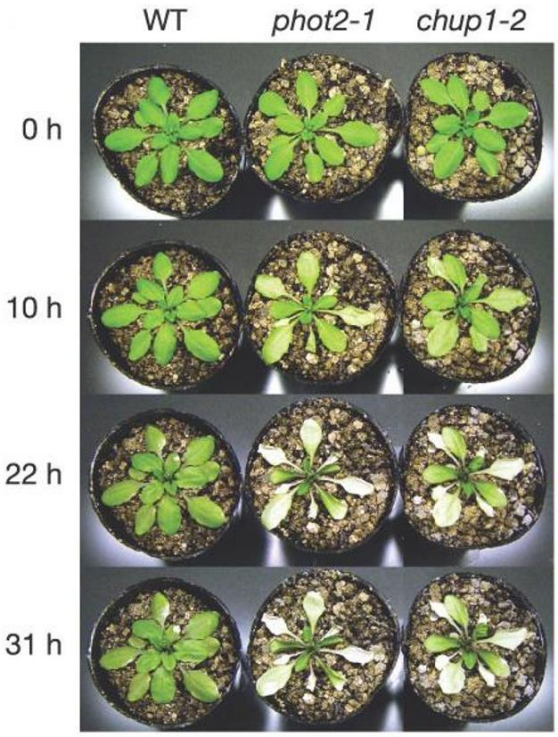
CHUP1: attaches chloroplast to the actin cytoskeleton and probably to PM. Probably helps polymerization of actin filaments

Signal transduction pathways are unknown



IMPORTANCE OF CHLOROPLAST MOVEMENTS

12 hours of high light treatment causes cell death in *phot2* and *chup1* mutants, which are unable to relocate their chloroplasts



ASSIMILATION OF INORGANIC CARBON

Photosynthesis provides:

- Energy in the form of ATP
- Reducing power in the form of NADPH

RUBISCO

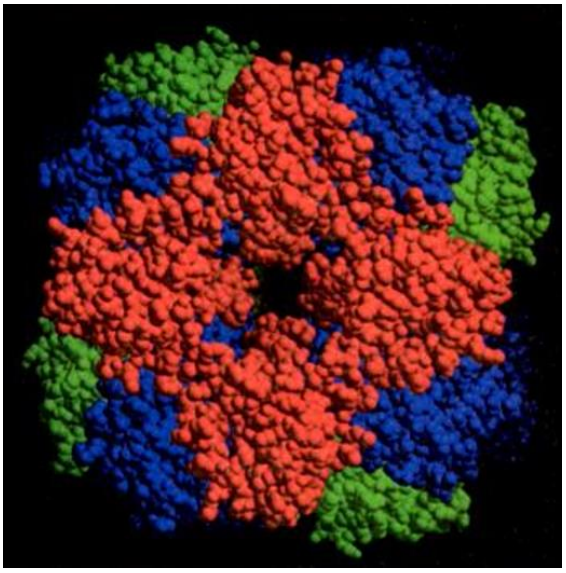
Rib**u**lose 1,5-**bis**phosphate **c**arboxylase/**o**xygenase

RUBISCO is the most abundant protein on the planet → RUBISCO is very slow so lots of it is needed

Predominant mechanism for net fixation of inorganic carbon

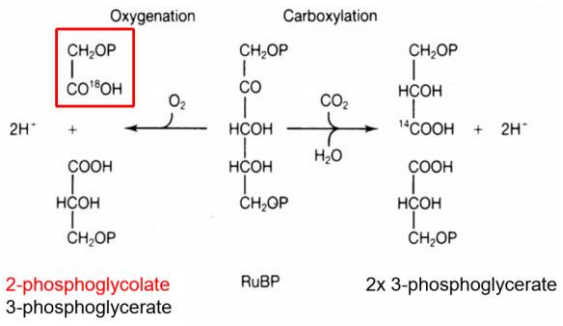
RUBISCO biogenesis requires:

- A multitude of chaperones
- The coordinated synthesis of plastid-encoded large and nuclear-encoded small subunits



RUBISCO is capable of catalysing two reactions:

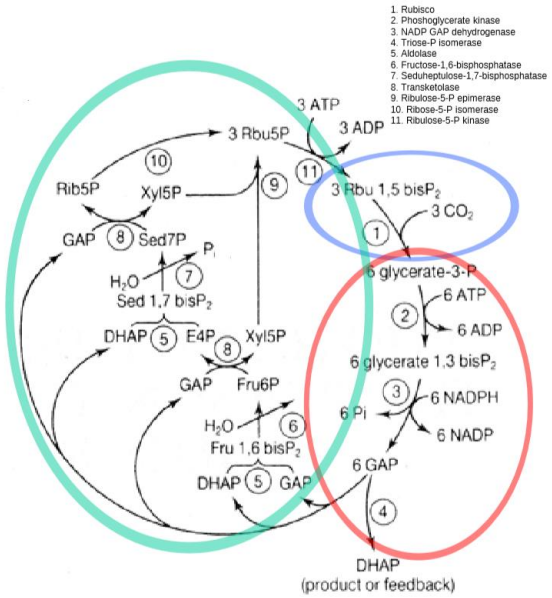
- Carboxylation of ribulose-1,5-bisphosphate (RuBP)
- Oxygenation of RuBP
  - Unwanted → no gain of carbon
  - Produces 3-P-glycerate → used to regenerate RuBP
  - Produces 2-P-glycolate → must be recovered via photorespiration



CALVIN-BENSON CYCLE

The Calvin-Benson cycle is often split into three phases:

- Carboxylation of ribulose-1,5-bisphosphate
- Reduction of 3-phosphoglycerate to glyceraldehyde-3-phosphate
- Regeneration of ribulose-1,5-bisphosphate from triose phosphates

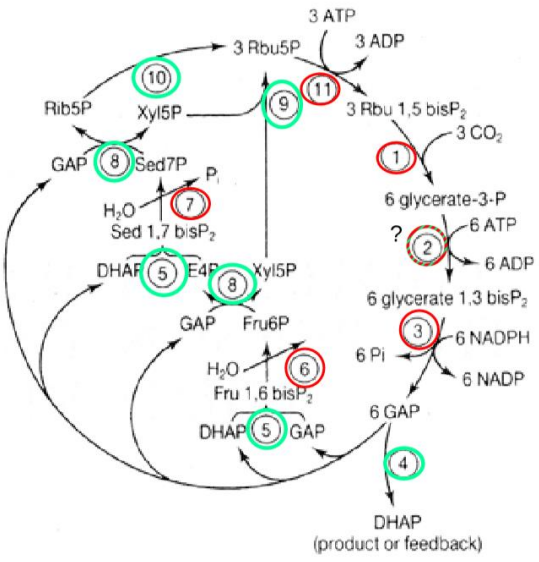


Enzymes with significant regulatory properties:

- Often catalyse irreversible reactions
- Activities similar to or slightly above flux
- Rate limiting steps

Enzymes lacking significant regulatory properties:

- Often catalyse readily reversible reactions
- Present at very high activities



How to remember the regeneration of RuBP from triose phosphates

5 x C3 are converted to 3 x C5

C4 is the smallest intermediate and C7 the largest

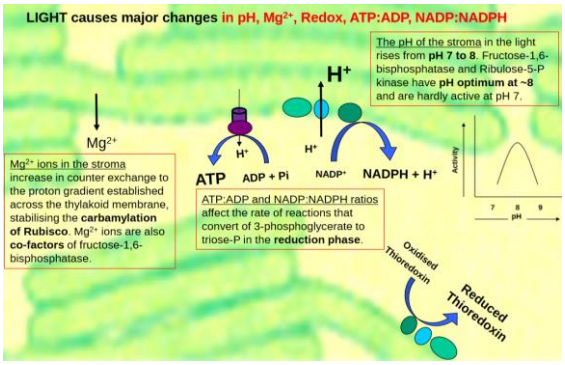
$$C3(1) + C3(2) = C6$$

$$C6 + C3(3) = C4 + C5(1)$$

$$C4 + C3(4) = C7$$

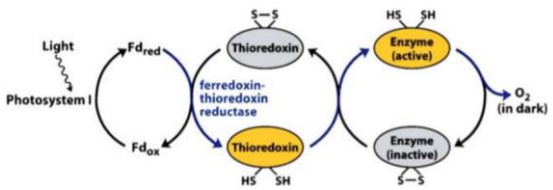
$$C7 + C3(5) = C5(2) + C5(3)$$

ROLE OF LIGHT IN THE CALVIN-BENSON CYCLE



REDOX REGULATION OF CALVIN-BENSON CYCLE ENZYMES

Change in redox potential of the stroma is communicated by the small, redox-active protein thioredoxin. The thioredoxin pool is reduced by the action of PSI and reduces sulfhydryl groups on glyceraldehyde-3-P dehydrogenase, fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase, cleaving disulfide linkages. This rapid, reversible, covalent modification alters the conformation of the proteins activating all three enzymes. RUBISCO activase is also activated this way. RUBISCO has a tendency to bind phosphorylated sugar (RuBP) → RUBISCO activase frees RUBISCO from RuBP



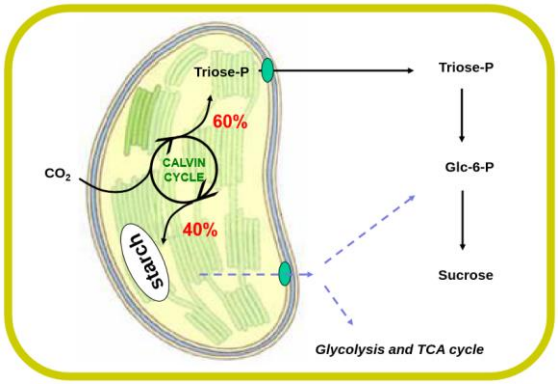
Regulation at multiple points via multiple mechanisms:

- Allows rapid changes in the rate of carbon fixation in line with changes in illumination
- Prevents fixation in the dark
- Allows co-ordinated regulation so intermediates of cycle are not depleted to zero

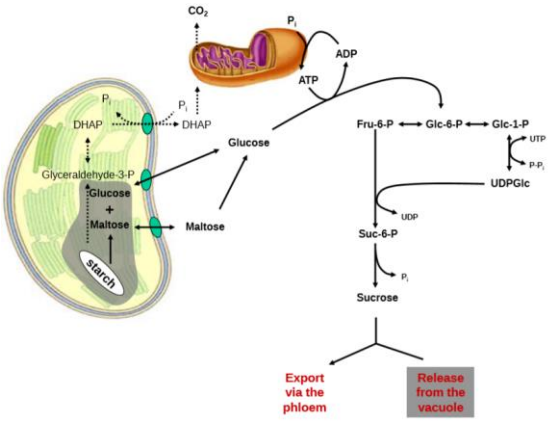
CARBOHYDRATE SYNTHESIS FROM PHOTOASSIMILATED CARBON

During the light, assimilated carbon is partitioned between sucrose and starch

During the dark, carbon stored in starch is used to continue sucrose synthesis and support



Sucrose synthesis during the night

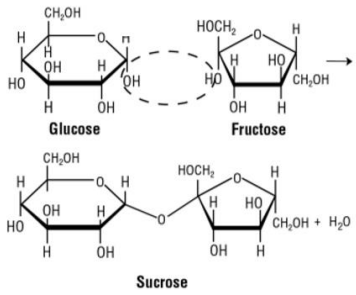


FUNCTIONS OF SUCROSE

Sucrose is:

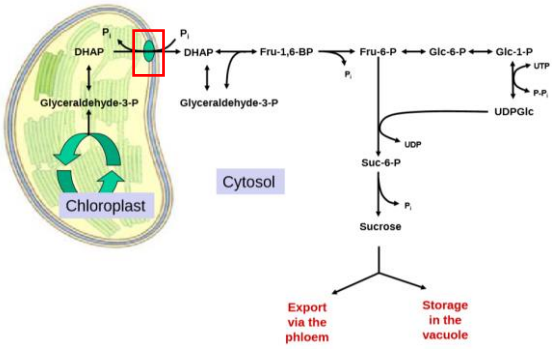
- The primary product of photosynthesis
- The form in which carbon is exported to the rest of the plant via the phloem

- A non-reducing disaccharide composed of one glucose and one fructose molecule
- Exclusively synthesized in the cytosol of plant cells, so assimilated carbon must be exported from the chloroplast



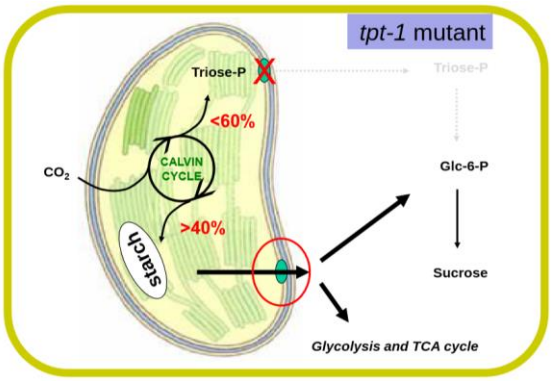
PATHWAY OF SUCROSE SYNTHESIS

Normally most sucrose is exported



Triosephosphate/phosphate translocator (TPT): This transport protein is central to photosynthetic carbon metabolism. It catalyses the strict counter exchange of triose phosphates and inorganic phosphate. Phosphate is returned to the chloroplast for ATP synthesis. Changes in free phosphate act as a signal for metabolic regulation at several points in carbon metabolism

tpt-1: viable. Why? The lack of phosphate transport in *tpt-1* is compensated by increased partitioning into starch, turnover of transitory starch during the day and export of starch-derived neutral sugars

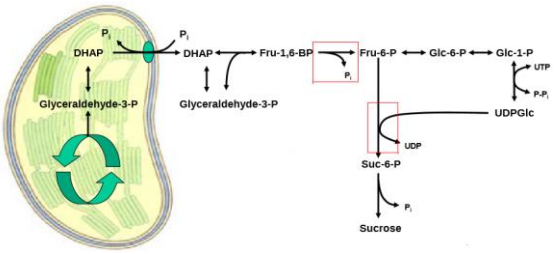


REGULATION OF SUCROSE BIOSYNTHESIS

Regulation at two key points:

- Sucrose-P synthase
  - Regulated allosterically and via protein phosphorylation
- Cytosolic FBPase
  - Allosteric regulation via a signal metabolite

At these two steps the supply of photosynthates from chloroplast (feed-forward control) and demand for substrate for sucrose biosynthesis (feedback control) are integrated

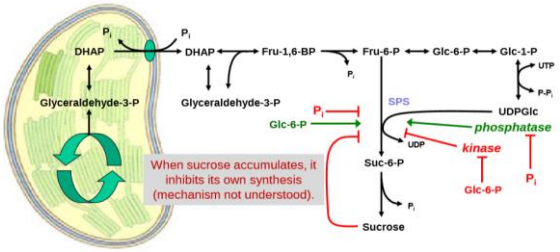


REGULATION OF SUCROSE BIOSYNTHESIS VIA SUCROSE-P SYNTHASE (SPS)

When photosynthesis takes place:

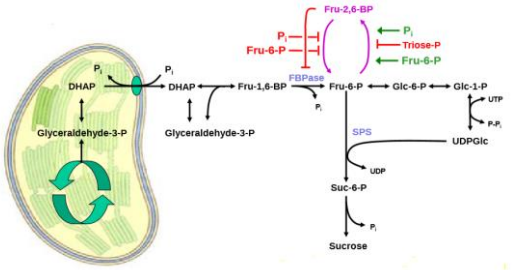
- Levels of free phosphate low
- Levels of cytosolic hexose-P high → sucrose-P synthase activity is stimulated (feed-forward control)

Sucrose-P synthase is also controlled by reversible phosphorylation (phosphorylated form is inactive). Glc-6-P inhibits the inhibitory kinase. Free phosphate inhibits the activating phosphatase



REGULATION OF FBPASE VIA FRUCTOSE-2,6-BISPHOSPHATE

Fructose-2,6-bisphosphate (Fru-2,6-BP) is a signal metabolite that is not part of any metabolic pathway. It is made from Fru-6-P and it inhibits fructose-1,6-bisphosphatase (FBPase) activity already at very low concentrations. When triose-P/phosphate ratio is high: Fru-2,6-Bp is low → FBPase is active



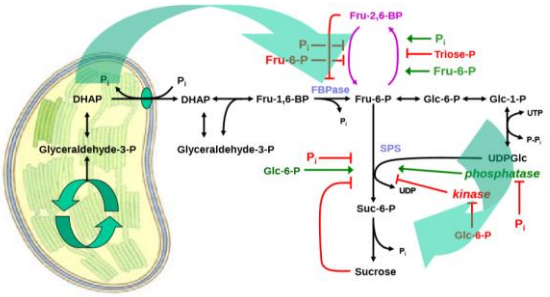
High ratio between triose-P/phosphate stimulates both FBPase and SPS and thus sucrose synthesis

SUCROSE BIOSYNTHESIS

At onset of day → start of carbon assimilation

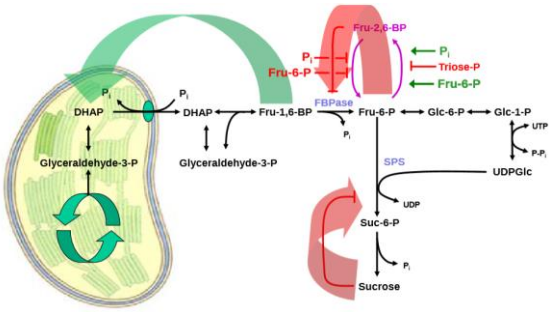
Triose phosphates increase, free phosphate decreases

- The levels of Fru-2,6-BP are low
- FBPase is therefore active
- Increases abundance of hexose-P
- These conditions stimulates SPS directly, and by preventing its phosphorylation (inactivation)
- Sucrose is produced
- However, continued high rate of sucrose synthesis requires a high rate of sucrose export

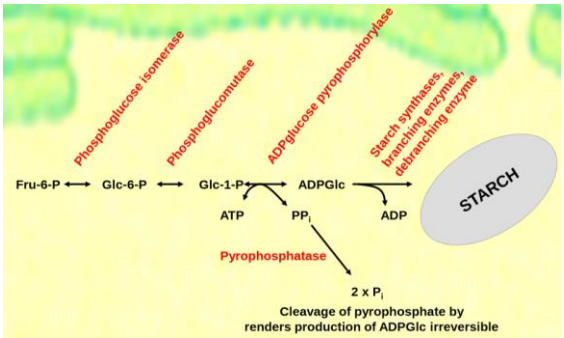


If sucrose synthesis exceeds export/storage demands (e.g. high lights)

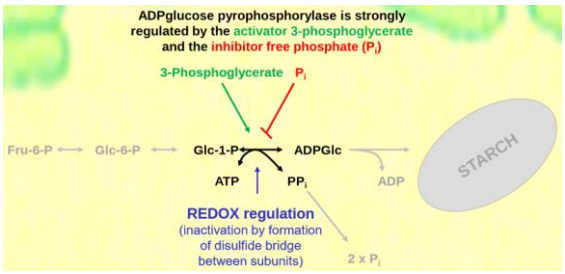
- Sucrose inhibits its own synthesis (unknown mechanism)
- Hexose phosphates rise to high levels
- Fru-2,6-bisphosphate becomes high (Fru-6-P overrides triose-P signals) → FBPase inhibited
- Triose-P accumulate in cytosol → less triose-P is exported into cytosol via the triosephosphate/phosphate translocator
- Triose-P in plastid increases → 3-phosphoglycerate in plastid increases → promotes partitioning of phosphosynthates into starch



PATHWAY OF STARCH SYNTHESIS IN CHLOROPLASTS

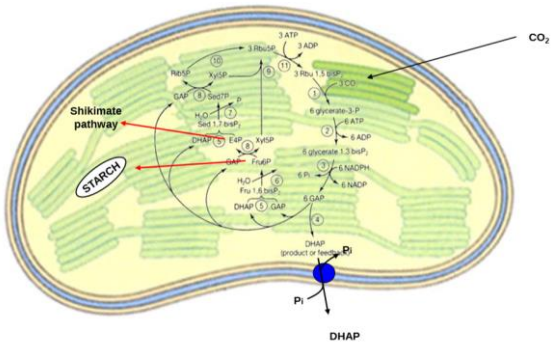


ADPglucose is synthesized when rates of photosynthesis and carbon assimilation are high



REMOVAL OF CARBON FROM THE CALVIN CYCLE

Traditionally triose phosphate is seen as the net product of the Calvin cycle, but intermediates can be removed at several stages depending on the partitioning of photosynthesis

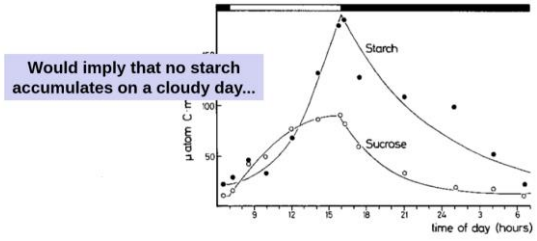


CONCEPT OF LEAF STARCH AS AN OVERFLOW FOR PHOTOSYNTHESIS

Starch as an insoluble store of carbohydrate allows photosynthesis to continue when the demand for sucrose has been met

If sucrose were the only product of photosynthesis: When the rate of photosynthesis exceeds the demand of sucrose in sink tissues and the capacity to store sucrose in the vacuole, an accumulation of intermediates would cause an inhibition of photosynthesis

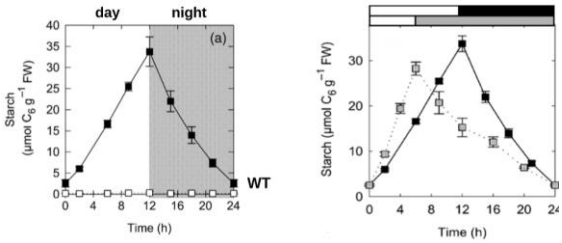
Example: Spinach leaves



Does not apply to all species

Some plants do not store significant sucrose in the leaf but partition carbon into starch at a fixed rate during the day

When grown in short days and long nights plants store a greater proportion of their fixed carbon in the leaf for use at night



TAKE HOME MESSAGES

- The Calvin-Benson cycle enzymes are regulated by light dependant mechanisms so that carbon fixation and light harvesting are co-ordinated. Regulation is via multiple mechanisms at multiple points to ensure effective regulation and the retention of Calvin-Benson cycle intermediates
- The export of assimilated carbon from photosynthesis is via the triose-P/phosphate translocator, which ensures that inorganic phosphate is returned to the chloroplast in stochiometric amounts
- Sucrose synthesis proceeds in the cytosol and is regulated by feed forward mechanisms from the supply of photosynthate and feedback mechanisms from sucrose accumulation
- Leaf starch serves as a deposit of carbohydrate and is made either as an overflow (when the demand for sucrose is met) or constitutively as a store of carbon and energy for metabolism during the night

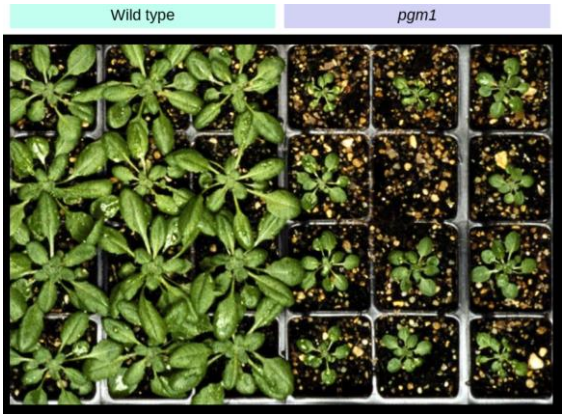
CENTRAL METABOLISM II

LEARNING GOALS

- The synthesis and structure of starch
- The breakdown of starch in leaves
- Starch metabolism in stomatal guard cells

PGM1 MUTANT

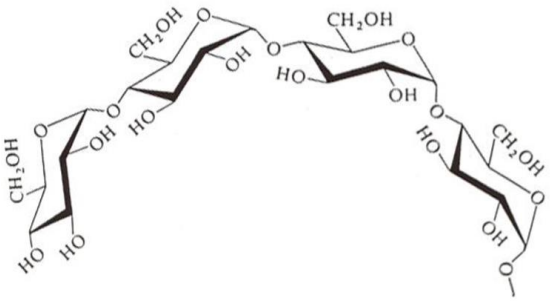
pgm1 mutant: is not able to synthesize starch. They are viable but grow slower as WT Arabidopsis. These plants degrade proteins and lipids instead when there is not enough energy. The longer the photoperiod, the less severe the phenotype. In continuous light (24h light), WT and mutant grow at the same rate

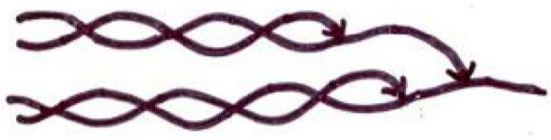


STARCH STRUCTURE AND METABOLISM

Starch is a glucose polymer with α-1,4 and α-1,6-linkages

α-1,4-linkage → curved chains that interact with themselves and neighbours to give helical structures in starch

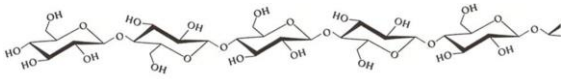




Double helices (in amylopectin)

Single helices (in amylose)

β-1,4-linkage → straight chains which interact with neighbours to give cellulose microfibrils. Extensive hydrogen bonding



COMPOSITION OF STARCH

Starch is composed of two glucan polymers: Amylose and Amylopectin

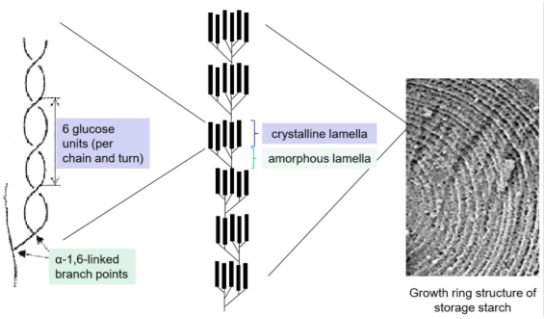
Amylopectin: 10'000 – 100'000 glucose units, makes up 70% or more of starch. Amylopectin forms double helices

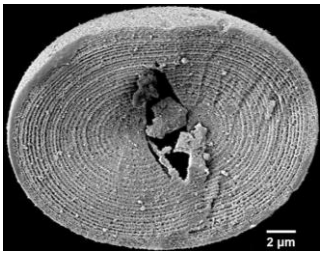
Amylose: 1'000 glucose units, comprises up to 30% of starch. Amylose is predominantly linear

Starch with almost only Amylopectin exists, but not with almost only Amylose

STRUCTURE OF STARCH

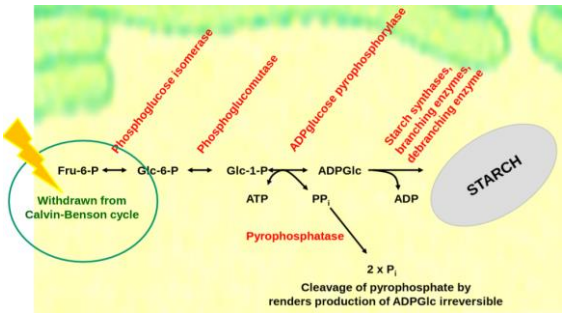
Adjacent amylopectin chains form double helices which pack into crystalline lamellae

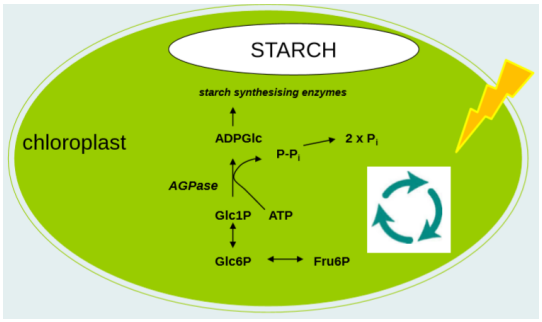




STARCH SYNTHESIS

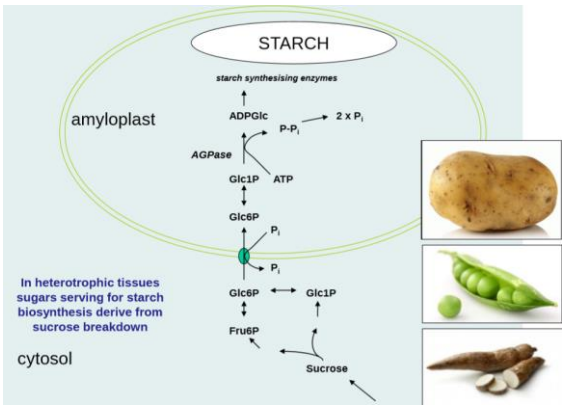
IN CHLOROPLAST





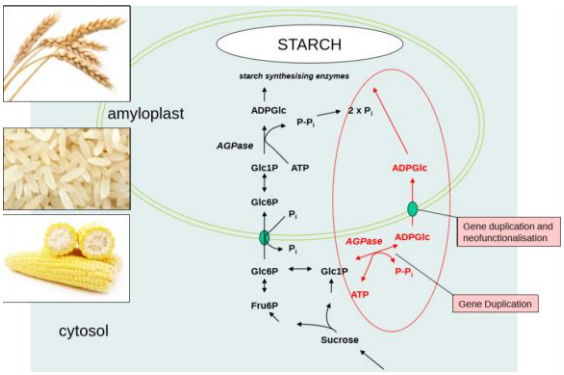
IN AMYLOPLAST

Amyloplasts are specialized to synthesize and store starch



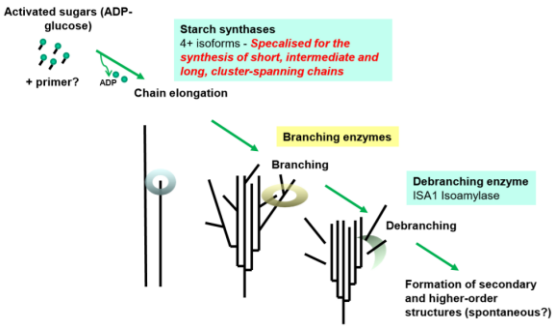
Example: Cereal

In cereal there is an additional, dominant pathway for starch production



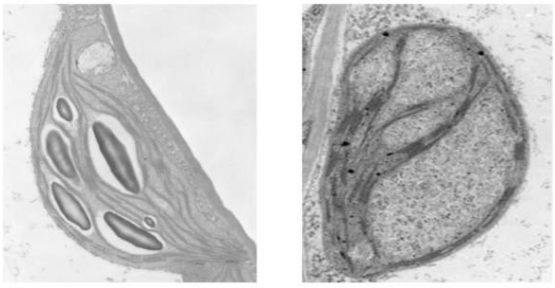
AMYLOPECTIN SYNTHESIS

Sugars are only added on existing chains → new chains can't be started



ISA1 isoamylase is believed to remove wrongly-placed branches that would interfere with the formation of secondary and higher-order structures

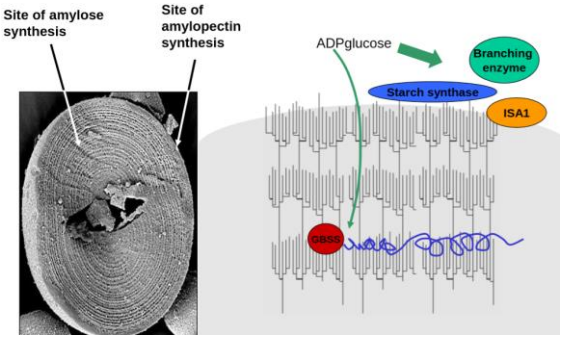
isa1 mutant: accumulates a different glucan. Accumulates a glucan which is more highly branched. The glucan lacks secondary structure and is soluble. It is more like glycogen than amylopectin → phytoglycogen. The mutants lack an isoform of the debranching enzyme



Wild type **Arabidopsis *isa1* debranching enzyme mutant**

HOW TO SIMULTANEOUSLY SYNTHESIZE BRANCHED AND LINEAR GLUCANS

Amylose is synthesized within the matrix formed by amylopectin by a granule-bound starch synthase (GBSS)

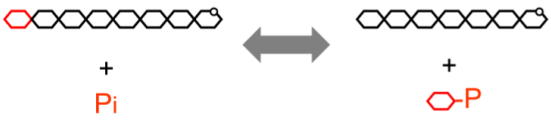


STARCH BREAKDOWN IN LEAVES

Two main enzymatic mechanisms

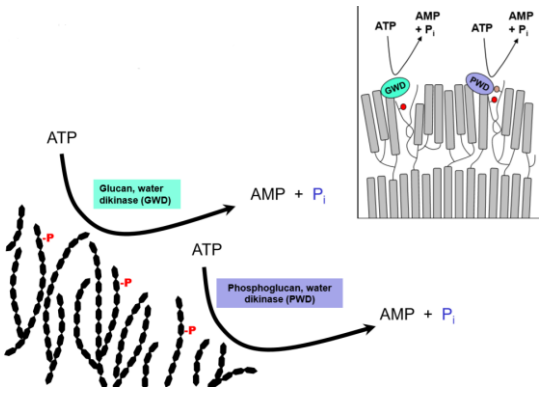
- Hydrolysis: α-1,4- (or α-1,6-linkage) is broken with the addition of water

- Phosphorolysis: α-1,4-linkage is broken with the addition of phosphate

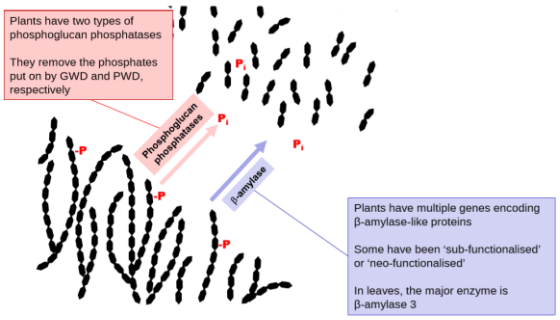


In leaves whether α-Amylase nor Phosphorylase are essential for starch degradation!

