Genomics, Epigenetics & Synthetic Biology

Lecture 4:
Self-organisation and reprogramming of multicellular systems

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Applications of Synthetic Biology

Lecture 3
Cell autonomous genetic circuits with self-regulating properties
e.g. microbial engineering,
enviromental and biomedical sensors
engineering novel metabolic pathways

Lecture 4
Morphogenetic circuits with self organising properties
e.g. microbial biofilms or self-organising communities for
bioremediation and bio catalysis
engineering novel plant varieties for bioproduction

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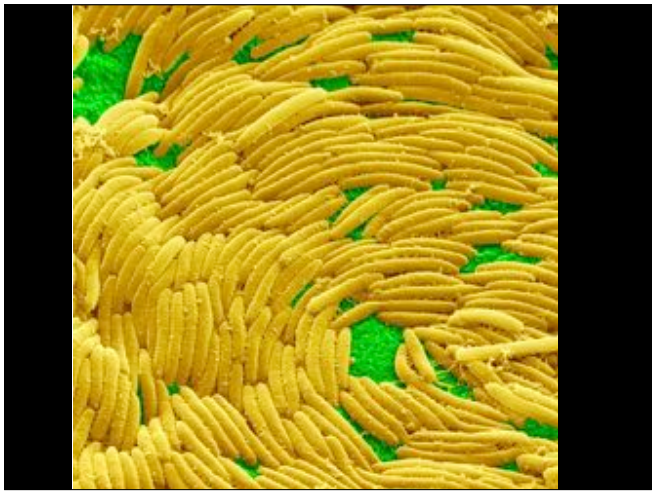
In this lecture, we will look at circuits which (i) act cell non cell autonomously, and (ii) work in a multicellular context and involve communication between cells.

3

Microbes have a social life. Myxococcus xanthi a slime bacterium which has a planktonic form that can scavenge for nutrients. When nutrient sources are poor, the bacteria congregate and form fruiting bodies. This image shows fruiting bodies on a substrate of dung.



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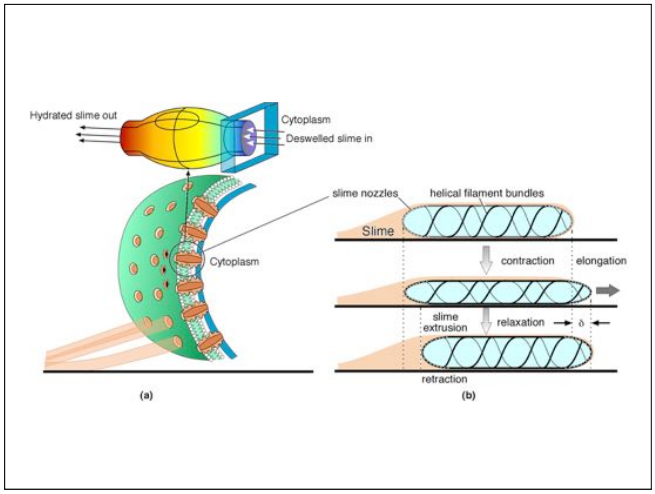
Formation fruiting bodies, individual bacteria congregate and swarm.



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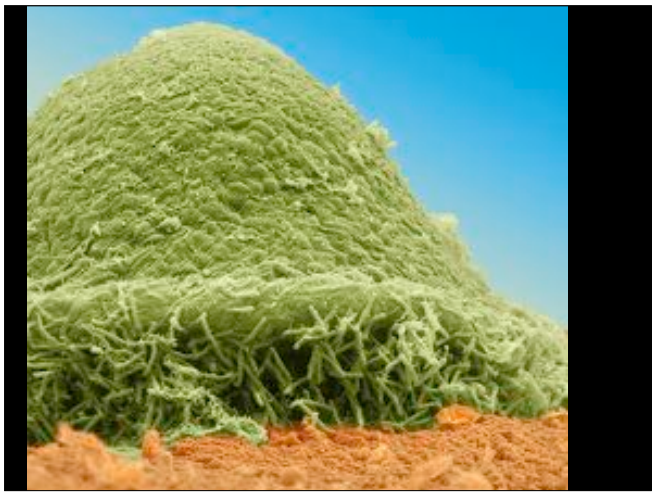
Cell movement is driven by molecular jet propulsion, caused by hydration of muco-polysaccharides from nano-scale pores at the termini of the bacterial cells.



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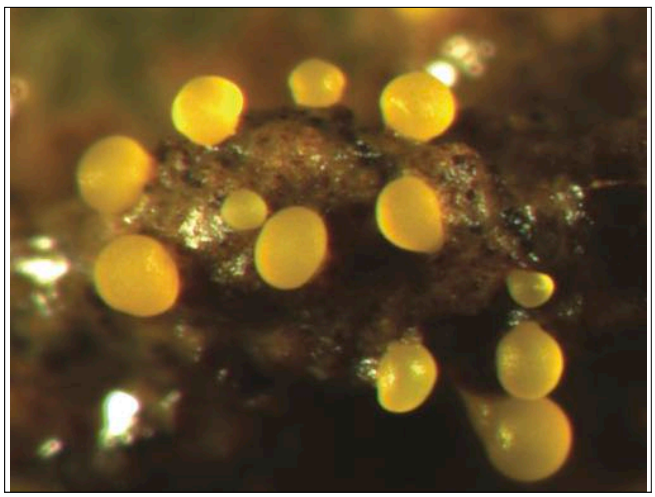
Chemotaxis and cell cell communication results in the formation of cellular mounds. As the cells climb over each other, some cells differentiate into spores and form fruiting bodies.



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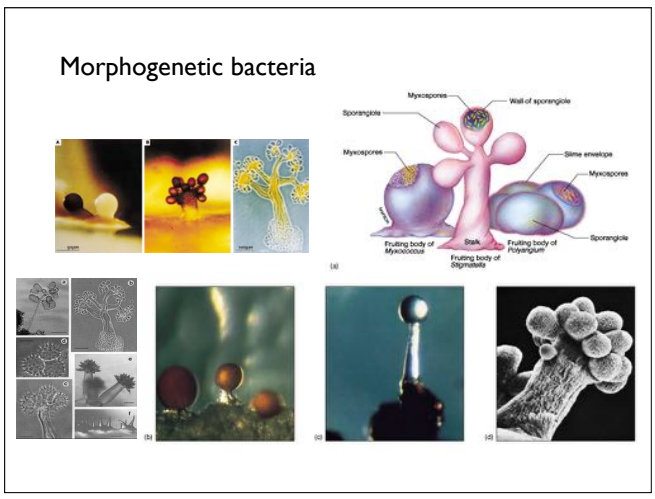
The spore bearing structures are elevated from the substrate to allow aerial dispersion of spores.



