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The shape of things to come: ovate family proteins regulate plant organ shape

Ashley Snouffer[1], Carmen Kraus[2] and Esther van der Knaap[1,2,3]

The shape of produce is an important agronomic trait. The knowledge of the cellular regulation of organ shapes can be implemented in the improvement of a variety of crops. The plant-specific Ovate Family Proteins (OFPs) regulate organ shape in Arabidopsis and many crops including rice, tomato, and melon. Although OFPs were previously described as transcriptional repressors, recent data support a role for the family in organ shape regulation through control of subcellular localization of protein complexes. OFPs interact with TONNEAU1 RECRUITMENT MOTIF (TRMs) and together they regulate cell division patterns in tomato fruit development. OFPs also respond to changes in plant hormones and responses to stress. The OFP-TRM interaction may work in conjunction with additional shape regulators such as IQ67 Domain (IQD) proteins to modulate the response to tissue level cues as well as external stimuli and stressors to form reproducible organ shapes by regulating cytoskeleton activities.

Addresses
[1] Center for Applied Genetic Technologies, University of Georgia, 111 Riverbend Rd, Athens GA, 30602 United States
[2] Institute for Plant Breeding, Genetics and Genomics, University of Georgia, 111 Riverbend Rd, Athens GA, 30602 United States
[3] Department of Horticulture, University of Georgia, 111 Riverbend Rd, Athens GA, 30602 United States

Corresponding author: van der Knaap, Esther (vanderkn@uga.edu)

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Introduction

During plant development and growth, the frequency and orientation of cell divisions must be strictly regulated to ensure proper organ shape as the cell wall prevents cell migration or reorganization within the tissue [1••]. The shape of organs on a tissue level is regulated by a multitude of hormones as well as internal and external signals, which ultimately must regulate the activities of individual cells to control plant organ shape. Microtubule structures within plant cells are essential for cell division, cell expansion, cell wall biosynthesis and cellular organization [2,3] and these each can impact organ shape [4].

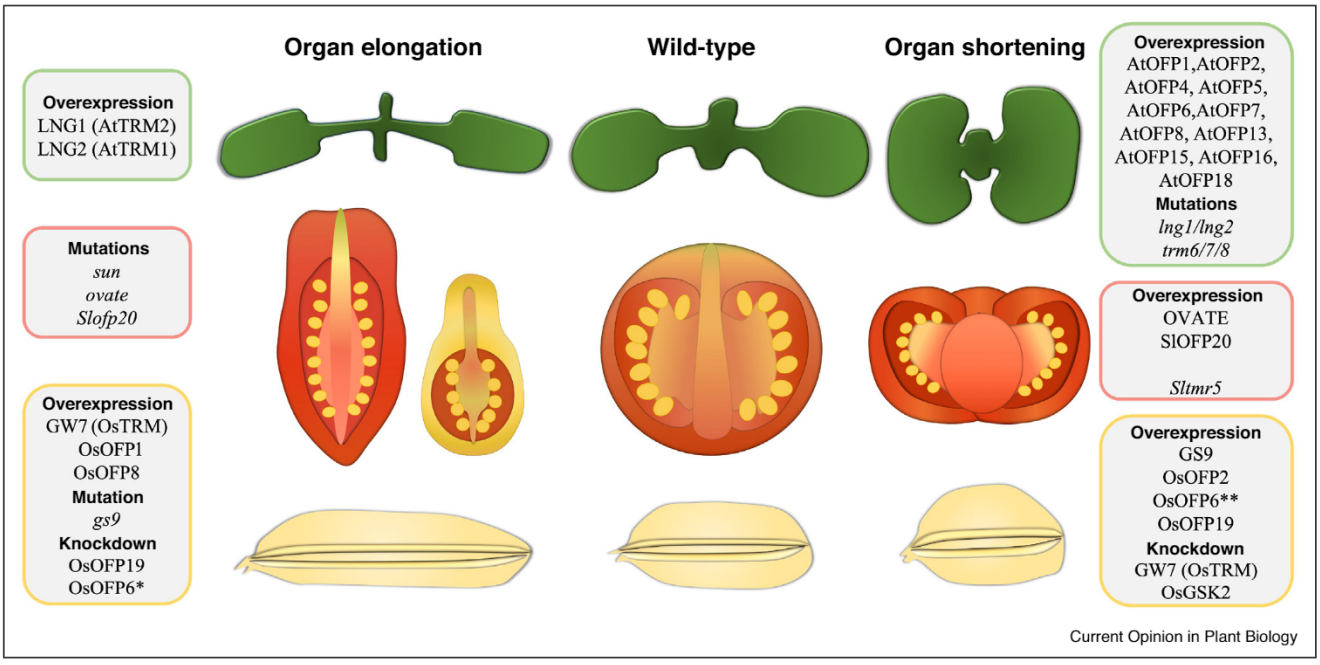
The harvestable organs of modern crops have been selectively bred for improvements in shape, size and appearance quality. Enlargement of organs and alterations to shapes have been several of the key defining features in modern crops. However, the developmental mechanisms underlying the beneficial alleles altering produce appearance have not yet been fully elucidated. Recently, Ovate Family Proteins (OFPs) have been implicated in the regulation of a number of plant organ shapes including tomato and melon fruits, rice grains, *Arabidopsis* siliques and leaves and potato tubers [5••,6–9,10•]. This review highlights the role of OFPs and proteins that genetically and biochemically interact with OFPs in crop organ shape regulation.

