On the Inside

Crispr Mutants Shed Light on Pectin's Role in Tomato Fruit Softening

Tomato (*Solanum lycopersicum*) fruits undergo pronounced softening during ripening. Softening is important for flavor development and overall palatability, but it also impacts fruit storage, transportability, and shelf life. Shelf life is a particularly important quality trait of tomato fruits affected by alterations in the cuticle properties of the ruits and the remodeling of fruit cell walls. Studies with transgenic tomato plants have indicated that a range of pectin-degrading enzymes are involved in fruit softening by way of cell wall remodeling. Until recently, pectin was thought to contribute to wall mechanics independently of other cell wall polymers such as cellulose and xyloglucan. However, the validity of this conventional role of pectin has been challenged by a series of recent discoveries that support the idea that pectin may directly contribute to the cross-linking of cellulose microfibrils in the cell wall, potentially to a greater extent than xyloglucan, the classical cross-linking hemicellulose. Previous molecular studies of ripening-related genes have usually involved silencing of only a single gene, and it has proved difficult to compare the effects of silencing these genes across the different experimental systems. **Wang et al. (pp. 402–414)** now report the generation of CRISPR-based mutants in the ripening-related genes encoding the pectin-degrading enzymes pectate lyase (PL), polygalacturonase 2a (PG2a), and β-galactanase (TBG4). Comparison of the physiochemical properties of the fruits from a range of *PL*, *PG2a*, and *TBG4* CRISPR lines demonstrated that only mutations in *PL* resulted in firmer fruits, although mutations in *PG2a* and *TBG4* influenced fruit color and weight. The presence of all three enzyme activities, however, is needed to allow normal ripening-related changes in pericarp cell-to-cell adhesion and solubilization of pectin from association with cellulose microfibrils. Pectin localization, distribution, and solubility in the pericarp cells of the CRISPR mutant fruits were also investigated. In toto, the data obtained indicate that PL, PG2a, and TBG4 act on separate cell wall domains.

The Terpene Synthases of Red Algae Have A Bacterial Origin

The red algae (Rhodophyta), encompassing over 8,000 species, are the richest source of marine secondary metabolites. Among red algae, many genera produce terpenes, which constitute the largest class of secondary metabolites. Despite the rich diversity of terpenes in red algae, little is known about how they are biosynthesized. This is in sharp contrast to our considerable knowledge of terpene biosynthesis in land plants. Land plants produce a large array of terpenes, whose skeletal diversity can be attributed largely to terpene synthases. Two types of terpene synthases are known: typical plant terpene synthases and microbial terpene synthase-like (MTPSL) enzymes. The genes encoding typical plant terpene synthase are well understood. In contrast, *MTPSL* genes were discovered only recently and are more closely related to terpene synthase genes from bacteria and fungi than to typical plant terpene synthase genes. By systematic sequence analysis of seven genomes and 34 transcriptomes of red algae, **Wei et al. (pp. 382–390)** have identified *MTPSL* homologs within one genome and two transcriptomes of red algae: no homologs of typical plant terpene synthase genes were found. Phylogenetic analysis showed that red algae MTPSLs group with bacterial terpene synthases. Analysis of the genome assembly and characterization of neighboring genes demonstrated that red algal *MTPSLs* are bona fide red algal genes and not microbial contaminants. *MTPSL* genes from *Porphyridium purpureum* and *Erythrolobus australicus* were characterized via heterologous expression and demonstrated to have sesquiterpene synthase activities. Expression of the *MTPSL* gene in *P. purpureum* was found to be induced by methyl jasmonate, suggesting a role for this gene in host defense. In summary, this study indicates that the formation of terpene carbon skeletons in red algae is carried out by MTPSLs that are phylogenetically unrelated to typical plant terpene synthases and most likely originated in Rhodophyta via horizontal gene transfer from bacteria.

Advances in Understanding Root Hair Formation

Root hairs greatly increase the surface area of roots, thereby facilitating the uptake of nutrients and water from the rhizosphere. They also serve as sites for plant interactions with soil microorganisms. Thus, elucidation of the molecular pathway for their development is important for potential modification of root hair morphology to produce crops with improved growth traits. ROOT HAIR DEFECTIVE SIX-LIKE (RSL) class II proteins are expressed preferentially in future root hair cells of Arabidopsis (*Arabidopsis thaliana*). **Moon et al. (pp. 558–568)** functionally characterized the seven members of the RSL class II subfamily in the rice (*Oryza sativa*) genome: six of these genes were preferentially expressed and four were strongly expressed in root hairs. Overexpression (OX) of each of the four highly expressed family members in rice resulted in an increase in the density and length of root hairs. These four members also possess a transcription activation domain and are targeted to the nucleus. Genetic mutation of one member (*Os07g39940*) caused a severe reduction in root hair length. Biochemical studies demonstrated that the RSL class II members interact with Root Hairless1 (OsRHL1), a key regulator of root hair development, and apparently assist in its translocation into the nucleus. A transcriptome analysis of *Os07g39940-OX* plants revealed that 86 genes, including class III peroxidases, were highly upregulated. Furthermore, reactive oxygen species levels in the root hairs were increased in *Os07g39940-OX*

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plants but were drastically reduced in the *os07g39940* and *rhl1* mutants. These results demonstrate that RSL class II members are essential regulators of root hair development in rice.