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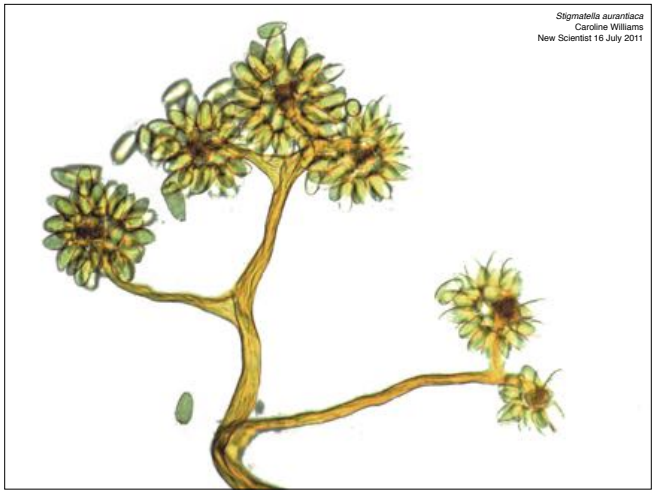
Different species of Myxobacteria form different spore-bearing structures.



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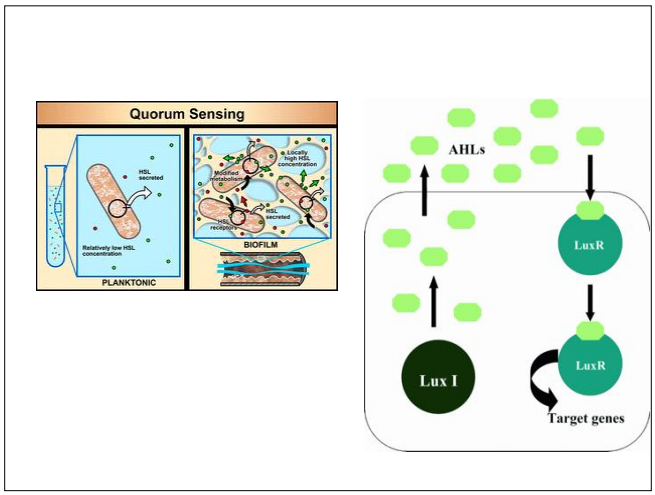
Some, like *Stigmatella aurantica* form microscopic tree-like forms, complete with branches and spore-containing "fruit". The fruiting bodies of slime bacteria are an extreme case of bacterial communication, but most microbes possess some rudimentary form of cell-cell communication.



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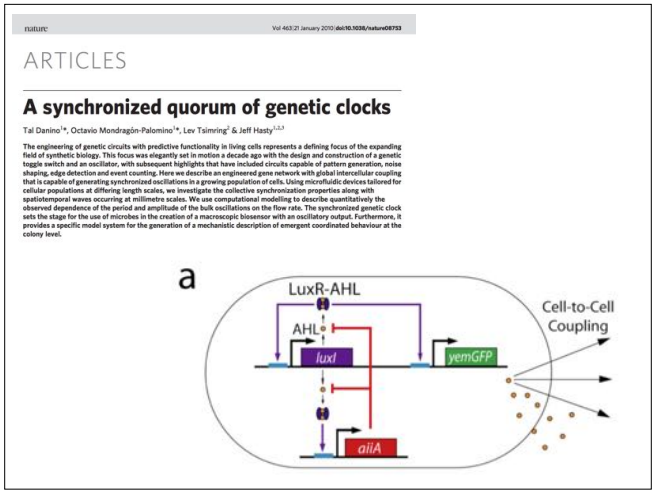
Processes of microbial pathogenesis or biofilm formation rely on cell-cell communication. In particular, the exchange of small molecules is a characteristic of quorum sensing. Here, bacteria produce and receive chemical signals that allow them to sense local population densities. These include some of the simplest known communication systems using molecules such as acyl homoserine lactone derivatives (AHL's), which are capable of directly passing in and out of cells. A single enzyme (e.g *Vibrio fisheri* LuxI) is required for synthesis, and a single regulatory protein (e.g. *Vibrio fisheri* LuxR) can dimerise on binding, bind to DNA, and activate gene expression.



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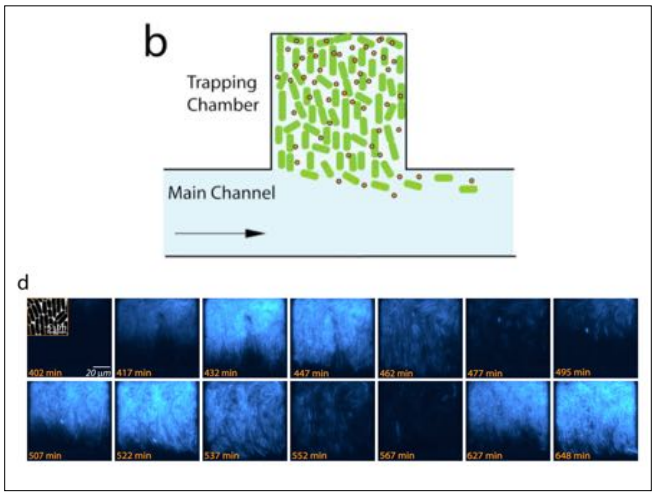
The Hasty lab use this simple communication system to create a non-cell autonomous oscillator. Presence of the inducer allows formation of an active LuxR complex, and triggers the production of Lux I. This in turn produces more AHL molecules in a positive feedback loop. The AHL molecules can diffuse from the cell and allow communication with neighbours. Presence of the active LuxR regulator drives expression of green fluorescent protein reporter gene, and a potent lactonase (aiiA). After a short delay due to the timing of gene activation, transcription and translation, active aiiA will destroy all AHL within the cell. This will turn off gene expression in the circuit, and a recovery period ensues before the cycle starts again.



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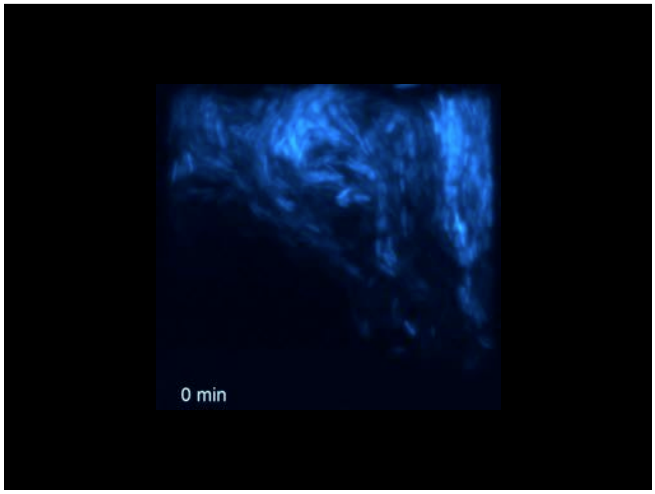
Small populations of cells carrying the circuit were contained in small chambers in microfluidic devices. Excess cells from the dividing population were washed away and GFP expression could be observed in the remaining population of cells. Rhythmic oscillations of GFP expression could be seen in time course observations.



