Strapline: Plant cytokinesis

Title (36c): Phragmoplast navigator needs high IQ

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Standfirst* (212c/27w)*: Polarity cues direct tissue patterning by defining the cell division plane. Proteins containing IQ67 calmodulin-binding domain govern cell division by establishing and maintaining cell polarity during cytokinesis.

Main text (900w):

Land plants partition daughter cells during cytokinesis by a membrane compartment filled with callose and other oligosaccharides known as cell plate. The cell plate is assembled by the phragmoplast, a structure composed of cytoskeletal polymers, membranes, and associated cytosolic proteins. Most cell plates exceed the phragmoplast size, with some spanning a length of 1 mm. This means that the phragmoplast should expand from the space between the daughter nuclei all the way to the parental cell wall (**Figure 1A**). While all organelles move along cytoskeletal tracks or hitchhike cytoplasmic streaming, the phragmoplast paves its own way, making it the largest known intracellular structure capable of self-propelled directional motility. This motility is guided by a bipartite navigation system. One part is the cortical division zone, a fine strip of the plasma membrane with adjacent cell wall that possesses a unique molecular composition (**Figure 1A**). From late G2 and until nuclear envelope breakdown, this region acquires a specialized microtubule array, the pre-prophase band. The cortical division zone defines the division plane in response to chemical and mechanical polarity cues that couple cell division patterns with plant morphogenesis. The second part of the navigation system is a microtubule-based engine within the phragmoplast that follows signals generated by the cortical division zone (**Figure 1B**). What these signals are and how they guide the engine remain unknown.

Our progress in understanding phragmoplast navigation is hindered by limited information about the composition of the cortical division zone. In this issue, Kumari et al. report that three members of the plant-specific IQ67 domain protein family—IQD6, IQD7, and IQD8 (IQD6,7,8)—localize to (i) the plasma membrane during interphase; (ii) the cortical division zone from late G2 until the end of cytokinesis; and (iii) phragmoplast microtubules, and the cell plate during cytokinesis[1]. Strong triple knockdown of the corresponding genes causes loss or disorganization of the preprophase band, deviation of the phragmoplast from the correct division plane, and loss of directional phragmoplast expansion (**Figure 1C**). Collectively, these phenotypes indicate defects in the navigation system.

IQD6,7,8 join a small group of known cortical division zone proteins[2]. Furthermore, IQD8 interacts with two of them, Phragmoplast Orienting Kinesin (POK) and Pleckstrin Homology GTPase Activating Protein (PHGAP), and is necessary for localization of PHGAP at the cortical division zone. IQD6 and IQD7 interact only with POK. Thus, IQD6,7,8 maintain molecular integrity of this region and contribute to governing both orientation and assembly of the preprophase band. What makes these proteins exciting is that the IQ67 domain, a run of 67 amino acids with two to three copies of the IQ motif, IQxxxRGxxxR or of its more relaxed

version [I,L,V]QxxxRxxxx[R,K], binds calmodulin in plants and animals[3]. The presence of IQ-domain proteins in the cortical division zone supports a role for Ca^{2+} signalling in phragmoplast navigation.

The phragmoplast could be navigated by a bridle of actin filaments or microtubules within cytoplasmic strands that connect the phragmoplast leading edge with the cortical division zone. Another mechanism could be chemical signals (**Figure 1B**). The strongest evidence for the latter is cell plate assembly without direct contact with the parental cell wall during endosperm cellularisation. What chemicals contribute to the long-range signalling during cytokinesis remains unknown. Ca^{2+} has been a long-term suspect since original identification in the cell plate by electron microscopy[4]. Incidentally, Ca^{2+} is essential for the catalytic activity of callose synthase, which localizes in the cell plate and produces the main cell plate oligosaccharide callose[5]. Subsequently, the existence of Ca^{2+} signalling in the phragmoplast was supported by analysis of Kinesin-like Calmodulin-Binding Protein (KCBP). Association with calmodulin in the presence of Ca^{2+} inhibits KCBP binding to microtubules *in vitro*[6]. KCBP resides in the cortical division zone[7] and binds microtubules distal to the cell plate[8], suggesting a Ca^{2+} gradient with the highest concentration in the phragmoplast midzone.

The next crucial step in understanding phragmoplast navigation will be determining whether IQD6,7,8 are Ca^{2+} effectors and how Ca^{2+} impacts their activity. Other members of the IQD family in *Arabidopsis thaliana* and *Oryza sativa* function as platforms for assembling complexes containing diverse molecules that include tubulin, microtubule binding proteins, actin-binding proteins, protein kinases, and membrane-bound proteins[3]. For example, *O. sativa* IQD protein GW5 binds and inhibits activity of glycogen synthase kinase 2 (GSK), a homologue of Arabidopsis BRASSINOSTEROID INSENSITIVE2 (BIN2) kinase, leading to up-regulation of downstream brassinosteroid signalling[9]. IQD6,7,8-PHGAP-POK complexes could recruit signalling proteins in the cortical division zone in a Ca^{2+}-dependent manner.

Another significant challenge is specifying which of the many IQD6,7,8 activities are essential for phragmoplast navigation. Considering their strong expression during interphase, IQD6,7,8 cold function in both establishing the cell division plane during G2 and maintaining integrity of the cortical division zone during cell division. Furthermore, IQD6,7,8 localize to phragmoplast microtubules and the cell plate. Over-expression of many IQDs causes stabilization of microtubules[3]. Consequently, IQD6,7,8 could regulate microtubule stability through interaction with other proteins or direct binding to microtubules. Another member of IQD family, IQD2, cooperates with NETWORKED3 in linking microtubules and microfilaments to endoplasmic reticulum (ER) and regulating ER morphology[10]. Plausibly, IQD6,7,8 function in connecting microtubules and microfilaments to the cell plate.

Solving the riddle of phragmoplast navigation will not only demonstrate how the cell division plane is governed by the mechanical and chemical cues, but also shed light on the biggest mystery in plant development – establishment and maintenance of polarity at all scales. Discovery by Kumari et al. takes us one leap closer to this goal.

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Competing interests

The author declares no competing financial interests.

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Figure 1. IQD6,7,8 proteins function in phragmoplast navigation.

A, The phragmoplast forms between daughter nuclei at the beginning of cytokinesis. Once the cell plate is mechanically strong, phragmoplast microtubules depolymerize and freed tubulin is used to construct phragmoplast at the cell plate edge leading to the next round of cell plate synthesis. The process continues until cell plate fuses parental cell wall in the region occupied by the cortical division zone. Then the phragmoplast disappears. Position of the cortical division zone is marked by the preprophase band from late G2 and until the nuclear envelope breakdown. After preprophase band disassembly, the cortical division zone maintains unique molecular composition.
B, Events in the regions marked as black rectangle in **A**. The phragmoplast engine consists of microtubule polymerization at the leading zone and depolymerization at the lagging zone. Cytokinetic vesicles containing cell plate building blocks move along microtubules. Microtubules depolymerize in the lagging zone upon cell plate reaching sufficient mechanical strength. Phragmoplast navigation could be driven by a chemical gradient produced in the cortical division zone and by physical links between the cortical division zone and the phragmoplast leading edge. The physical link could be facilitated by microtubules and microfilaments. IQD6,7,8 interact with POK2 and PHGAP in the cortical division zone.
C, *iqd6iqd7iqd8* triple knockdown perturbs assembly and position of the preprophase band, orientation of the phragmoplast, and directional phragmoplast expansion.