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Plasmodesmata Formation: Poking Holes in Walls with *ise*

Secondary plasmodesmata are cytoplasmic channels connecting adjacent plant cells that arise after cell division. How membrane-delimited channels penetrate cell walls is unknown, but now two genes, *ISE1* and *ISE2*, are shown to be required for pathways that limit their formation.

Dong-Keun Lee
and Leslie E. Sieburth

Cell–cell communication is central for developmental decision making. In plants, an important vehicle for cell–cell communication is plasmodesmata. These channels provide a cytoplasmic bridge between adjacent cells; their structure, still under intense investigation, includes a centrally located desmotubule (a narrow strand of endoplasmic reticulum) surrounded by a cytoplasmic sleeve and attendant proteins, and bounded by plasma membrane (Figure 1A). Plasmodesmata carry out selective cell-to-cell trafficking of proteins and mRNAs as well as non-selective trafficking of small molecules, including nutrients and hormones (reviewed recently in [1,2]). Both types of transport are developmentally regulated. One element of regulation is the control of size exclusion limit. During plant development, sets of coupled cells (called symplastic domains) are dynamically regulated [3–8]. However, the biogenesis of plasmodesmata and developmental regulation of plasmodesmata-based movement are still largely unknown.

There are two distinct pathways for the biogenesis of plasmodesmata (Figure 1B). Plasmodesmata can arise from remnants of endoplasmic reticulum left within the phragmoplast of a dividing cell, and ones formed this way are called primary plasmodesmata. By contrast, secondary plasmodesmata arise independently of cell division; they are inserted into a pre-existing cell wall by a process requiring cell wall thinning and membrane insertion, presumably in conjunction with deposition of secreted cell wall material [9,10]. Although the hormone cytokinin has been shown to increase secondary plasmodesmata production [11], the molecular basis for secondary plasmodesmata biogenesis remains completely unknown. A paper in this issue of *Current Biology* from Burcher-Smith *et al.* [12] makes an important step toward dissecting this pathway. Two genes previously shown to be required for normal *Arabidopsis* embryogenesis and for embryonic symplastic domain establishment — *ISE1* and *ISE2* — have now been shown to be required for negative regulation of secondary plasmodesmata numbers and structure.

The structure of plasmodesmata ranges from simple, characterized by a single sheath of cytoplasm, to complex, characterized by branched, H-shaped, and twinned structures (Figure 1A). These structures also appear to be developmentally regulated, as young tissues commonly have simple plasmodesmata, with complex plasmodesmata arising later, after cell expansion [9]. The molecular basis for forming these distinct plasmodesma morphologies is also unknown, but again, *ise1* and *ise2* might provide clues, as both mutants have higher proportions of the complex plasmodesmata [12].

The identification of *ise1* and *ise2* resulted from the screening of *Arabidopsis* embryos for mutants with defects in the regulation of symplastic domain size [3]. Isolated torpedo-stage embryos were incubated in fluorescent tracers, e.g. a 10 kDa fluorescent-conjugated dextran, which infiltrated through breaks. Normal mid-torpedo embryos establish distinct symplastic domains by reducing their plasmodesmata size exclusion limit; however, *ise* mutants (which stands for Increased Size Exclusion limit) continued to allow free movement of the 10 kDa tracer.

A connection between ISE1 and ISE2 functions and complex plasmodesmata formation came about from an in-depth transmission electron

microscopy analysis of *ise1* and *ise2* mutants reported by Burch-Smith *et al.* [12] in this issue. Disruption of *ISE1* and *ISE2* causes severe developmental defects, necessitating analysis of embryos. Two surprising findings were made: wild-type embryos were found to produce the complex twinned and branched class of plasmodesmata in a developmentally regulated manner, and in *ise1* and *ise2* mutants, the relative abundance of complex plasmodesmata was strongly increased. To address whether defects in *ise1* or *ise2* mutants had a differential effect on primary and secondary plasmodesmata, the group required a system in which cell lineage would be highly predictable. This led them to examine leaves, which arise at the flank of shoot apical meristems. In dicotyledonous plants, the shoot apical meristem's outer two layers (L1 and L2) both undergo largely anticlinal divisions (Figure 2) [13]. The L1 gives rise to the leaf epidermis, while the mesophyll largely comes from the L2. This means that plasmodesmata connecting the epidermis and mesophyll cells must arise *de novo*, that is, by the secondary plasmodesmata pathway.