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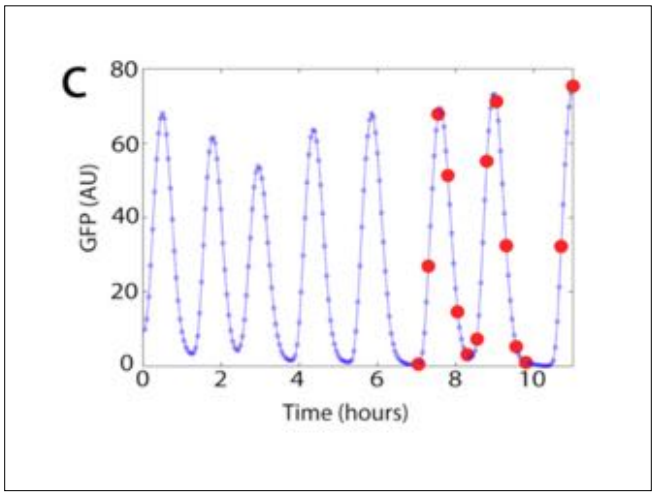
Video of oscillations



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GFP expression levels could be easily quantified. The simple oscillator showed regular period and amplitudes due to the quorate nature of the system.

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Video of a growing biofilm and spontaneous oscillations that act as travelling waves.

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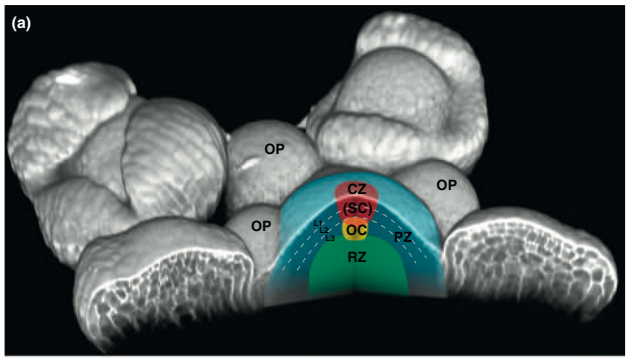
Travelling waves in an extended biofilm, showing different magnifications of the microfluidic track. The use of a simple communication system allows traffic of information across extended fields of cells.



Engineering multicellular architectures in plants

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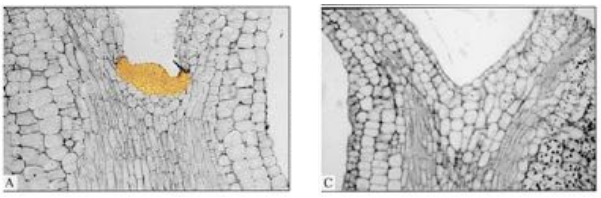
In order to reprogram plant systems, it will be necessary to include cell cell communication. This will be required to initiate, maintain and coordinate patterns of gene expression in a multicellular context. We will look at an example of manipulation of shoot growth in a higher plant.

WUSCHEL (WUS) is a homeodomain gene that is required for formation and maintenance of the shoot apical meristem in Arabidopsis.



wt

wuschel (wus)

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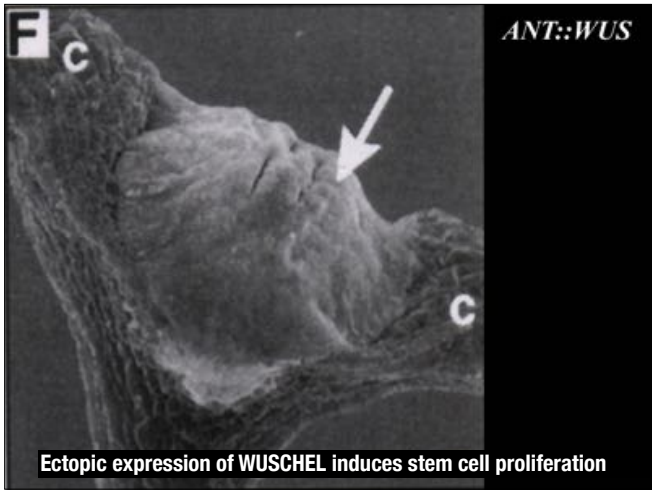
The growth of the apical meristem in high apical meristem in higher plants is controlled by opposing sets of regulatory proteins. We will look at two of these, as characterised in the model plant Arabidopsis. First, Wuschel is a homeodomain gene that is required for maintenance of the stem cells within the shoot apical meristem. In a wuschel mutant, the cells within the apical meristem fully differentiate, and the meristem terminates.



Ectopic expression of WUSCHEL induces stem cell proliferation

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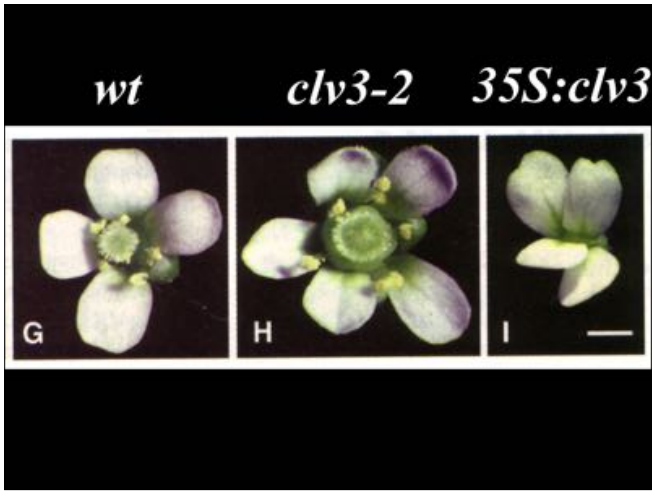
Overexpression of the Wuschel gene results in the hyper-proliferation of stem cells within the meristem. Wuschel is sufficient for the production of indeterminacy in targeted cells.



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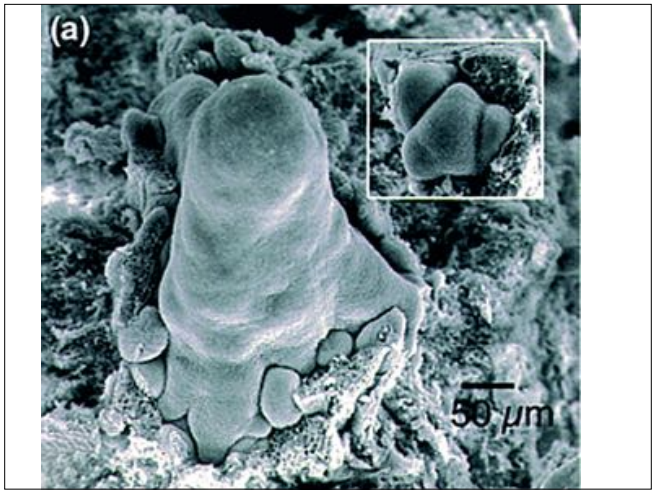
In contrast, the Clavata3 gene plays a role in suppressing the size of the meristem. In its absence the meristem enlarges (which can result in the oversize Arabidopsis flowers shown here). Overexpression of Clavata3 results in loss of meristem activity (and undersized flowers).



