Lecture 4: Radial Patterning and Intercellular Communication.

Summary:
Description of the structure of plasmodesmata, and the demonstration of selective movement of solutes and large molecules between cells. Regulatory proteins such as KNOTTED1 and SHORTROOT act non cell autonomously, and appear to move between cells.

Recommended reading:

Plasmodesmata and intercellular connectivity.
Cell walls separate individual plant cells. To enable essential intercellular communication, plants have evolved membrane-lined channels, termed plasmodesmata, that interconnect the cytoplasm between neighboring cells. A plant’s entire interconnected cell contents is termed the symplast, and the extracellular space is termed the apoplast.

Historically, plasmodesmata were viewed as facilitating traffic of low-molecular weight growth regulators and nutrients critical to growth. Evidence for macromolecular transport via plasmodesmata was solely based on the passage of plant virus particles through plasmodesmata during infectious spread. Now plasmodesmata are known to transport endogenous proteins, including transcription factors important for development. Two general types of proteins, non-targeted and plasmodesmata-targeted, have been shown to move through plasmodesmata channels. The size and subcellular location of proteins influence non-targeted protein movement. Superimposed on these cargo-specific parameters, the aperture of the plasmodesmata fluctuates between closed, open, and dilated. Closed plasmodesmata result in symplastic isolation, open plasmodesmata allow the traffic of small molecules, say less than molecular weight 1000, and dilated plasmodesmata allow the traffic of macromolecules such as proteins. Plasmodesmata alter their transport capacity temporally during development and spatially in different regions of the plant. It is increasingly apparent that plasmodesmata regulate the exchange of metabolites and signaling molecules during development, growth and pathogen response.
Architecture of plasmodesmata.
Plasmodesmata form channels containing two membranes. The plasma membrane between adjacent cells defines the outer limit of the channel. The axial center of the plasmodesmata, termed the desmotubule, is made up of appressed endoplasmic reticulum. The region between the desmotubule and the plasma membrane is the cytoplasmic sleeve, the major conduit through which molecules pass from cell to cell. The cytoplasmic sleeve likely contains components that regulate and facilitate plasmodesmatal transport; evidence for other transport routes, such as through the lumen or along the lipid bilayers of the desmotubule, is limited. Electron dense substructures line the cytoplasmic sleeve between the DT and the plasma membrane. Both globular particles and elongated spokes appear to interconnect these two membranes and may act to expand or contract the cytoplasmic space to increase or restrict transport. Cross-sectional views reveal that PD are subdivided into 2.5-nm diameter microchannels.

Plasmodesmatal regulation.
Regulation of PD transport may occur along their entire length or at the neck region by manipulation of structural components or deposition or removal of addition factors. Two possible mechanisms for rapidly constricting or expanding the PD channel have been suggested. First, the presence of actin and myosin along the length of PD provides for a possible contractile mechanism to regulate PD transport potential. Helically arranged actin filaments may link the desmotubule to the plasma membrane, contraction or relaxation of which would alter the aperture of the cytoplasmic sleeve. In fact, actin-disrupting agents increase movement of large dextrans through PD, whereas phalloidin, which stabilizes actin filaments, prohibits intercellular exchange. Another possible function of an actin-myosin-based mechanism is to serve as an elongated track to facilitate transport along the length of PD. Alternatively, PD aperture may be controlled by the contraction of centrin nanofilaments since centrin localizes to the neck region of PD. Calcium, possibly in concert with calreticulin, may regulate PD aperture by controlling the contraction of centrin nanofilaments.

Using fluorescent tracers to visualise intercellular connectivity.
Symplastic domains have been visualized within shoot apices of Arabidopsis, by monitoring fluorescent symplastic tracers. Tracers were directly microinjected into meristematic cells or
loaded through cut leaves and distributed via the vascular tissue to the apex. The distribution of dye to symplastically connected cells within vegetative apices indicated that there are symplastic domains within the shoot meristem.

The root of *Arabidopsis* is small, transparent and has a simple architecture - all of which makes it an attractive model system to observe local intercellular communication using fluorescent probes. The extent to which the constituent cells in the root meristem are symplastically coupled has been measured by direct injection of fluorescent tracer dyes and by dye permeability studies using near-infrared femtosecond pulses from a Titanium:Sapphire laser. Two-photon excitation is limited to the focal plane of the specimen. The laser was used to induce localised cell damage, and to allow the permeation of fluorescent propidium iodide dye into selected cells within the root meristem. The spread of the dye to adjacent cells through plasmodesmata could be directly measured. All undifferentiated cells are dye-coupled. When the dye is permeated into the central cells, it rapidly moved into the adjacent initials of the columella, cortex, pericycle and stele. However, when either of the initials were loaded with the dye, it never moved into any of the central cells. Amongst the epidermal cells, the differentiated hair cells are symplastically isolated. (1) There is different dye-coupling behaviour between quiescent centre cells and the neighbouring initials; (2) Cells in the root are coupled during stages at which the cell-lineage pattern is formed and become progressively secluded as they differentiate and the pattern is fixed.

