On the Inside

Auxin Transport Synchronizes Division Pattern

The tobacco cell line VBI-0 (*Nicotiana tabacum*) provides a simple model system to study the role of intercellular communication in patterning. In this system, singular cells divide axially to produce linear cell files of distinct polarity. A curious feature of these cell files is that they almost always consist of an even number of cells. In a strictly binary system of cell division, files consisting of 2^n cells (2, 4, 8, 16. . .) should be most frequent. In the VBI-0 system, however, files consisting of 6 cells are highly frequent, even more than files consisting of 8 cells, which is difficult to reconcile with a strictly binary system. Thus, **Campanoni et al. (pp. 1251–1260)** conjecture the existence of some form of information exchange (coupling) between the cells that serves to coordinate and synchronize cell division. To test whether the observed deviation from a binary system could be caused by coupling, they developed a simple mathematical model to simulate the dynamics of cell number. They report that the VBI-0 cell culture system can best be described as a one-dimensional array of coupled oscillators, where the number of oscillators is not conserved over time. Instead, a new oscillator is generated and inserted adjacent to the "parent" cell, whenever a certain oscillator has finished one cell cycle. The model indicates that a given oscillator senses interference with just one neighboring oscillator not two (unilateral coupling). This unilateral or polar interference may be related to the polar transport of a coordinating signal such as auxin. In agreement with this model, the authors report that treatment of the cell cultures with low concentrations of 1-N-naphthylphthalamic acid (NPA), an inhibitor of auxin efflux carriers, equalizes the frequencies of files with even and uneven cell numbers. These results show that *intra file* auxin flux mediates pattern formation in this simple system. Auxin *transport*, not the mere presence of exogenous auxin, synchronizes the cell cycle.

Guard Cell Surface Area

Guard cells must maintain the integrity of the plasma membrane during the large and relatively rapid changes in volume they undergo. Since plasma membranes are only about 5% elastic, stretching alone cannot be responsible for maintaining membrane integrity. In this issue, **Shope et al. (pp. 1314–1321)** explore the question of what happens to the plasma membrane of guard cells during a massive decrease in volume? Does the guard cell protoplast simply shrivel with no reduction area in the surface area of the plasma membrane? Or does the excess plasma membrane become internalized? To distinguish between these two possibilities, the authors determined the surface area of intact guard cells of *Vicia faba* as they underwent changes in volume in response to changes in the external osmotic potential. They also employed a membrane-specific fluorescent dye to measure membrane internalization by these cells. Surface area decreased by as much as 40% when external osmotic potential was increased from zero to 1.5 MPa, and surface area varied linearly with volume. Membrane internalization was found to increase approximately linearly with decreases in the cell's surface area. The changes in surface area, volume, and membrane internalization were reversible when the guard cells were returned to a buffer solution with an osmotic potential near zero. The data show that intact guard cells undergo changes in surface area that are too large to be accommodated by plasma membrane stretching and shrinkage, and suggest that the plasma membrane is reversibly internalized to maintain cell integrity.

Wounding-Induced Trichome Production in Arabidopsis

Many plant species, including Arabidopsis, respond to insect damage by increasing the density of trichomes on new leaves. Gibberellin has already been shown to play a role in constitutive trichome production in Arabidopsis, and in this issue, **Traw and Bergelson (pp. 1367–1375)** demonstrate that two plant defense-related chemicals, notably jasmonic acid and salicylic acid, also influence trichome production. The results are interesting because salicylic acid and jasmonic acid are known to play key roles in regulating the induction of other types of herbivore resistance. Herbivore damage and artificial wounding both cause rapid increases in jasmonic acid and, as the authors demonstrate, an increase in trichome production. The *jar1–1* mutant exhibited normal trichome induction following treatment with jasmonic acid, suggesting that adenylation of jasmonic acid is not necessary. Salicylic acid, which negatively regulates the jasmonate-dependent pathway in many plants including Arabidopsis, had a negative effect on trichome production and consistently reduced the effect of jasmonic acid, suggesting negative crosstalk between the jasmonate and salicylate-dependent defense pathways. Interestingly, the effect of salicylic acid persisted in the non-inducible immunity mutant *nim1–1*, suggesting that the *Npr1/Nim1* gene, which encodes a nuclear protein that is necessary for downstream activity of the salicylate-dependent pathway in other systems, is *not* downstream of salicylic acid in the negative regulation of trichome production. Finally, they report that gibberellin and jasmonic acid had a synergistic effect on the induction of trichomes, suggesting important interactions between these two compounds.