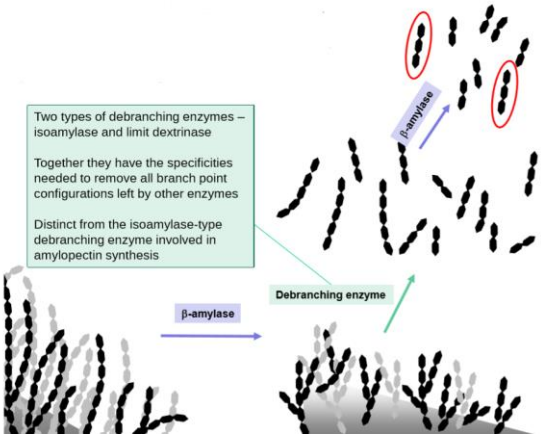
In chloroplasts starch degradation is initiated by glucan phosphorylation. It is proposed to disrupt the semi-crystalline packing of amylopectin



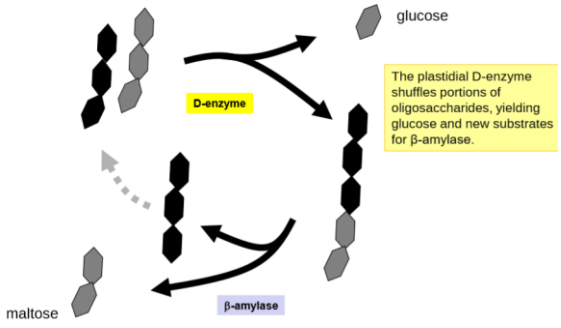
β-Amylases attack exposed linear chains, yielding maltose. However, they cannot pass phosphate groups. Phosphoglucan phosphatases remove the phosphates, aiding β-amylases



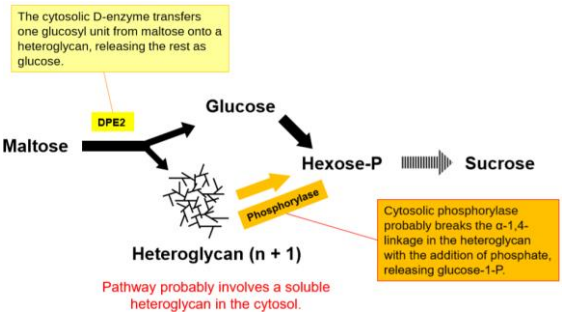
β-Amylases attack exposed linear chains, yielding maltose. However, they also cannot pass branch points. Debranching enzymes remove branch points from the β-limit surface



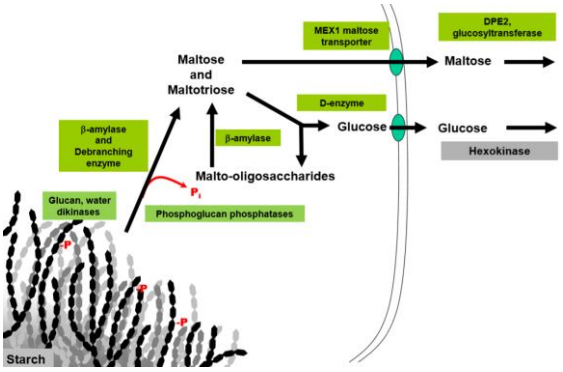
Also, maltotriose (3 glucose units) is too short for β-amylase to attack. The disproportionating enzyme (D-enzyme) DPE1 metabolizes such short oligosaccharides



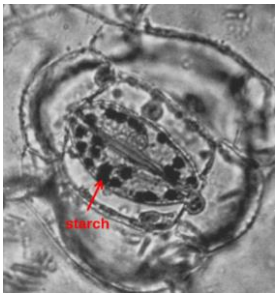
DPE2 in the cytosol



Complete pathway

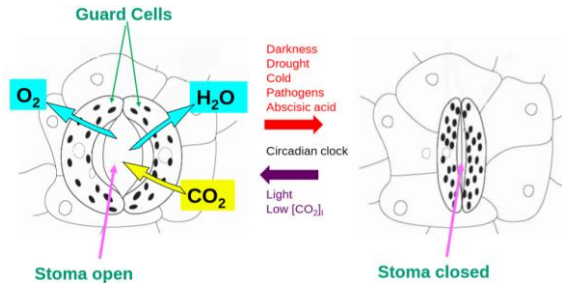


STARCH METABOLISM IN STOMATAL GUARD CELLS



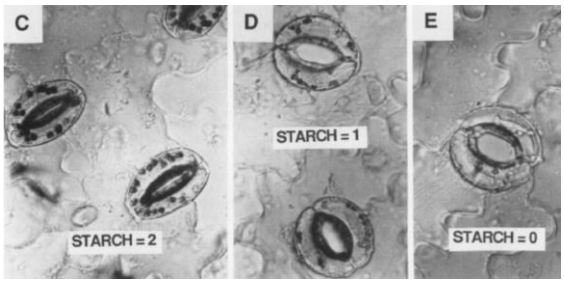
GUARD CELLS FORM ESSENTIAL WATER AND CARBON GATE

Balance between photosynthesis and transpiration

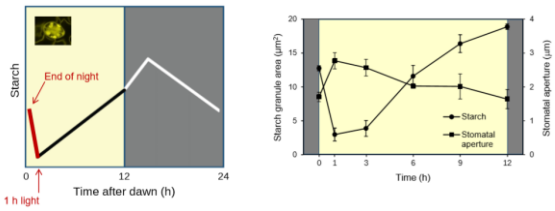


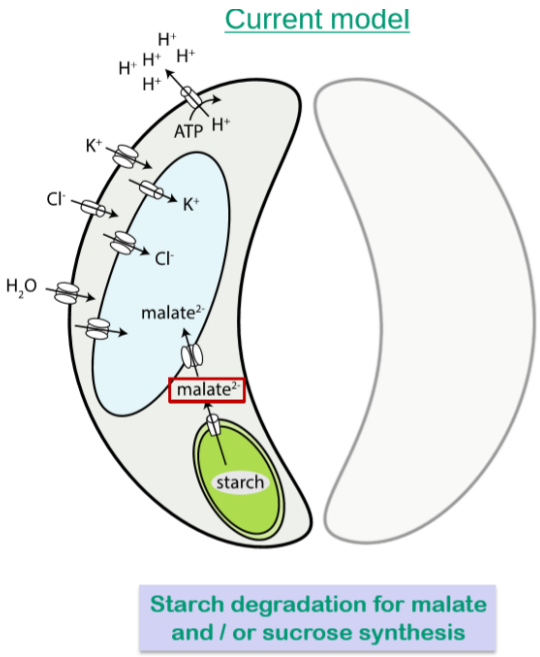
GUARD CELL STARCH DEGRADATION

Starch is degraded during stomatal opening

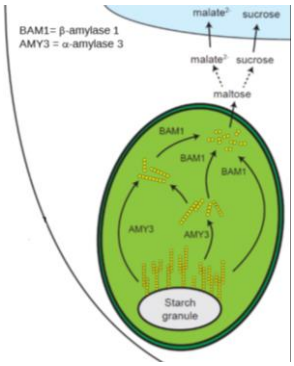


Synthesis and degradation of starch occur at different times and rates in stomatal guard cells. Starch dynamics in guard cells are inversely correlated to stomatal aperture



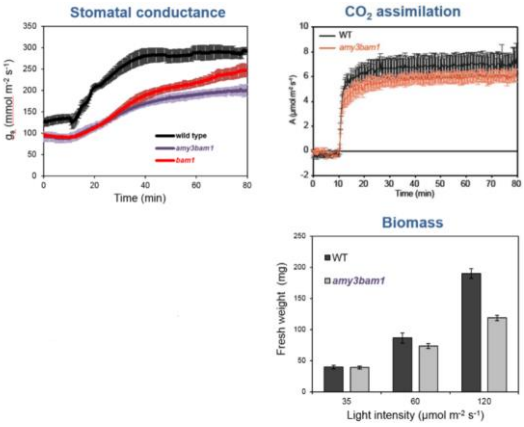


The enzymes β-amylase 1 and α-amylase 3, which are not required in overall leaf starch metabolism, are specialized for starch metabolism in guard cells



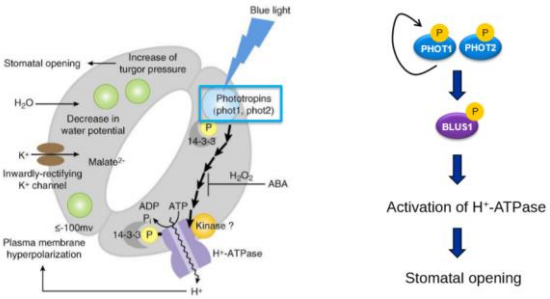
INFLUENCE OF GUARD CELL STARCH DEGRADATION AND STOMATAL FUNCTION

Stomatal conductance (gas flow rates) and CO_2 fixation rates at the onset of light are reduced in *amy3bam1* mutants, leading to growth deficits

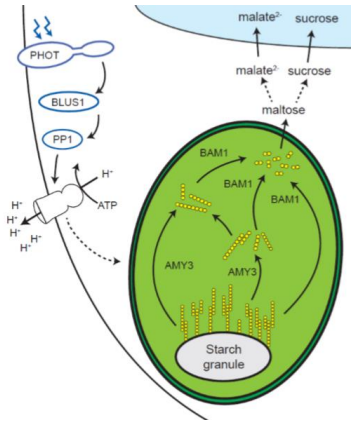


REGULATION OF STARCH DEGRADATION IN GUARD CELLS

The opening of stomata is mediated by blue light



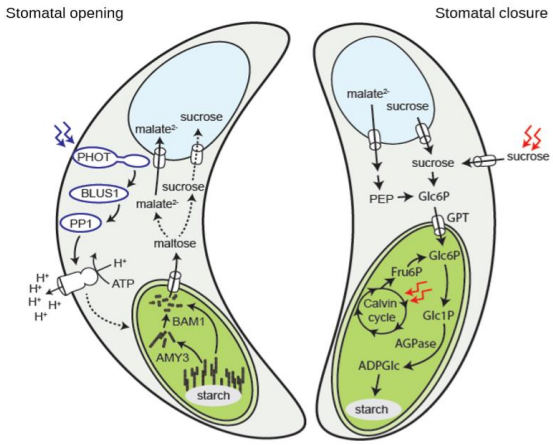
Activation of starch degradation is largely PHOT dependant but mesophyll starch degradation in *phot* mutants is normal



H⁺-ATPase1 (AHA1) is required for guard cell starch degradation

aha1 mutant: impaired in starch degradation and stomata opening

SUMMARY

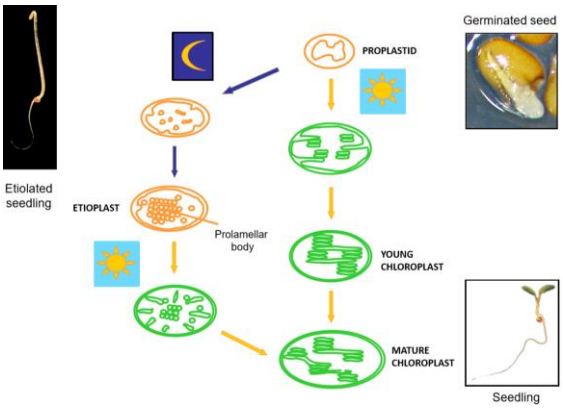


CHLOROPLAST DEVELOPMENT & CHLOROPHYLL BIOSYNTHESIS

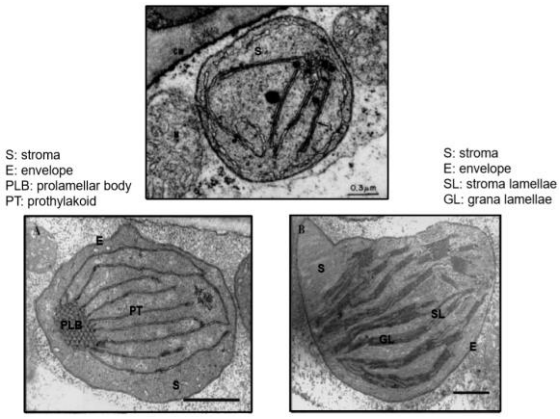
LEARNING GOALS

- Chloroplast biogenesis
- Chloroplast protein import
- Chloroplast division
- Chlorophyll synthesis

DEVELOPMENT OF CHLOROPLASTS

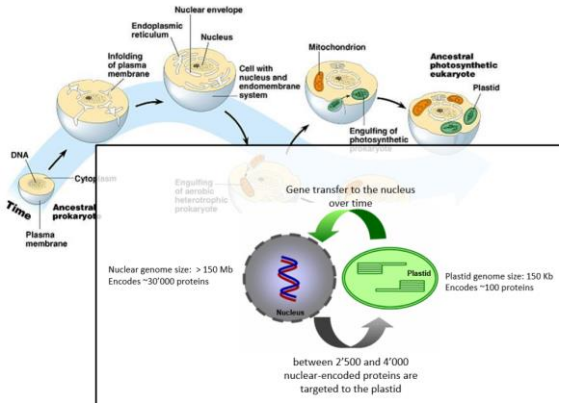


In the etioplast more and more chlorophyll accumulates

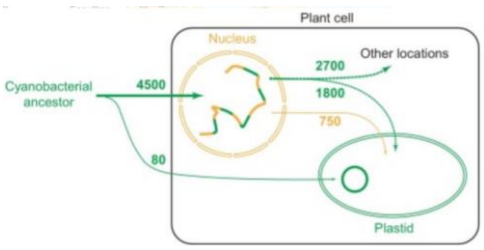


(Top: Proplastid, Left: Etioplast, Right: Chloroplast)

CHLOROPLAST DNA

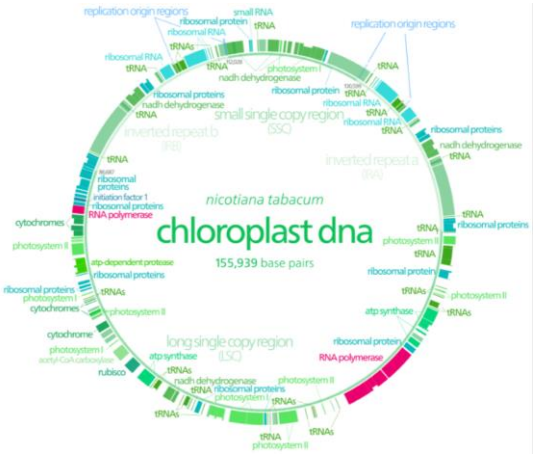


Genes from the nucleus are redirected to the plastid or here to maintain the chloroplast genome



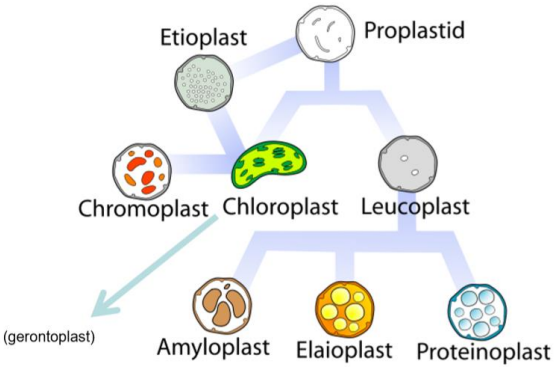
Many parasitic plants don't have chloroplasts

Several RNAs from the chloroplast are transspliced

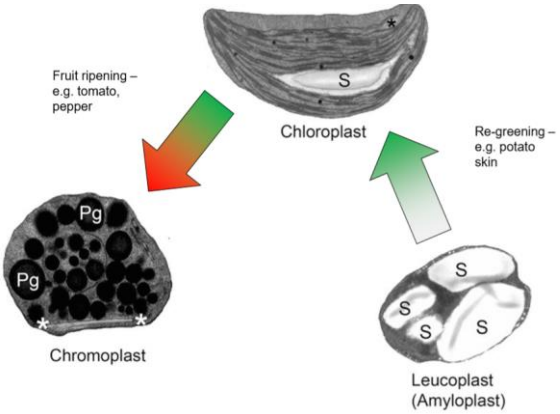


TYPES OF PLASTIDS

Chromoplasts are very common in fruits and are responsible for fruit colour. Leucoplasts are in non-green tissues of the plant

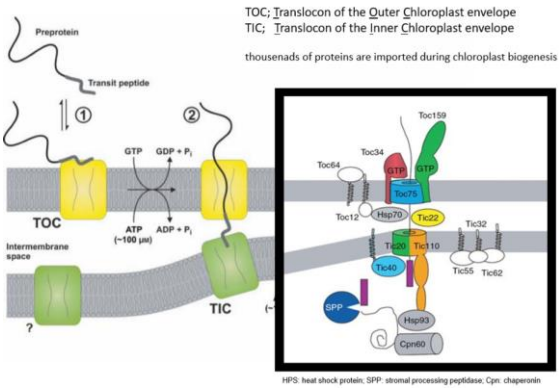


During plant development plastid types can be interconverted



PLASTID PROTEIN IMPORT

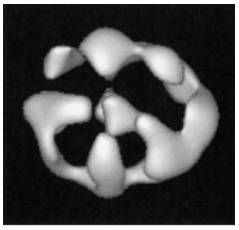
THE TOC-TIC SYSTEM

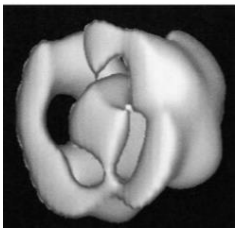


(The transit peptide is cut afterwards)

VISUALISATION OF THE TOC TRANSLOCON

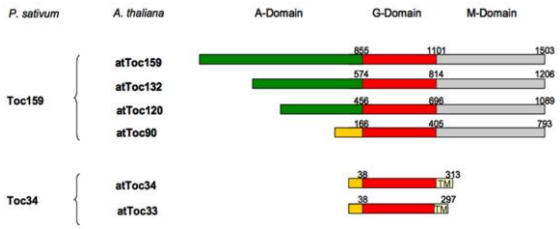
The complex was purified from pea chloroplast envelopes and stained with heavy metals. The complex was visualized using transmission electron microscopy and hundreds of images were used to reconstruct the complex in silico



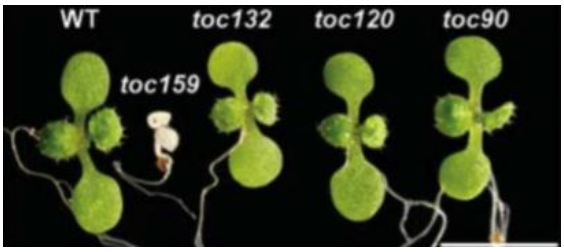


TOC GENES

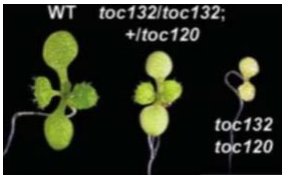
A small gene family encodes TOC159-like proteins. Each could recognize/import subcategories of chloroplast proteins



toc159 mutant: severe phenotype. Mutants can grow better when supplied with sucrose, but they still have a very severe, chlorophyll-free phenotype. The plastids would be functional but they don't photosynthesize

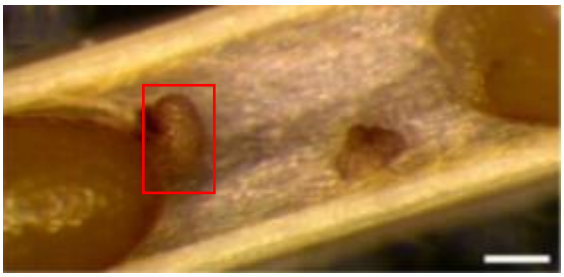


Combinatorial mutants of TOC159 homologues: severe, low-chlorophyll phenotype



Mutations that affect key components of the TOC-TIC translocon are embryonal lethal

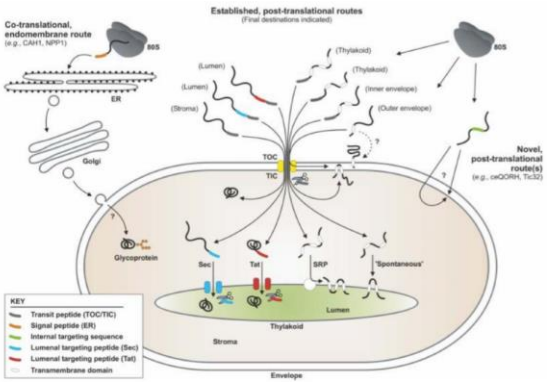
toc75 mutant: embryos arrest at the 2-cell stage → functional plastids are essential



(Aborted embryo)

SUB COMPARTMENTS OF CHLOROPLASTS

The chloroplast has multiple sub compartments. There are now thought to be multiple pathways for chloroplast protein import



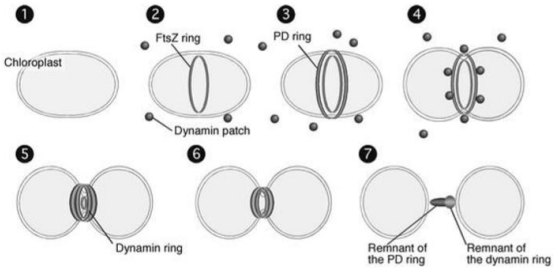
CHLOROPLAST DIVISION

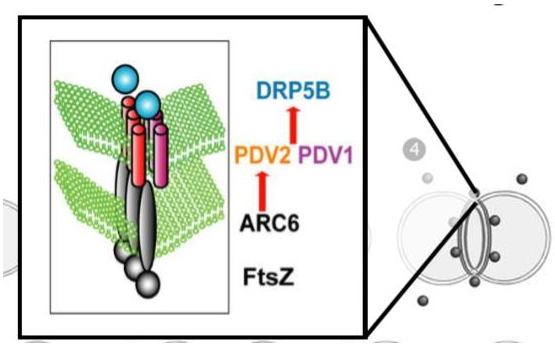
Plastids divide by fission:

- Cells in young Arabidopsis leaves have about 10 chloroplasts
- Cells in mature leaves have 50-100 chloroplasts
- Chloroplasts enlarge and undergo 2 or 3 rounds of fission

Division process:

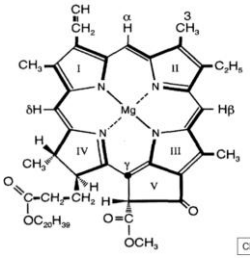
1. Undivided chloroplast
2. An FtsZ ring forms inside the chloroplast
3. An external PD ring forms outside the chloroplast
4. Dynamin is attracted to the PD ring
5. The dynamin forms a ring and constricts
6. Eventually the ring pinches the chloroplast in two
7. The remnants of the PD ring remain after division

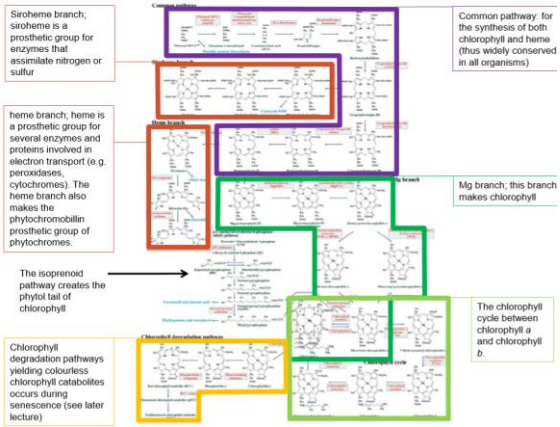




Mutants or over-expressors of plastid division proteins have less or more chloroplasts, respectively. However, chlorophyll content is the same in all lines suggesting independent control of photosynthetic capacity

SYNTHESIS OF CHLOROPHYLL



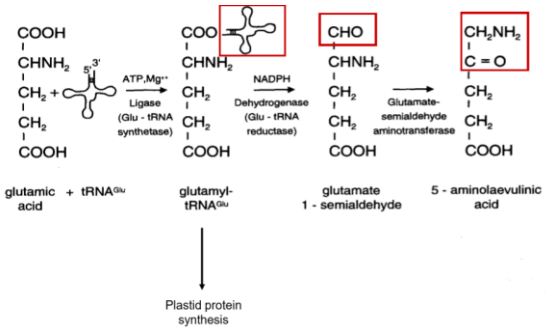


(Just an overview, don't memorize all structures)

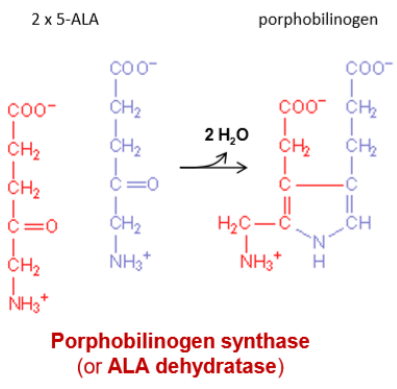
THE COMMON PATHWAY

5-aminolaevulinic acid

RNA is cleaved off of glutamyl $tRNA^{Glu}$ and amino group is moved to end of chain (glutamate 1-semialdehyde → 5-aminolaevulinic acid)

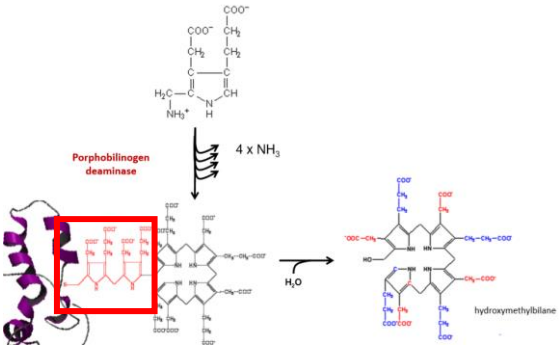


Porphobilinogen



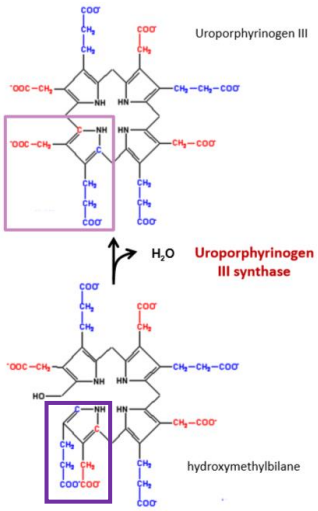
Hydroxymethylbilane

Part marked by red rectangle gets hydrolysed and stays on the enzyme. The resulting hydroxymethylbilane is still a linear molecule



Uroporphyrinogen III

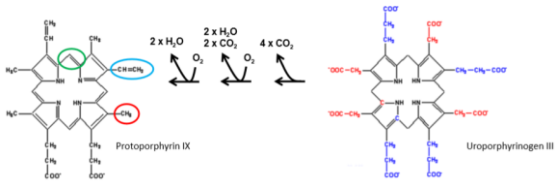
The parts marked by violet rectangle are flipped



Protoporphyrin IX

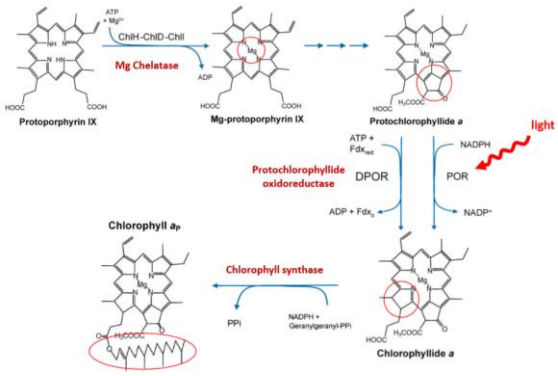
The side chains are modified.

