Unlocking the Secret of Fruit Size: Tomato FW2.2/CNR Regulates Fruit Size via Plasmodesmata Callose Deposition

Thu M Tran [1,2], **Kumari Billakurthi** [1,3]

1 Assistant Features Editor, Plant Physiology, American Society of Plant Biologists, Rockville, USA

2 Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA

3 Department of Plant Sciences, Downing Street, University of Cambridge, Cambridge CB2 3EA, UK. (0000-0003-4468-2106).

How do we grow a bigger fruit? This question excites everyone from backyard gardeners growing giant pumpkins for local Fall festival contests to large-scale agriculture companies producing commercial fruit crops. Fruits are essential for plant reproduction, as they protect the developing seeds and aid in the dispersal of mature seeds. In agriculture, fruits are harvested and consumed. Despite the functional and agricultural importance of fruit size, its underlying genes and mechanisms still need to be better understood.

Tomato (*Solanum lycopersicum*) presents a wide variety of fruit sizes and shapes which contributes to its popularity as one of the most highly consumed vegetables in the world. In addition to their fruit phenotypic diversity, tomatoes have extensive genetic resources and efficient genome editing, making them ideal systems for studying the mechanism of fruit size (Alonge et al., 2020). Thirty years ago, Frary et al. first isolated the gene underlying *FW2.2*, a major Quantitative Trait Loci (QTL) that accounts for up to 30% of tomato fruit size (Frary et al., 2000). *FW2.2* encodes a CELL NUMBER REGULATOR (CNR) family protein. CNR family proteins harbor an ancient eukaryotic PLAC8 (placenta-specific) domain, and they exhibit diverse functions, includes heavy metal transport, calcium uptake and signaling, and regulating organ size (reviewed in Beauchet et al., 2021). Their roles in regulating organ size, particularly in tomatoes, maize, and rice, are well-established (Cong et al., 2002, Guo et al. 2010, Ruan et al. 2020). These previous studies suggest that CNR proteins act as negative regulators of the cell cycle in controlling organ size, although their precise molecular mechanisms remain to be elucidated.

In this issue of *Plant Physiology*, Beauchet et al. (2024) investigated the cellular and molecular mechanism underlying the mode of action of *FW2.2*. Using transient expression in tobacco leaves and stable transformation in tomato, they demonstrated that FW2.2 is enriched at plasmodesmata (PD) with N- and C-termini facing the apoplast. PD are plant-specific structures: plasma membrane-lined cytoplasmic channels connecting adjacent cells. These structures are essential for transporting nutrients, metabolites, and signaling macromolecules from cell-to-cell. The localization of FW2.2 suggested its potential involvement in cell-to-cell transport through PD.

To examine the roles on FW2.2 in cell-to-cell transport, the authors applied Drop-ANd-See (DANS) assays (Cui et al., 2015). The DANS assay utilizes membrane permeable, non-

fluorescent CFDA dye, which is converted to fluorescent membrane-impermeable CF upon cleavage by cellular esterases. This assay allows visualization and quantification of PD permeability in plant leaves via fluorescent microscopy. The authors observed an enhanced diffusion of fluorescent dye in leaf cells of overexpressing *FW2.2* lines, demonstrating the role of FW2.2 in controlling PD permeability.

PD permeability is controlled in part by the deposition and degradation of callose around the neck of PD. To validate if FW2.2 controls PD permeability through callose deposition, the authors examined the callose level in the leaves of the overexpression of FW2.2 and loss-of-function lines. The results revealed that overexpression of *FW2.2* reduces callose accumulation at PD, potentially enhancing cell-to-cell diffusion, while loss-of-function *fw2.2* mutations show no impact on callose levels. These findings suggest that FW2.2 plays a role in modulating PD callose deposition.

The authors established that FW2.2 controls accumulation of callose at PD, and now ask how does FW2.2 contribute to the fruit size? To answer this question, the authors investigated the fruit development in genotypes with altered levels of FW2.2. The results reveal that overexpression of *FW2.2* led to reduced fruit weight, locule number, and pericarp callose deposition compared to the wild-type, while loss-of-function mutations showed opposite phenotype effects. These observations support the hypothesis that FW2.2 negatively regulates callose deposition at PD within the fruit pericarp, thus the mobile signals that inhibit cell division may be reaching the fruit tissue when callose was reduced due to overexpression of *FW2.2*.