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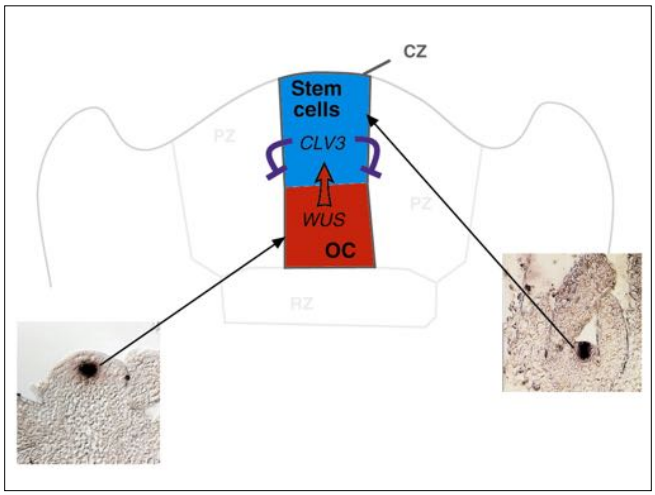
Scanning electron micrographs of wild-type (inset) and clavata3 mutant meristems. Loss of a clavata3 activity results in gross enlargement of this floral inflorescence meristem.



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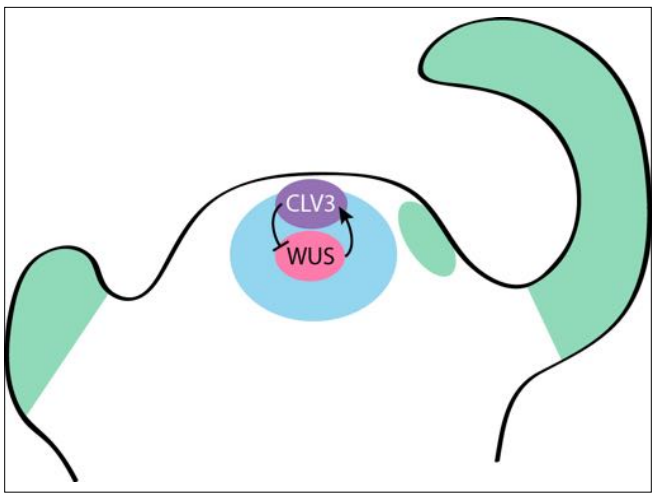
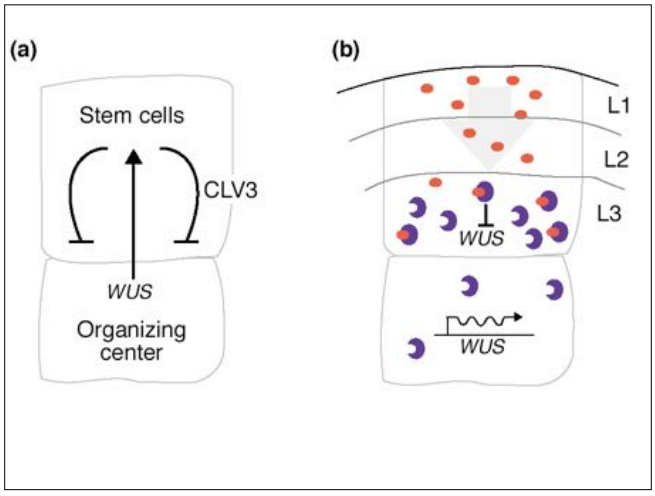
The expression of both Wuschel and Clavata3 is tightly localised to the shoot apex of the Arabidopsis plant. They possess overlapping expression patterns, with Clavata3 expression positioned to the outside of the meristem. Wuschel expression is localised inside the meristem.



23

Wuschel and Clavata3 form part of a feedback loop that regulates meristem size in Arabidopsis. Wuschel gene activity promotes cell proliferation, and Clavata3 acts in a negative feedback loop to suppress meristem growth.



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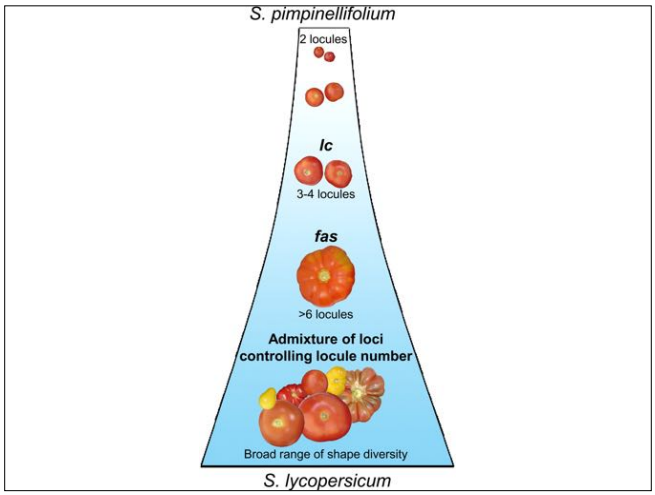
Clavata3 encodes a peptide that is produced on the outside of the meristem, diffuses to the cells on the inside, binds to receptors on these cells, and and inhibits Wuschel expression via the MAP kinase pathway. These mechanisms of action have been discovered through work with the model plant Arabidopsis. However, the same mechanisms are at work in most other higher plants.

25

The domestication of a crop plant like the tomato, has been accompanied by the selection and breeding of a wide variety of variants. These include plant varieties with profound differences in fruit size and shape, and plant architecture.



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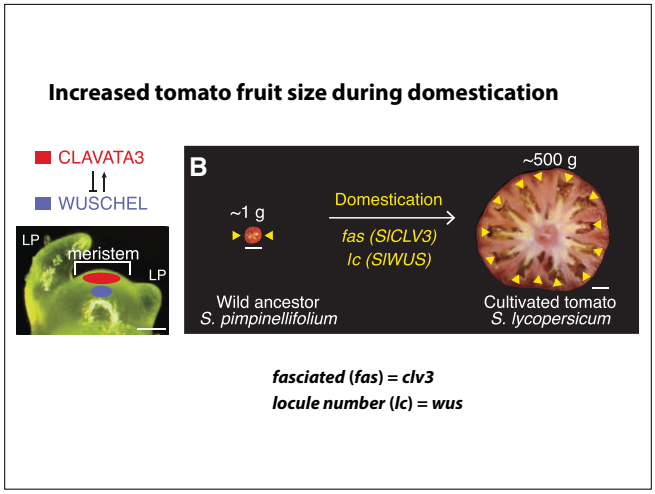
Modern tomato varieties emerged from wild Peruvian species (*Solanum pimpinellifolium*). Two mutant alleles which have played a major role in the breeding of large fruit sizes are *locule number* (*lc*) and *fasciated* (*fc*).