Ovate family proteins (OFPs)

OFPs have diverse roles in regulating plant growth and development [11,12]. The founding member of the protein family, OVATE, was identified in tomato [13,14]. OFPs are found in all land plants examined so far and are identified by the conserved OVATE domain [15,16]. Much of the foundational investigation of OFP functions were elucidated in studies focusing on *Arabidopsis*, where AtOFPs were proposed to function as transcriptional repressors [6,7]. However, as will be outlined below, recent findings suggest that this may be a misconception and the OFP family likely functions in controlling the subcellular localization of protein complexes that impact distinct cellular processes [4,5••]. Furthermore, the cellular functions of OFPs seem to be conserved in crop growth and development. Functional disruption of a single OFP generally results in mild or aphenotypic plants suggesting there is likely functional redundancy within the OFP family [6,7,17,18]. The *ovate* mutation in tomato is a notable exception, but the elongated fruit phenotype is dependent on genetic background in cultivated tomatoes [19]. Moreover, in the wild species *Solanum pimpinellifolium*, the effect of *ovate* is more subtle than in cultivated accessions [5••].

Overexpression of OFPs generally results in dwarfed plants with shorter and thicker/wider organs such as kidney shaped cotyledons or rounder leaves and fruit [5••,6,7,17,20] (Figure 1). Overexpression of *OVATE* and *SlOFP20* in tomato resulted in smaller floral organs and leaflets similar to the overexpression of *AtOFP1* [5••,14,20]. Furthermore, overexpression of several rice *OFPs* showed similar pleotropic phenotypes as seen in

Figure 1



Overview of OFPs and OFP interactors studied in organ shape regulation in *Arabidopsis*, tomato and rice. Modulation of OFP expression and expression of OFP interactors has been found to affect *Arabidopsis* aerial organs, including cotyledons (green boxes), tomato fruit and leaf shape (red boxes) and rice grain shape (yellow boxes) in addition to plant height in all three species [5••,8,9,14,21,22].
See Supplemental Table 1 for references for each gene.
*reduced grain width.
**increased grain width.

Arabidopsis and tomato with reduced plant height, panicle size, seed width and leaf width [5••,8,9,14,21,22] implying that OFPs have a role in regulation of organ shape during development of many plant species. Additionally, overexpression of rice *OsOFP6* resulted in plants that were less sensitive to cold and drought stress while RNAi knockdown of *OsOFP6* resulted in more cold-sensitive and drought-sensitive plants [10•]. Together, these misexpression results suggest that modulating *OFP* expression can be used to modify organ shapes within vegetables, crops and other plant organs, and that OFP members may function redundantly to mitigate changes to organ shape as a result of external stressors, such as cold and drought. It is important to note that two naming systems for rice (*Oryza sativa*) OFPs have been published [15,23].