- Decarboxylation of all 4 acetyl side chains to methyl groups
- Oxidative decarboxylation of 2 of the 4 propionyl side chains to vinyl groups
- Oxidation adds more double bonds

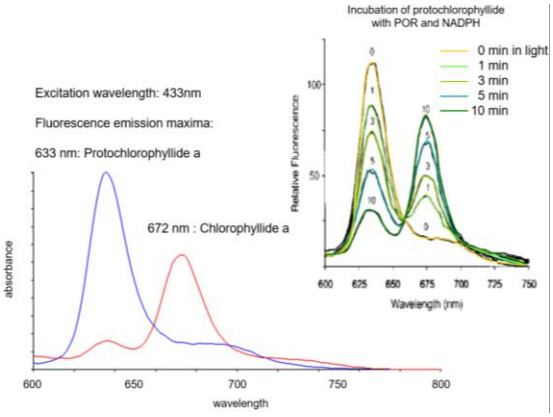


THE MG BRANCH VIA MG-PROTOPORPHYRIN IX

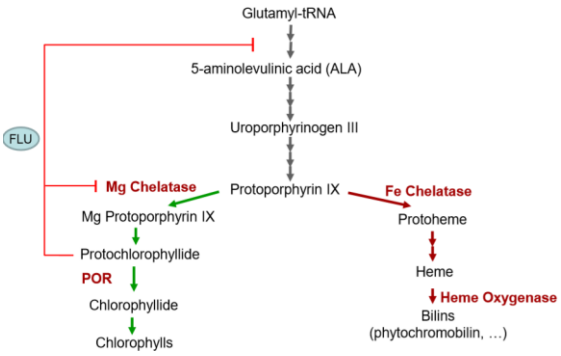
Protochlorophyllide a → Chlorophyllide a: the proplast turns green



EXCITATION WAVELENGTHS



REGULATION DURING CHLOROPHYLL BIOSYNTHESIS



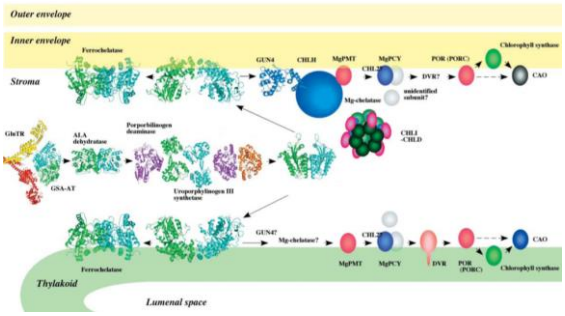
flu mutant: over accumulates protoporphyrin IX and dies when exposed to light

hy1 mutant: Heme Oxygenase is affected

LOCALISATION OF TETRAPYRROLE BIOSYNTHESIS

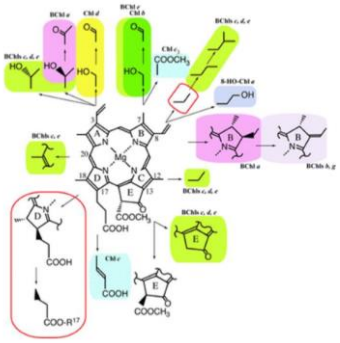
Some of the proteins of tetrapyrrole synthesis are stromal, some are associated with both the thylakoids and the chloroplast inner envelopes. Half of the proteins are well known, other half of

proteins are almost unknown. Half of the proteins are also abundant in human or bacteria

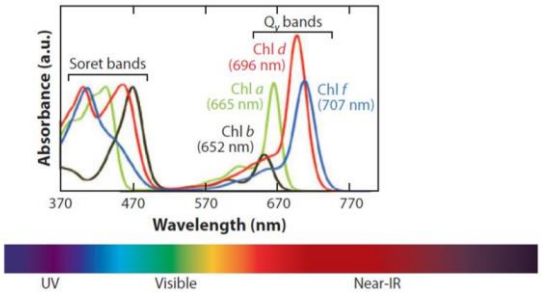


DIVERSITY OF CHLOROPHYLL FORMS

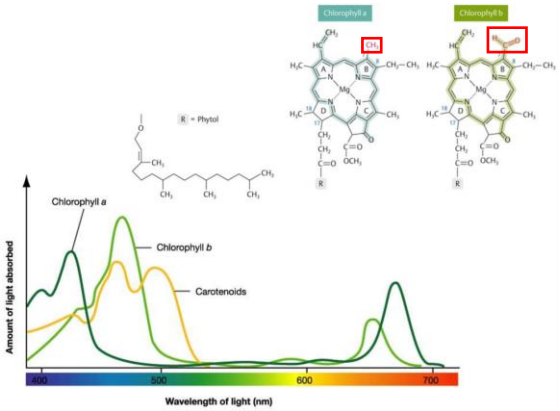
Almost 100 different variants of chlorophyll have been reported, most of them from bacteria and algae



Variants of chlorophyll allow oxygenic and anoxygenic photosynthesis to occur. In bacteria and algae outside the range of wavelengths used by vascular plants

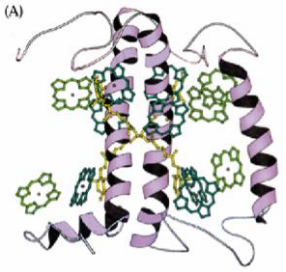


In plants: chlorophyll a and chlorophyll b

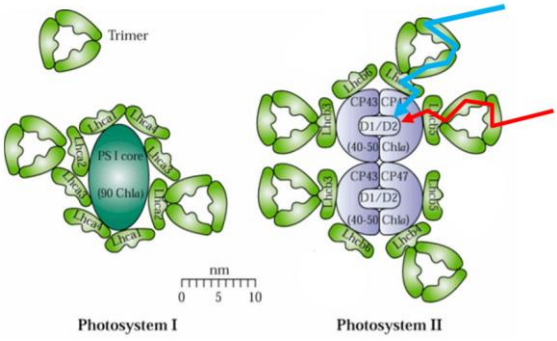


ASSEMBLY OF THE LIGHT HARVESTING COMPLEX (LHC)

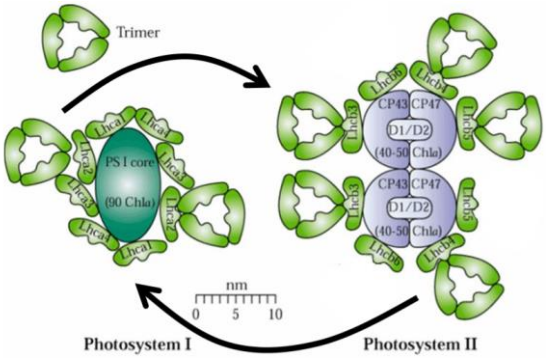
One LHC proteins binds approximately 12 chlorophyll molecules and several carotenoids



LHC trimers associate with the photosystems and light energy captured by the resident chlorophylls is delivered via resonance energy transfer



LHC trimers can move between the photosystems to an extent to balance the light energy captured each (a state called state transition)



ADDITIONAL CONSIDERATIONS ABOUT CHLOROPLAST BIOGENESIS (NOT EXAM RELEVANT)

- Production of potentially cytotoxic tetrapyrroles. Must be tightly coordinated with apoprotein synthesis
- Co-ordinated protein and lipid biosynthesis to create the thylakoid membranes and protein components
- Co-ordination of 2 genomes. Some components of the photosystems are nuclear genome encoded and others are plastid genome encoded
- There is communication between nucleus and plastid (anterograde signalling) and between plastid and nucleus (retrograde signalling)
- Different cell types within the leaf have very different plastids (e.g. non-photosynthetic plastids in epidermal cells). In some plants there may even be different types of plastids within a single cell
- Accompanied by peroxisome and mitochondrial biogenesis - compartments all involved in normal C3 photosynthesis

INTEGRATION OF METABOLISM

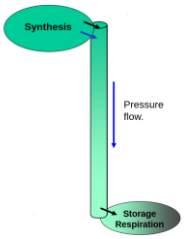
LEARNING GOALS

- Sugar transport
- Sugars acting as signals
- Integration of carbon and nitrogen metabolism
- (Metabolomics and metabolite sensors)

PHLOEM TRANSPORT AND SUCROSE UTILISATION

Active uptake of sugars into the phloem against a concentration gradient. Water follows by osmosis. High pressure

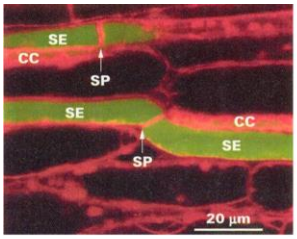
Passive unloading of sugars into sink tissues with concentration gradient. Lower pressure

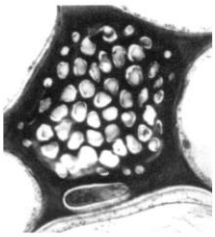


CONSIDERATIONS ABOUT PRESSURE FLOW

- Energy dependant as loading occurs against a concentration gradient: sucrose concentration in the apoplast is low mM range, whereas in the phloem it can be up to 1M
- Flow is a passive process down a pressure gradient. Flow rates can be 1m/h. Diffusion over 1m is 300’000 times slower!
- Sieve plates have open pores to allow net flow (may be a different system operating in Gymnosperms where sieve plate pores are occluded by membranes)
- Also transported in the phloem: amino acids, organic acids, inorganic ions, proteins, RNA,…

SIEVE ELEMENTS

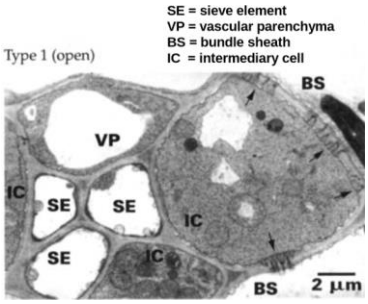




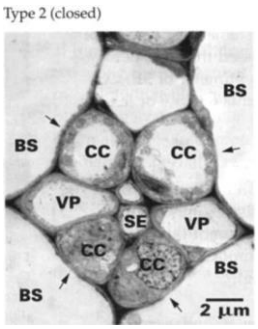
SE = sieve element
SP = sieve plate

Sieve elements are live, nucleated cells with companion cells, separated from surrounding cells

Type 1 phloem: occurs often in tree species, and cucurbits. Intermediary cells (IC) are connected to both mesophyll and sieve elements via plasmodesmata

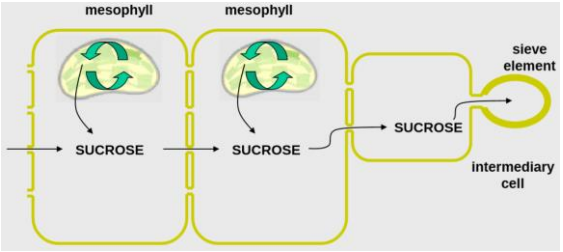


Type 2 phloem: in herbaceous species (including most crops). Symplastically isolated

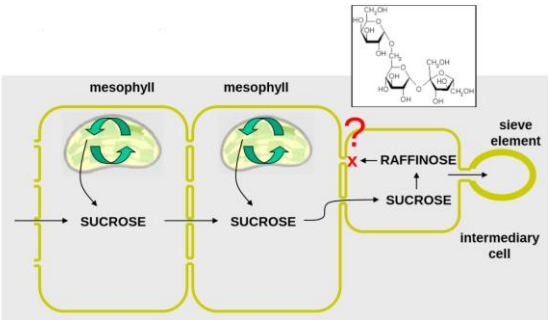


EXPORT OF SUCROSE FROM THE LEAF

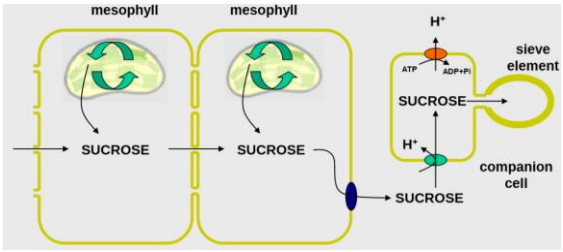
Type 1 phloem: transport is thought to be symplastic. Sucrose is transferred to intermediary cells and then flows directly to the sieve elements, always through plasmodesmata. Passive loading → slow transport



Sucrose is converted into larger sugars (e.g. raffinose) in intermediary cells. It has been proposed that the larger sugars cannot return through the small plasmodesmata, a so called sugar-trap mechanism. This may or may not be correct

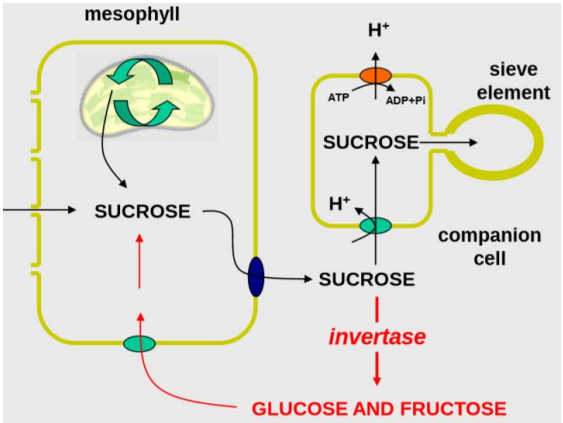


Type 2 phloem: sucrose transport is apoplastic. In bundle-sheath cells sucrose is unloaded into the apoplast down its concentration gradient via SWEET transporters. Sucrose is taken up via the action of a sucrose/proton symporter



EXPERIMENTAL APPROACH TO INTERFERE WITH PHLOEM LOADING

A yeast invertase (which cleaves sucrose into glucose and fructose) was expressed in tobacco plants. The invertase was engineered to contain an N-terminal signal peptide, targeting it to the secretory pathway



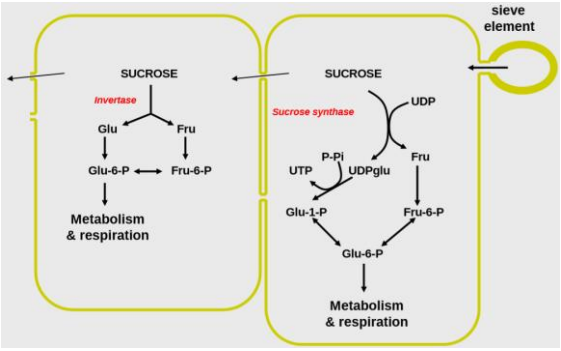
Dramatic effect on plant growth, confirming the importance of the uptake of sucrose via the apoplast: Severe reduction in growth, accumulation of carbohydrate in source leaves, development of chlorotic lesions in leaves as they undergo the source to sink transition

UNLOADING OF SUCROSE IN SINK TISSUES

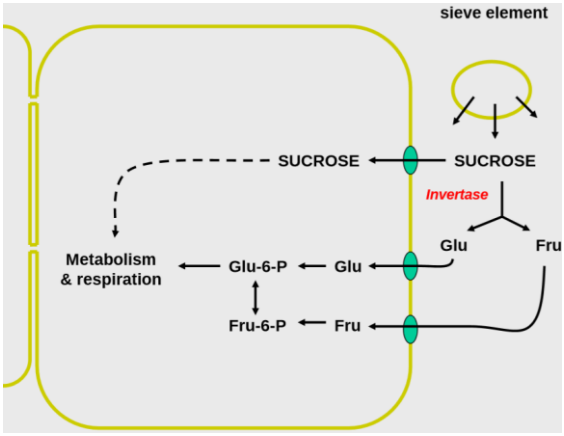
Unloading of transported metabolites into sink tissues is usually symplastic, through plasmodesmata. Unloading is passive (no energy directly required), but depends on the removal of translocated metabolites to maintain a concentration gradient (and thus pressure gradient) within the phloem. In some cases unloading is thought to be apoplastic. E.g. at the site of seed development, there is no symplastic connection between maternal and embryonic tissues

Two possible routes for sucrose metabolism:

- Symplastic unloading of sucrose from the phloem

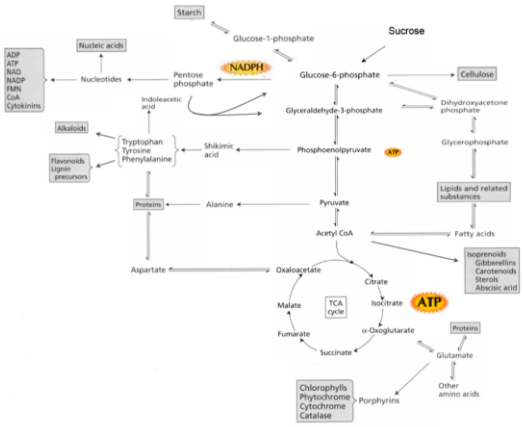


- Apoplastic unloading from the phloem



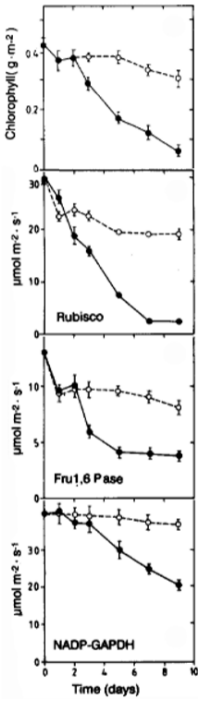
Used for:

- Energy
- Reducing powers
- Carbon storage
- Membranes
- Cellulose
- Amino acid synthesis
- Proteins
- DNA/RNA and co-factors
- Secondary metabolites and hormones



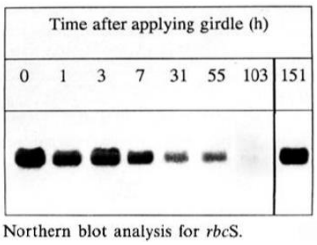
SUGARS AS SIGNALS

Accumulation of carbohydrates correlates with decreases in chlorophyll and the activity of photosynthetic enzymes



(● in glucose, ○ in water)

Similar experiments conducted by fitting a cold girdle to the petiole of growing spinach plants (3cm aluminium tube perfused with cold water). This cold girdle inhibits phloem export, an active, energy-requiring process, along the whole phloem path. Girdled leaves accumulate carbohydrate

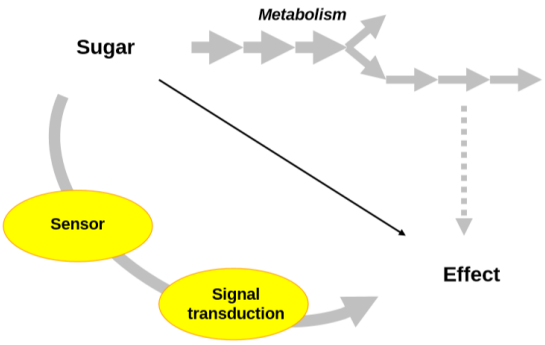


Northern blot analysis for *rbc*S.

(151: after removal of girdle)

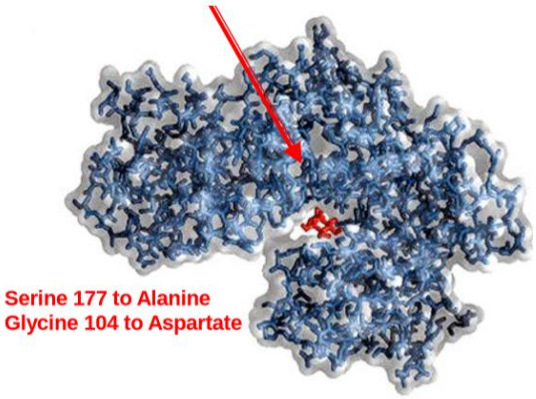
PROBLEM WHEN TRYING TO INVESTIGATE SUGAR SENSING

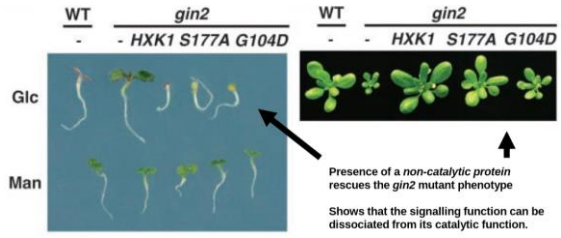
Sugars are substrates as well as signals → to demonstrate signalling it has to be dissociated from the effects of downstream metabolism



Approach used to demonstrate sensing function in yeast: construction of altered versions of the hexokinase protein

Strategy: introduce mutations in the conserved catalytic amino acids so that the protein is present, but not catalytically active

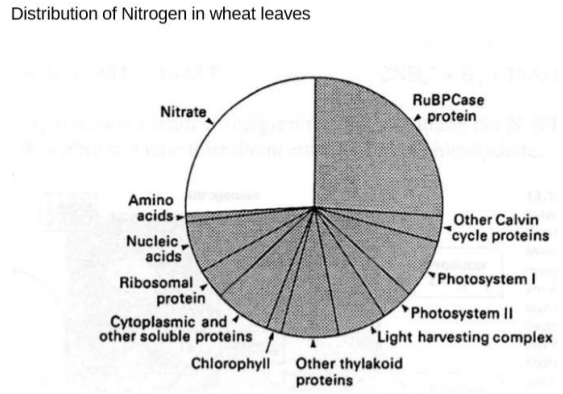


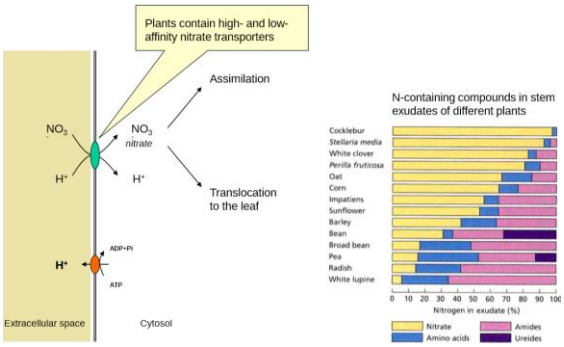


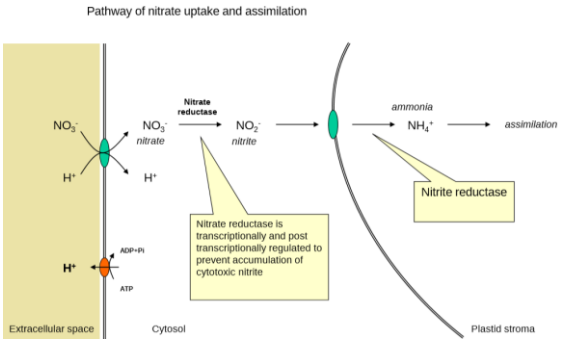
Sugars that are or might be sensed:

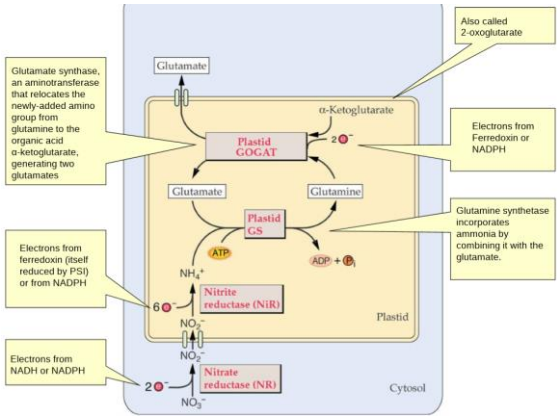
- Sucrose
- Glucose
- Fructose
- Maltose?
- Oligogalacturonides
- Amino acids
- Nitrate
- Phosphate
- Sulphate

INTEGRATION OF CARBON AND NITROGEN METABOLISM



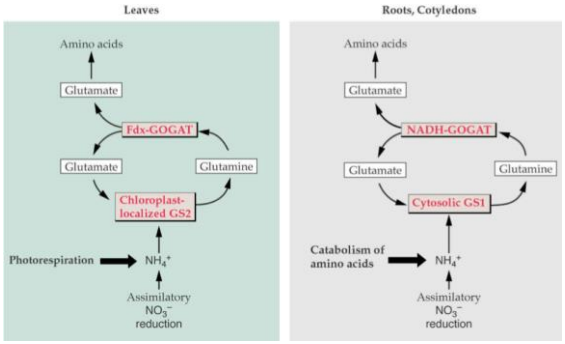


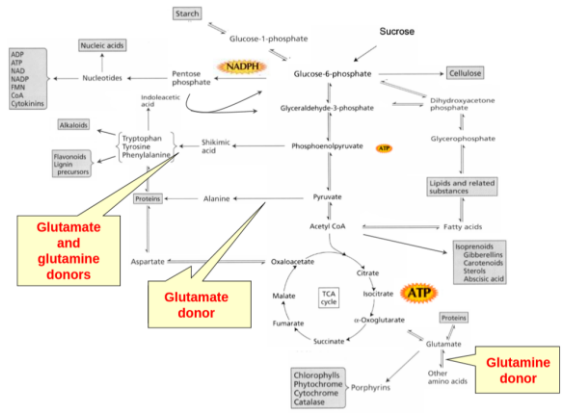




(α-Ketoglutarate: carbon skeleton needed to accept nitrogen)

Other sources of N for (re-) fixation in photosynthetic and non-photosynthetic tissues

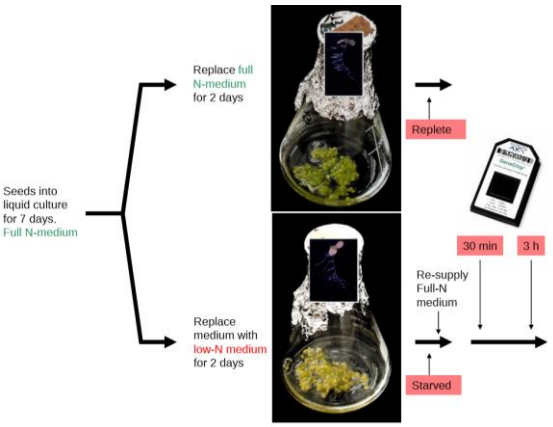


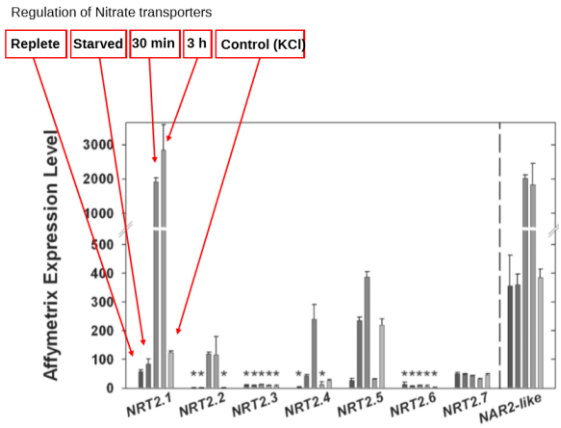


Assimilation of nitrate requires:

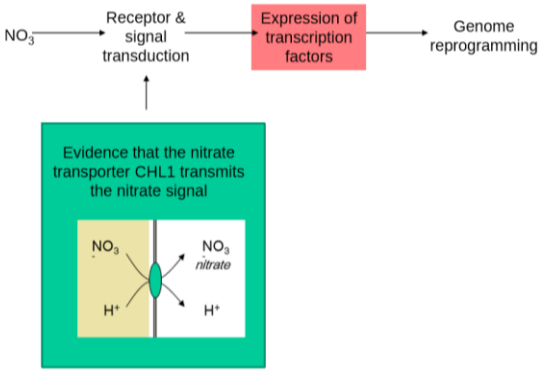
- Reducing power (either from photosystem I or from respiration)
- Carbon skeletons as acceptors for ammonia
- Organic acids to prevent alkalisation

Evidence for the reprogramming of carbon metabolism by nitrate supply





Hypothetical signal transduction pathway



TAKE HOME MESSAGES

- Phloem export from the leaf requires energy-dependant loading and passive pressure flow to sink organs

- Phloem unloading is a passive process, but metabolism of imported sugars is required to maintain sucrose gradient
- Sugars and other metabolites are sensed and regulate gene expression
- Nitrogen (nitrate) is reduced to ammonia and assimilated via GS-GOGAT system, yielding glutamate which is used in transamination reactions to produce other amino acids
- Respiratory pathways supply carbon skeletons for biosynthesis (e.g. amino acids)

SENESCENCE

LEARNING GOALS

- Patterns of senescence and its control
- Deconstruction of the chloroplasts
- Chlorophyll degradation
- Autophagy of the chloroplasts
- Re-allocation and cell death

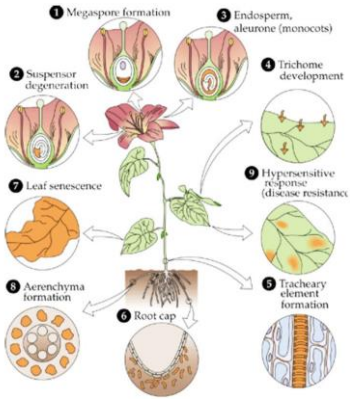
WHAT IS SENESCENCE?