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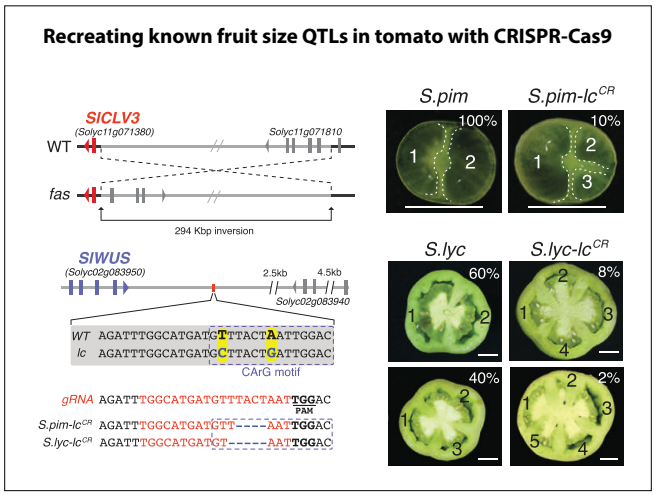
Fas encodes *Solanum lycopersicum* CLV3, and *Lc* encodes WUS. The conserved CLV3-WUS negative feedback circuit controls meristem and fruit size in tomato.
(LP, leaf primordia). (B) The fasciated (fas) and locule number (*lc*) fruit size QTL both contributed to increased tomato fruit size and locule number during domestication. Yellow arrowheads, locules.



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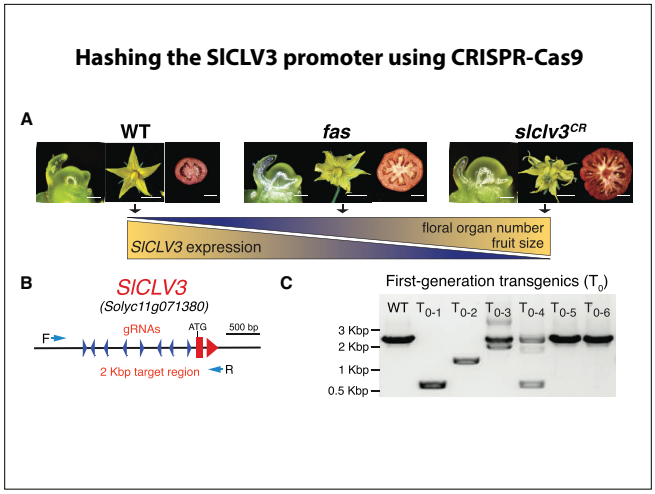
1. The fas mutation is caused by an inversion with a breakpoint 1 Kbp upstream of SlCLV3. (D) The lc QTL (red rectangle) is associated with two SNPs (in bold) in a putative repressor motif (CArG, blue-dashed square) 1.7 Kbp downstream of SlWUS.
2. CRISPR/Cas9-induced deletions in the CArG repressor motif (blue-dashed square) of Solanum pimpinellifolium (S. pim) and Solanum lycopersicum (S. lyc). The gRNA target sequence is highlighted in red and the PAM site underlined.
3. S.pim-lcCR plants produce fruits with more than two locules. S.pim-fasNIL S.pim-lcCR double mutants synergistically increase locule number.



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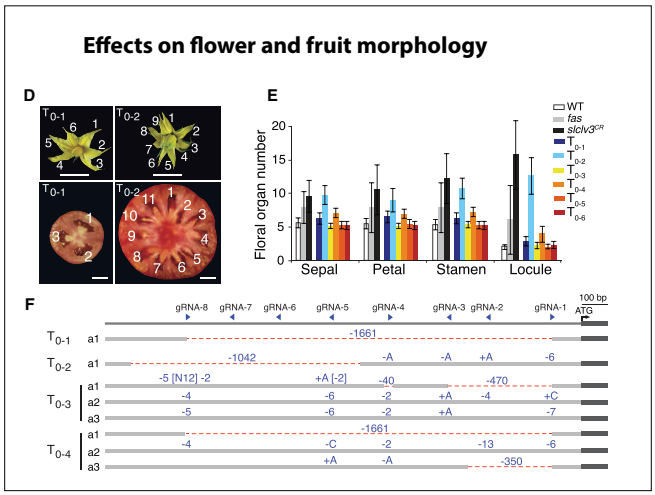
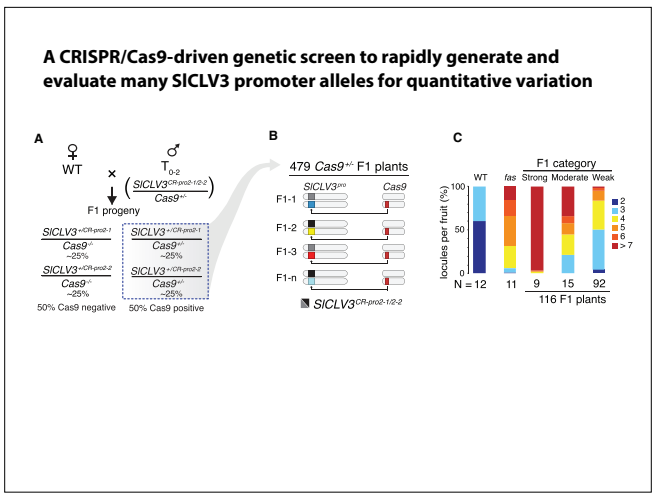
A. Model showing how an allelic series of SlCLV3 transcriptional alleles could provide a range of quantitative effects on floral organ number according to a simple linear relationship of reduced expression resulting in increased phenotypic severity. (WT, fas, and clv3CR are shown as reference points in this hypothetical continuous relationship).
B. Schematic of SlCLV3 promoter targeted by eight gRNAs (numbered blue arrowheads). Blue arrows, PCR primers.
C. PCR showing multiple deletion alleles in four T0 plants. Amplicons were obtained using primers spanning the entire target region.



30

Weak and strong effects on flower morphology and fruit size were observed among T0 lines. Number of floral organs and locules are indicated. (E) Quantification of floral organ number (mean ± SD; n>10) in T0, WT, fas, and slclv3CR plants. (F) Sequencing of SlCLV3 promoter alleles for all T0 plants. Deletions (–) and insertions (+) indicated by numbers or letters. T0-5 and T0-6 contained only WT alleles (data not shown). Blue arrowheads, gRNAs; a, allele.



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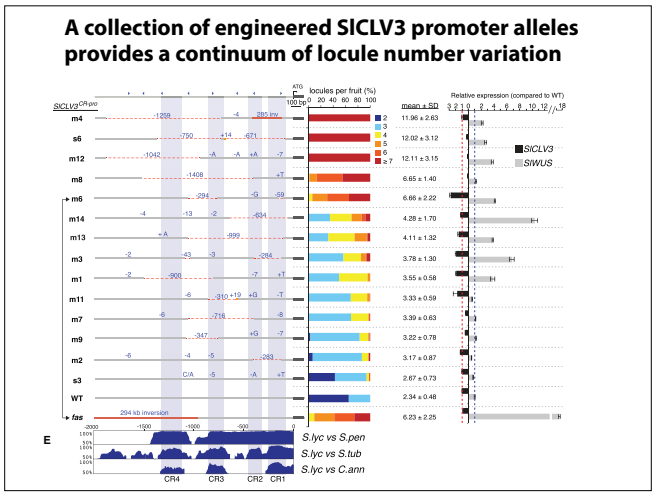
A. Crossing scheme for generating a sensitized F1 population heterozygous for an inherited allele and segregating for a CRISPR/Cas9 transgene (blue-dashed square). Expected segregation frequencies are indicated (%).
B. Model showing how Cas9 activity in Cas9+/ hemizygous plants creates new mutant alleles (colored boxes) by targeting the WT SlCLV3 promoter (SlCLV3pro) introduced from the cross. Alleles derived from T0-2 are shown as black or dark gray boxes. The transgene containing the CRISPR/Cas9 cassette (Cas9) is shown (red box).
C. Locule number for WT, fas, and F1 plants grouped into three phenotypic categories: strong, moderate, weak.