OFP protein interactions in hormone signaling regulate plant organ shape

Alterations of OFP expression has been found to impact the expression of genes involved in a number of phytohormone signaling and biosynthesis pathways including gibberellins, auxin, ethylene and ERECTA [20,21,23–26]. However, the fold change in expression levels of the impacted genes is generally minor. Furthermore, analysis of transcriptome data in overexpression mutants and protoplast systems may not represent the biological function of OFPs in a given tissue, and a clearly conserved role for OFPs in hormone and transcriptional regulation across plant species has not been elucidated. Given these caveats, it is unlikely OFPs function as transcriptional repressors; however, the disruption of organ shape that result from changes to OFP expression may provide feedback to modulate hormone and signaling pathways within the developing organ. Current biochemical evidence suggests that OFPs may function in this feedback through interactions with three amino acid loop extension (TALE) proteins, as well as through interactions with additional proteins within signaling pathways.

AtOFP interactions with the TALE proteins, KNOX and BELL, have been found to regulate secondary cell wall biosynthesis [17,27], embryo sac development [28], the timing of the transition from vegetative to reproductive phases [18] and the subcellular localization of TALE proteins [20]. Rice OsOFPs and cotton GhOFP4 also interact with TALE proteins and function in secondary cell wall biosynthesis [21,29] as well as vascular bundle positioning and hormone responses in rice [8,9,21].

Recent work has further established a role for OsOFPs in brassinosteroid (BR) response, including an OsOFP

interacting with a KNOX protein [8]. The BR hormone is associated with initiating cell elongation at lower concentrations and inhibiting cell elongation at higher concentrations [30]. In rice, BR signaling regulates plant height, leaf angle and grain size [31–33]. *OsGSK2* (BIN2 in *Arabidopsis*) encodes a kinase, which negatively regulates BR signaling through phosphorylation of DWARF AND LOW-TILLERING (DLT) protein [34]. OsOFP8 interacts with and is phosphorylated by OsGSK2 which may serve to modulate the feedback loop of BR signaling to regulate hormone response within a tissue [22]. OsOFP8 and OsOFP14 also interact with another protein involved in organ shape, Grain Shape Gene on Chromosome 9 (GS9). The GS9–OsOFP8–OsGSK2 complex is implicated in regulating grain shape through control of cell divisions [35•].

OsOFP19 can also modulate BR signaling through interaction with the OsGSK2 targets, DLT and *O. sativa* homeobox1 (OSH1) proteins. *OSH1* encodes a KNOX protein which promotes expression of BR catabolic genes [8,36]. Similar to OsOFP8, OsOFP1 interacts with OsGSK2 as well as DLT thereby positively regulating BR response. Even though the function of the latter interaction is to regulate plant architecture and not grain shape, the results suggests that OFPs have tissue-specific interactions and functions [9]. Different OFPs within the large family may function in separate pathways and/or in different tissues to broadly modulate signaling response in developing plant organs. OFPs may also function in feedback mechanisms to further modulate signaling in response to the organ shape at specific stages of development.

OFPs interact with TRM proteins to control cell division and organ shape

Recent work in tomato has elucidated a conserved shape regulatory module between OFPs and TONNEAU1 RECRUITMENT MOTIF (TRM) proteins, providing some insight into the mechanisms by which OFPs regulate cell divisions and overall organ shape. Two tomato OFPs, OVATE and SlOFP20, genetically and physically interact with members of a subset of the TRM family containing the M8 motif [5••,37]. Specifically, a knockout mutation in *Sltrm5* partially rescues the elongated fruit shape controlled by the *ovate/slofp20* mutation. TRM proteins were first identified in *Arabidopsis* and some TRMs regulate aerial organ shape [38,39]. Overexpression of *AtTRM1* and *AtTRM2* (*LONGIFOLIA2* and *LONGIFOLIA1* respectively) resulted in elongated organs while a double mutant for *trm1* and *trm2* had organs of decreased length as a result of reduced cell elongation rates (Lee *et al.* [39]). These two AtTRMs contain the M8 motif (Supplementary Figure 4 in Wu *et al.* [5••]) suggesting that the phenotype is controlled by interactions with OFPs.