- Senescence in plants is an internally regulated and orderly degeneration leading to the death of single cells, organs or even whole plants during their life cycle
- Leaf senescence is a regulated process of programmed cell death (PCD) coordinated with the development of other plant organs such as seeds, more apical leaves or storage structures
- Leaf senescence can be defined as nutrient remobilisation, a process that accompanies the decline in photosynthetic activity in ageing leaves

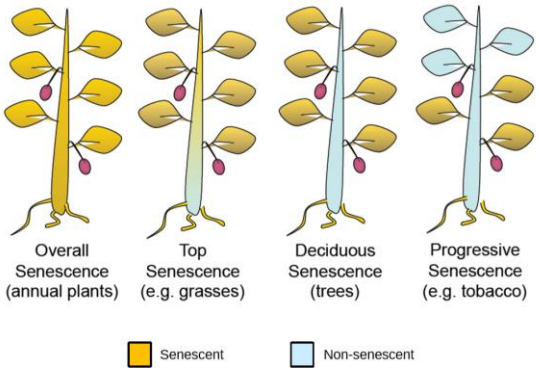
SENESCENCE AS A KEY PROCESS

Senescence of:

- Nutritive tissue: e.g. cotyledons of seeds exhibiting hypogeal germination
- Roots of nutrient-depleted soils
- Leaves: e.g. in response to external stress conditions, darkness, drought, in response to seasonal changes, daylength, temperature
- Spent floral tissue: e.g. petals
- Whole vegetative plant during seed set



SENESCENCE PATTERNS



Monocarpic: Plants that flower once during their life cycle and then die

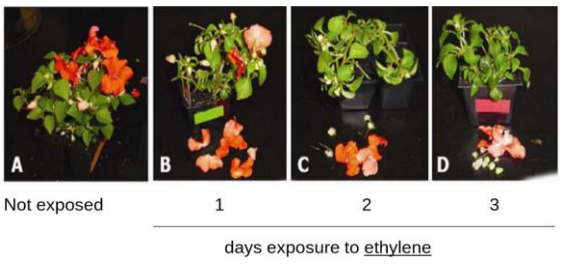
Polycarpic: Plants that repeatedly flower during their life cycle

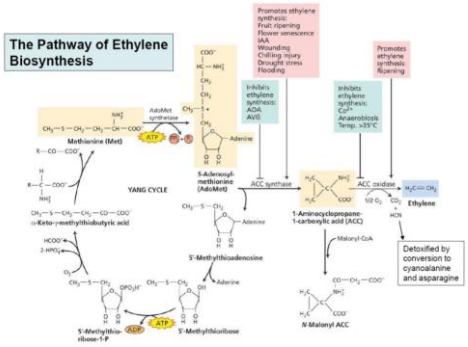
CONTROL OF SENESCENCE

HORMONES

Abscisic acid: originally named as it can promote abscission of leaves in some species. However, in most plants it has a minor role compared to other hormones

Ethylene: This volatile hormone is required to induce the senescence of vegetative tissues (and ripening to fruits). Alone, it is not sufficient





Cytokinin: A strong senescence inhibitor, which can induce re-greening (re-production of chloroplasts) of a leaf well into the senescent program

Auxin: Acts as an inhibitor of senescence

Jasmonic acid: Promotes senescence

Salicylic acid: Promotes senescence and is involved in cell death

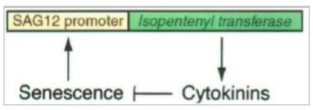
DEVELOPMENTAL EVENTS

Removal of the gynoecium prevents fertilisation of the flower. This removes the trigger for petal senescence. Fertilisation causes induction of ethylene biosynthetic enzymes and a burst in ethylene production

Fruit-set dependent senescence in soybean plants. Soybean plants that have set seeds induce whole plant senescence. In soybean plants from which developing seeds were removed, senescence is not induced

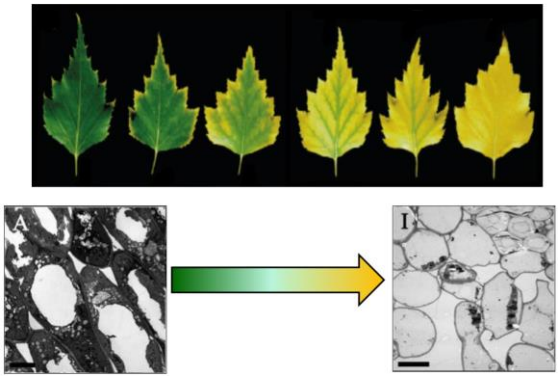
PREVENT SENESCENCE

An engineered feedback loop of senescence-induced cytokinin biosynthesis prevents senescence



LEAF SENESCENCE

Senescence initiates in the mesophyll cells and later affects other cell types. Senescence initiates at the leaf tip and margins, affecting the veins last. Within cells, chloroplasts are the first organelles to be affected. Nuclei, mitochondria and vacuoles remain intact and involved in the senescence process

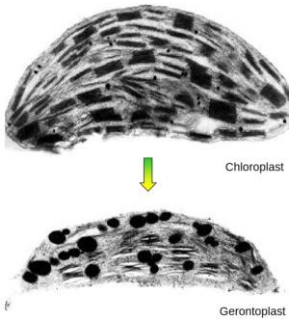


Senescence is a very active, regulated process. Transcriptome analysis in the naturally senescing leaves reveals almost 850 induced genes (SAG genes or SEN genes), a large fraction of which are regulatory proteins. Many genes are repressed e.g. those encoding photosynthetic proteins. The pattern of induced genes varies depending on the mechanism of senescence induction

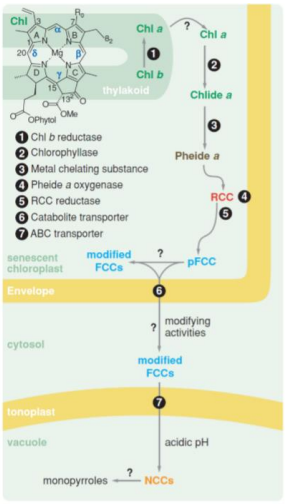
Chlorophyll declines steadily during senescence. Photosynthesis remains active until 75% to the chlorophyll has gone. Anthocyanins accumulate in the vacuole and may serve a photo-protective role during chlorophyll degradation

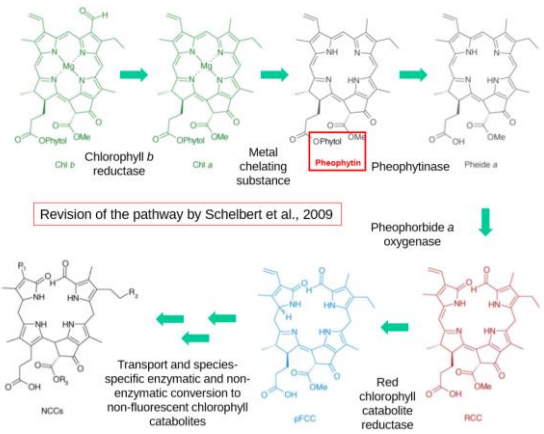
CHLOROPLAST DEGRADATION

- Reduced thylakoid system
- Increase in the number and size of plastoglobuli
- Chloroplasts become rounder in shape (usually)
- The number of chloroplasts decreases
- Outer membrane to the chloroplasts eventually breaks or the plastid is engulfed by the vacuole



CHLOROPHYLL DEGRADATION





PROBLEMS WITH CHLOROPHYLLASE

Chlorophyllase: Enzyme that will remove phytol tail from chlorophyll in vitro. However, GFP-furion proteins of the two chlorophyllase isoforms of Arabidopsis do not localize within the chloroplast. Mutants lacking chlorophyllase degrade their chlorophyll normally

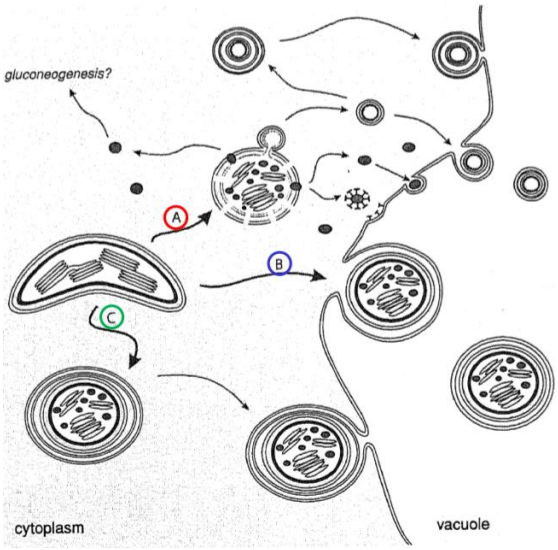
The pheophytinase (PHH) in Arabidopsis is in the chloroplast and mutants lacking PPH have a stay-green phenotype

Stay-green mutants may be commercially useful:

- Functional stay-green mutants
- Possible to extend the duration of photosynthesis during the growing season of annual crops
- Cosmetic stay-green plants
- Useful for grass/turf which is exposed to drought

AUTOPHAGY

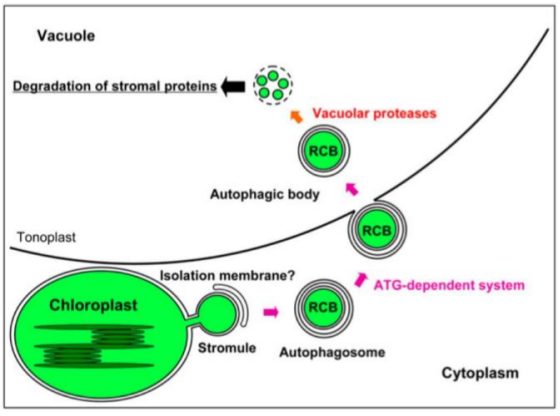
a. Chloroplasts may release material into the cytoplasm (vesicles containing soluble stromal material and plastoglobules containing thylakoid-derived material)
b. Chloroplasts may be engulfed by the vacuole
c. Chloroplasts may be included in autophagosomes which may fuse with the vacuole



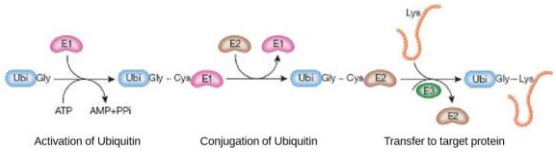
Enlarged, lipid filled plastoglobules bleb from the gerontoplasts. The fatty acids will be metabolized through β-oxidation. Acetyl-CoA can be used for respiration (TCA cycle), supporting the leaf in the absence of photosynthesis, or for gluconeogenic formation of sugars for export to the rest of the plant

Vesicles in the cytoplasm contain RUBISCO (RUBISCO-containing bodies (RCBs))

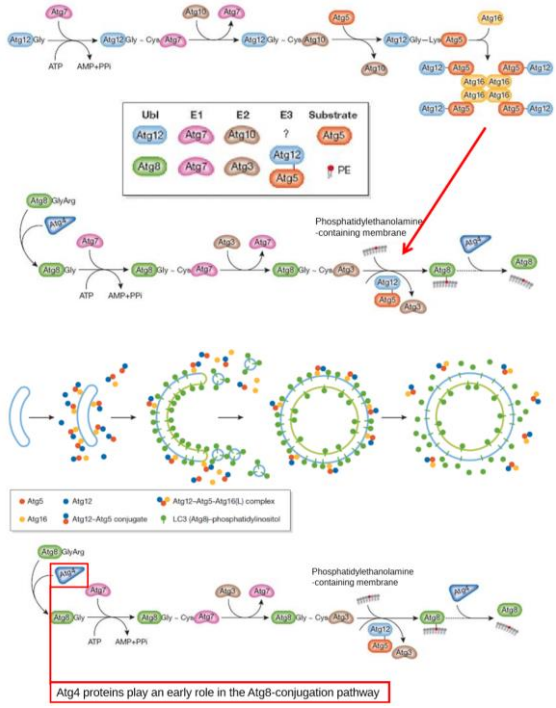
Stromules might be involved in the generation of RCBs



Autophagy uses a system of targeting proteins comparable to the Ubiquitin system



In autophagy, two conjugation systems operate in parallel, resulting in Atg8-tagged membranes



EXPORT

- Throughout senescence protein degradation products are exported as free amino acids, principally glutamine, asparagine, serine and proline
- Small peptides may also be exported
- Nitrogen released from other sources (e.g. nucleic acids) as ammonia is incorporated into amino acids via GS-GOGAT system and the GSH-GOGAT system
- Sugars derived from residual photosynthesis, and form gluconeogenesis are also exported
- Sulphur (in the form of glutathione)
- Phosphorus (as phosphate?)
- K^+, Mg^{2+}, Ca^{2+} and micronutrients (confliction reports)

CELL DEATH

- Mitochondria degrade
- DNA fragments
- Vacuole and plasma membrane loose integrity, resulting in ion leakage and cell death

TAKE HOME MESSAGES

- Senescence is a slow (and painless) form of programmed cell death
- Senescence is age-dependant, but can be induced or delayed by external factors
- Senescence is under hormonal control, principally ethylene (inductive) and cytokinin (repressive)
- In leaves the biggest and most obvious cellular change is in the chloroplast, which contains most protein
- There are multiple biochemical and cell-biological pathways (e.g. chlorophyll breakdown) for the recycling of cellular components (e.g. autophagy)

PART VOINNET

GENERAL PRINCIPLES OF RNA SILENCING

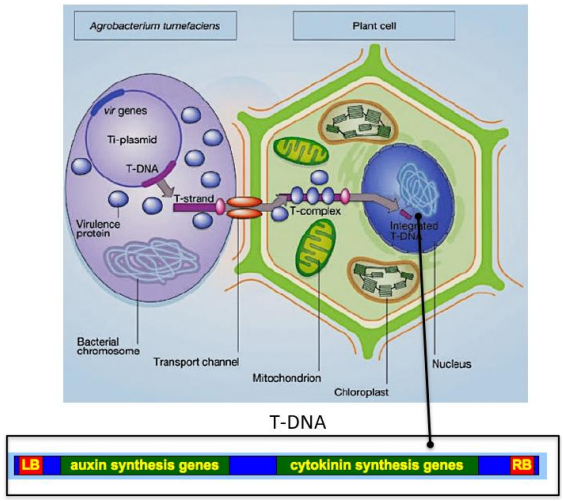
POINTS COVERED IN LECTURE

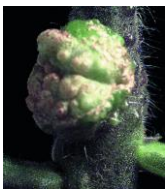
Here we are transporting ourselves in the early days of RNAi and its historical perspective. These are essentials to understand the paths that led to the various discoveries and the identities and universality of the RNAi effector components

AGROBACTERIUM TUMEFACIENS

Agrobacterium tumefaciens is a natural tool for plant transformation

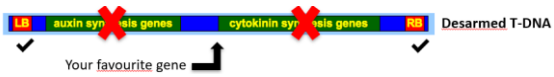
Auxin and cytokinin contribute to produce the typical tumors caused by *A. tumefaciens*





DISARMED T-DNAs: GATEWAYS TO TRANSGENESIS

In the 1980s, scientists developed methods for introducing genes into plant genomes, using the bacterium *A. tumefaciens.* Scientists engineered harmless T-DNAs devoid of any tumor-inducing genes but containing the RL and LB. These are called disarmed T-DNAs.

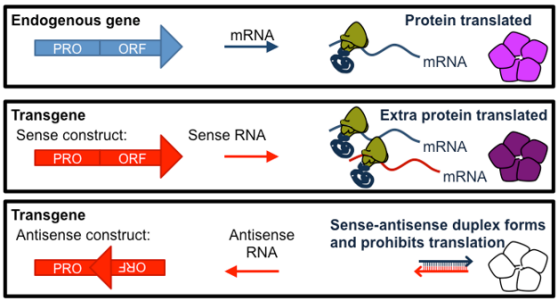


Using recombinant DNA technologies, they could then introduce their favourite piece of genetic information into disarmed T-DNA. Induced genes were then called transgenes. Experiments to modify flower colour in petunia gave early evidence of RNA silencing

MANIPULATION OF *CHALCONE SYNTHASE* EXPRESSION TO MODIFY PIGMENTATION

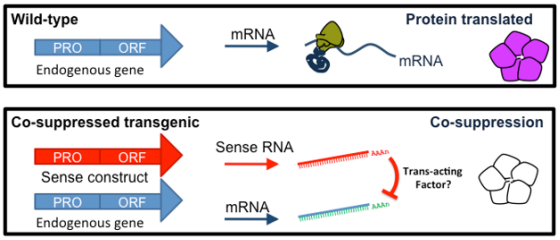
Chalcone synthase (CHS): key enzyme at the beginning of the biosynthetic pathway for anthocyanins

By introducing extra transgenic copies of *CHS* researchers thought they would achieve darker flower pigmentation and antisense production would block pigmentation



Surprisingly, both antisense and sense gene constructs could inhibit pigment production in a fraction of transformants! Both the endogenous and transgenic CHS mRNA were degraded. This phenomenon, in which both the introduced sense transgene and the endogenous gene are silenced, was coined “co-suppression”

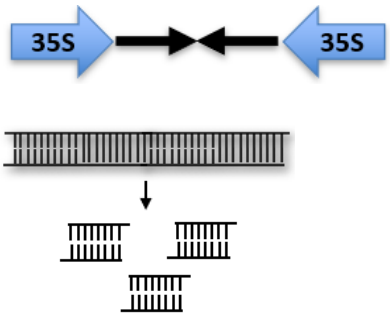
Nuclear run-on analyses confirmed that CHS silencing occurs at the post-transcriptional level. A transgenic trans-acting factor must cause degradation of the endogenous mRNA. Sense transgene RNA induces degradation of endogenous sense RNA. Therefore, this factor is likely an antisense RNA



DISCOVERY OF SILENCING small RNAs

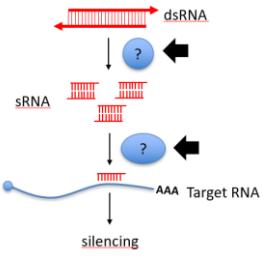
In all cases, an antisense RNA, sequence-specific for the target mRNA must be involved, but searches for a long antisense RNA were unsuccessful. In all cases 21-25nt long RNAs with sequence of the silenced mRNA accumulate specifically in silenced tissue

Sense small RNAs also accumulate in co-suppressed lines. This suggests that the small RNA precursor is a long dsRNA, but how would such a molecule be made from a sense transgene? In nearly all cases, the transgenes had been rearranged during the *A. tumefaciens* transformation process to form inverted repeats. Transcription of inverted repeats leads to long dsRNA production



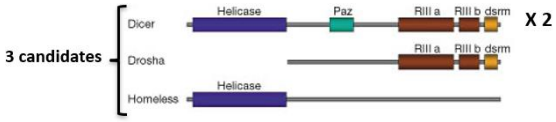
RELATED PHENOMENON IN *C. ELEGANS*

In vitro transcribed antisense RNA homologous to the *unc-22* gene was micro-injected into worm in an attempt to study its function. Silencing of *unc-22* causes loss of muscle control. The antisense approach was only modestly effective (10 – 20% effect). Andy Fire and Craig Mello looked at the phenomenon and found that the preparations of sense RNA were contaminated with a bit of antisense RNA with the potential to form dsRNA. Fire and Mello then decided to inject directly dsRNA into *C. elegans* and achieved nearly 100% *unc-22* inactivation. dsRNA targeting any ORF of any tested gene could achieve potent and nucleotide-sequence specific silencing → RNA interference (RNAi) was discovered



IDENTIFICATION OF DICER

Lysates from fly embryo or S2 cell recapitulate the small RNA processing step of RNAi. The only known enzymes capable of digesting long dsRNA are RNaseIII. Tagged recombinant proteins were then incubated with labelled dsRNA and only "Dicers" could produce sRNAs. Immunoprecipitates of Dicer-1 from fly S2 cells could reproduce the raction in strict ATP-dependant manner. Co-depletion of Dicer-1 and Dicer-2 in S2 cells resulted in an 8-fold reduction of RNAi efficacy implicating Dicer as the enzymes that makes sRNA



Dicer was found in all organisms supporting RNAi:

- Plants
- Fungi
- Invertebrates
- Vertebrates
- Mammals

Four Dicers in Arabidopsis:

- DCL1: 20 – 25nt miRNA
  - Development
  - Many biological processes
- DCL3: 24nt siRNA
  - DNA methylation
  - Heterochromatin
  - TGS
- DCL4: 21nt siRNA
  - TasiRNA
  - RNAi
- DCL2: 22nt siRNA
  - Redundant
  - Target cleavage?

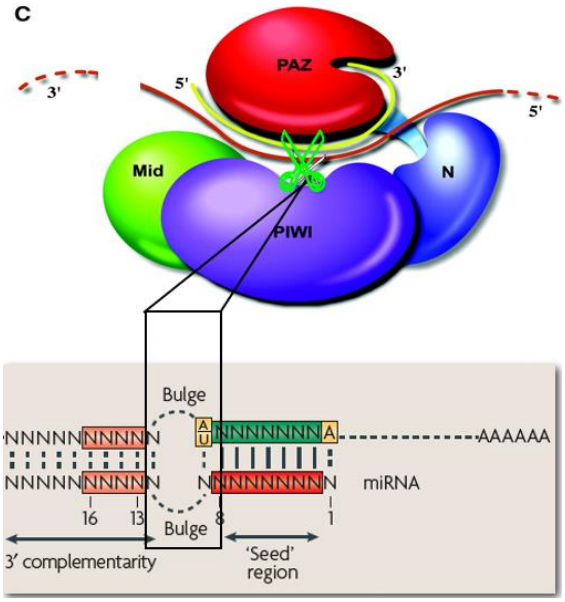
IDENTIFICATION OF THE RNA-INDUCED SILENCING COMPLEX (RISC)

Differential centrifugation isolates the S10 fraction as the only one capable of silencing the target lacZ mRNA, yet both S10 and S100 fractions display Dicer activity, implying that the RISC is distinct from the Dicer complex. A core complex of the RISC is identified as an ARGONAUTE protein that

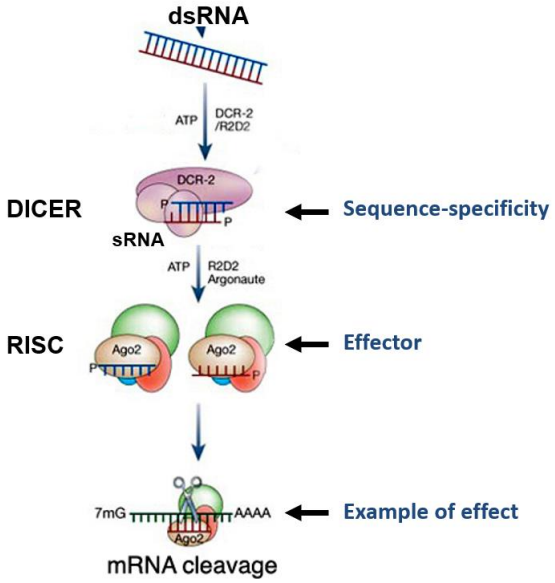
co-fractionates exactly with the silencing nuclease activity

ARGONAUTES (AGO): CORE COMPONENTS OF RISCS

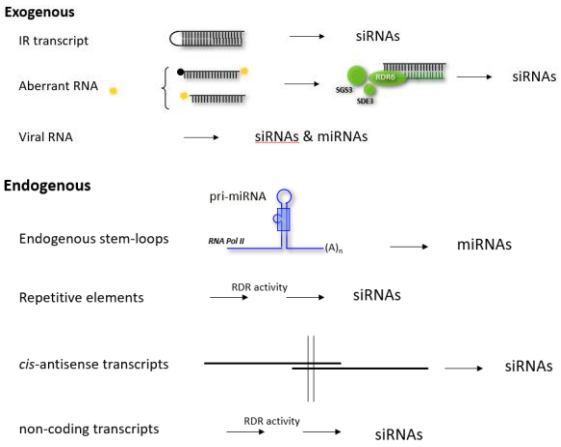
AGO bind sRNAs at their 3' end via the PAZ domain. The 5' end of the small RNA, called "seed" allows the micro nucleation to the target RNA. AGO are endonucleases that use catalytic residues (DDH) to cleave target small RNA hybrids exactly at the 10-11nt. This reaction is called "slicing". Sliced RNA can't be ligated again, slicing is irreversible



BASIC MECHANISM OF RNA SILENCING



FOREIGN AND CELLULAR SOURCES OF dsRNA



Endogenous dsRNA is tolerated, exogenous dsRNA is not

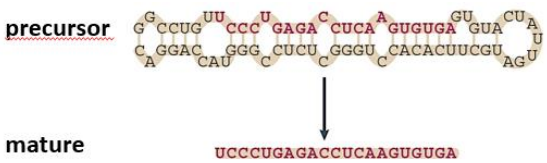
microRNAs: DISCOVERY, PRINCIPLE OF BIOGENESIS AND ACTION

POINTS COVERED IN THE LECTURE

Here we look at the historical perspective of miRNA discovery first in *C.elegans* and later in many eukaryotic organisms including plants. We learn how miRNA are synthesised, how they operate and how the pathway self-regulates in higher plants

DISCOVERY OF microRNAs

A forward genetics screen had been initiated in *C. elegans* to identify heterochronic mutants displaying developmental timing defects. An allelic series that prevents the L1-to-L2 transition was found to affect a single non-protein coding gene, lin-4. In 1993, lin-4 is isolated and found to encode a non-protein coding RNA. lin-4 products accumulate under two main forms: a rare, larger precursor that displays a fold-back structure and an abundant mature form of 21nt



The lin-14 mRNA levels are normal in lin-4 mutants, but the lin-14 protein accumulates extopically. lin-14 displays many putative lin-4 binding sites. Fusing the lin-14 3'UTR to a reporter gene is sufficient to induce its lin-4-dependant translational repression → lin-4/lin-14 interactions

are important to regulate lin-14 protein production. In 2001, a second heterochronic *C. elegans* mutant was identified with mutations in the let-7 gene, which also encodes a non-protein coding stem loop RNA precursor. Let-7 accumulates mostly as a 21nt small RNA imperfectly complementary to two target sites in the 3'UTR of the lin-41 mRNA, which is also regulated at the protein production level. A point mutation in let-7 comprises its binding to the lin-41 mRNA, supporting a direct sense/antisense effect. Lin-4 and let-7 accumulation is inversely correlated to that of their targets allowing exquisite control on the timing of their expression and allowing adequate larval-to-adult phase transition. Strikingly, let-7 and lin-4 are both 21nt in length which is exactly within the size range of Dicer products in RNA silencing model organism. RNAi of Dicer in *C. elegans* leads to heterochronic defects and a decrease in let-7 and lin-4 accumulation at their cognate larval stages. Both small RNA precursors are cut precisely by Dicer in vitro. Dozens of small RNA-encoding loci were discovered and their mature products called "microRNAs". Many were conserved in the relates species *C. briggsae* and even in flies and human, which was confirmed by parallel deep sequencing analyses in those organisms

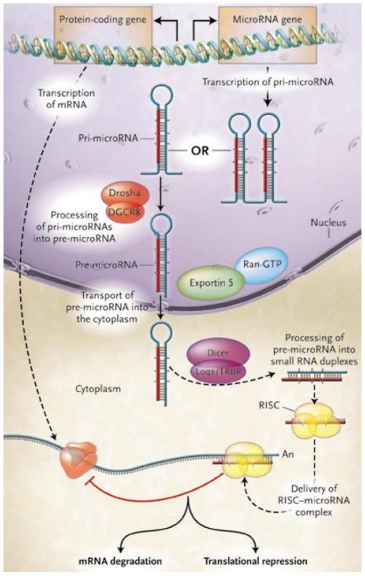
ROLE OF miRNAs

About 800 miRNA genes in human:

- Regulate virtually all biological processes
- Some are key to acquisition and/or maintenance of cell identity
- Many miRNA recognized as oncogenes or tumor suppressor genes
- miRNA dysfunction at the core of many genetic disorders

Major roles for microRNA regulation:

- Networking and fine-tuning of gene expression
- Facilitaiton of rapid switching to new developmental programs
- Guardians of established cellular fates

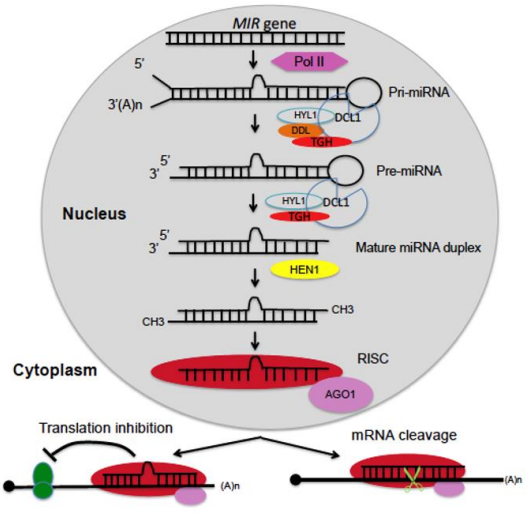


Very tight control of gene expression is suggested. miRNA appear cell-autonomous and are expressed in fully developed tissues. Both in plants and in animals, many miRNAs guard established differentiated states to the point that target and miRNA expression patterns are often mutually exclusive

PLANT *MIRNA* GENES

Many *MIRNA* loci were found in plants. Their lengths and shapes are more variable than those of their metazoan counterparts. Many are conserved among plant species, but others are species-specific

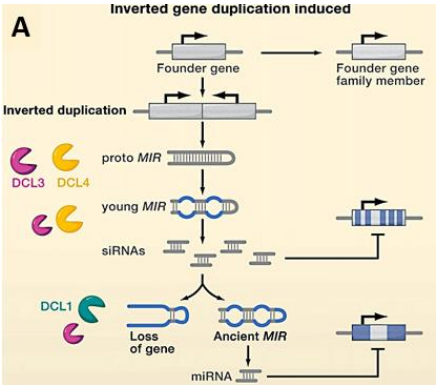
In plants there is no Drosha. In mutants the miRNA is not unabundant, it is just not stable



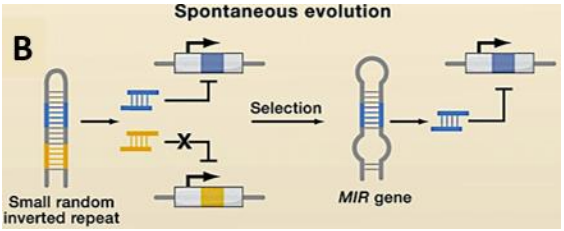
(for the mRNA bulk remaining post-slicing)

POSSIBLE ORIGINS

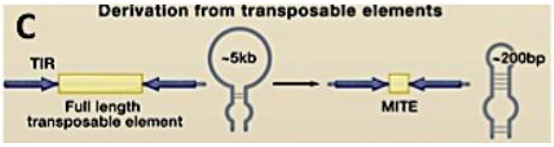
In model A, the *MIRNA* gene originates from its "to be" target gene. Gene duplication is then followed by drift mutations accumulating in a young miRNA precursor but keeping the sequence of the mature miRNA intact



In model B, acquisition of promoter sequences allows random small inverted repeats to be transcribed into protomiRNAs

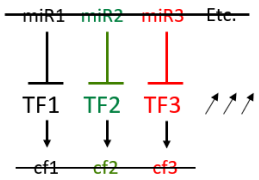


In model C, the young miRNA precursor is formed naturally during evolution of miniature inverted-repeat transposable elements (MITES)



miRNA EXPRESSION

Removing miRNA would increase the transcription factor (TF) production



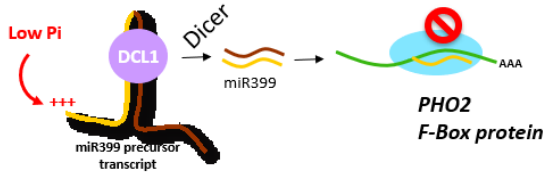
miRNA overexpression leads to their accumulation in tissues where they should not be expressed. Therefore, their mRNA targets become silenced in tissues where they should not be expressed

miRNAs do not move, they stay at the site of transcription

Unlike siRNAs, miRNAs are produced as discrete small RNA species. Sometimes they individually accumulate to very high levels (50'000/cell)

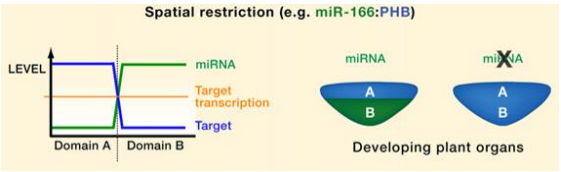
STRESS ADAPTION

PHO2 is a negative regulator of plant phosphate assimilation. Adaption is therefore achieved by repressing a repressor

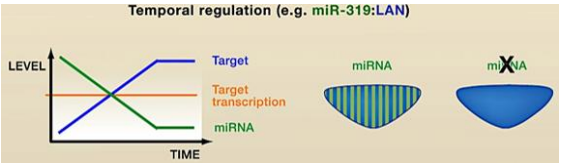


REGULATION BY PLANT miRNAs

In the spatial restriction mode, the miRNA target accumulates in domain A where the miRNA is not present and the miRNA accumulates in domain B, from which the target is depleted. This mode of regulation is typically observed with HD-ZIP TFs regulated by miR-165/166 paralogs in Arabidopsis or Maize

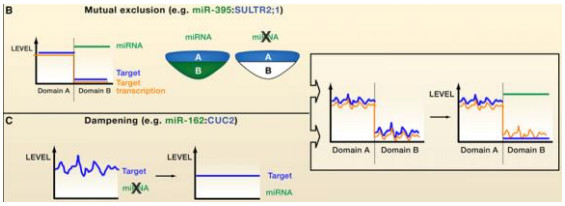


In temporal regulation, a gradient of miRNA expression generates an opposing gradient of its target over time. The LANCEOLATE (LAN)-miR-319 interaction in the tomato meristem is an example of this type of regulation



In mutual exclusion, the miRNA and its target have spatially separated expression domains. The miRNA acts as a backup to ensure the strict transcriptional confinement of the target within its cognate expression domain. Dampening or "thresholding" entails the reduction and stabilization of target gene expression levels in coincidence with miRNA expression. Dampening

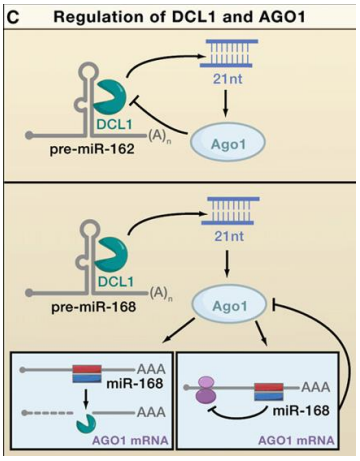
and mutual exclusion could possibly also cooperate to further refine the output of miRNA target interactions



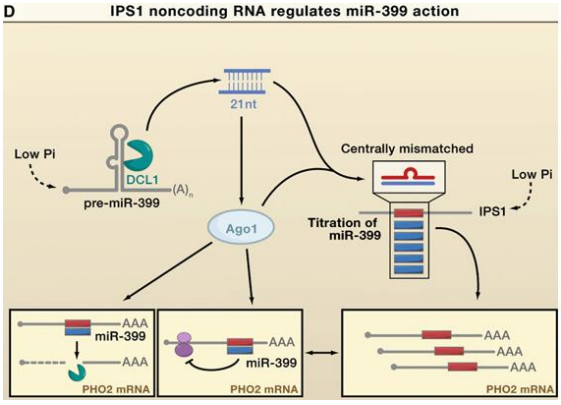
REGULATION OF miRNA TRANSCRIPTION, PROCESSING AND ACTIVITY

miR-166 paralogs in the maize shoot apical meristem are exquisitely spatially partitioned. This suggests that each miR-166 paralog is controlled by its own specific promoter bound by a distinct cell layer-specific transcription factor

Both DCL1 and AGO1 are regulated each by specific miRNA. miR-162 regulates the levels of DCL1, the enzyme that processes nearly all plant miRNAs, in AGO1-dependant manner. miR-168 regulates the levels of AGO1, the factor that operates nearly all plant miRNA action. miR-168 control of AGO1 levels is achieved in AGO1-dependant manner at the mRNA stability and protein production levels. These feedback regulations allow DCL1 and AGO1 levels to be maintained at the right levels. The miRNA machinery is thus controlled via homeostasis



The IPS1 noncoding RNA provides an efficient means of modulating the activity of miR-399 and of its target, PHO2, in a sequence-specific manner. Because both miR-399 and PHO2 are induced by a shortage in inorganic phosphate, this type of regulation could be seen as an example of miRNA (as opposed to target) dampening



(SMALL)RNA-DIRECTED DNA METHYLATION

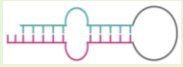
POINTS COVERED IN LECTURE

Here we look at the Arabidopsis DCL3-dependent pathway and how it allows to tame transposon expression to the point of debilitation and how the host genome might eventually exploit the ensuing transposon remnants for the sake of regulating neighbouring gene expression

siRNAs

Most endogenous siRNAs are made by DCL3. siRNAs are produced as populations

siRNA VS. miRNA

siRNA	miRNA
Small/short interfering RNA	microRNA
Not conserved throughout species	Highly conserved
21 -23nt RNA duplex, dinucleotide 3' overhang 	19 – 25nt RNA hairpin 
Exogenous dsRNA, taken up by cell, also encoded by heterochromatin regions and transposons	Endogenous ssRNA, non-coding
Needs exact complementary mRNA sequence	Can bind imperfectly to mRNA or to 3'UTR
AGO2 needed	Any AGO protein

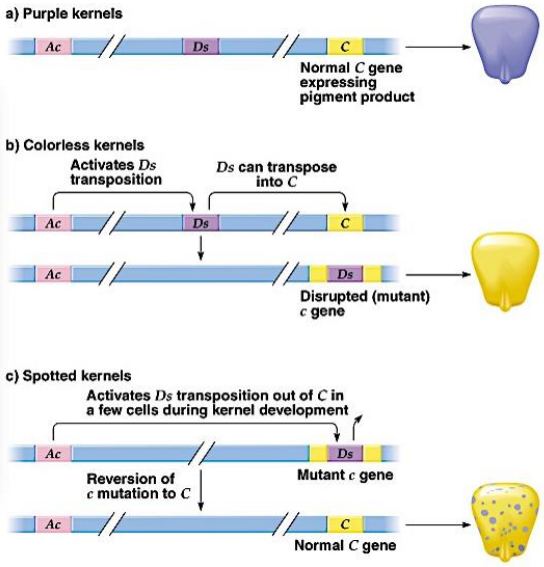
Regulates different genes	Regulates similar genes as they originate from
Epigenetic modifications	
Viral defence, genome stability	Endogenous gene expression regulator
Not in mammals	

https://geneticeducation.co.in/sirna-vs-mirna-10-major-differences/

TRANSPOSONS

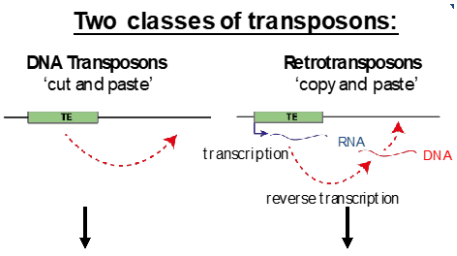
Transposon: Fragments of DNA that can insert into new chromosomal locations. Some copy themselves and increase in number within the genome. Responsible for large scale chromosome rearrangements and single-gene mutagenic events

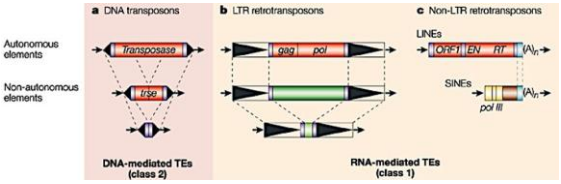
Ac element required for transposition of Ds element



MAJOR CLASSES OF TRANSPOSABLE ELEMENTS

The main threat to genome integrity, by far, comes from retrotransposons. Retrotransposons are similar to retroviruses, but they are not infectious





Transposons make up a major content of eukaryotic genomes:

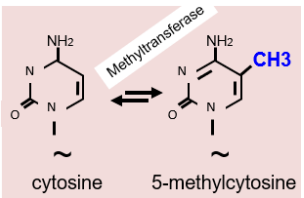
- 50% of genome of human, chimp, mouse, ape
- 75% of genome of maize
- 85% of genome of barley
- 98% of genome of iris

HOW DO ORGANISMS LIVE WITH TRANSPOSABLE ELEMENTS (TEs)?