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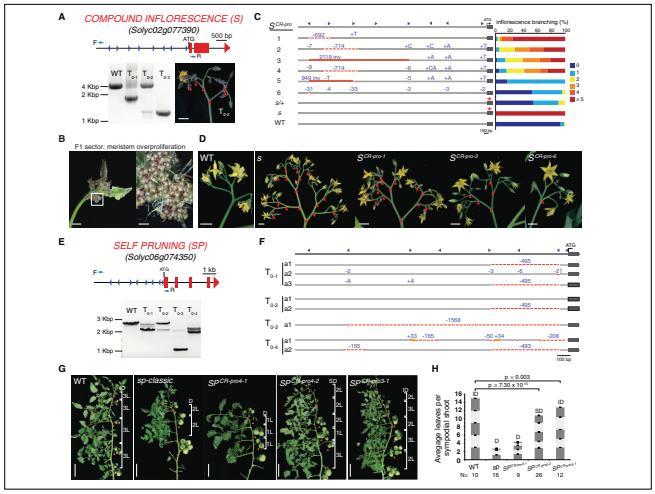
1. Sequences of 14 new SlCLV3pro alleles. Deletions (–) and insertions (+) indicated as numbers or letters. gRNAs, blue arrowheads. Parental F1s marked at right.
2. qRT-PCR of SlCLV3 and SlWUS from reproductive meristems for WT, fas, and each SlCLV3CR-pro allele. Dashed lines mark WT levels for SlCLV3 (red) and SlWUS (blue)



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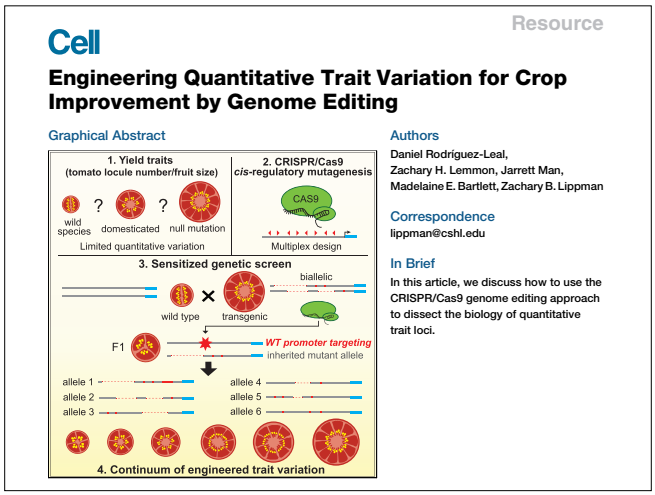
CRISPR/Cas9 Targeting of the Compound Inflorescence (S) and Self Pruning (SP) promoters results in a further range of modified inflorescence and plant architectures.



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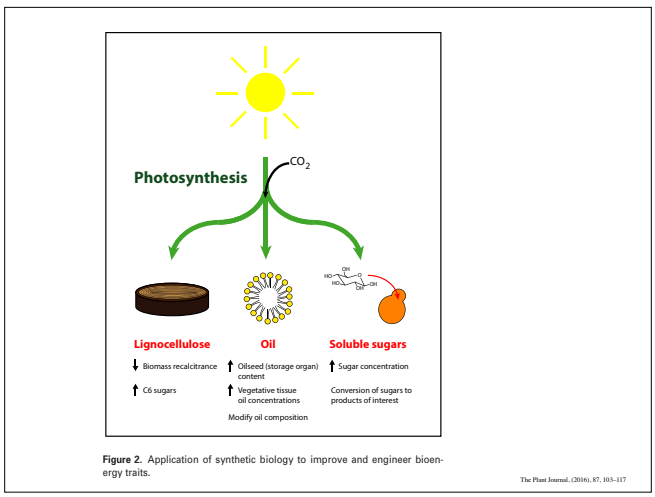
1. CRISPR/Cas9 targeting of a cis-regulatory motif recreated a domestication QTL.
2. CRISPR/Cas9 drove mutagenesis of promoters to create a continuum of variation.
3. Phenotypic effects were not predictable from allele type or transcriptional change.
4. Selected promoter alleles in developmental genes could improve yield traits.



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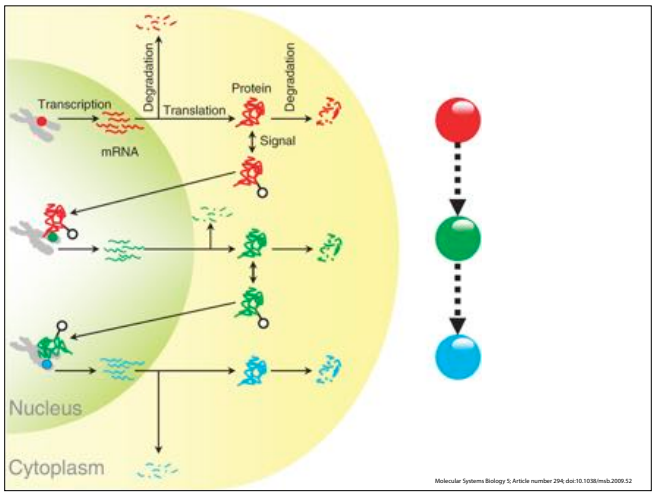
Can one also manipulate regulatory proteins in order to manipulate plant products that are produced by metabolic networks?



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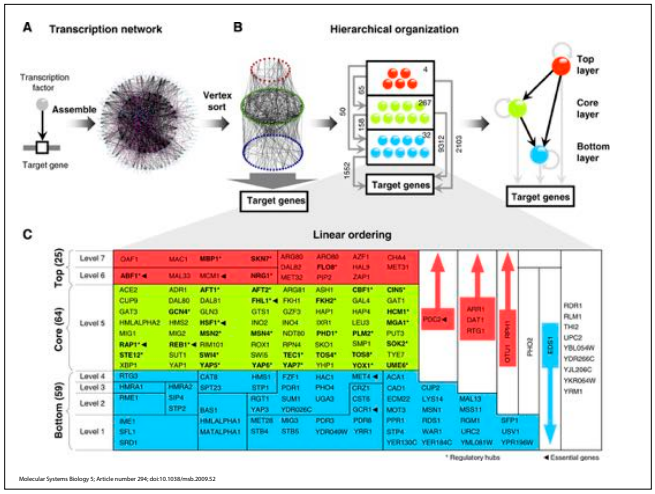
Transcriptional cascades underpin gene regulatory networks. Transcription factors (TFs), denoted as nodes in a network (red and green circles), represent several entities (gene, mRNA, and protein) and events (transcription, translation, degradation, etc) that are compressed in both space and time. The series of regulatory events can be conveniently represented as a node in the network, although this does not capture the dynamics of these entities and the biological processes.