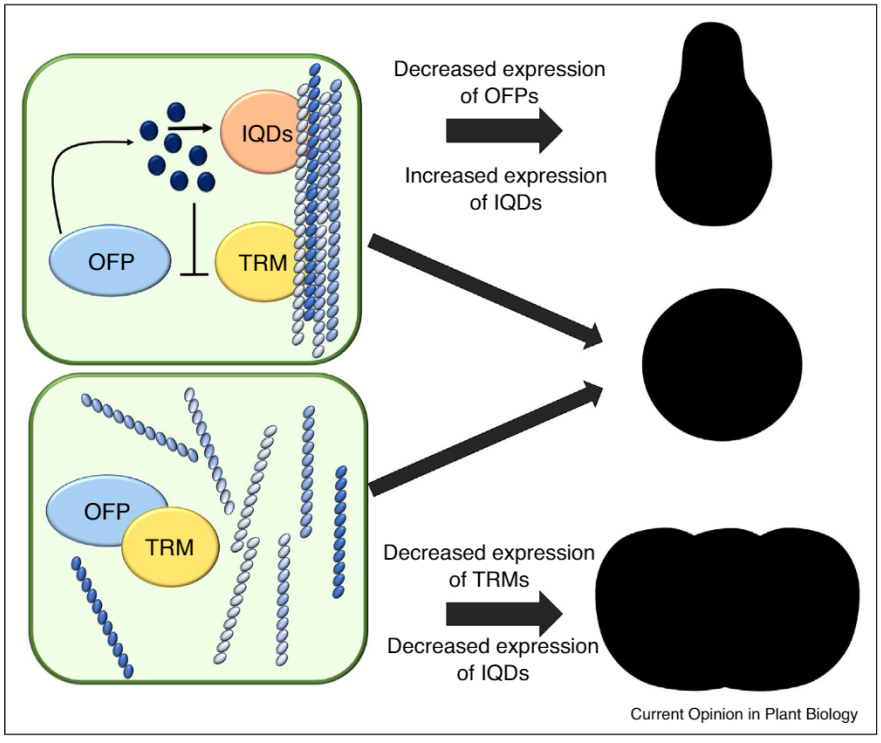
TRMs were identified and named after their interaction with TONNEAU1 (TON1). This interaction is essential for recruitment of the TTP (TON1–TRM–PP2A) complex to microtubules [38,40,41]. The TTP complex is recruited to a ring of cortical microtubules that forms around the periphery of the nucleus of a premitotic cell called the preprophase band (PPB). The PPB is a cytological marker of the future division plane and predictably forms at the future cortical site where the new cell plate will attach [38,41,42]. Interestingly, although plants do not have formal microtubule organizing centers, TON and TRM proteins share sequence similarity with animal centrosomal proteins [38] suggesting they may have a role in microtubule organization.

Natural variation in OFP and TRM families impacts melon and cucumber fruit shape as well as potato tuber shape [5••,43,44]. *GL7* (also known as *GW7*) in rice encodes a TRM protein and interacts with the rice TTP complex and was found to regulate grain shape and quality [45–48]. This rice TRM contains an M8 motif and falls in the AtTRM1-5 subclade that contains AtTRM1, AtTMR2 and SlTRM5 implying that this subclade of TRMs is associated with plant organ shapes [5••]. These results suggest that the interactions between OFPs and TRMs may function as a conserved shape regulation module in many plant species, including the harvestable organs of important fruit and vegetable species [4,5••].