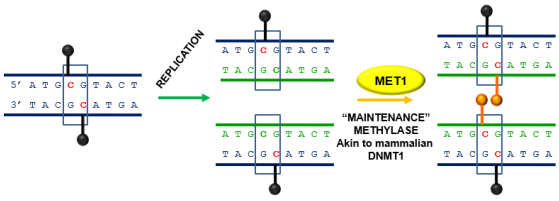
- Most TEs are broken (cannot transpose → "fossils", "remnants"
- Active TEs evolved to insert into "safe-heavens" including themselves (tendency to insert into themselves)
- Host regulates TE transcription and movement by epigenetics means
- TEs can provide advantages to gene expression control
- TEs are usually inactive but stress conditions may activate TEs
- Active TEs increase mutation frequency
- Most mutations caused by TEs are neutral and sometimes harmful
- Rare TE-induced mutations (or rearrangements) may be adaptive
- The major threads posed by TEs are intra- and inter-chromosomic rearrangements, caused by their highly repetitive nature
- DNA and histone methylation cause heterochromatin formation at TE loci, which reduces or prevents recombination and rearrangements

DNA METHYLATION

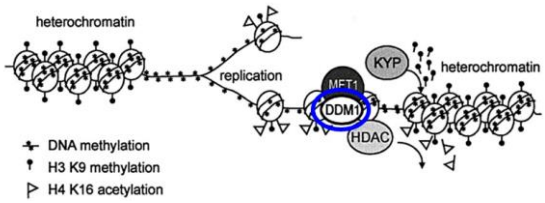
DNA can be covalently modified by cytosine methylation



Cytosines can be methylated in all sequence context in plants: CG, CHG, CHH. Only CG and CHG methylation can be propagated during DNA replication

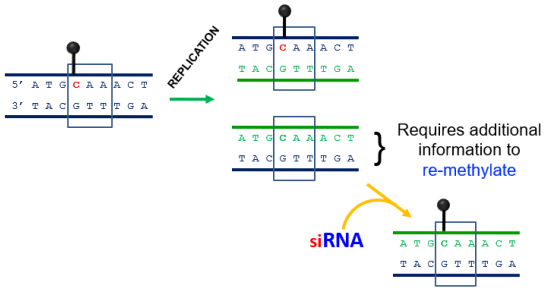


DNA methylation is necessary to silence transposons, it is necessary to maintain genomic integrity. DDM1 is a chromatin remodelling protein



ASYMMETRIC METHYLATION

Asymmetric methylation sites are maintained (and initiated) by information on associated histones and an RNA-based mechanism, RNA-directed DNA methylation (RdDM), that directs de novo DNA methylases to these sites. RdDM is also required to explain how transposons and repeats become methylated for the first time once detected as foreign in the genome

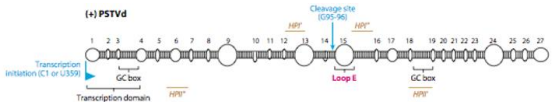


INITIATING AND MAINTAINING SILENCING

Maintaining transposon silencing is an active, dynamic process that requires ongoing siRNA production and epigenetic "vigilance" via RdDM

VIROIDS

Viroids: plant pathogens that consist of a short stretch (a few hundred nucleobases) of highly complementary, circular, ssRNA e.g. potato spindle tuber viroid (PSTVd)



Viroids cause devastating diseases and are economically important. The viroid RNA does not code for any protein. Thus, viroids rely on their host entirely for their replication as well as for their cell-to-cell and systemic movement

DISCOVERY OF RdDM

In an attempt to contain PSTVd infection, the concept of pathogen-derived resistance (PDR) was evaluated in an experimental host. Two infectious PSTVd genomes organised in tandem were transformed into tobacco and transgenic lines were established. Most transformants were expected to produce replicating viroid at low levels but several did not. In those transformants, the transgene region corresponding exactly to the inserted viroid genome was found resistant to methylation-sensitive restriction enzymes: the transgene had thus become methylated de novo. New lines were engineered with the same, non-replicating copies of PSTVd, but none of the transformants exhibited DNA methylation in that case. However, when these lines were infected with exogenous viroid, the transgene became methylated. Since viroids are non-coding, pure RNA entities, de novo methylation was therefore a new RNA-mediated process. It was coined RNA-directed DNA methylation (RdDM)

Not the viroid itself mediates methylation, it is the product of the viroid that mediates methylation

Key features:

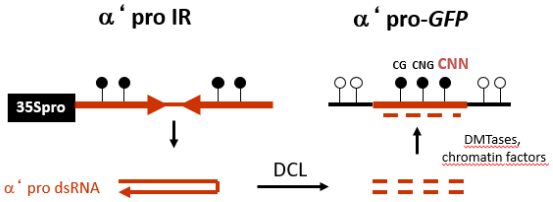
- RdDM could be detected in tissues that were fully developed at the time of PSTVd infection, indicating that DNA replication is not necessary for this process
- RdDM was extremely potent and could methylate cytosines in all sequence contexts (CG, CHG, CHH
- RdDM was strictly confined to the regions of homology between the transgene and the inoculated viroid
- Using new transgenic lines, it was found that transgene sequence as small as 30bp could undergo efficient methylation,

implying that the elusive viroid-derived guide RNA could be as small or even smaller than 30nt

MAKING SENSE OF RdDM

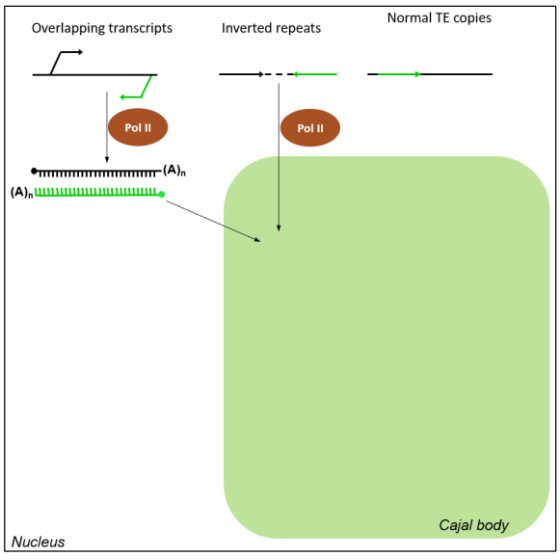
The near double-stranded RNA structure of the viroid genome makes it an ideal template for Dicer activity, making it possible that silencing systems were found to silence transgenes exhibiting promoter sequence homology: e.g. 6b5. The 6b5 locus had low GUS mRNA levels owing to low transcription and was found to produce 35S siRNA owing to a 35S promoter inverted duplication the accumulation of which correlated with RdDM of targeted 35S promoters

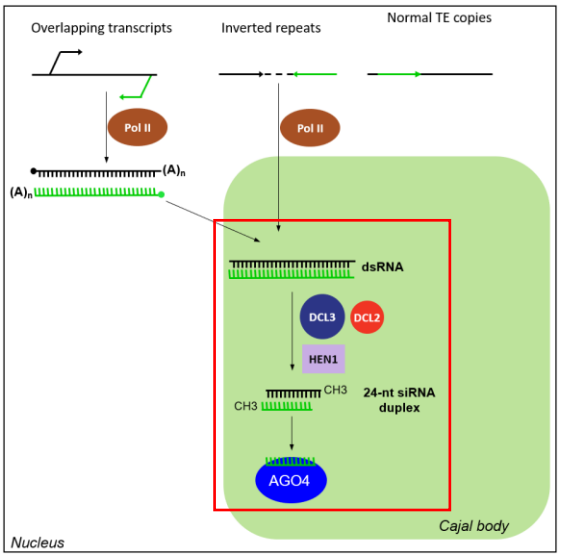
RdDM IN ARABIDOPSIS

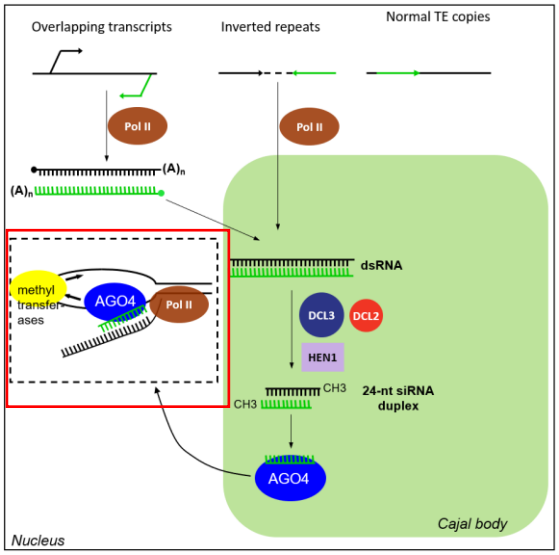


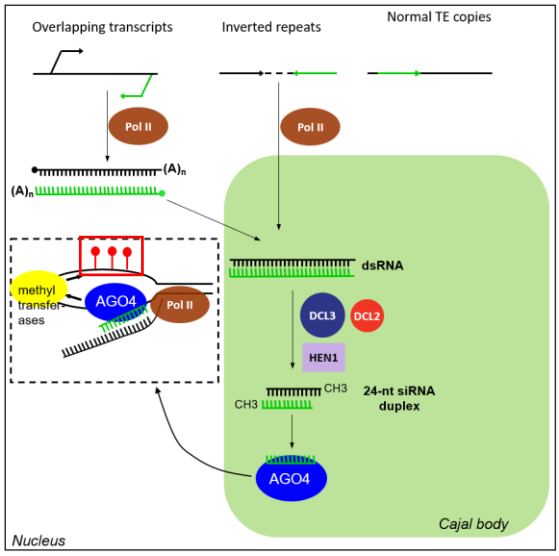
THE "1 HOUR SLIDE"

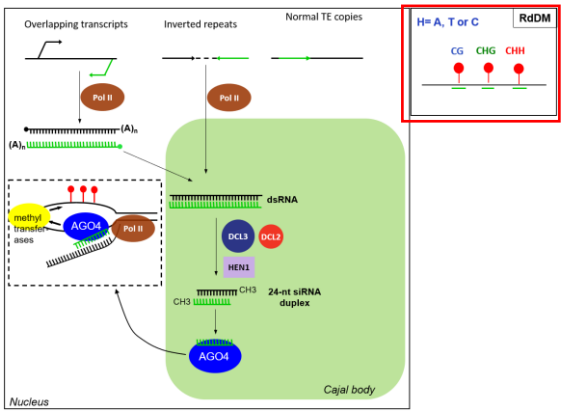
- The aberrant TE loci that genetically initiates epigenetic silencing are no longer required to maintain the process
- The original aberrant loci may be segregated away via out-crossing leaving no tractable origins of the whole process
- The PolIV/PolV/RDR2 process active on all copies now maintains the system dynamically

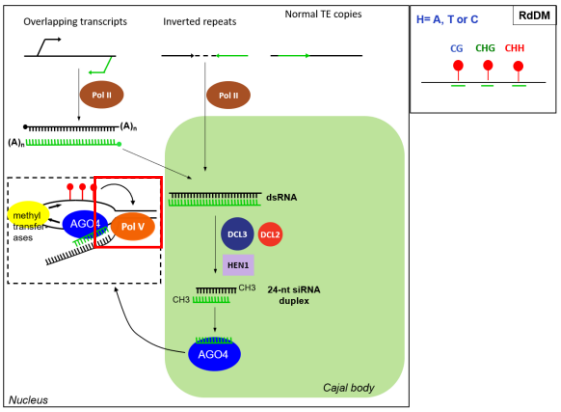




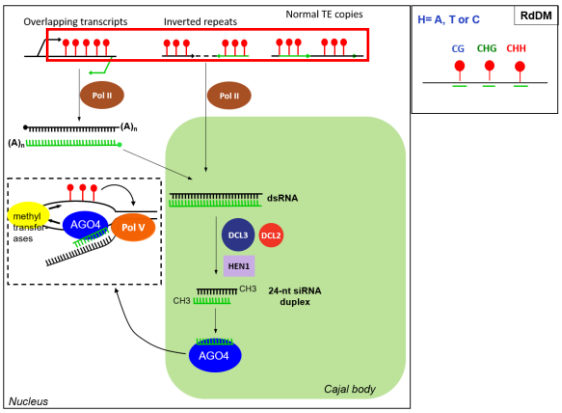


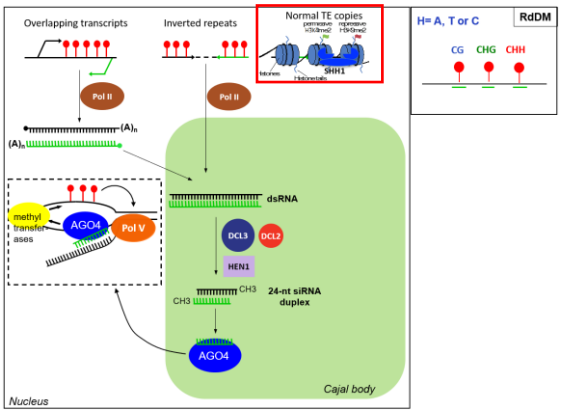




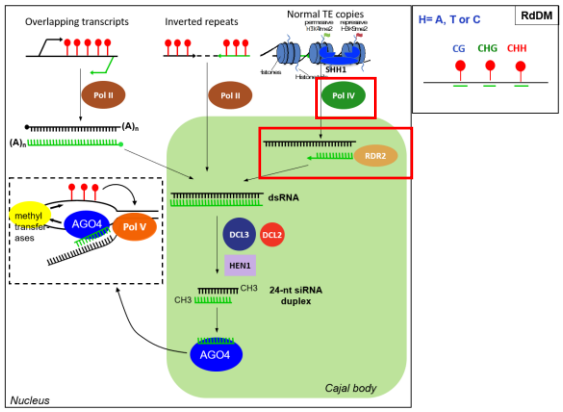


All transcripts become de novo methylated



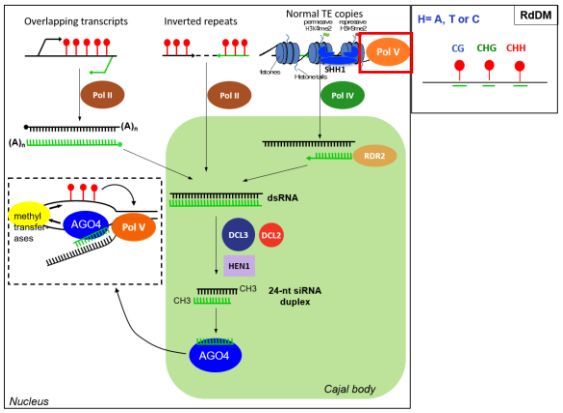


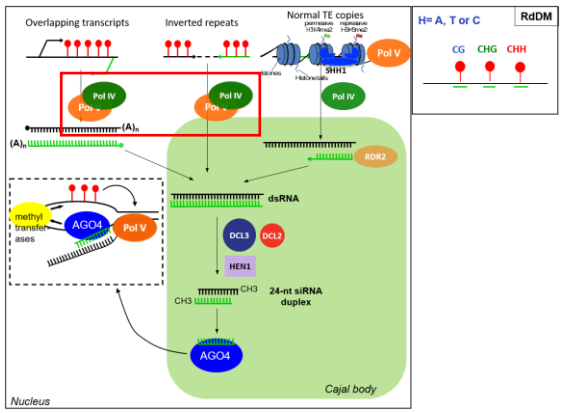
Methylated transcripts are sensitive to histone mehtylation



Pol IV is recruited by SHH1

30-40nt long in length, not for scaffolding. Is a template for RDR2. Is only needed as an initial trigger





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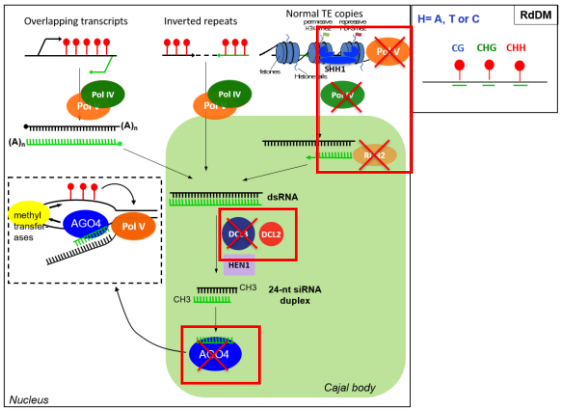
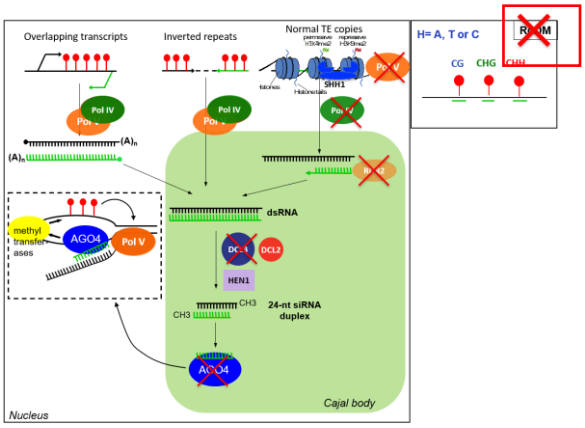
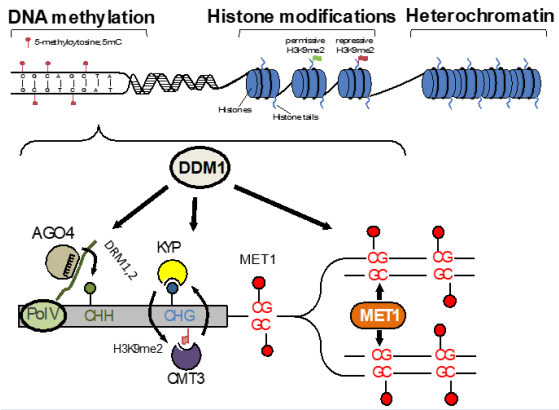


DLC2 takes over when DLC3 isn't there anymore



INITIATING AND MAINTAINING SILENCING AT REPETITIVE DNA

Epigenetic silencing has a genetically tractable origin and is dynamic. Paradoxically, epigenetic transcriptional silencing requires active transcription

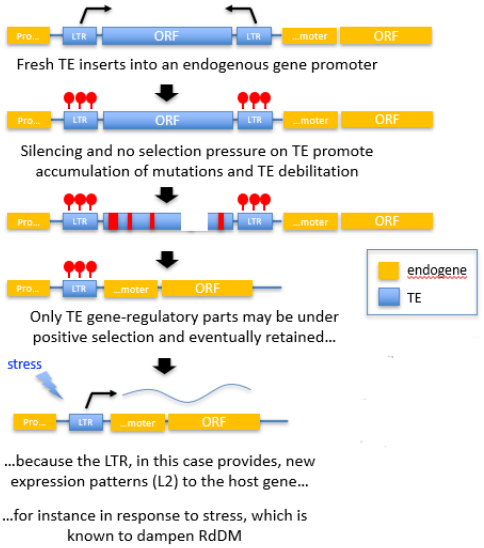


The loci that are not transcribed accumulate mutations because there is no selection → this is the state of most transposons in most organisms

GENETIC AND EPIGENETIC CONSEQUENCES ON ENDOGENOUS GENE EXPRESSION

An initial genome invader and its many copies have been tamed by the host via epigenetic silencing. The now silent threat is undergoing selection to retain only useful remnants of the invader and to use dynamic RdDM imposed on such remnants to modulate endogene expression

The more methylated a locus is, the lower the polymerase affinity



LEVELS OF TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

- Euchromatin is ready to transcribe, heterochromatin isn't
- Transcription can be turned on or off
- Transcription can be reduced with epigenetic control
- Epigenetics is no magic! It is a very fine-tuned process

ANTIVIRAL RNAi IN PLANTS

POINTS COVERED IN LECTURE

Here we discuss one of the most ancient functions of RNA silencing i.e. to provide sequence-specific immunity to exogenous viruses. We look at it from a historical and contemporary perspective

THE CONCEPT OF PATHOGEN-DERIVED RESISTANCE (PDR)

In 1985, Sanford and Johnston published a paper entitled "The concept of pathogen derived resistance: deriving resistance genes from the parasite's own genome". Pathogens produce

molecules that are critical and unique for their own specific pathogeny. Thus, if pathogen-specific molecules are expressed in a modified (dysfunctional) form by a host cell genome, these dysfunctional pathogen-derived gene products could act to inhibit the pathogen. In this way, a pathogen-specific resistance gene could theoretically be generated from just a portion of a pathogen's own genetic material. Recombinant bacteria expressing an altered form of the Qβ phage replicase were resistant to the phage. In fact, the altered replicase was competing with the native form for replication sites of the pathogen's genome

As the replicase of viruses are the essential components of their biology, strategies aimed at interfering directly with this function were investigated in plants. Curiously, the highest levels of resistance were achieved with the lowest expressing plant, questioning the assumption that the effect was protein-mediated

The experiment was repeated with untranslatable form of the replicase. Several resistant plants were isolated. The resistance was extreme, as no symptoms were observed in plants and accumulation of PVX was drastically reduced. Resistance was also highly virus strain-specific: only effective against PVX strains sharing high nucleotide sequence homology with the strain from which the engineered replicase was derived

Surprisingly, the replicase mRNA was undetectable in the resistant lines. Yet the replicase mRNA was efficiently transcribed. Resistance was achieved by RNAi → plant viruses can be targeted by transgenic RNA silencing

VIRAL NATURE OF THE SILENCED TRANSGENE IS NOT A PREREQUISITE FOR RESISTANCE TO OCCUR

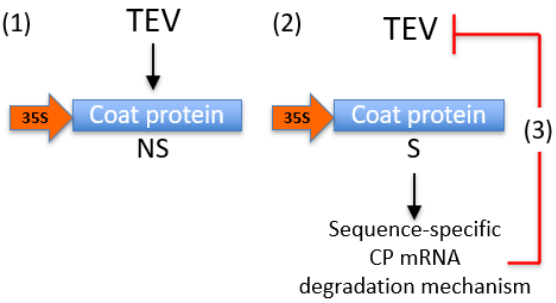
PVX can be made recombinant to produce proteins of interest. Non-silenced GUS transgenic plants are sensitive to PVX-GUS. Silenced GUS transgenic plants are immune to PVX-GUS. Silenced and non-silenced GUS transgenic plants are sensitive to PVX-GFP → a non-viral transgene can silence a recombinant virus

VIRUS-INDUCED RECOVERY IN TRANSGENIC PLANTS

An attempt of PDR was made against *Tobacco etch virus* using the coat protein. Transgenic plants initially displayed severe symptoms, but later developed new leaves that were symptom free and contained no detectable virus

The recovery is caused by virus-induced silencing of the coat protein transgene. The CP transgene mRNA was found specifically silenced in recovered tissue, yet the coat protein mRNA was efficiently transcribed

The recovered state coincides with post-transcriptional gene silencing induced by TEV that is then turned back onto the virus to degrade it in a sequence-specific manner. Therefore, non-recombinant plant viruses can trigger post-transcriptional gene silencing of homologous transgenes



RECOMBINANT VIRUSES SILENCE ENDOGENOUS GENES

PVX can be used as a vector to express genes of interest. The phytoene desaturase (PDS) protects plants against photobleaching. The tobacco PDS ORF was inserted into PVX to increase PDS content in leaves: PVX-PDS. Infected plants developed the opposite phenotype and were photobleached (by gene silencing). Similar results were obtained with nonsense, antisense and even portions of PDS. The effect was sequence-specific as PVX-GFP infection did not cause photobleaching. Recombinant plant viruses trigger silencing of homologous endogenes: VIGS

VIRUS-INDUCED GENES SILENCING (VIGS) AS A TOOL FOR FUNCTIONAL GENOMICS

Many mutations are homozygous lethal and many plants cannot be transformed. Hence, the need to

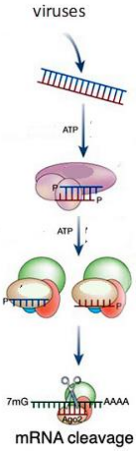
develop inducible gene silencing tools that bypass transgenesis. VIGS can be used as a powerful functional genomics tool

NON-RECOMBINANT VIRUSES INDUCE A NATURAL FORM OF RECOVERY

Use of recombinant PVX demonstrates the nucleotide sequence-specificity of the phenomenon. Some viruses without sequence homology to the host genome trigger an RNA silencing-based mechanism leading to recovery

THE FINAL PROOF

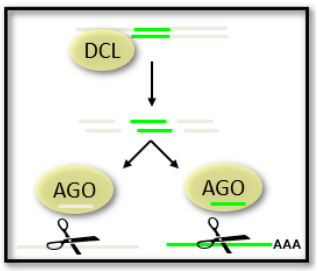
The landmark feature of all RNA silencing systems is the accumulation of siRNAs. It is a true innate immune system mechanism. The response is not programmed by the host genome but rather by structural and sequence features of the pathogen's genome



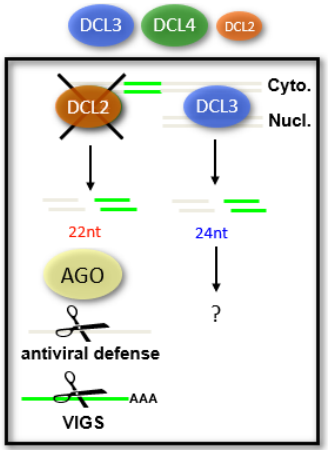
DECIPHERING THE ANTIVIRAL SILENCING GENETICALLY

DCL4-dependant 21nt siRNA are sufficient to mediate VIGS. DCL3-dependant 24nt siRNA seem dispensable for VIGS. Novel 22nt siRNAs that accumulate *dcl4* seem sufficient for VIGS

Dicing alone isn't enough for silencing → AGO is necessary



24nt siRNAs are ineffective. The 22nt siRNAs are DCL2-dependant and incorporate into a RISC to mediate VIGS.DCL2 functionally substitutes DCL4 and both DCL2 and DCL4 mediate antiviral defence against TRV and many other viruses. There is no role for DCL1



AGO1 IS AT LEAST ONE ANTIVIRAL AGO

There are 10 distinct AGO's in Arabidopsis. AGO1 mutants are hypersusceptible to several plant viruses. AGO1 is one antiviral effector and loads 21nt viral siRNAs. AGO1 is also the major miRNA effector. miRNA and antiviral pathways converge at AGO1 → AGO1 deals with siRNAs (viral) and miRNAs (own)

SUPPRESSION OF ANTIVIRAL SILENCING

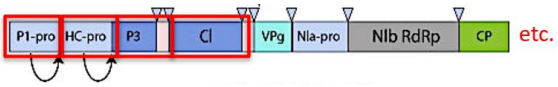
POINTS COVERED IN LECTURE

In this final course, we look at ways by which plant viruses circumvent or directly oppose the effects of antiviral RNAi from their hosts. This is achieved by dedicated viral-encoded suppressors of RNAi, or VSRs and provide yet another illustration of the never-ending molecular arms race opposing pathogens to their hosts