Non-specific trafficking of proteins between source and sink tissues.
The green fluorescent protein (GFP) gene has been expressed in *Arabidopsis* and tobacco under the control of a promoter from the AtSUC2 sucrose-H+ symporter gene that limits expression to phloem companion cells. Analysis of the AtSUC2 promoter-GFP plants demonstrated that the 27-kD GFP protein can traffic through plasmodesmata from companion cells into sieve elements and migrate within the phloem. With the stream of assimilates, the GFP is partitioned between different sinks, such as petals, root tips, anthers, funiculi, or young rosette leaves. Eventually, the GFP can be unloaded symplastically from the phloem into sink tissues, such as the seed coat, the anther connective tissue, cells of the root tip, and sink leaf mesophyll cells. In all of these tissues, the GFP can move cell to cell by symplastic transport. The plasmodesmata of the sieve element-companion cell complex, as well as plasmodesmata into and within the analyzed sinks, allow trafficking of the 27-kD nonphloem GFP protein. The size exclusion limit of plasmodesmata can change during organ development.

Targeted movement of proteins through plasmodesmata.
GFP reporters have been used to demonstrate two forms of protein movement across plasmodesmata, non-targeted and targeted. Although leaves have generally been considered closed to non-specific intercellular transport of macromolecules, 23% of the cells were found to have plasmodesmatal channels in a dilated state, allowing GFP that was not targeted to plasmodesmata to move into neighbouring cells. GFP fusions that were targeted to the cytoskeleton or to the endoplasmic reticulum did not move between cells, whereas those that were localized to the cytoplasm or nucleus diffused to neighbouring cells in a size-dependent manner. Superimposed upon this non-specific exchange, proteins that were targeted to the
plasmodesmata could transit efficiently between 62% of transfected cells. A significant population of leaf cells contain plasmodesmata in a dilated state, allowing macromolecular transport between cells. Protein movement potential is regulated by subcellular location and size.

Intercellular traffic of homeodomain proteins involved in meristem maintenance.

The maize homebox gene KNOTTED1 (KN1) is expressed in vegetative and floral meristems and is down-regulated at the site of primordia formation. Members of this gene family are expressed early in embryogenesis, providing molecular markers for meristem initiation. Ectopic expression of either KN1 or a related Arabidopsis gene, KNAT1, causes dramatic alterations in leaf morphology. Meristems form on the leaf, producing small shoots. Misexpression of KN1 confers different phenotypes in simple and compound leaves. KN1 induces leaf malformations but fails to elicit leaf ramification in plants with inherent simple leaves such as Arabidopsis, tobacco or mutant tomato plants with simple leaves. However, up to 2000 leaflets, organized in compound reiterated units, are formed in tomato leaves expressing KN1. Moreover, the tomato KN1 ortholog, unlike that of Arabidopsis, is expressed in the leaf primordia. The two alternative leaf forms are conditioned by different developmental programs in the primary appendage that is common to all types of leaves.

KNOTTED1 acts non cell autonomously.

The knotted1 mutation was first isolated as a dominant mutation in maize and produced a “knotted” leaf phenotype. The mutation was located in the promoter of the kn1 gene, and resulted in ectopic expression of the gene in the vascular tissues of the leaf. This aberrant expression produced disturbed cell proliferation in the tissues surrounding the vasculature, i.e. in cells not expressing KN1. Similar evidence for non cell autonomous behaviour of the KN1 gene was seen in wild-type maize embryos and meristems. Comparison of the patterns of KN1 mRNA expression and protein accumulation shows that they do not coincide. While expression of the mRNA is excluded from the outer L1 cells in the embryo and shoot meristem, localisation of the KN1 protein shows that it is (i) nuclear localised, and (ii) is present at high levels in the L1 layer.
Intercellular movement of KNOTTED1 protein and mRNA shown by microinjection.

Microinjection studies showed that plasmodesmata facilitate the cell-to-cell transport of KNOTTED1. KNOTTED1 protein can also mediate the selective plasmodesmal trafficking of KN1 sense RNA. Mutant forms of the protein are not capable of active transport between cells. Cell fate is determined at least in part by intercellular signals, allowing cells to responding to cellular position as well as lineage. One of the mechanisms that enables the intercellular communication appears to involve transfer of informational molecules, such as KNOTTED1 protein and mRNA, through plasmodesmata.