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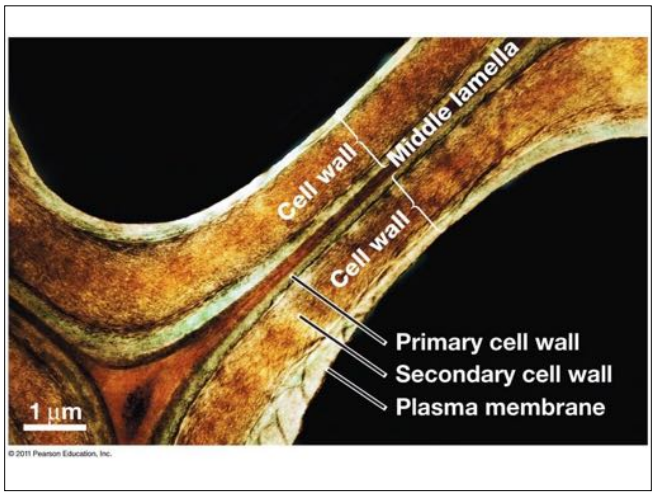
An example: hierarchical structure in the yeast transcription regulatory network. The organization of transcription factors in the network naturally clusters into three basic non-overlapping layers: the top (red), core (green), and bottom (blue). Thirty-two regulatory hubs are highlighted in bold and marked with a star (*), and nine essential TFs are marked with an arrow.



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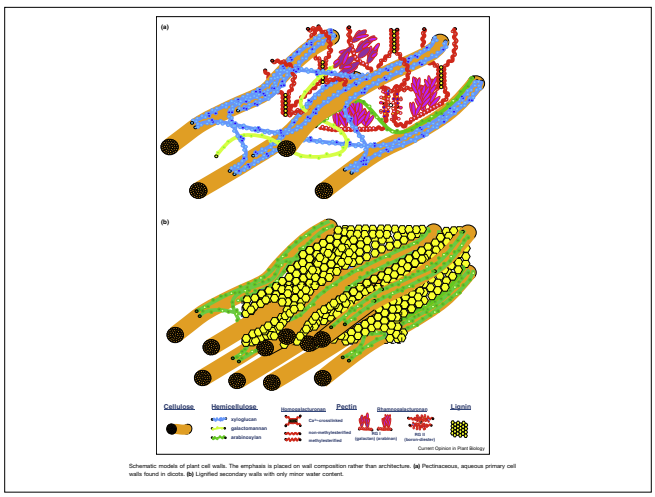
As an example, we will look at the engineering of transcription factors to modify cell wall composition in plants. The development of improved feedstocks for bioenergy production has focused on maximising primary cell wall and cellulosic material, and limiting amounts of lignin compounds during secondary wall growth.



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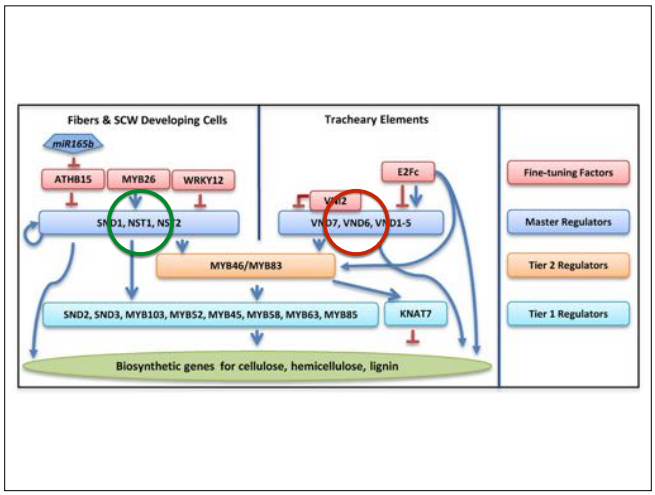
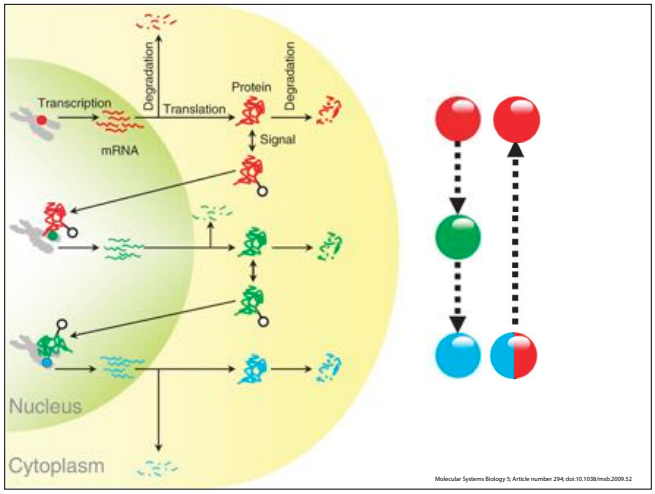
Primary cell walls are composed mainly of cellulose micro fibrils and cross-linked hemicellulose and pectins. During secondary wall deposition lignin is deposited which results in cell wall material that is chemically resistant, difficult to digest for biofuel production.



40

A hierarchical transcriptional network controls biosynthetic genes that are responsible for timing, synthesis and balance of different cell wall carbohydrates. This is been best characterised in Arabidopsis. Locque and colleagues have developed a technique for reprogramming this transcriptional hierarchy - and increasing the balance of resources devoted to (i) fibres and secondary cell wall material vs. (ii) lignified tracheary elements.



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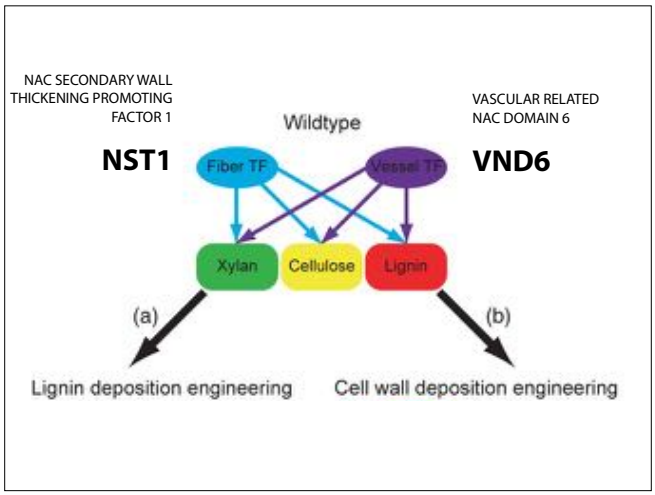
In this scheme, a gene from downstream in the regulatory hierarchy is identified. Its promoter (blue) is fused to a copy of the master regulator (red). Therefore when the master regulator is switched on during the normal course of development, there is a cascade of transcriptional control events that results in triggering of housekeeping genes, and in addition, triggering of a new copy of the master regulator. Results in expansion of the normal pathway.