Tobacco and viral-induced gene silencing were used to knock down *ISE1* and *ISE2* expression, and this knock-down tissue was used for electron microscopy. By comparing plasmodesma density and structure between adjacent epidermal cells (primary plasmodesmata) and between epidermal and sub-epidermal mesophyll cells (secondary plasmodesmata), the authors found increased numbers of secondary plasmodesmata and an increased proportion of plasmodesmata with complex structures.

Because *Arabidopsis ise1* and *ise2* mutants were isolated based on altered plasmodesmata-mediated transport (excess transport), transport in the tobacco *ISE1* and *ISE2* knock-downs was also tested. GFP-based tracers introduced into individual cells moved much further in the knock-down plants, both between epidermal cells and from the epidermis into the mesophyll cells. These observations indicated roles for *ISE1* and *ISE2* in plasmodesma numbers, structure, and movement, and suggested that plasmodesmata with complex structures might allow higher rates of movement.

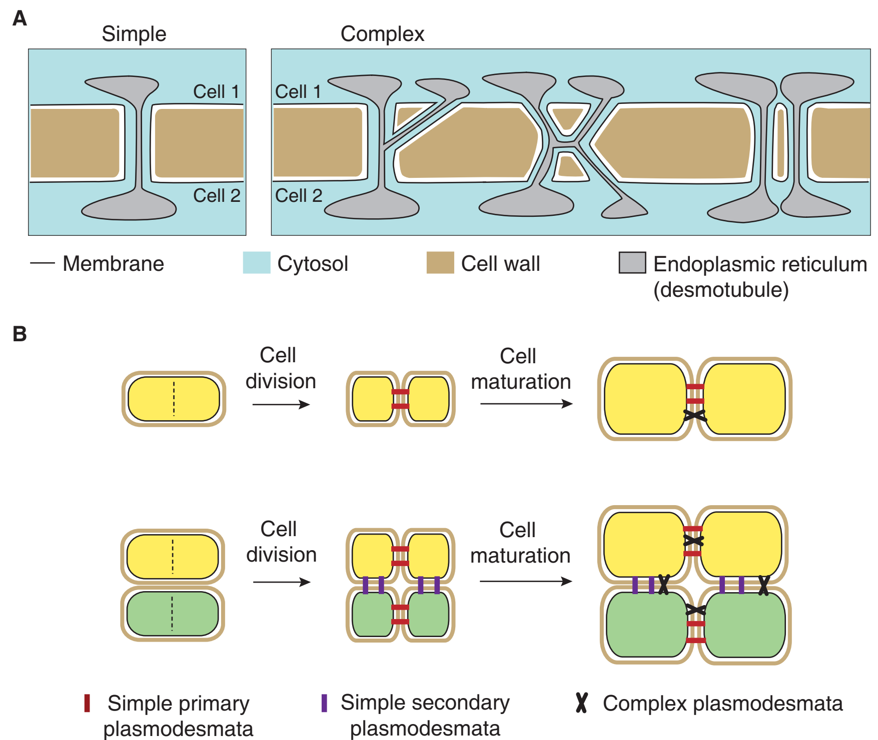

Figure 1. Structure and origin of plasmodesmata.

(A) The structure of plasmodesmata ranges from simple to several different complex forms, including branched, H-shaped, and twinned. (B) Two types of plasmodesmata are defined by their origin. Primary plasmodesmata are generated during cell division, whereas secondary plasmodesmata develop entirely *de novo* in existing cell walls . Both primary and secondary plasmodesmata tend to be simple early in development, and to develop into complex structures later.

Insight into the genetic control of plasmodesma structure and transport has also come from analysis of the *Arabidopsis gat1* (for GFP Arrested Trafficking 1) mutant [14]. Like *ise1* and *ise2*, *gat1* mutants have an increased proportion of complex plasmodesmata, but in contrast to *ise1* and *ise2*, plasmodesma transport is reduced in *gat1*. These different results indicate that there is no simple relationship between plasmodesma structure and transport rates.