Cellular mechanism of OFPs and TRMs in organ shape regulation

OFPs and TRMs regulate organ shape in a number of species and mutations in these genes can impact cell elongation and cell division in *Arabidopsis*, rice and tomato. The changes in the cell division patterns organized by OFPs and TRMs therefore control the overall organ shape (Figure 2) [5••,6,8,35•], but the cellular mechanisms they use to control these processes remains to be determined. Mutations in TTP components disrupt PPB formation and results in dwarfed plants with severely altered organ shapes [41]. Recent investigation of the triple mutant, *Attrm678*, resulted in only the loss of the PPB in the root and shoot meristems without disruption of interphase microtubule structures. The *Attrm678* mutants had a more subtle decrease in silique and root elongation than other TTP mutants that impact additional microtubule structures. The overall organization of the root tissue in *Attrm678* was generally normal in the absence of PPBs, although there was increased variation in cell wall placement and some disruption of cell files. These surprising results imply that the PPB has only a minor role in regulating the robustness of cell divisions in these tissues but can still impact organ shape [1••]. Furthermore, these data suggest an important role for the TTP complex, or its components, in additional microtubule structures, and indeed, mutations in components of the TTP complex

Figure 2



Regulation of OFP-TRM interactions promote microtubule organization to balance shape elongation. Levels of a secondary messenger, such as Ca^{2+}, hormone or regulation of the expression levels of OFPs and TRMs themselves over developmental time impacts the organization of microtubules and ultimately cell divisions or elongation. OFPs control the localization of TRMs to the microtubules. Mutations that result in reduced or no expression of OFPs results in mis-localization of TRMs and elongated organs. Furthermore, reduced or no expression of TRMs results in organ flattening. These shapes can be further regulated by other shape regulators such as IQDs.

also disrupt the organization of interphase microtubule arrays [40,41,49,50]. For example, *Arabidopsis trm4* mutants have disordered microtubules resulting in improper cellulose deposition in the seed epidermal cells [8]. CELLULOSE SYNTHASE complexes (CSC) are responsible for cellulose biosynthesis and are guided around the cytoplasmic column on cortical microtubules [51]. The organization of microtubules is important for the direction of CSC transport and for cellulose deposition in distinct locations around the plant cell [4,51,52,53•]. AtTRM4 contains the M8 motif, implying that it may function through interactions with OFPs. Interestingly, secondary cell wall formation is impacted by mutations in *AtOFP4* and *KNAT7* demonstrating that a TRM as well as an OFP could function in the movement of CSC [8,17].

The TRMs along with the TTP complex likely function to organize microtubule structures throughout the cell cycle, and the subcellular localization of these proteins is crucial for proper cell division and deposition of cellular components, both of which can impact organ shape [4]. Changes in cell numbers and cell elongation have been reported in a number of OFP and TRM mutants [1••,5••,6,10•,39,45–47].

Additionally, tomato SlOFPs regulate the subcellular localization of SlTRMs in *Nicotiana benthamiana* leaf epidermal cells when co-expressed [5••]. Expressed individually, OVATE and SlOFP20 are localized to the cytosol and the cytosol and nucleus, respectively, while SlTRM5 localizes to the microtubules. Co-expression of OVATE and SlTRM5 results in the majority of cells relocalizing SlTRM5 to the cytosol, suggesting OVATE prevents microtubule binding of SlTRM5 and potentially association with the TTP complex. Interestingly, co-expression of SlOFP20 with SlTRM5 results in recruitment of SlOFP20 to the microtubules, co-localizing with SlTRM5, suggesting that different combinations of OFPs and TRMs may have distinct regulatory functions and may serve to respond to a broad range of developmental and environmental conditions (Figure 2) [4,5••].

Protein–protein interactions likely regulate the function of OFPs, and there is also evidence for an impact of post-translational modifications regulating OFP function. There are putative MAPK phosphorylation sites in AtOFP15 and AtOFP18, and overexpression of a phosphomimetic mutant of OFP15 resulted in a more severely dwarfed plant with blunt-ended siliques suggesting the

Figure 3

The IQD gene, *SUN*, in tomato genetically interacts with OFP-TRM5 in *S. pimpinellifolium*. Tomatoes with increased expression of *SUN*, or mutant for *ovate* and *ovate/slofp20* produce elongated fruit [4,5••,37,64]. The addition of the *trm5* mutant to an *ovate/slofp20* [5••] *sun*, or *ovate/slofp20/sun* background partially rescues the elongated phenotype, suggesting these proteins genetically interact to control tomato fruit shape. Scale bar =0.5 cm Tomato lines generated and pictures taken by Carmen Kraus.

phosphorylation at these sites can regulate OFP function [26]. Together, these results suggest that endogenous localization of these proteins may be impacted by other protein–protein interactions or be controlled through post-translational modifications of OFPs themselves. Furthermore, changes in the subcellular localization of OFPs and interacting proteins may be functionally important in regulating organ shape.