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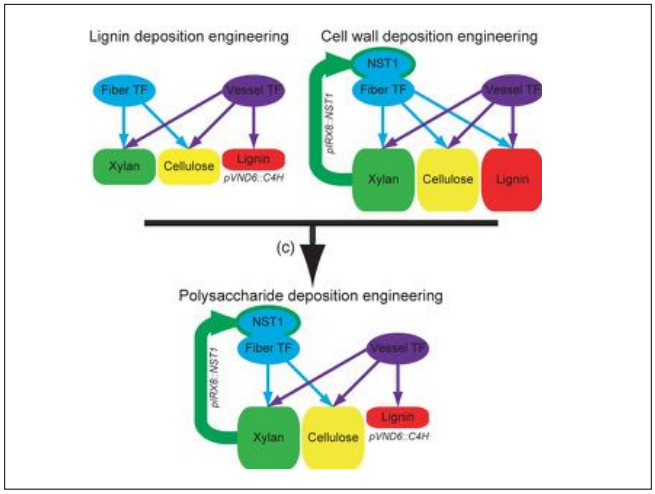
The two "arms" of vascular cell development are regulated by two transcription regulators, NAC Secondary Wall Thickening Promoting Factor 1 (NST1) and Vascular Related NAC Domain 6 (VND6). These genes show expression patterns that are limited to fibre and vessel cells, respectively.



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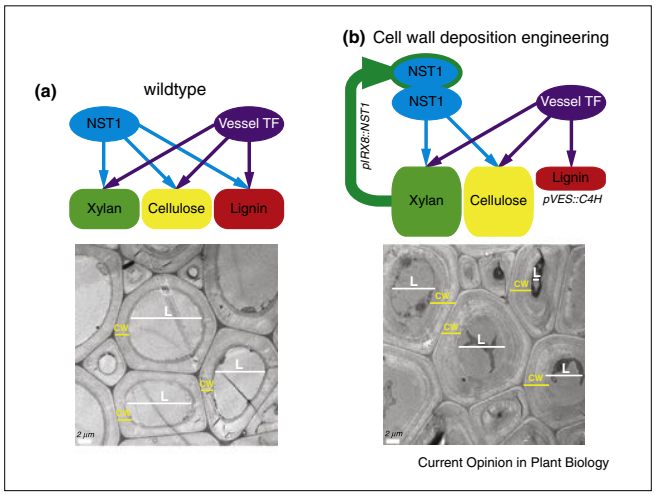
In a two-way approach, Loque and colleagues interfered with the synthesis and deposition of lignin. (i) The promoter of a key lignin gene, *C4H*, was replaced by the vessel-specific promoter of transcription factor *VND6* in a *c4h* mutant. This rewired lignin biosynthesis specifically for vessel formation while disconnecting C4H expression from the fibre regulatory network. (ii) The promoter of the *IRX8* gene, a secondary cell wall glycosyltransferase, was used to express a new copy of the fibre transcription factor *NST1*, and as the *IRX8* promoter is induced by *NST1*, this creates an artificial positive feedback loop



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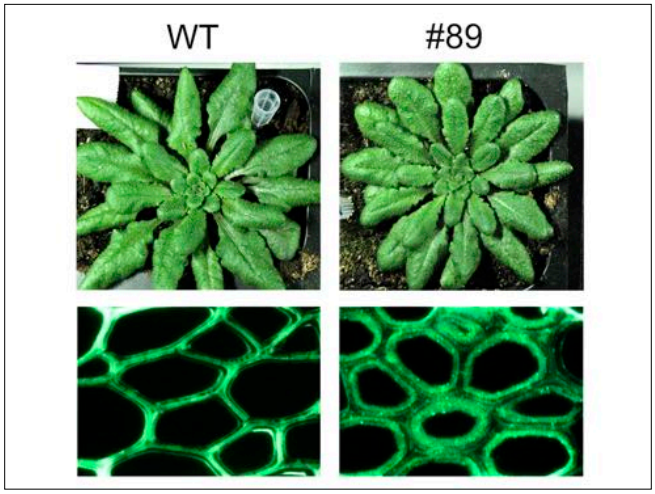
Engineering of increased cell wall density and decreased lignin in Arabidopsis. Schematic of simplified regulatory network controlling secondary cell wall biosynthesis in vessel and fibre cells in plants and images from wildtype (a) and engineered (b) Arabidopsis plants depicting interfascicular tissues composed of fiber cells. Engineered plants were generated from a c4h defective mutant (mutant affected in the second lignin biosynthesis step) that was transformed with the wild-type version of the mutated C4H gene driven by a vessel-specific promoter which rescued the negative effect of low lignin content. Generated plants were further transformed with a construct (pIRX8::NST1) that led to higher expression of master transcription factor controlling secondary cell wall biosynthesis in fiber cells (e.g. NST1).



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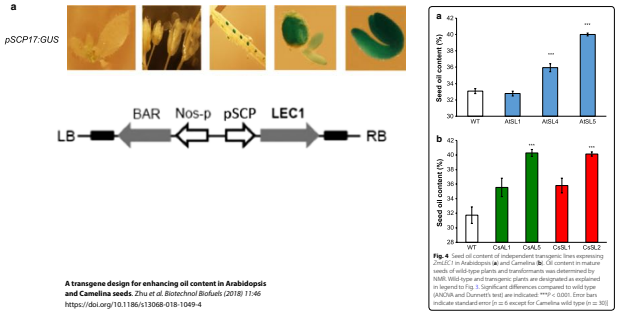
Production of transgenic plants with increased xylan content and decreased lignin content.

Engineering of LEC1 for increased oil content



A transgene design for enhancing oil content in Arabidopsis and Camelina seeds. *Zhu et al. Biotechnol Biofuels (2018) 11:46* https://doi.org/10.1186/s13068-018-1049-4

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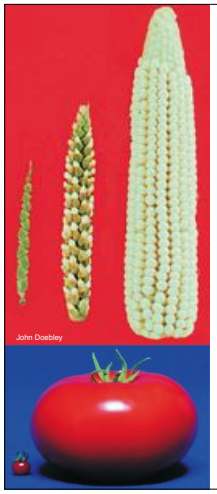
A similar approach has been used to elevate oil content in a Arabidopsis and Camelina plants by overexpression of the Leafy Cotyledon (Lec1) gene, using a downstream, seed specific promoter (pSCP).



Reprogramming plants

- Plants provide proven, global, low-cost technology for gigatonne scale bioproduction
- We need faster, simpler multicellular systems for engineering form and metabolism
- Synthetic biology offers breakout technologies

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Modern approaches to gene editing and reprogramming of the expression of master regulators allows rapid modification of plant architecture and other properties for crop improvement and possible domestication of new species.

Lecture 1: Genetic modification in agriculture and the advent of Synthetic Biology.
Lecture 2: Genetic circuits and genome scale DNA engineering.
Lecture 3: Engineered logic and the control of gene expression.
Lecture 4: Self-organisation and reprogramming of multicellular systems.

1. **Organisation of natural microbial populations**
2. **Coupling of genetic circuits using cell-cell signalling systems**
3. **Meristematic growth in plants**
4. **Tomato domestication**
5. **Creating new alleles with CRISPR**
6. **Reprogramming local regulatory networks**

Additional resources: **http://www.haseloff-lab.org** (Education)

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