However, identifying FW2.2's function in callose deposition raises another question: How does FW2.2 regulate callose deposition if it does not have callose synthesis enzyme activity? Using an IP-MS/MS proteomics approach, the authors found that FW2.2 is part of a protein complex containing various **callose synthases (CalS)** known to regulate callose homeostasis at PD. This interaction between FW2.2 and CalS suggests a mechanism for balancing callose synthesis at PD, with FW2.2 potentially negatively regulating CalS activity **(Figure 1).**

Even though more work is necessary to understand the mechanism by which FW2.2 interacts and regulates CalS activity the study by Beauchet et al. provides a mechanism for FW2.2, an important QTL to regulate tomato fruit size through regulation symplastic transport and improves our understanding of the genomic basis for the complex fruit size trait. Some questions remain: how does FW2.2 regulation of PD permeability affect fruit size? Moreover, how is FW2.2 regulation of PD permeability associated with cell division? And which signaling molecules are diffusing through PD? One hypothesis is that FW2.2 may interact with cell cycle repressors, such as Kip-Related Proteins (KRPs), to control cell division, as demonstrated in rice by Ruan et al. (2020).

Figure 1: FW2.2 regulates callose deposition at PD via interactions with the callose synthase protein complex. FW2.2 negatively regulate callose synthase activity, thus impacting PD permeability and facilitating cell-to-cell symplastic transport. The figures are adapted from Beauchet et al. (2024)

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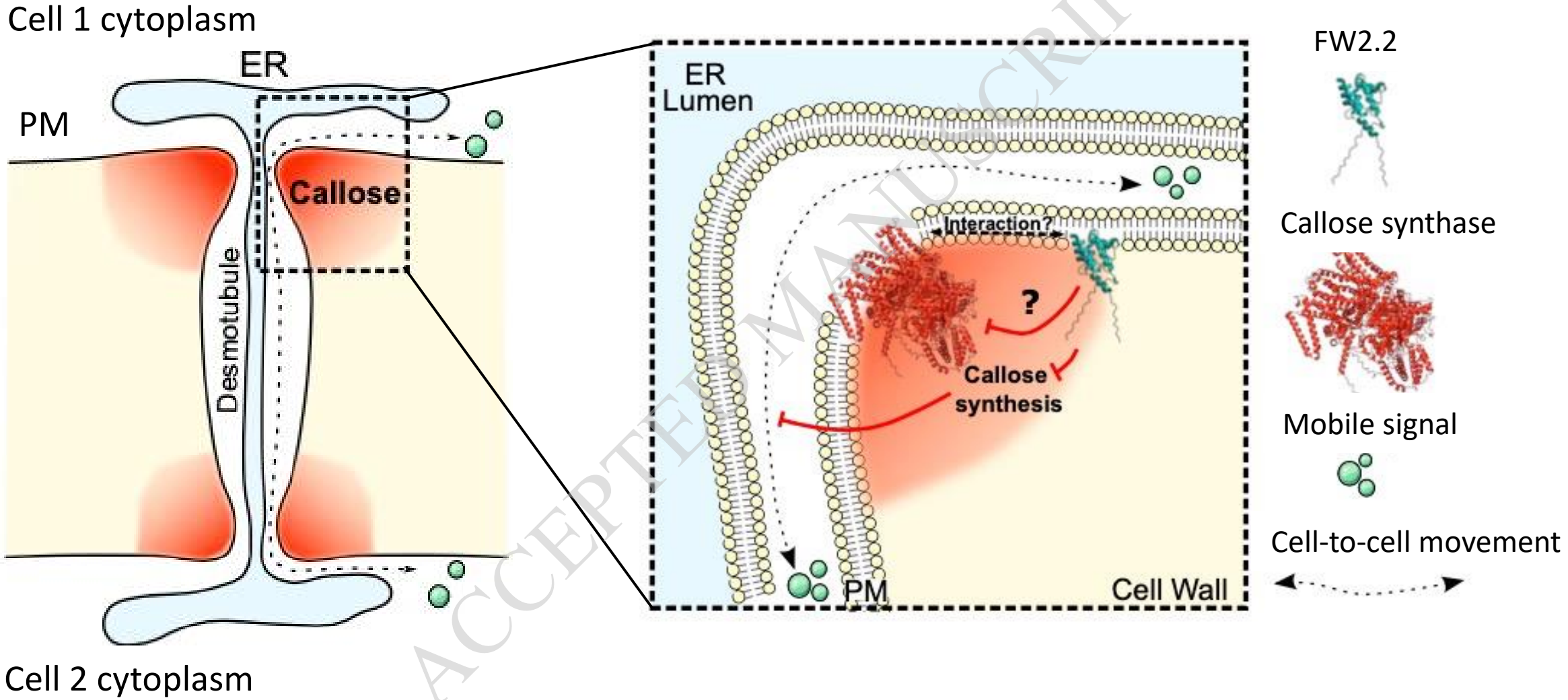

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