The characterization of the *gat1* mutant also highlighted another emerging player in plasmodesma transport: callose, which is a cell wall polysaccharide. Callose has been known to be deposited adjacent to the plasmodesma's neck region [15], and a candidate for callose regulation is the degrading enzyme β-1,3-glucanase. This protein localizes to plasmodesmata, and its ectopic expression leads to both reduced callose deposits and higher plasmodesma transport; β-1,3-glucanase mutants have more callose and reduced transport [16,17]. Both callose and H_2O_2 were found to be increased in the *gat1* mutant. *GAT1* encodes a thioredoxin, which has been proposed to function in redox regulation of callose deposition. The elevated transport rates in *ise1* and *ise2* mutants suggests that callose might be relatively depleted, but so far this is not known.

Increased secondary plasmodesmata formation in *ise1* and *ise2* mutants offers the tantalizing possibility of molecular insight into this complex process. However, molecular identification of these genes failed to make any clear prediction. *ISE1* encodes a mitochondrially localized DEAD-box RNA helicase [18] and *ISE2* encodes a cytoplasmic DEVH-box RNA helicase that localizes to cytoplasmic stress granules [19]. The diverse processes carried out by ISE1 and ISE2 suggest that their roles in secondary plasmodesmata formation might be indirect.

Formation of secondary plasmodesmata is a perplexing

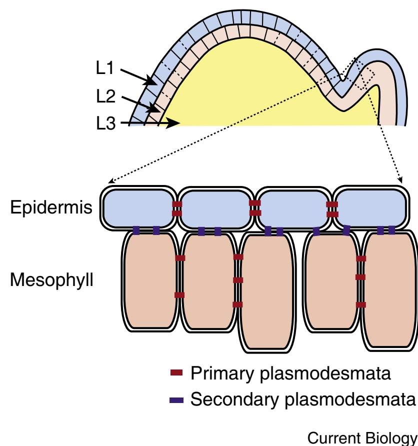

Figure 2. A system for specific monitoring of secondary plasmodesmata.

The shoot apical meristem (SAM) is composed of three clonally distinct layers, which in dicots are called L1, L2, and L3. Epidermal L1 and sub-epidermal L2 undergo predominantly anticlinal cell divisions. Leaf primordia are recruited on the flanks of the SAM, and the epidermis derives from L1 cells and mesophyll is predominantly derived from L2 cells. This means that the leaf epidermal cells connect to each other through primary plasmodesmata, but their connection to the mesophyll is exclusively through secondary plasmodesmata.

process. Somehow, membrane delimited channels penetrate across the cell wall, and their symmetric organization with respect to the joined cells would appear to require cell–cell communication [9]. Chimeric plants, in which loss of *ise1* or *ise2* was limited to only L1 or only L2, might provide useful insight into the direction and sufficiency of putative signals regulating secondary plasmodesmata formation.

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Working Memory: The Importance of Theta and Gamma Oscillations

Working memory is the ability to actively hold information in the mind. Recent results demonstrate that working memory is organized by oscillatory processes in the theta and gamma frequency range.

John Lisman

When told a phone number, how do we hold it in our mind? Experiments suggest that this process, termed working memory, depends on the persistent firing of neurons [1]. There is converging evidence that this persistent firing has an oscillatory character and that the frequencies involved are in the theta (4–8 Hz) and gamma (30–100 Hz) range. Two recent papers [2,3] add to this evidence. Fuentemilla *et al.* [2] reported recently in *Current Biology* that the sites where oscillations occur depend on the particular information stored. Furthermore, the firing of individual cells tends to occur at a particular phase of the theta oscillation [3]. Importantly, both studies show a relationship of these oscillatory processes to successful memory recall, indicating a causal role of the oscillations in the memory process.

It has long been clear that working memory, also termed short-term memory, is fundamentally different from long-term memory. Whereas the amount of information that can be stored in long-term memory is very large, the amount of information that can be stored in short-term memory is limited; for example, approximately seven digits can be held in short-term memory [4]. Physiological experiments strongly suggest that long-term memory is stored by changes in the strength of synapses, whereas