**Fig. 4.** KN1 protein-mediated cell-to-cell transport of kn1 RNA-TOTO. (A) Coinjection of KN1 and kn1 sense RNA-TOTO into a tobacco mesophyll cell revealed movement of the kn1 sense RNA-TOTO into cells in the vicinity of the injected cell (arrow) after 1 min. IAS, intercellular air space. (B) Kn1 antisense RNA-TOTO failed to move out of the target cell when coinjected with KN1. The false-color image was taken 15 min after coinjection, at which time fluorescence had accumulated in what appeared to be the nucleus (arrowhead). (C) Tobacco mesophyll cell coinjected with KN1 and CMV RNA-TOTO (18 after 15 min. Fluorescence remains confined to the injected cell. Although KN1 would presumably have trafficked into surrounding cells, it failed to transport the CMV RNA-TOTO. (D) Coinjection of CMV 3a movement protein and kn1 sense RNA-TOTO into a tobacco mesophyll cell (arrow) resulted in extended CMV 3a movement protein–mediated transport of the kn1 sense RNA-TOTO into the surrounding cells 2 min after injection. Scale bar in (A), 35 μm; (B), 20 μm; (C), 25 μm; and (D), 50 μm.

Non autonomous cell signalling in the root meristem.

Common initial cells give rise to both the cortex and endodermis in the Arabidopsis root meristem. Each initial goes through a repeated set of (i) proliferative anticlinal, and (ii) asymmetric periclinal cell divisions to generate the cortex and endodermis files. Radial patterning mutants short-root scarecrow have been identified that posses defects in the division and/or specification of the endodermis and cortex.
Patterning mutants that affect formation of the endodermis and cortex.
Both the SHORT-ROOT (SHR) and SCARECROW (SCR) genes encode members of the plantspecific GRAS family of putative transcription factors, and defects in these two genes affect radial patterning of the endodermis and cortex.

SCARECROW.
The *scarecrow* mutation results in roots that are missing one cell layer owing to the disruption of the asymmetric division that normally generates cortex and endodermis. Tissue-specific markers indicate that a heterogeneous cell type is formed in the mutant. SCR is expressed in the cortex/endodermal initial cells and in the endodermal cell lineage. Tissue-specific expression is regulated at the transcriptional level. Analysis of the shoot phenotype of *scr* mutants revealed that both hypocotyl and shoot inflorescence also have a radial pattern defect, loss of a normal starch sheath layer, and consequently are unable to sense gravity in the shoot. Analysis of *SCR* expression and the mutant phenotype from the earliest stages of embryogenesis revealed a tight correlation between defective cell divisions and *SCR* expression in cells that contribute to ground tissue radial patterning in both embryonic root and shoot.

SHORTROOT
*SHORTROOT* is responsible for specifying endodermis. It is also needed for transcriptional activation of *SCR* and division of the initial daughter cell to form and cortex and endodermis. As would be expected, *SCR* is expressed in the initial daughter prior to division. Surprisingly, *SHR* RNA is found not in these cells, but only in the pericycle and vascular tissue internal to these cells. This argues for a non-cell-autonomous mode of action by *SHR* in order to activate *SCR* transcription.

Intercellular movement of SHORTROOT protein.
Ectopic expression of *SHR* results in supernumerary layers of cells external to the root vascular tissue. Most of these additional cells exhibit endodermal markers indicating that SHR is sufficient for cell division and cell specification. This suggests a simple pathway for radial patterning in which SHR is made in the centrally located vascular tissue and pericycle,
and that it signals the adjacent tissue to divide and specifies the inner layer of the newly divided cells as endodermis. The apparent movement of SHR-GFP proteins suggests that this signalling occurs by direct traffic of the SHR protein. Shoot phenotypes in scr and shr mutants as well as expression data suggest that a similar pathway acts in the upper parts of the plant to specify the cell layer surrounding vascular bundles.

Left: SCARECROW promoter driving GFP expression in the Arabidopsis root meristem. Right: SHORTROOT promoter driving GFP expression. ep=epidermis, co=cortex, en=endodermis, CEI=cortex/endodermis initial, QC=quiescent centre, st=stele.

Left: SHORTROOT promoter driving expression of a SHORTROOT-GFP fusion protein. Inset: expression pattern of SHORTROOT promoter. The SHORTROOT-GFP protein is capable of movement to adjacent cells in the endodermis, initials and quiescent centre. Right: Ectopic expression of the SHORTROOT protein causes proliferation of the endodermis, marked by a β-glucuronidase reporter gene.

Similar mechanisms for intercellular transport in animal and plant cells?