Coordination of additional shape modulators in the OFP-TRM interaction

Shape regulation and growth of organs requires individual cells to interpret and integrate a plethora of cues. Interpretation of essential conditions such as cellular positioning in the overall tissue or external stress can be done through signaling fluctuations, and this input can be used to determine cell division rates, expansion, or differentiation [54–57]. Complex integration of both internal and external stimuli is facilitated through the use of secondary messengers such as calcium signaling (Ca^{2+}). Ca^{2+}-signaling regulates the microtubule cytoskeleton [58] likely through binding of Ca^{2+}-sensors such as calmodulin (CaM) and then calmodulin binding to microtubule-associated proteins (Figure 2) [4,59,60,61••]. The tomato SUN protein is a member of the IQ67 Domain (IQD) family that have been found to localize to membranes and microtubules by binding CaM in a Ca^{2+}-dependent manner [61••,62]. IQDs regulate organ shape and cell number, as well as hormone and environmental stress response in Arabidopsis, tomato, cucumber and rice [25,37,61••,63–73].

The Ca^{2+}binding proteins CENTRIN1 and CENTRIN2, similar to calmodulin, were found to interact with TON1 [40]. Centrins in animals are important for the function of the microtubule organizing center, the centrosome [74]. Although it is unclear how CENTRIN binding to TON1 impacts its function in the TTP complex or binding TRMs, it may provide a link between Ca^{2+}, OFPs-TRMs and microtubule organization in plants. These data suggest that Ca^{2+}signaling may function to regulate multiple shape modulating factors in response to external stimuli (Figure 2).

Genetic evidence in multiple species also suggests that organ shape regulation can be coordinated through interactions between different protein families, and that pyramiding of beneficial alleles for multiple genes may lead to further crop improvement. Combination of the *SUN* and *ovate* mutations result in extremely elongated tomato fruits, suggesting a synergistic interaction between these genes (Figure 3) [4,12,37]. In rice, the TRM gene GL7 interacts with beneficial alleles of GS3, GW8, TGW6 and GS5 to further regulate grain shape and appearance quality [47]. Although the molecular mechanisms underlying the coordination in shape regulation between these different protein families remains elusive, further investigation may lead to better understanding of organ shape regulation and facilitate breeding of improved varieties that enhanced appearance quality in a number of agriculturally important harvestable organs.

Conclusions

Plant organ shape regulation during development and growth is dependent on the coordination and integration of numerous signals and stimuli. The OFP-TRM regulatory module may function to enact a cellular response and to ensure proper organ growth in the appropriate dimensions. Furthermore, OFPs may provide feedback on shape regulating signaling to further modulate organ

shape during development [21,22,35•]. The large protein families for both OFPs and TRMs may function to precisely regulate organ shapes under a variety of external conditions, including buffering organ shape against external stress such as drought and cold [10•]. The OFP-TRM organ shape regulatory mechanism may rely on distinct subcellular localization of different OFPs and TRMs in response to external and internal signals over developmental time [5••]. The precisely controlled localization then promotes specific microtubule architecture and divisions to ensure precise growth of organ shape.

Additional shape-modulating proteins such as IQDs that interact with microtubules may coordinate organ development with OFPs. Although these proteins families have not been found to directly interact biochemically, they can genetically interact in tomato fruit (Figure 3). Furthermore, both OFPs and IQDs can alter microtubule structure in response to hormones, external stimuli, or stress to impact cell divisions and cell elongation to regulate shape. OFPs and IQDs may also serve a role in the feedback loops of the hormone signals for additional control of organ shape and hormone response within a developing tissue. Further understanding of the cellular function of OFPs and TRMs and the interaction with additional shape modulators, such as IQDs, in organ shape regulation will allow for the manipulation of produce morphology to facilitate breeding efforts in a variety of crop species.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pbi.2019.10.005.

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- • of special interest
- •• of outstanding interest

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