Urea Transport by Aquaporins

Although urea is the major form of nitrogen (N) in most plant fertilizers and is also an important N metabolite in plants, the mechanisms that underlie urea transport in higher plants are not well understood. Transport assays have previously suggested that two mechanisms are at work in characean algae: At low concentrations, an active, energy-dependent pathway drives the uptake of urea, whereas at higher external concentrations, urea uptake follows a linear concentration depen-

dency, indicating a second passive or diffusion-controlled transport pathway. Higher plants have been shown to transport urea actively by the H^+-urea cotransporter AtDUR3, which is preferentially expressed in roots under N deficiency Also, in some systems, aquaporins facilitate urea transport although this is not a general property of all aquaporins. To better understand the molecular basis for urea transport in higher plants, **Liu et al. (pp. 1220–1228)** adopted a yeast (*Saccharomyces cerevisiae*) complementation approach to isolate genes encoding urea transport proteins in Arabidopsis. Here, they report that this approach led exclusively to the isolation of tonoplast intrinsic protein (TIP)-related genes. The urea-transporting properties of these aquaporins were characterized in yeast and *Xenopus laevis* oocytes and found to differ fundamentally from the recently characterized secondary active urea transport mediated by AtDUR3. In contrast to the high-affinity H^+/urea symporter AtDUR3, these AtTIPs provide a less concentration- and pH-dependent transport pathway for urea. The identified AtTIPs could potentially facilitate urea transport either from the external growth medium into the cytosol or from the cytosol into the vacuole, for example, for the storage or detoxification of excessive urea. Transcriptional up-regulation of the isolated AtTIPs under N deficiency in roots further supports a role for aquaporins in urea transport.

Arabidopsis Transcriptome Responses to 2,4,6-Trinitrotoluene (TNT)

The manufacture, processing, and storage of explosives, such as 2,4,6-trinitrotoluene (TNT) during the past century has led to soil and groundwater contamination in some areas. Unlike many other nitroaromatic compounds, including pesticides and various feedstock chemicals, the energetic nitroaromatics are highly resistant to degradation and may persist in the environment for decades. Certain plant species have the ability to accumulate TNT from their surroundings and, thus, offer a potential means for removing these compounds from the environment by phytoremediation. Few of these species, however, are capable of tolerating the high contamination levels typically encountered in those sites most in need of remediation. Unfortunately, a lack of information on the biochemical mechanisms involved in TNT uptake and metabolism limits our ability to genetically modify plants specifically for this task. In this issue, **Ekman et al. (pp. 1397–1406)** report on their use of Serial Analysis of Gene Expression (SAGE) to profile transcript levels in Arabidopsis roots and assess their responses to TNT exposure. Among the proteins that were most highly transcribed in response to TNT exposure included a glutathione S-transferase, several cytochrome P450 enzymes, an ABC transporter and a probable nitroreductase. Analyses also revealed an oxidative stress response upon TNT exposure as well as the repression of some transcript levels. Although many of these findings were expected based on current models of xenobiotic metabolism in plants, evidence for an unsuspected anthranilate conjugation pathway was also noted. Identifying transcriptome-level responses to TNT exposure will better define the metabolic pathways plants use to detoxify this xenobiotic compound, which should help improve phytoremediation strategies directed at TNT and other nitroaromatic compounds.

Does the Krebs Cycle Reduce Photosynthesis?

Although the operation and location of the Krebs cycle was demonstrated in plant cells decades ago, many fundamental questions remain concerning how its activity is integrated with other plant processes. For example, controversy exists over whether the Krebs cycle operates in illuminated photosynthetic tissue and if it contributes to the energy requirements for the synthesis of Suc in photosynthetic tissues. In this issue, **Carrari et al. (pp. 1322–1335)** describe the molecular and genetic analysis of *Aco-1*, a *Lycopersicon pennellii* accession that is deficient in aconitase, the Krebs cycle enzyme that catalyzes the reversible interconversion of citrate and isocitrate. As expected, the mutation resulted in lowered expression of the *Aco-1* transcript and lowered levels of both cytosolic and mitochondrial aconitase protein and activity. Biochemical analysis of leaves of the *Aco-1* accession suggested that they exhibited a restricted flux through the Krebs cycle and reduced levels of Krebs cycle intermediates but were characterized by elevated adenylate levels and an enhanced rate of CO_2 assimilation. Furthermore, the analysis of both steady state metabolite levels and metabolic fluxes revealed that this accession also exhibited elevated rates of photosynthetic Suc synthesis and a corresponding increase in fruit yield. The enhancement of photosynthesis and fruit yield in this aconitase-deficient mutant is somewhat stunning given the limited success in achieving similar results through direct modification of the Calvin cycle.

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