PLANT VIRAL SYNERGISM

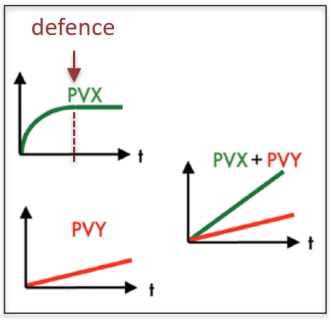
Synergism: an infection by two viruses causes symptoms that are more severe than those caused by either infection alone. Synergism is unidirectional and benefits only one of the two viruses. In this example it is PVX

A factor derived from PVY helps PVX to overcome a host defence mechanism that normally restricts PVX



HcPro is necessary and sufficient to recapitulate synergistic symptoms. No effect of untranslatable insert → protein-mediated effect

- HcPro suppresses the host defence that limits PVX
- PVX accumulation is restricted by RNA silencing

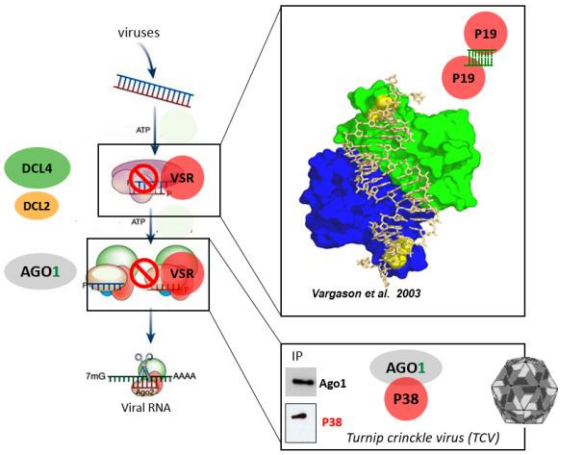


HcPro

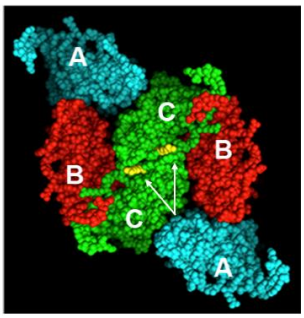
HcPro is a viral suppressor of RNA silencing (VSR)

Silencing suppression s a property of virtually all families of plant viruses and o some invertebrate viruses. E.g. “mottling” in soybean seeds results for persistent infections by poty virus and cucumoviruses of cultivars naturally displaying RNAi of *CHS* due to an inverted duplication of the gene

VSRs COMPLICATE THE GENETIC DISSECTION OF ANTI-VIRAL INFECTIONS

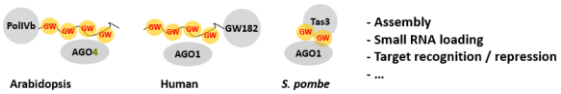


P38 co-IP with AGO1 in up to 800 mM NaCl but not AGO4 nor with AGO7. Nearly all of P38 cofractionates with AGO1 in gel filtration → P38 binds AGO1 and therefore prevents loading of AGO1. Multiple P38 units seem to interact with AGO1. P38 interacts with AGO1 at least as C:C homodimers



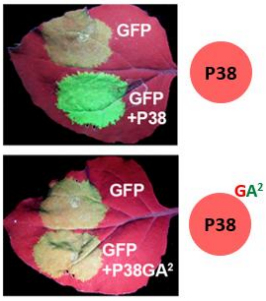
CELLULAR GW-REPEAT PROTEINS

Cellular GW-repeat proteins are key components of RISCs in various organisms

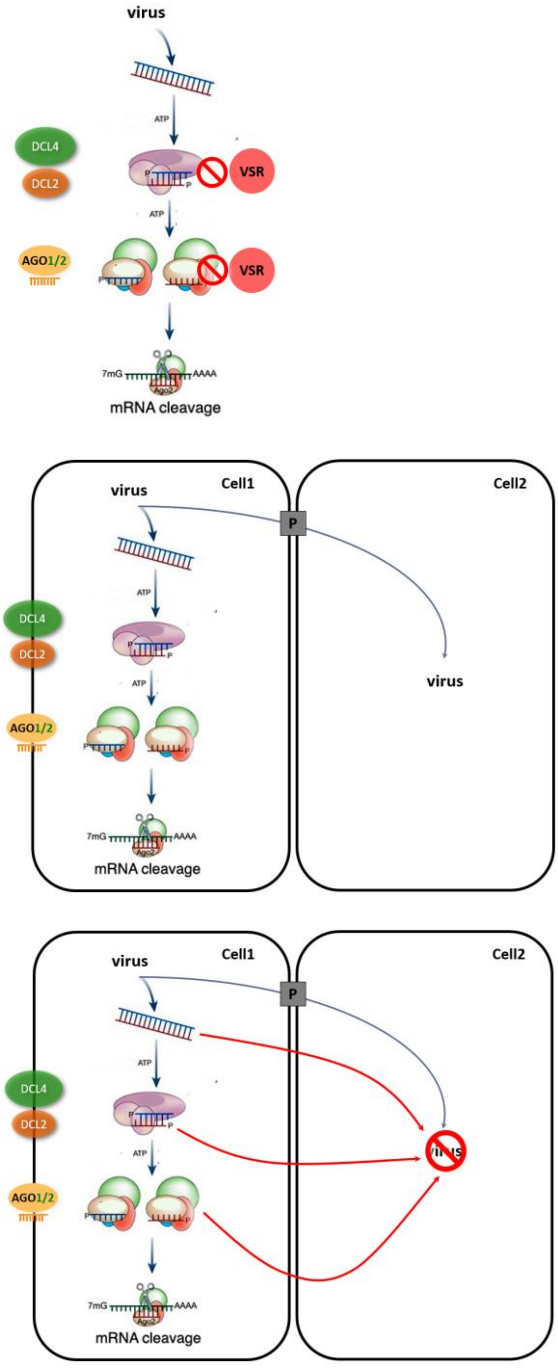


P38 is a pathogen-encoded GW-repeat protein that maybe anchors AGO → P38 bind AGO1 and therefore prevents loading of AGO1

Single or dual GA mutations abolish P38 VSR function. TCV GA^2 (two amino acid mutations) virulence is restored in *ago1-27* physical and genetic interactions. The GA^2 allele is unlikely to affect C:C dimer formation

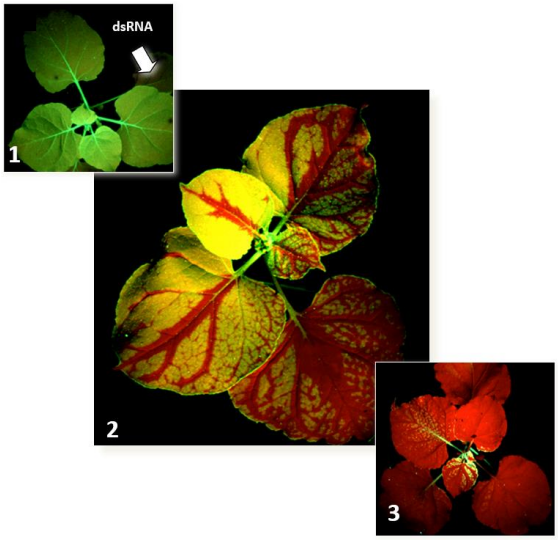


VIRUS PROBABLY EVADE SILENCING



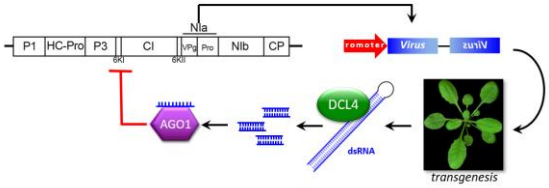
SYSTEMIC TRANSGENE RNAi

The systemic arm of RNAi



Sequence-specific signals, phloem movement, cell-cell movement, amplified nucleic acids (RNA)

NUCLEOTIDE SEQUENCE-SPECIFIC IMMUNITY



If the plant is equipped with viral dsRNA, the viruses couldn't infect the plants anymore, but if those plants get infected with other viruses, the virus silences the plant silencing machine and the plant dies

- Widespread use throughout the world
- Proved useful many times, but confronted to difficult public acceptance of GMOs

PART SANCHEZ-RODRIGUEZ

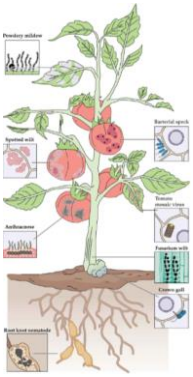
PATHOGEN ATTACK

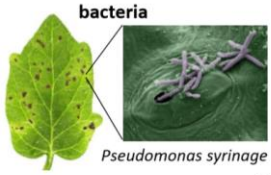
PLANT MICROBES

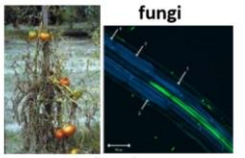
Pathogen: Any organism that completes part of its life cycle inside the plant, often with detrimental effects

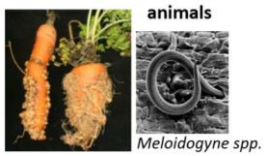
Plant pathogens and pests cause 25 – 35% yield losses annually

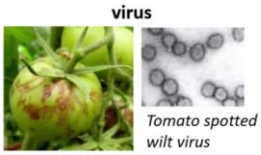
Examples:







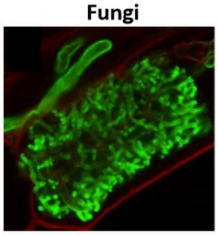




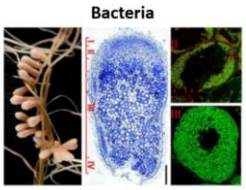
Symbionts:

Not all organisms which interact with plants are pathogens

Examples:



An arbuscule of *R. irregularis* (green) in a cortex cell of *P. hybrida* (red)



Structure of nitrogen-fixing root nodules formed in *S. meliloti – M. truncatula* symbiosis

COLONIZATION STRATEGY

	Biotrophic	Hemibiotrophic	Necrotrophic
Attack strategy	Intimate intracellular contact with plant cells	Initial biotrophic phase, then necrotrophic phase	Secreted cell wall-degrading enzymes, host toxins, or both
Specific features of interaction	Plant cells remain alive throughout the infection. Minimal plant cell damage.	Plant cells alive only in the initial stages of the infection. Extensive plant tissue damage at late stages.	Plant tissue killed and then colonized by the pathogen. Extensive tissue maceration.
Host range	Narrow; often only a single species of plant is attacked.	Intermediate	Broad
Examples	Fungal mildews and rusts; viruses and endoparasitic nematodes; *Pseudomonas spp. bacteria*	*Phytophthora infestans* *(causal agent of potato late blight disease)*	Rotting bacteria (e.g., *Erwinia* spp.); rotting fungi (e.g., ***Botrytis* cinerea)**

MICROBE PENETRATION

1. Reach the host and attach

A successful microbe must enter the host, avoid plant defence responses, reproduce and spread

Reach the host

wind (spores), water (bacteria), insects (virus), chemotaxis (soil living pathogens)

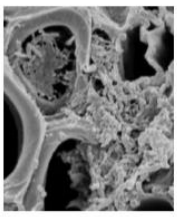
Citrus canker (*Xanthomonas axonopodis*)

Hurricanes regularly hit Florida's citrus growing regions, spreading citrus canker

Attachment

Biofils (bacteria)

Attaching proteins (fungus and oomycetes)



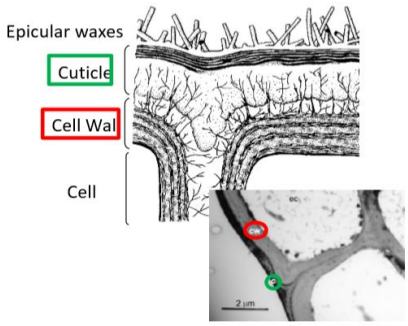
Xylella fastidiosa in a xylem vessel

Magnaporthe grisea (Hydrophobin)

2. Gain entry

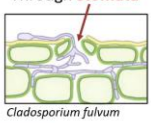
Microbes must be able to penetrate or circumvent the plant barriers: physical and chemical

Plant physical barriers



The microbes alter the plant cell wall and this activates defence responses.

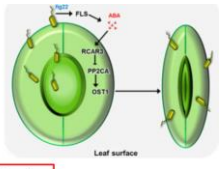
Through stomata



Cladosporium fulvum

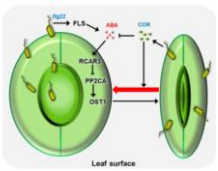
The pathogens adapt to the time of stomatal opening

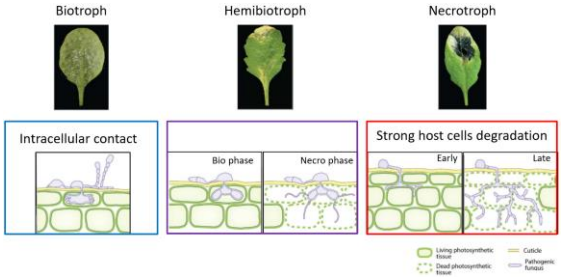
Plant defense at the pre-invasive stage : Stomata closure



The pathogen evolved...

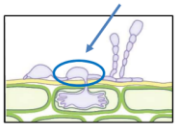
Bacteria produce coronatine (COR) a structural homolog of the plant jasmonates, which suppress ABA signaling, promoting stomata reopening

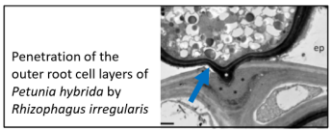




Turgor pressure+ Plant Cell wall degrading enzymes

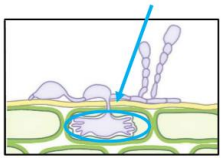
Appressorium: hyphal «pressing organ»

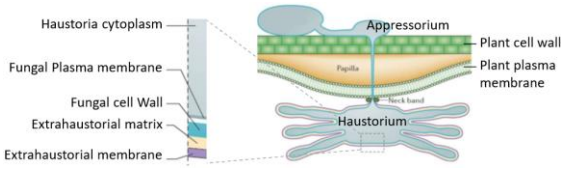




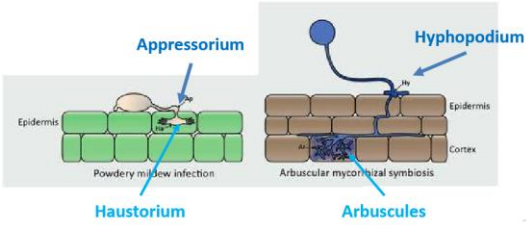
Turgor pressure+ Plant Cell wall degrading enzymes

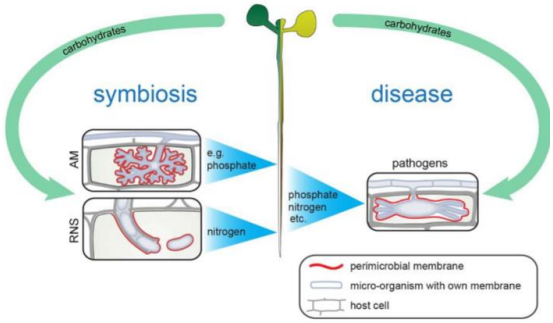
Haustorium: «interaction organ»



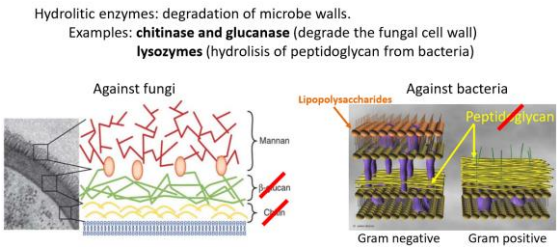


Symbiotic fungi → similarities with biotrophic fungi

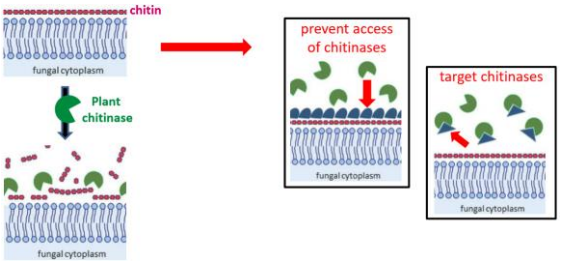




Plant preformed chemical barriers:



Chitinase:

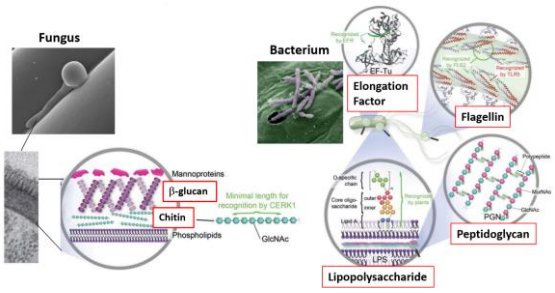


MICROBE RECOGNITION

MICROBE ASSOCIATED MOLECULAR PATTERNS (MAMPS)

Conserved molecules in microbes that can be recognized by the host and induce an immune response

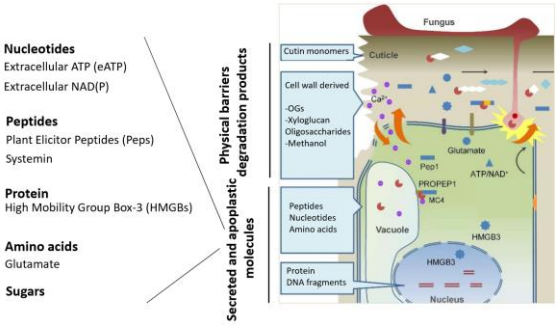
Initially found in pathogens, so called Pathogen Associated Molecular Patterns (PAMPs), they are also present in non-pathogen microbes



DAMAGE ASSOCIATED MOLECULAR PATTERNS (DAMPS)

Own signals when being attacked by microbes and herbivores, or after physical damage

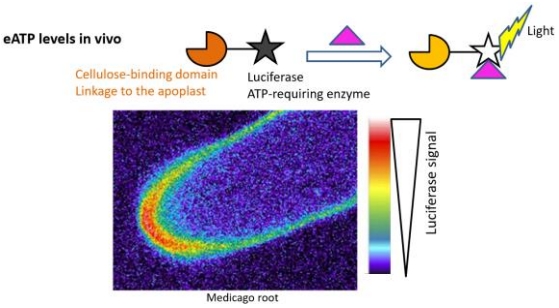
Some DAMPs are products of the own plant cellular damage



EXTRACELLULAR ATP (EATP)

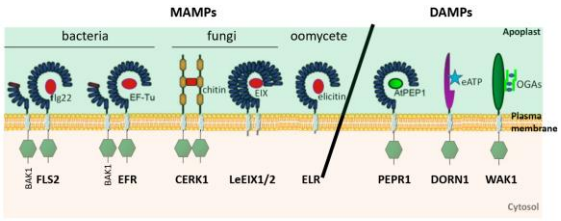
Identified as a signal molecule in 1929 (heart muscle contraction)

Involved in plant growth and development. ATP is actively released from plant cells in response to abiotic stress, fungal elicitors and mechanical stimuli. Low eATP levels induce growth, while high eATP levels block it. The levels are regulated by ecto-apyrases



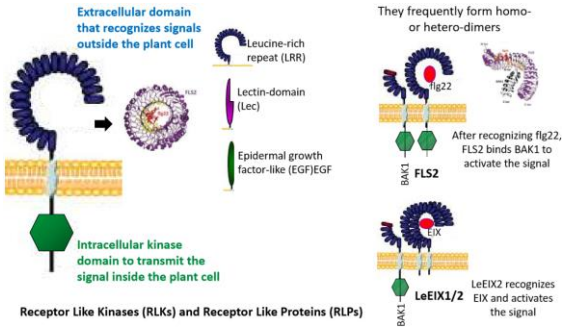
PLANT PATTERN-RECOGNITION RECEPTORS (PRRS)

MAMPs and DAMPs are recognized by Plant Pattern-recognition receptors (PRRs) located at the plant plasma membrane



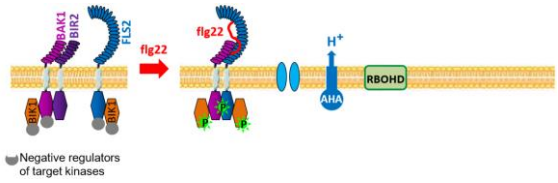
Flg22: 22 aa flagelling peptide
EIX: ethylene-inducing xylanase

PRRs recognize microbes or their effects outside the plant cell. This recognition initiates defence responses

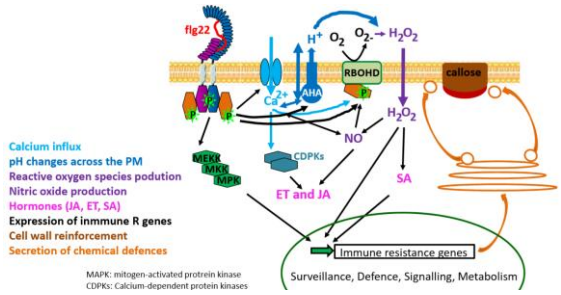


PATTERN TRIGGERED IMMUNITY (PTI)

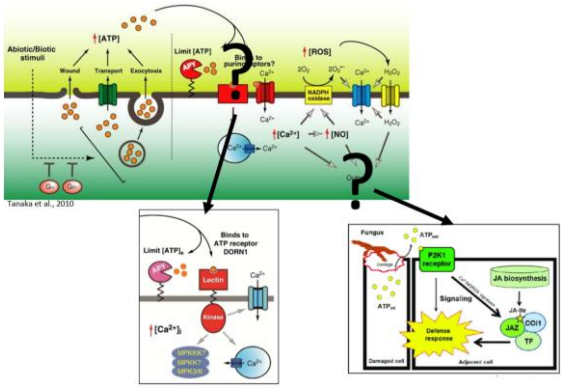
Upon microbe perception (through recognition of MAMPs and DAMPs), a plant immune response is activated to block the infection

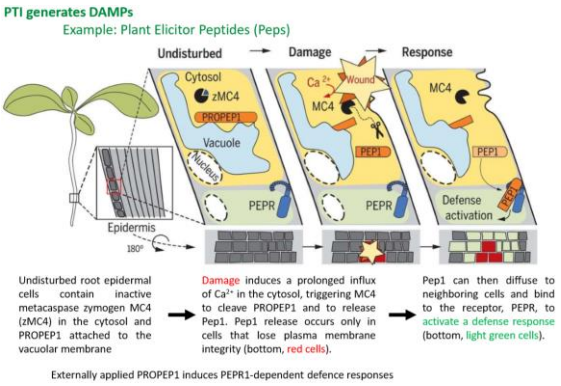


(AHA: Arabidopsis H^+-ATPase, RBOHD: NADPH/respiratory burst oxidase protein D)



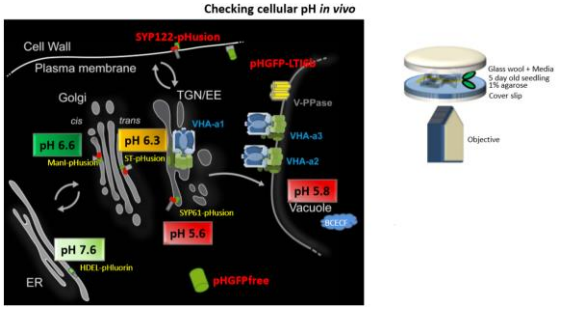
eATP perception and downstream signalling



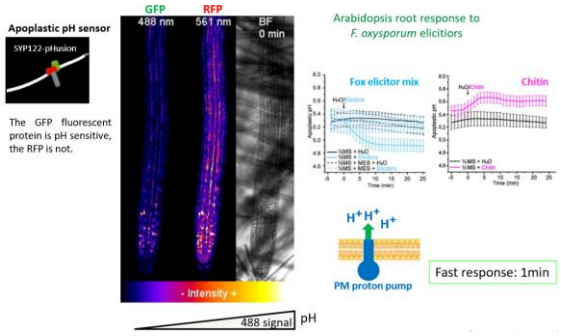


MAIN SIGNALS OF STRESS IN PLANTS

pH changes across the plasma membrane

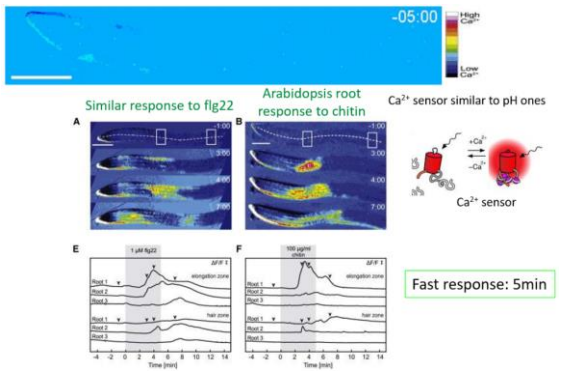


Microbe MAMPs/PAMPs induce fast changes in the apoplastic pH



Calcium influx

Microbe MAMPs/PAMPs induce $[Ca^{2+}]_{cyt}$ transients in the root originating from the elongation zone



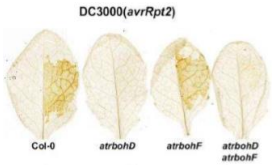
Reactive oxygen species (ROS)

- Hostile environment for the pathogen outside the plant cell
- Cell wall reinforcement
- Defense gene activation

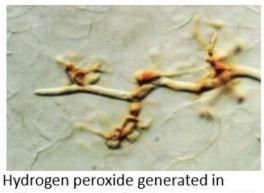
The Arabidopsis NADPH oxidase AtRBOHD is responsible for the production of most ROS in response to pathogens

H_2O_2 Histochemical detection

3,3'-Diaminobenzidine (DAB) is oxidized by H_2O_2 in the presence of peroxidases and produces reddish brown precipitate.



Hydrogen peroxide generated in Arabidopsis leaves after infection with avirulent bacteria



Hydrogen peroxide generated in lettuce cells attacked by incompatible isolates of downy mildew pathogen *Bremia lactucae*

All these signals are also responses to many developmental cues and are required for plant growth and development

ROS: intercellular and long-distance communication, root architecture, polar growth and organ senescence

$[Ca^{2+}]_{cyt}$: pollen tube elongation, cell division, seed germination, apoptosis, circadian rhythm, auxin responses

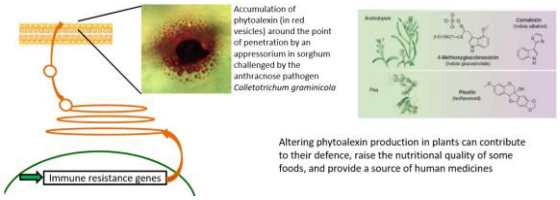
NO: seed germination, root organogenesis and elongation, hypocotyl elongation, pollen tube growth, leaf expansion, senescence, programmed cell death

pH changes across the membrane: cell elongation (acid growth theory), iron and sugar import

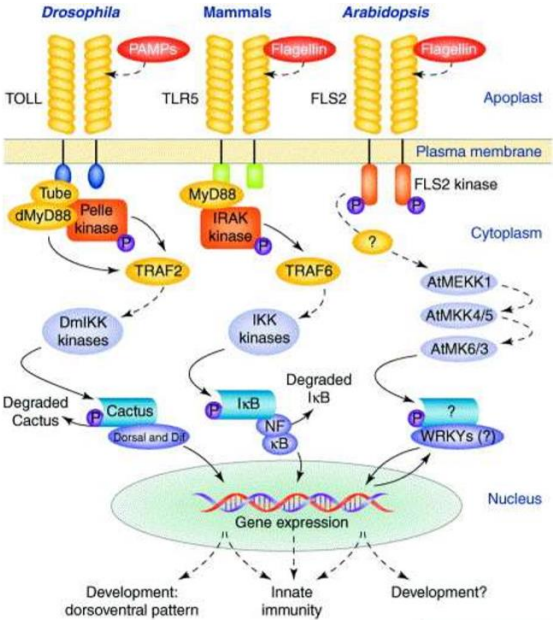
SECRETION OF CHEMICAL DEFENCES

Example: Phytoalexins

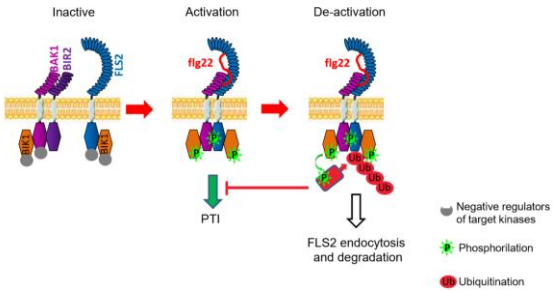
- Antimicrobial and often antioxidative substances synthesized de novo in response to pathogen attack, chemical injury, mechanical injury (low specificity for induction)
- Low molecular weight antimicrobial compounds synthesized by healthy cells next to infected (damaged) cells
- Chemically diverse, with different phytoalexins characteristic of different plant species, i.e. host-specific rather than pathogen-specific

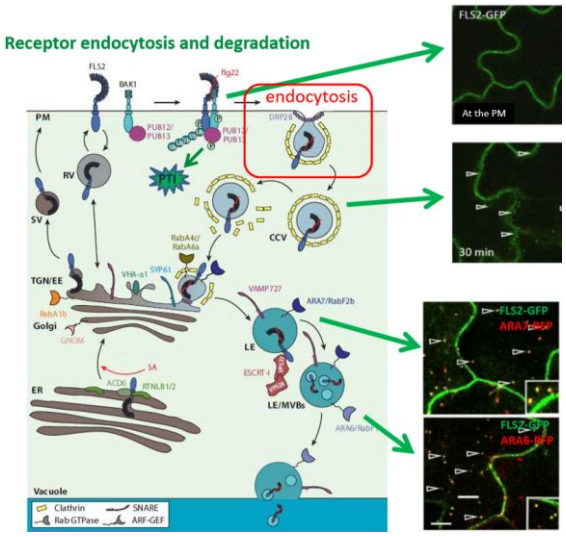


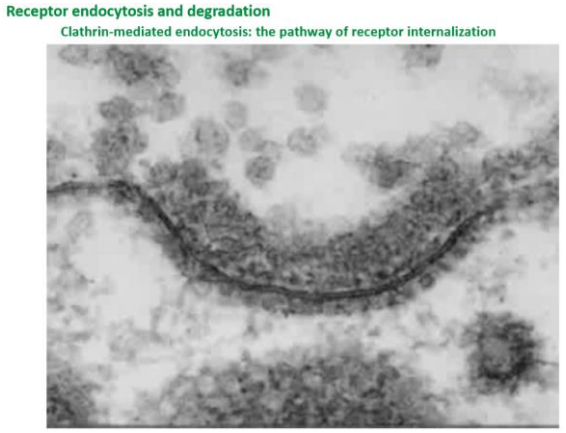
There are surprising similarities between how animals and plants perceive microbes