Insights into the Trans-Golgi Network and Protein Secretion

In eukaryotic cells, the movement of cargo between single membrane-bound organelles such as the endoplasmic reticulum, Golgi apparatus, trans-Golgi network (TGN), endosomes, lysosomes, and vacuoles is mediated by membrane trafficking. At the donor organelle, cargo molecules are loaded into transport vesicles, which then become tethered to and fuse with the target organelle membrane to discharge and deliver their cargos to a destination compartment. The coordination required for membrane fusion events and for the delivery of cargos to their correct destinations depends on specific interactions between members of the SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) family proteins. The post-Golgi interface, including the TGN, is a pivotal hub for two major trafficking pathways. Golgi-associated TGNs are adjacent to and function jointly with the Golgi apparatus. On the other hand, free TGNs, which are cellular components described only in plant cells, originate from Golgi-associated TGNs but are thought to operate independently from the Golgi apparatus and Golgi-associated TGNs. **Uemura et al. (pp. 519–532)** now show that free TGNs are associated with two SNARE proteins, VESICLE-ASSOCIATED MEMBRANE PROTEIN721 (VAMP721) and VAMP722, which are known to be responsible for protein secretion and for extracellular defense. The authors report that the perturbations of Golgi-associated and free TGN integrity induced by the *syp42syp43* double mutation caused impaired transport of VAMP721 to the plasma membrane, thereby directly connecting free TGNs to secretory pathway components. In addition, quantitative proteomic analyses of leaf apoplastic fluid revealed that SYP4 and VAMP721 are needed for the constitutive and pathogen-inducible secretion of cell wall modification enzymes that are important for plant growth and extracellular defense.

Auxin Affects Capitulum Pattern Formation

The flower head (capitulum) is a morphological feature that defines the family Asteraceae (the daisy or sunflower family). A typical capitulum consists of many flowers (florets) and phyllaries (modified bracts) compressed into a single structure that mimics a single flower. Capitula commonly have two types of florets: ray and disc florets. Disc florets are usually perfect flowers. In most cases, pattern formation of the capitulum is precisely controlled, with phyllaries (leafy bracts), ray florets, and disc florets positioned in a centripetal order in the capitulum, which mimic sepals, petals, and anthers, respectively. The formation of phyllaries and florets is typically asynchronous and acropetal (forming from the margin to the center of the capitulum). Although the patterning mechanisms underlying the structure of capitula remain elusive, it has recently been reported that LEAFY (LFY), a key regulator of floral meristem identity, plays a role in capitulum development. *lfy* mutants make secondary inflorescences with cauline leaves instead of flowers in Arabidopsis (*Arabidopsis thaliana*). It has been also shown that the auxin pathway interacts with the LFY pathway, suggesting a possible role for auxin in controlling capitulum patterning. In Arabidopsis, auxin accumulation preceded *LFY* expression, and application of auxin onto inflorescences up-regulated *LFY* mRNA and protein. Auxin has been previously suggested to play a morphogen-like or a morphogenic trigger role in plant development. An auxin gradient was reported in several plant tissues such as the secondary vasculature, the female gamete, and the root tip. This suggests that auxin can provide positional cues for tissue specification in a concentration-dependent manner. **Zoulias et al. (pp. 391–401)** now show that auxin provides a developmental patterning cue for the capitulum of *Matricaria inodora*. During capitulum development, a temporal auxin gradient occurs, regulating the formation of distinct florets and phyllaries. Auxin also regulates floral meristem identity genes, such as *M. inodora RAY2* and *LFY*, which determine floret and phyllary identity. This study suggests that hormone gradients are important in development and evolved independently in plants and animals.

Hydraulic Regulation of Stomata in Ferns

Stomatal responses to environmental and endogenous signals in vascular plants are critical for regulating plant gas exchange with the atmosphere. In addition, stomatal closure is vital for minimizing water loss and preventing lethal embolisms during drought. The vast majority of studies concerning stomatal physiology have focused on the angiosperms, where it is well established that abscisic acid (ABA) is the major factor regulating stomatal responses to changes in leaf water status: the situation is much less clear in nonseed plants. For example, when fern species are drought stressed and naturally synthesize ABA, this endogenous ABA is ineffectual in closing stomata. In contrast, a recent article observed stomatal closure by 15% when measuring gas exchange in leaves that were sprayed with high levels of exogenous ABA in the fern species *Athyrium filix-femina*. However, this response was only observed in plants acclimated to low vapor pressure difference (VPD) in a growth cabinet and not in plants of this species grown under high VPD or to any significant degree in two *Dryopteris* species. **Cardoso et al. (pp. 533–543)** have measured the stomatal response to changes in VPD in two natural forms of the fern species *A. filix-femina*, recently suggested to have stomata that are regulated by ABA. These two varieties have considerable variation in foliar anatomy and consequently leaf hydraulic properties, meaning similar changes in VPD should have very different effects on leaf water status The authors report that the two forms considerable differences in key hydraulic traits, including leaf hydraulic conductance and capacitance, as well as the kinetics of stomatal response to changes in VPD. In both forms, however, the stomatal responses to VPD could be accurately predicted by a dynamic, mechanistic model that assumes guard cell turgor changes in concert with leaf turgor in the light and not via metabolic processes including the

level of ABA. During drought, endogenous ABA did not play a role in stomatal closure, and exogenous ABA applied to live, intact leaves did not induce stomatal closure. These results indicate that functional stomatal responses to changes in leaf water status in ferns are regulated by leaf hydraulics and not metabolism.

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