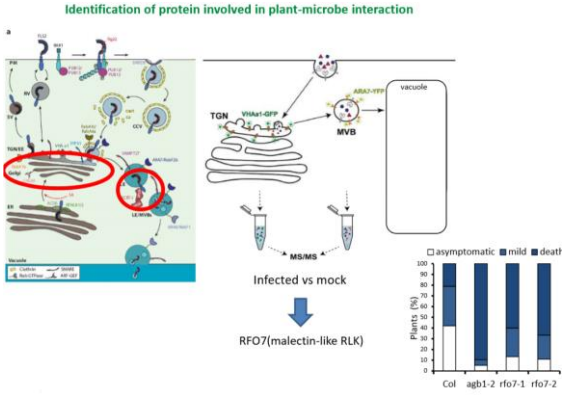


The PTI has to be switched off by the plant cell





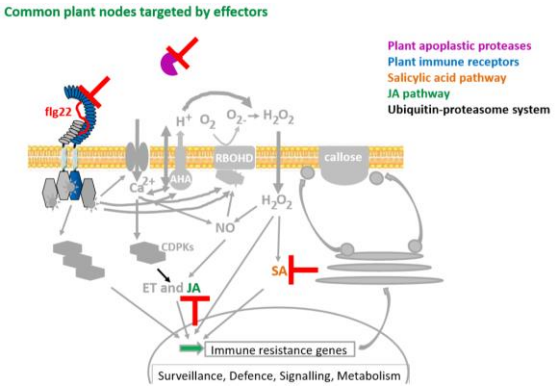




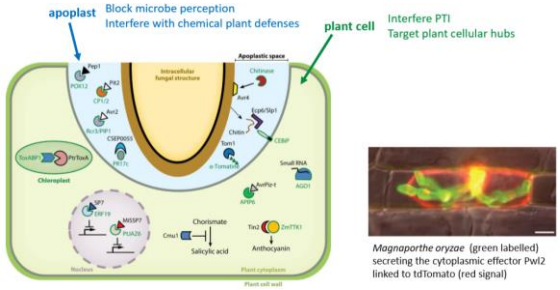
MICROBE EFFECTORS

Microbes interfere with PTI using effectors

Effector: molecule secreted by the microbe, which either promote the virulence of pathogens or allow symbionts to colonize a plant

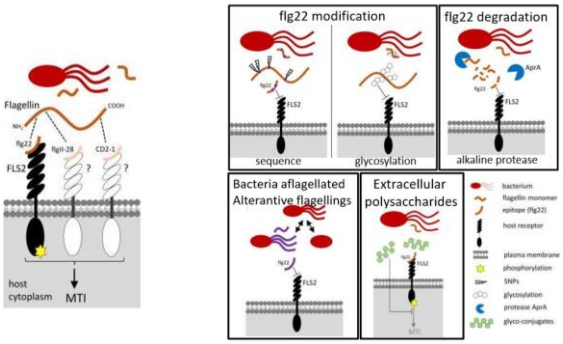


Effectors can act in the plant apoplast or inside the host cells

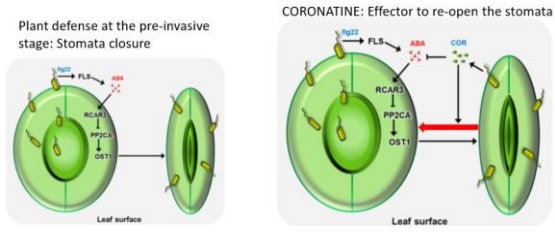


APOPLASTIC EFFECTORS

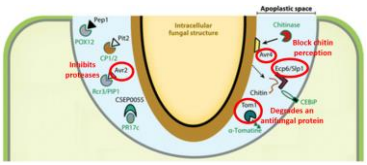
Example: bacteria flagellin preceptor



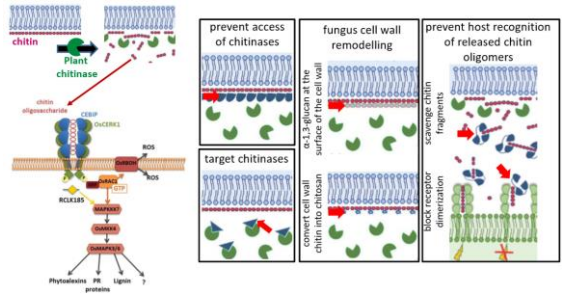
Example: bacteria opening stomata



Example: fungus



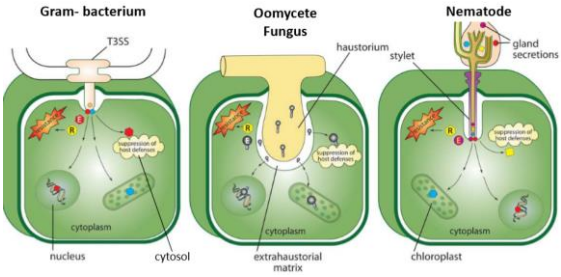
Example: fungi chitin perception



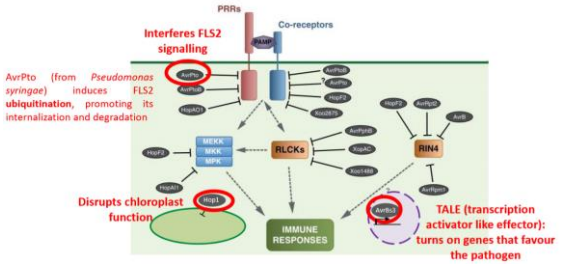
PLANT INTRACELLULAR EFFECTORS

They act in many cell compartments inside the cell

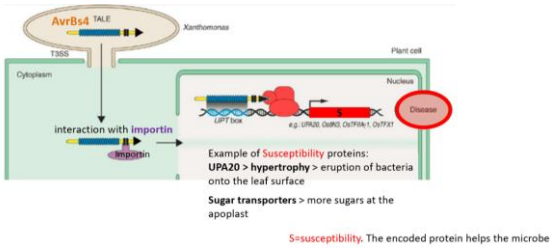
The effectors might interfere with PTI or target plant cellular hubs (such as the chloroplast)



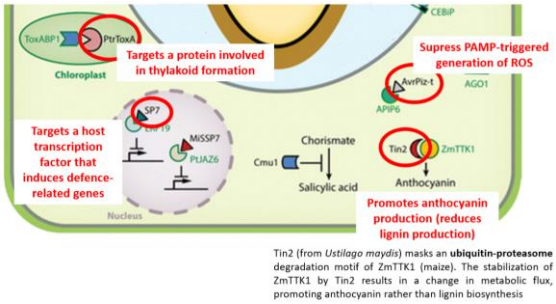
Example: bacteria



Example: bacteria TAL (transcription activator like) effectors



Example: fungi



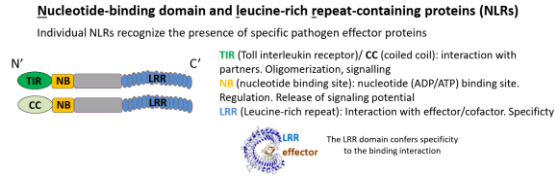
Tin2 (from *Ustilago maydis*) masks an **ubiquitin-proteasome** degradation motif of ZmTTK1 (maize). The stabilization of ZmTTK1 by Tin2 results in a change in metabolic flux, promoting anthocyanin rather than lignin biosynthesis

PLANT RESPONSE

EFFECTOR RECOGNITION

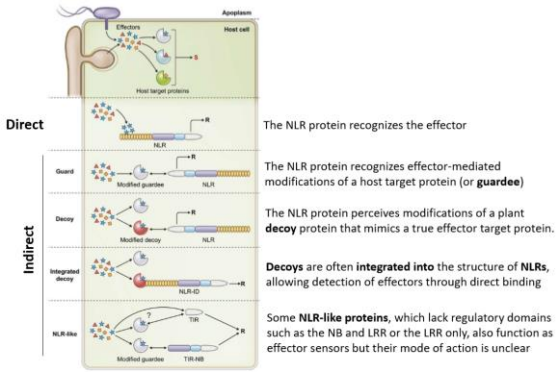
Effectors are recognized by intracellular immune receptors: the NLRs or Resistant proteins

Gene-for-Gene model: Flor observed that *R* genes respond specifically to the products of pathogen genes, which he dubbed avirulence genes (the protein is recognized by the host and confers lack of virulence). Now we know that many *Avr* genes (encoding effectors) promote virulence in hosts that do not express the corresponding *R* gene

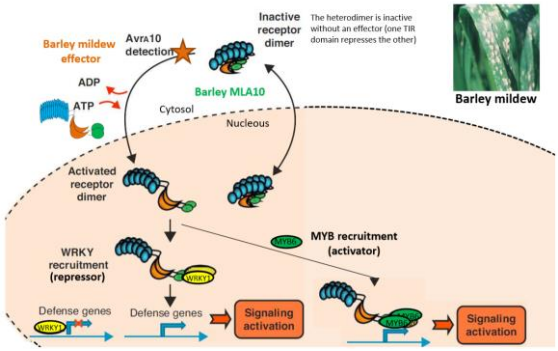


ATTENTION! "The functions of most plant NLRs have not been defined, the only function that has been definitively associated with them is as R-proteins. It is therefore not strictly correct to use the terms NLR protein and R-protein interchangeably as is often seen in the literature (e.g. Gao et al., 2018) since it is likely that many NLRs are not R-proteins. Furthermore, some R-proteins are not NLRs; there are a number of classes of R-proteins that do not have the NLR structure (Kourelisand van der Hoorn, 2018)."

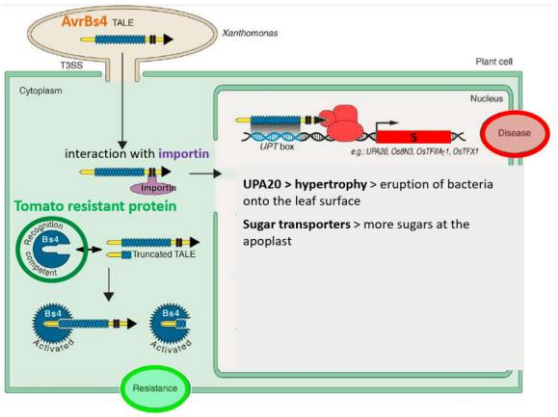
How do NLRs recognize effectors?



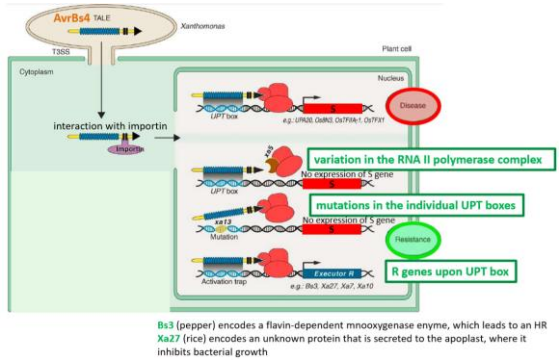
Example: R protein activation



Example: effector inactivation by R protein recognition

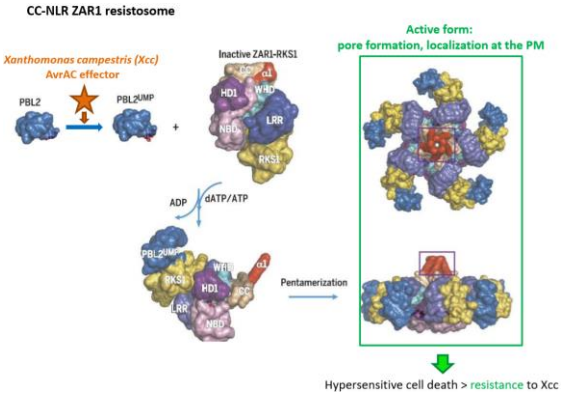


Example: suppression of S genes



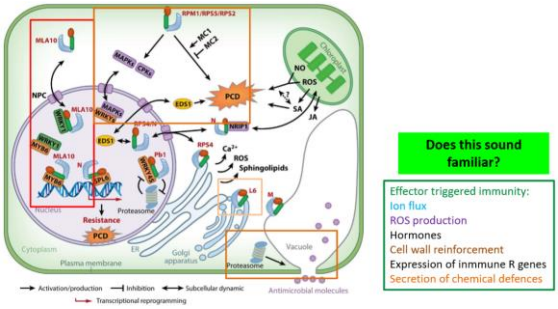
Bs3 (pepper) encodes a flavin-dependent mnooxygenase enyme, which leads to an HR
Xa27 (rice) encodes an unknown protein that is secreted to the apoplast, where it inhibits bacterial growth

Cooperative assembly formation for immune activation



EFFECTOR TRIGGERED IMMUNITY (ETI)

ETI: Effector recognition by R proteins leads to enhanced defence



HYPERSENSITIVE RESPONSE

Hypersensitive response (HR): Rapid localized cell death that occurs at the point of pathogen penetration and is associated with disease resistance. It prevents spread of pathogen to other cells. It is the most obvious manifestation of the defence response associated with NLRs

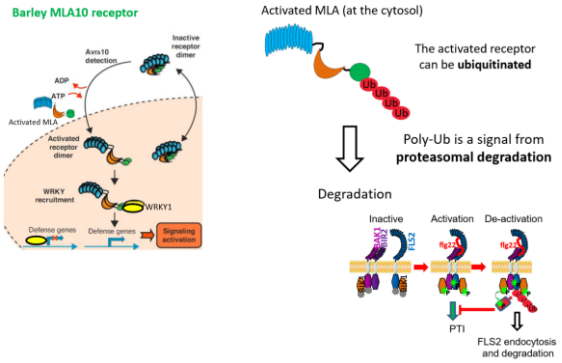
Activation of R genes upon recognition of elicitor leads to:

1. Oxidative burst (ROS, NO,…) a stronger peak than that generated from PTI → cellular lipid damage
2. Ion fluxes
3. Cell wall reinforcement (lignin and callose) of cells surrounding the infection. Creating a barrier to block the infection spread
4. Synthesis and secretion of antimicrobial compounds by cells surrounding the lesion
5. Salicylic acid production → systemic acquired resistance (SAR)

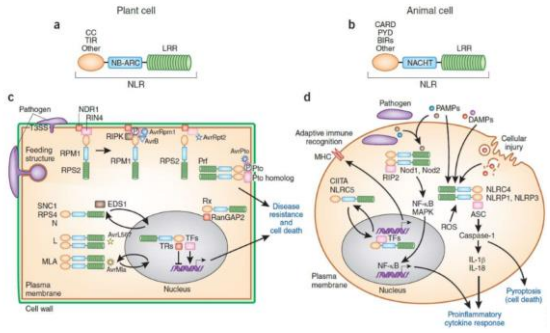
6. Cell death and formation of local lesions

- Operates for biotrophic and hemi-biotrophic fungi
- Analogous to the innate immune system of animals
- This is the resistance involved in Gene-for-Gene interactions, the type often selected by plant breeders

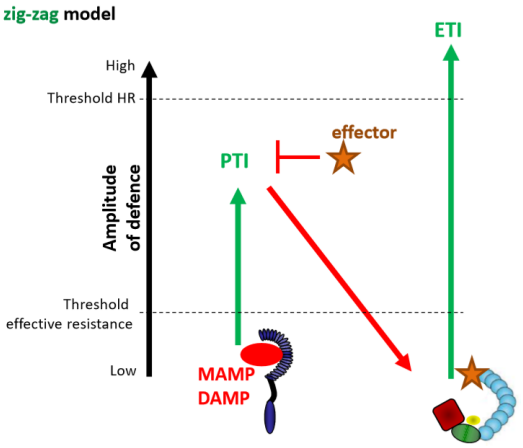
The ETI has to be switched off by the plant cell



There are surprising similarities between how animals and plants perceive and respond to pathogen effectors



ETI is regarded as an amplified, accelerated and more prolonged PTI response



EVOLUTIONARY WAR VIRULENCE GENES VS. R GENES

Effective pathogens show a high rate of effector innovation: large genome full of duplications and repetitive elements; effector are in regions that generate recombination and diversity

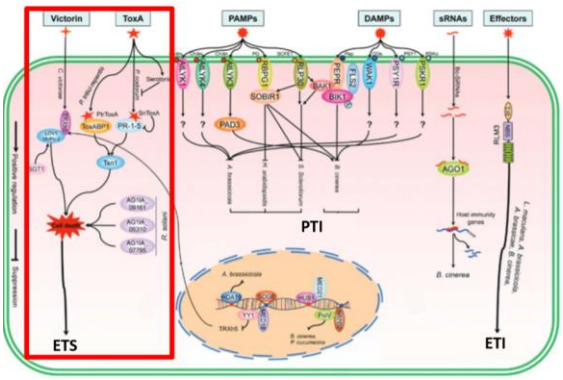
Resistant plants have many and very diverse NBS-LRR genes (>400 in rice, poplar and potato)

Example: re-emergence of wheat stem rust

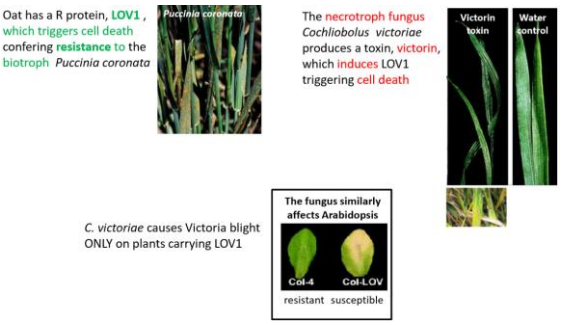
Puccinia graminis f.sp. Tritici	vs	wheat
Steam rust epidemics reported in Europe, Asia, Australia, and the USA	→	
	←	Wheat with steam rust resistance R genes (Sr31, among others), incorporated worldwide
Wheat stem rust strain Ug99 has broken *Sr31* resistance. Spreading across Africa, Asia and the Middle East	→	
	←	Newly developed Ug99- resistant wheat varieties are being cultivated in Ug99-affected areas

THE NECROTROPH PATHOGENS TAKE ADVANTAGE OF THE HR

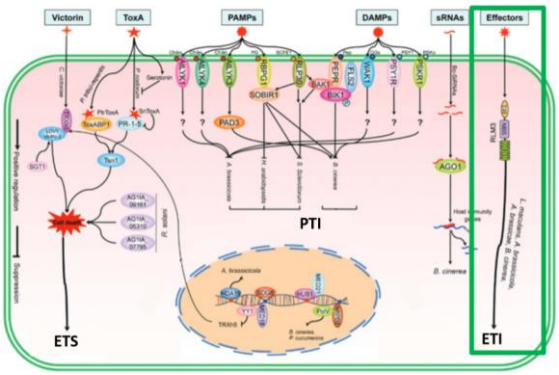
Host specific necrotrophes produce specific toxins necessary for their pathogenicity. Sometimes they target R proteins, in a counterintuitive manner, to induce programmed cell death and Effector Triggered Susceptibility



Example: Victoria blight

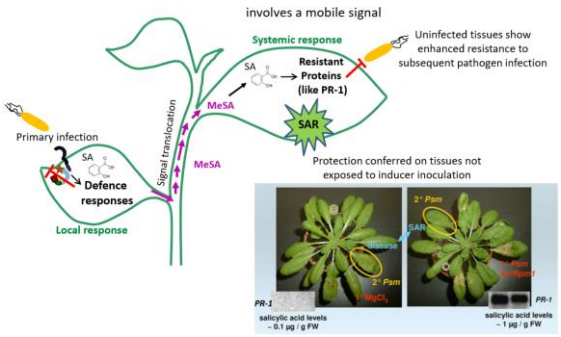


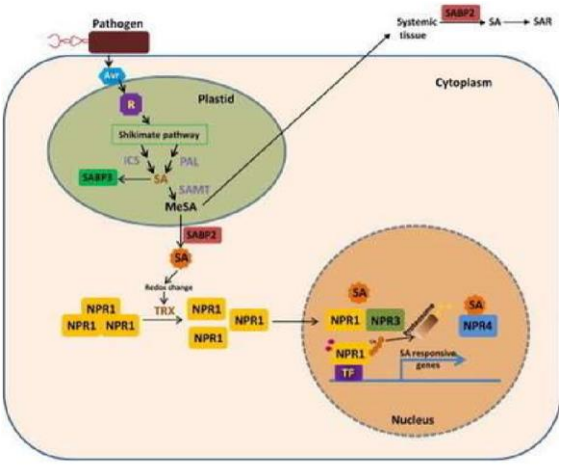
There are few cases of ETI against necrotrophs. There are few known *R* genes that confer resistance to necrotrophic pathogens, therefore breeding resistance to necrotrophs is more challenging than biotrophs



HORMONES AND DEFENCE

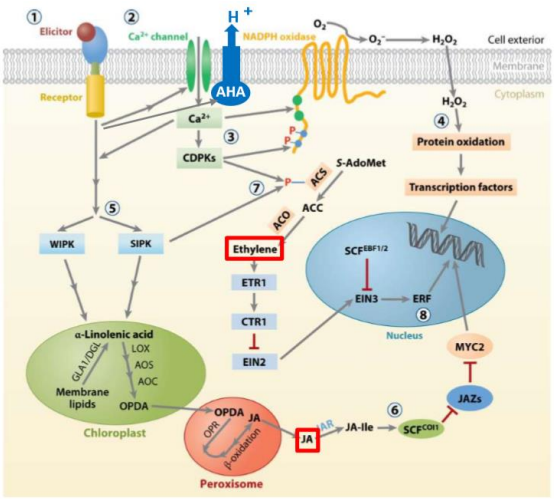
SALICYLIC ACID AND SYSTEMIC ACQUIRED RESISTANCE (SAR)





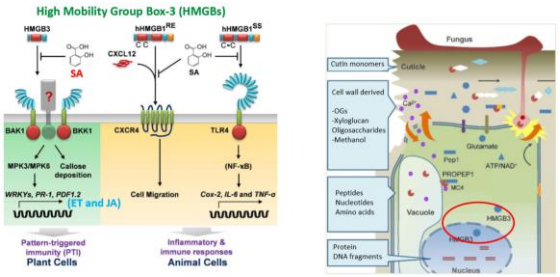
JASMONIC ACID AND ETHYLENE

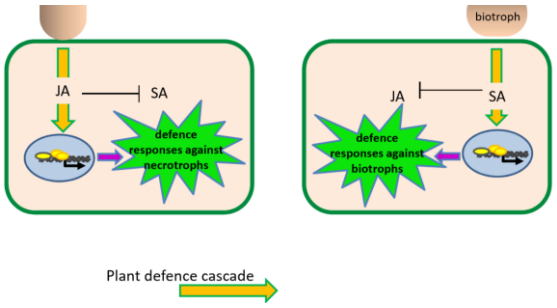
Some pathogens, wounding and herbivores can promote the synthesis of JA and ET



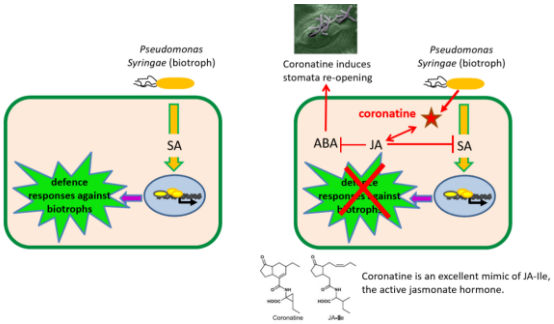
HORMONE ANTAGONISM

Salicylate and jasmonate can be mutually antagonistic

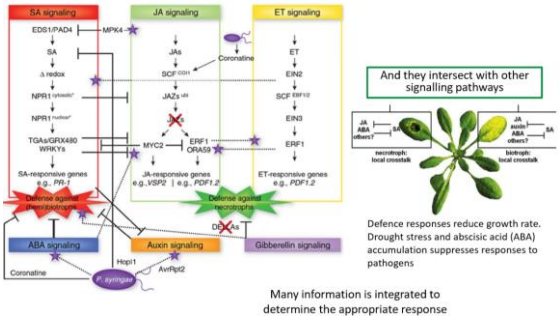




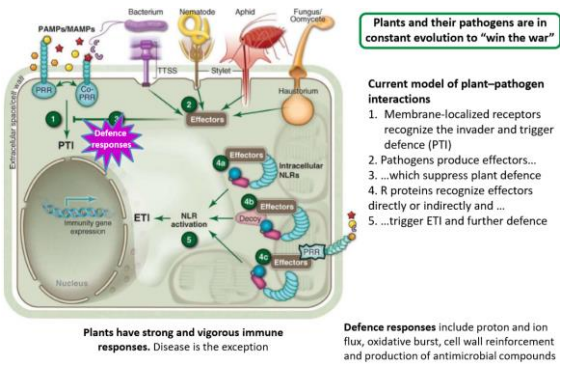
Some pathogens take advantage of the defence signal cross-talk



As a very general rule: SA is involved in defence against biotrophs, JA and ET are involved in defence against necrotrophs



Summary plant immune response



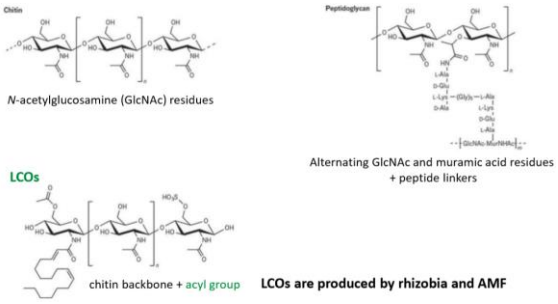
PLANT-SYMBIONT INTERACTION

Not all organisms which interact with plants are pathogens → symbionts

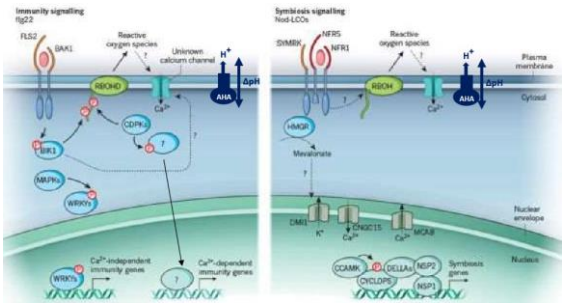
The symbiont must avoid plant defences → avoiding perception as pathogen and suppressing PTI

SYMBIONT IS NOT PERCEIVED AS A PATHOGEN

The microbial signals that are necessary for the establishment of arbuscular mycorrhizal symbioses (the Myc factors) and root-nodule symbioses (the Nod factor) are derivatives of chitin oligosaccharides. Important difference: the presence of lipid modifications on the chitin-oligosaccharides backbone of these symbiotic signals, which transforms them into lipochitooligosaccharides (LCOs)



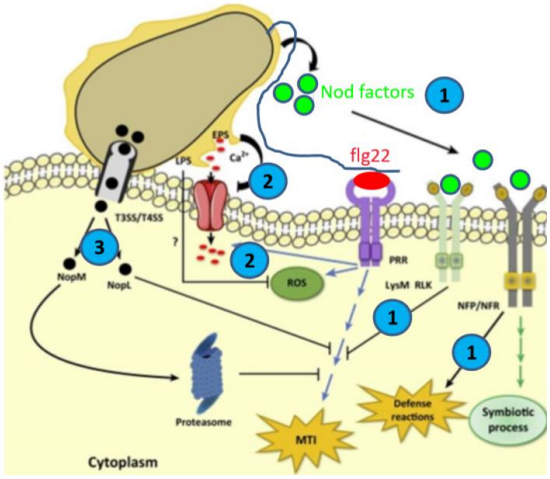
The recognition of LCOs activates calcium oscillations in the nucleus. The 3-hydroxy-methylglutaryl-CoA reductase (HMGR) that associates with SYMRK is involved in the production of mevalonate, which may function as a secondary messenger to the nucleus. Several channels that are located at the nuclear membrane coordinate the release of calcium from the nuclear envelope and endoplasmic reticulum: a complex of DMI1 and CNGC15 regulate counterflows of potassium and calcium, enabling these ions to flow without impinging on membrane polarity, and the calcium ATPase MCA8 pumps calcium back into the nuclear envelope. Nuclear calcium oscillations activate CCAMK, which phosphorylates CYCLOPS to promote the induction of symbiosis gene expression. CYCLOPS may form part of a large complex that contains a number of transcription factors that are also necessary for the expression of symbiosis genes



ACTIVE SUPPRESSION OF PLANT DEFENCES THROUGH THE ACTION OF EFFECTORS

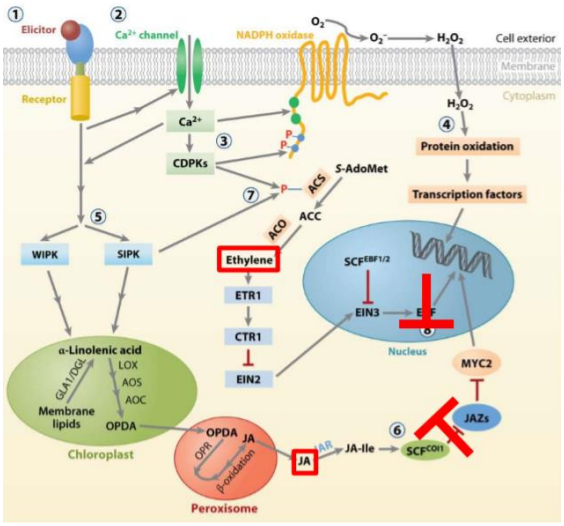
Rhizobia can escape MTI/PTI:

- Through nodulation factor (Nod factor)-mediated suppression of MTI/PTI
- By inhibiting the defence reactions through extracellular calcium chelation using bacterial exopolysaccharides and/or through ROS production inhibition using bacterial lipopolysaccharides
- By symbiotic effectors (for example Nop proteins) into the host cytoplasm to short circuit immune signalling, directly or via the proteasome pathway

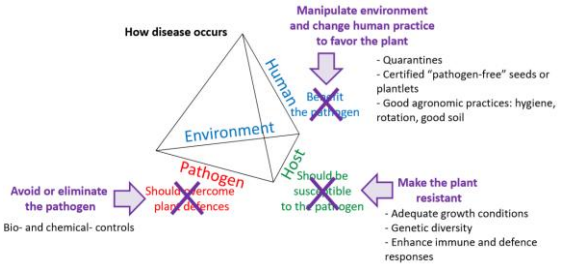


AM fungi can escape MTI/PTI by interfering with hormone pathways:

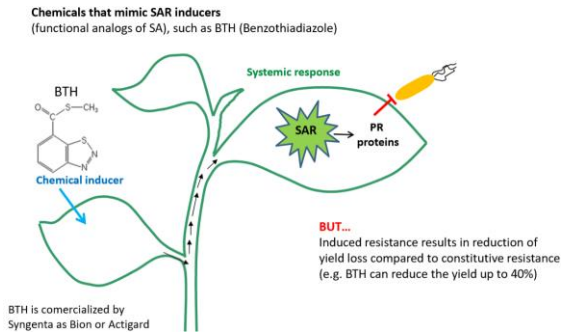
- SP7, a repetitive effector from the AM fungus *Glomus intraradices* blocks activity of the ERF19 transcription factor, leading to a downregulation of PTI in *M. truncatula*
- The *Laccaria bicolor* MiSSP7 effector interacts with PtJAZ6, a negative regulator of JA induced gene regulation in *Populus trichocarpa*. MiSSP7 prevented JA dependant degradation of PtJAZ6, resulting in the repression of JA induced genes



MAKE USE OF BIOTECHNOLOGY TO MANAGE PLANT DISEASES



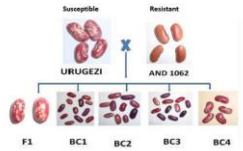
SAR CAN BE USED IN AGRICULTURE



GENETIC APPROACHES TO MAKE THE PLANT RESISTANT

Classical approaches: R genes and quantitative disease resistance

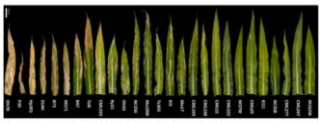
R gene introgression can confer complete resistance by a single gene. Introgression requires backcrossing (BC) to susceptible parental line to restore desired traits



BUT...
Within a few years (variable) of the introduction of a R protein into commercial production, often the gene ceases to protect the crop from disease, and severe epidemics occur

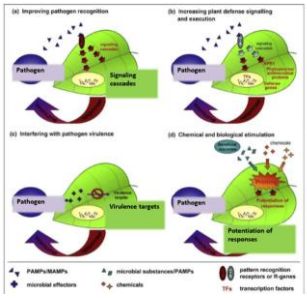
Why?

Quantitative disease loci (QDL) are loci that each promote partial disease resistance. Examples include protein kinases and transcription factors



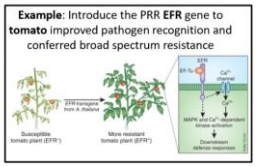
Recombinant inbred lines showing different levels of susceptibility to northern corn leaf blight through different suites of QDLs

Biotechnological approaches



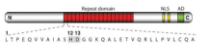
How?
a) Introduce or modify PRR
b) Overexpress or modify signalling intermediates to stimulate response
c) Detoxify or sequester effectors and virulence factors
d) Biological controls or chemical induction of priming

Example: Introduce the PRR **EFR** gene to **tomato** improved pathogen recognition and conferred broad spectrum resistance



Example: bacteria TAL effectors

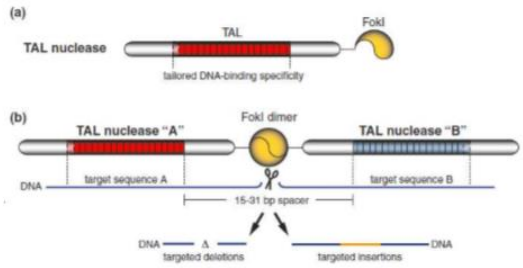
Example: Bacteria TAL effectors

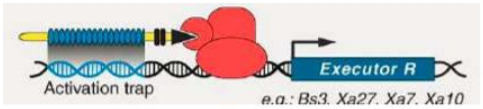


NLS: nuclear localization signals
AD: activation domain, to function as transcriptional activators.
Repeat domain (17.5 repeats) : confers DNA-binding specificity. It binds to the **effector-binding element (EBE)**

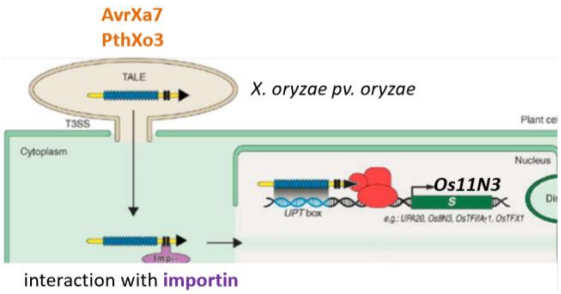
Biotechnology uses:

- TAL nucleases (TALN) for genome editing: Two TALN, each one has a TAL+endonuclease domain (FokI). Each TAL binds neighbourng DNA boxes. FokI+FokI=endonuclease, which cleaves the DNA in the spacer region between domains. The TALs are designed to make the FokI dimer cut the EBE domain. Cleaved DNA is filled by the plant, changing the EBE and preventing the expression of the susceptible gene by pathogen TALN
- Transcriptional remote controls: Induction of target genes that trigger resistance against pathogens that inject TALEs into the plant cells during infection. By knowing the EBE domain of the TALEs and introduce them into promoters driving resistance genes





- Enhance rice resistance to bacterial blight: bacterial blight, using its endogenous TAL effectors AvrXa7 or PthXo3, induces a rice susceptibility gene (Os11N3) encoding a member of the SWEET sucrose-efflux transporter family. Thus, the bacteria diverts sugars from the plant cell so as to satisfy the pathogen's nutritional needs and enhances its persistence. Goal: block expression of Os11N3 regulated by AvrXa7 and PthXo3



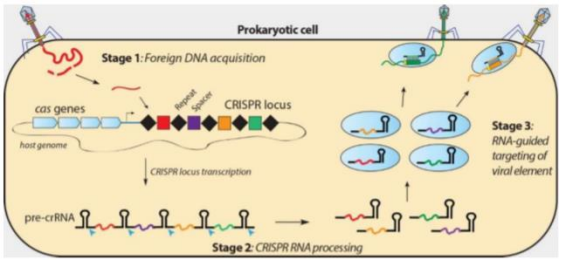
Example: CRISPR/Cas

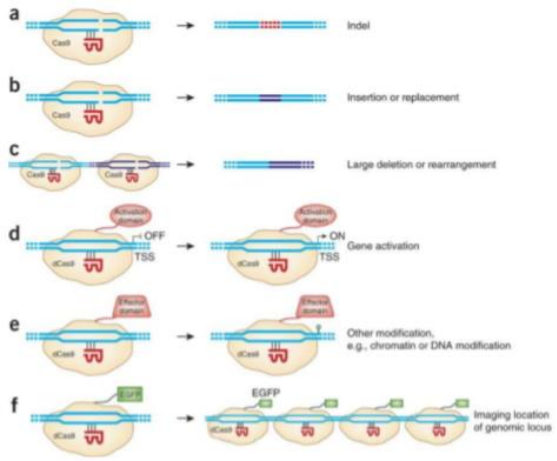
CRISPR: Clustered regularly interspaced short palindromic repeats

Cas: CRISPR associated proteins. Nuclease (Cas9 was the first nuclease discovered)

Genome editing via introduction of point mutations, insertions or deletions (a – c)

Transcriptional regulation via CRISPR interference, activation, repression or epigenetic modulation (d – f)





(dCAS9: Catalytically inactive or dead Cas9)

- Enhance blast resistance in rice: Knockdown of expression of the rice ERF gene *OsERF922* enhanced rice resistance to *M. oeyzae* (hemibiotroph), indicating that *OsERF922* act as a negative regulator of blast resistance in rice. Goal: KO OsERF922 by CRISPR/Cas9

ERF: ethylene response factor

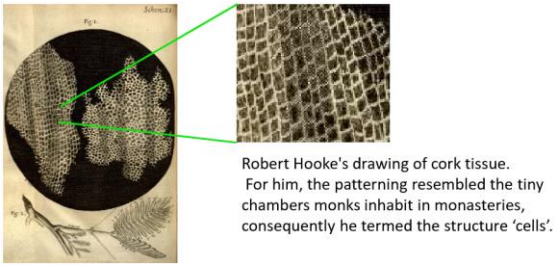
FIRST LAYERS OF PLANT DEFENCE IN DETAIL

THE PLANT CELL WALL

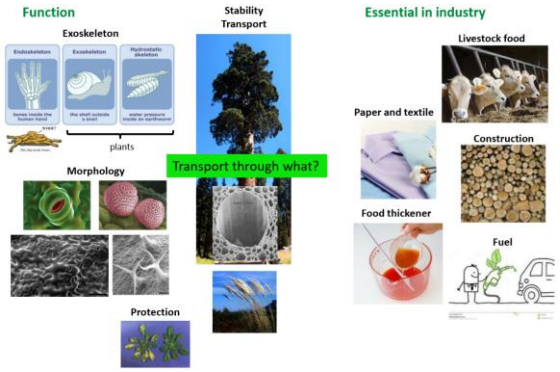
Cell walls → plant cells can't migrate

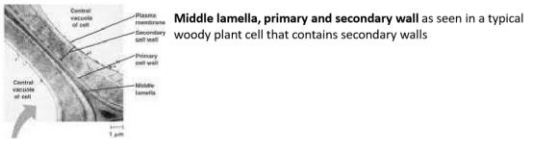
Growth: They have to adapt to the division and expansion of the neighbour cells

Defence: Lack of defence cells (phagocytes and leukocytes). All plant cells should be able to perceive and response to a microbe attack

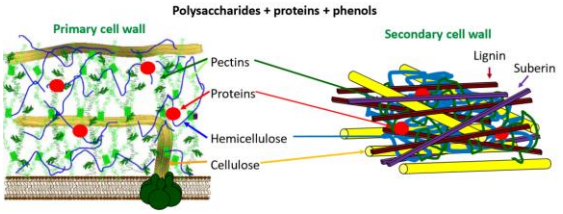


Robert Hooke's drawing of cork tissue. For him, the patterning resembled the tiny chambers monks inhabit in monasteries, consequently he termed the structure 'cells'.

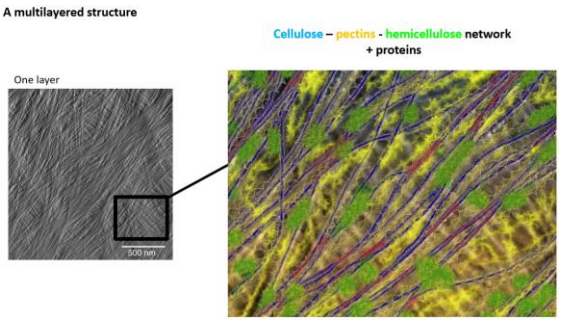


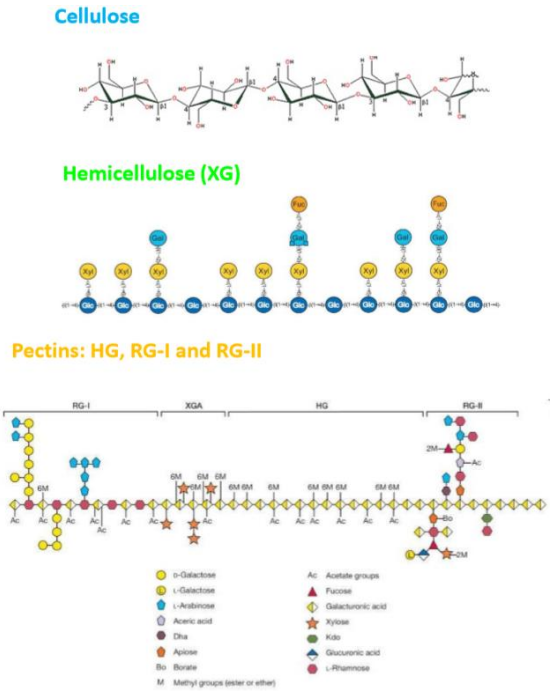


Middle lamella, primary and secondary wall as seen in a typical woody plant cell that contains secondary walls

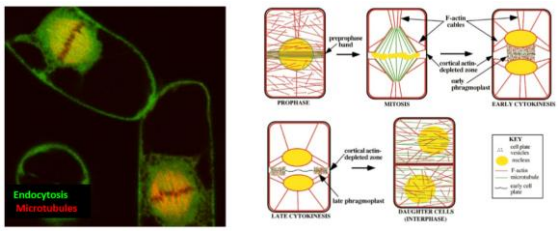


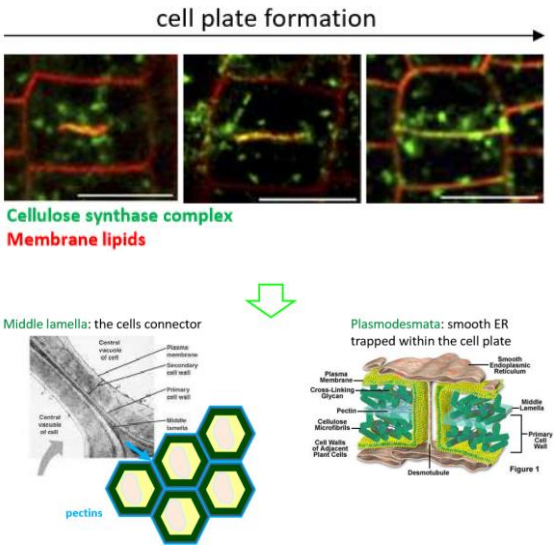
The cell wall is strong to resist tensile stresses (10MPa in the plane of the wall) and flexible to expand irreversibility (100 times more than the size of their meristem initial)





The first plant cell wall is formed during division. Polymers from the plasma membrane and the Golgi are transported to make the cell plate. Mainly pectin and cellulose, followed by cellulose synthesis start to form the primary cell wall

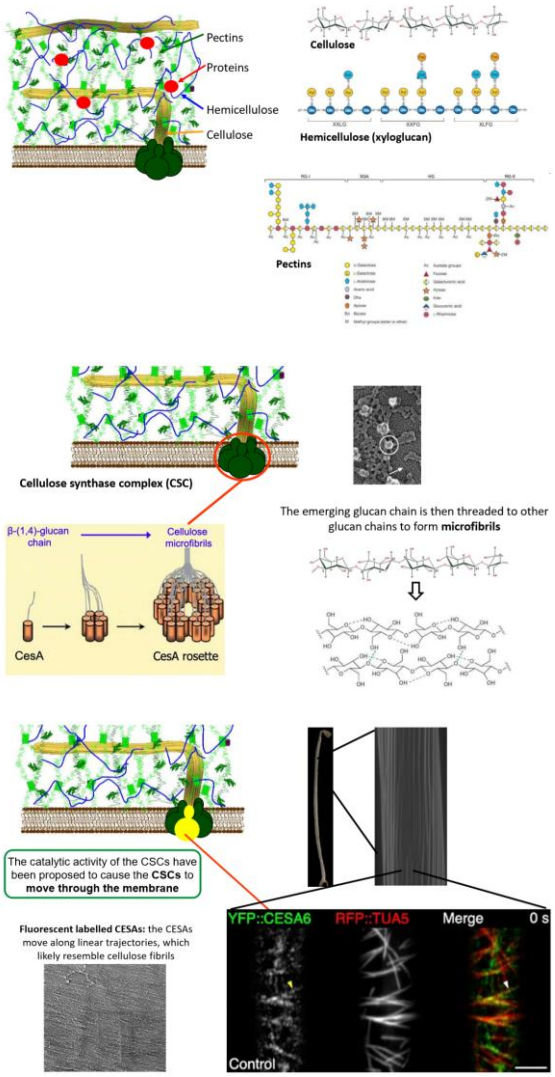




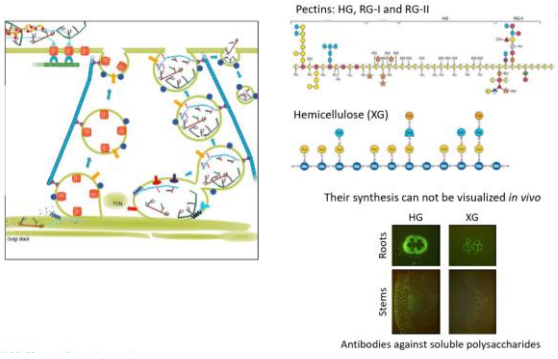
CELL WALL BIOSYNTHESIS

PRIMARY CELL WALL

Framework of cellulose cross-linked with soluble polysaccharides (hemicellulose and pectins)



Soluble polysaccharides: pectins and hemicelluloses

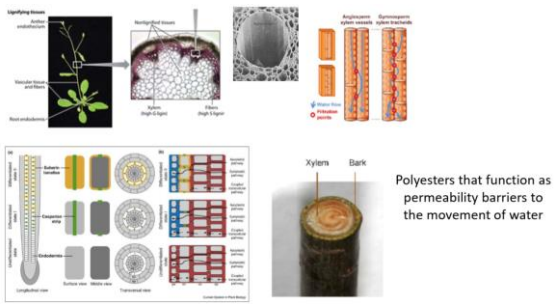


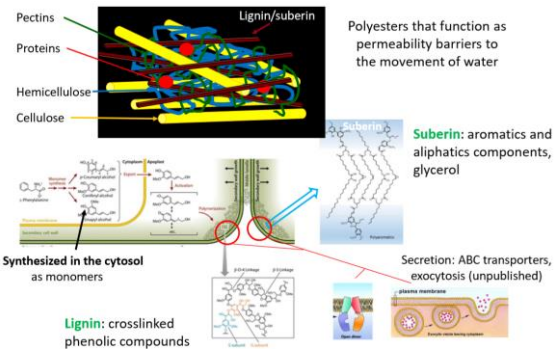
SECONDARY CELL WALL

Xylem (lignin)

Endodermis (casparian bands → lignin and suberin)

Bark (lignin and suberin)

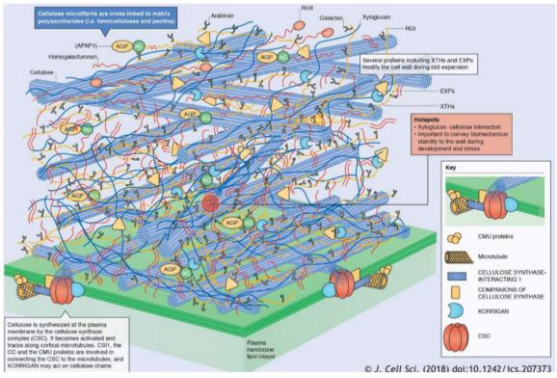




PRIMARY VS. SECONDARY CELL WALL

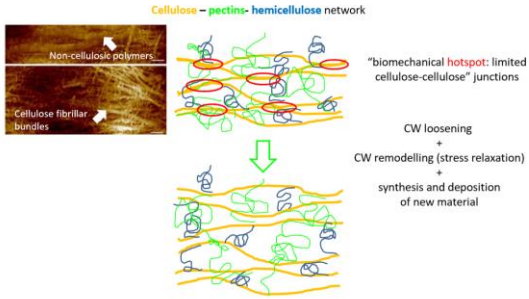
	Primary	Secondary
What? Composition	Celllulose Hemicellulose Pectin Proteins	Cellulose Hemicellulose Pectin (few) Lignin Proteins
Where? In which cells	Starts after the cell plate has formed in divided cells	Typically, around cells that need structural support
Why? Function	Protection Adhesion Turgor pressure Allow for cell expansion > Flexible	Rigidity, strength, protection Transport Main source of biomass in plants
How? Structure	Cellulose microfibrills influence cell shape; cross-linked by hemicellulose and pectins.	Cellulose microfibrills cross-linked by hemicellulose. Lignin forms covalent bonds with hemi-cellulose (difficult to break during industrial process)

ASSEMBLY AND REMODELLING



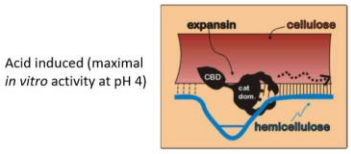
CELL WALL LOOSENING AND REMODELLING

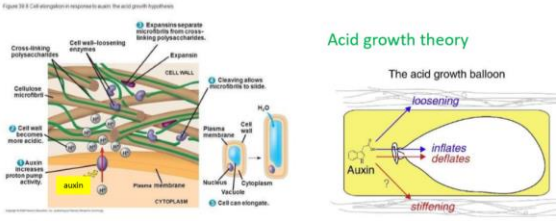
The cell wall is strong to resist tensile stresses and flexible to expand irreversibility



CELL WALL LOOSENING

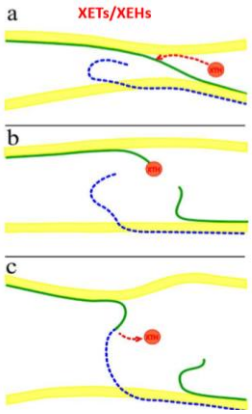
Expansins: Modify the H-bond between cellulose and hemicellulose





CELL WALL REMODELLING

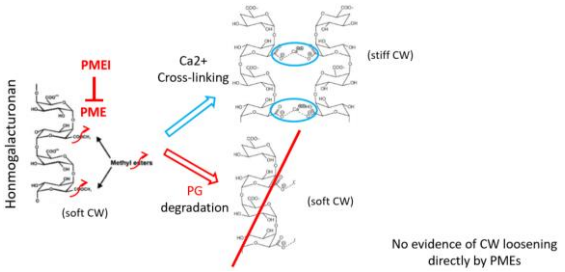
Xyloglucan endotransglycosilases (XETs) and hydrolases (XEHs): cut xyloglucan (hemicellulose) and join them (reducing to non-reducing end)



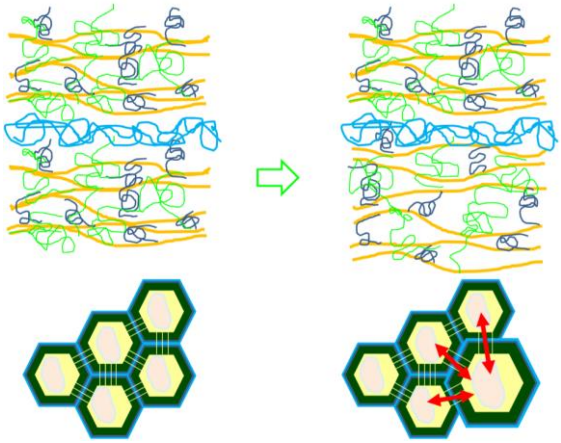
Not sufficient for CW loosening

Turnover or remodelling function

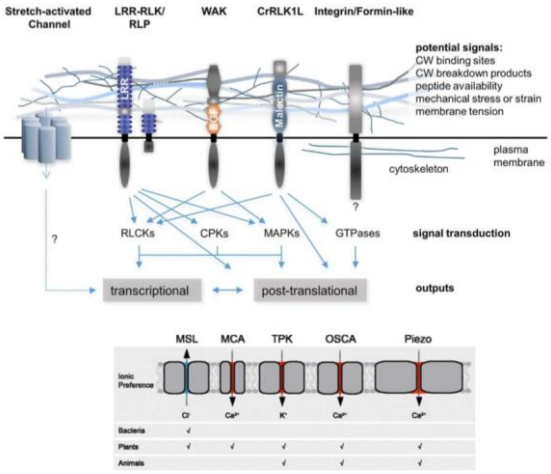
Pectin methylesterases (PME), PME inhibitors (PMI) and polygalacturonases (PG): pectin modification



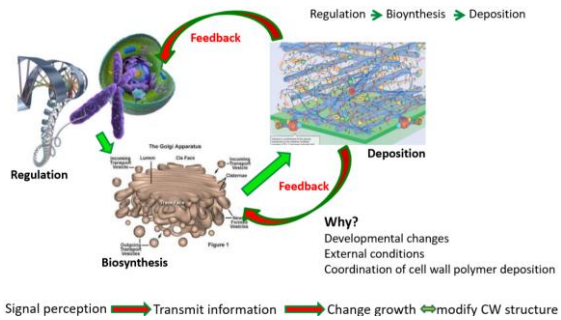
CELL WALL INTEGRITY SENSING



Cell wall integrity sensors



Plants must adapt their growth to their environment



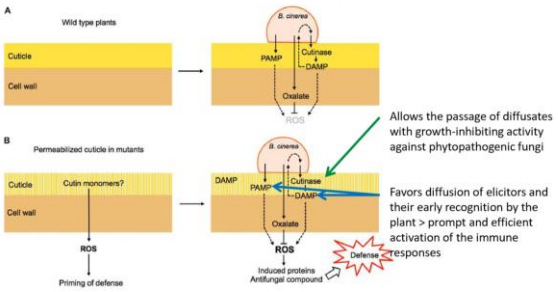
CELL WALL ROLE IN PLANT-MICROBE INTERACTION

Physical barrier for the microbe. Source of nutrients for the microbe and signals for the plant

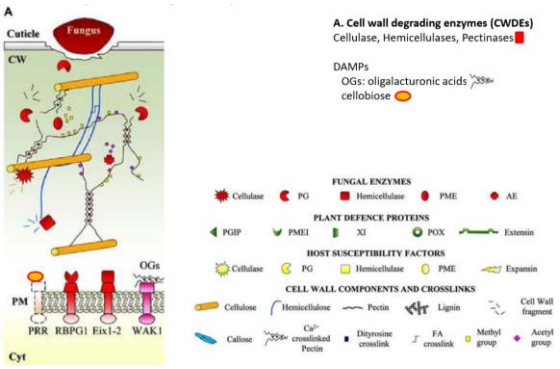
CUTICLE

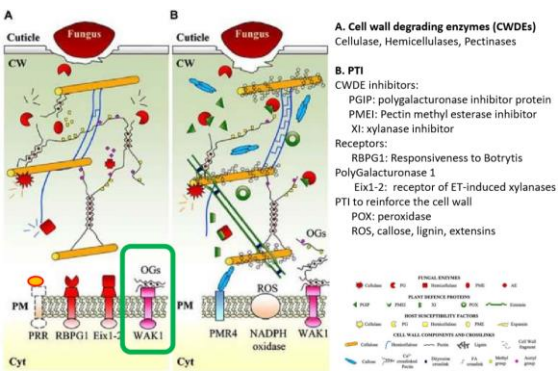
Cuticle: cutin and cuticular waxes. Only in aerial parts of the plant

A more permeable cuticle protects the plant against certain pathogens

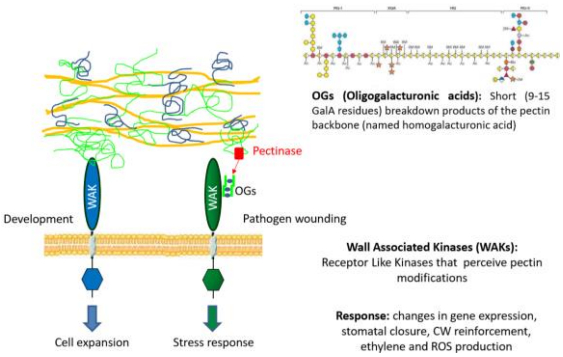


CELL WALL DYNAMICS DURING NECROTROPHS INVASION

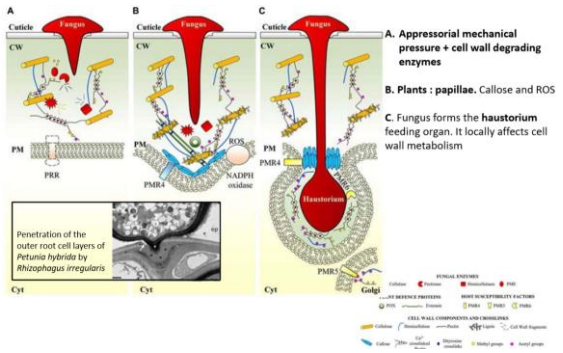




OLIGOGALACTURONIC ACIDS (OGS)



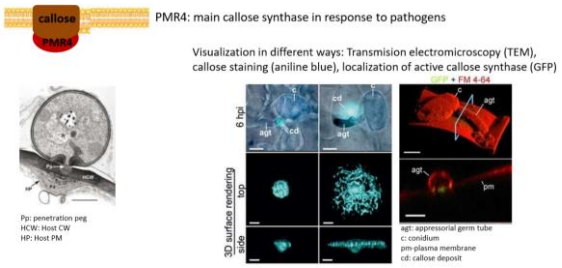
CELL WALL DYNAMICS DURING BIOTROPHIC FUNGI INVASION



CELL WALL REINFORCEMENT UPON BIOTROPH PENETRATION

Papillae formation: Callose deposition and cross-linking of proteins and phenolics (via ROS)

Papillae: Unique to plant cells because they are generated by focal deposition of de novo synthesized cell wall material, including callose, a (1 → 3)-β-D-glucan and cellulose, between the inner cell wall and the plasma membrane (paramural space)



PLASMODESMATA (PD)

Primary plasmodesmata form when the endoplasmic reticulum crosses the phragmoplast during cell division

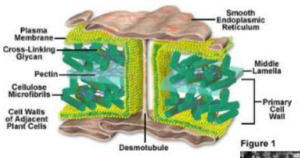
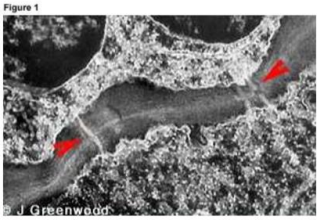
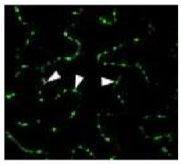


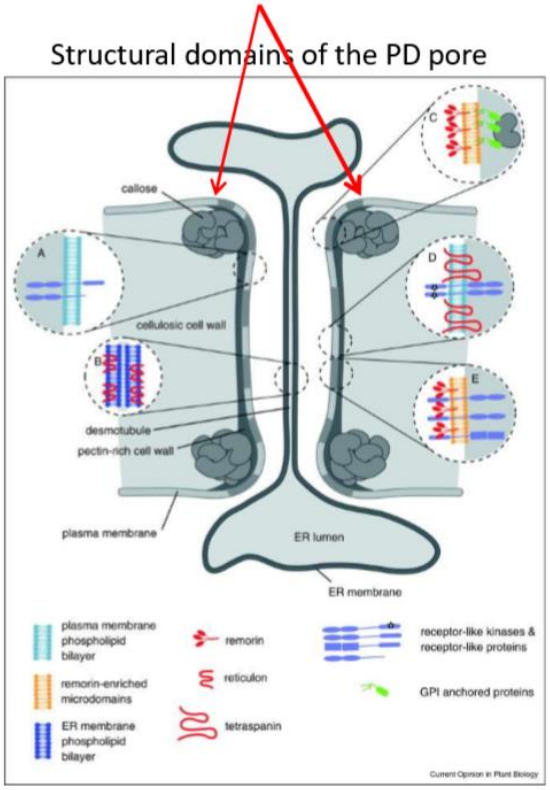
Figure 1



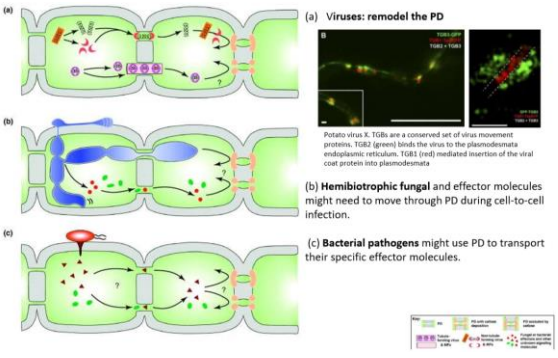


False-colored confocal image of aniline blue fluorescence shows plasmodesmata as punctate signals (arrowheads) at the epidermal cell junctions of *Arabidopsis* leaf tissue

Callose deposition regulates open/close

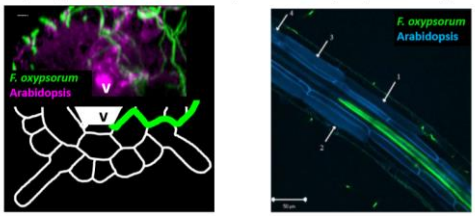


Role of PD in plant-pathogen interaction

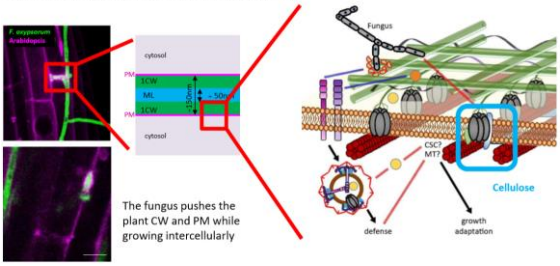


ONGOING SCIENCE FROM THE LAB

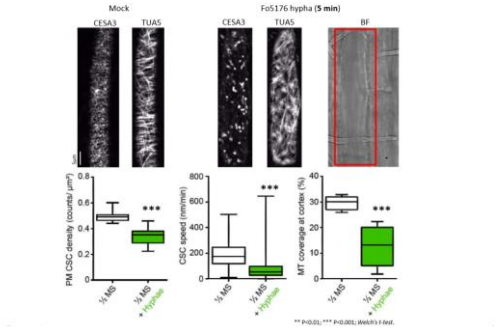
Vascular pathogens advance intercellularly towards the xylem, where they proliferate



Intercellular growth: intimate relation with the host walls



The cellulose synthesis machinery and cortical MT array respond drastically and immediately to *F. oxysporum*



Congratulations! You reached the end of this summary! After reading 21’598 words and studying 539 images you now deserve a break from learning.

Good luck at the exam!