Phosphorylation of Topoisomerase I from Pea (Pisum sativum)

DNA topoisomerases may play a role in regulating gene expression by maintaining the superhelical density of DNA and controlling the interconversions of DNA’s topological states. In this issue, Tuteja et al. (pp. 2108–2115) present evidence for the phosphorylation-dependent regulation of topoisomerase I from pea (Pisum sativum). The purified enzyme was phosphorylated by endogenous protein kinases present in pea nuclear extracts and by exogenous casein kinase 2 (CK2) and protein kinase C (PKC; from animal sources). Only Ser residues were phosphorylated. Topoisomerase I activity increased following phosphorylation with exogenous CK2 or PKC. These phosphorylation reactions were inhibited by heparin (an inhibitor of CK2) and calphostin (an inhibitor of PKC), suggesting that pea topoisomerase I is a bona fide substrate for these kinases. This study provided evidence that these classes of kinases may contribute to the physiological regulation of DNA topoisomerase I activity in plants.

Subcellular Targeting of Calcium-Dependent Protein Kinases

Calcium-dependent protein kinases (CDPKs) are Ser/Thr protein kinases that are only found in plants and some protozoans. They consist of four domains: a variable N-terminal domain, a catalytic domain, an auto-inhibitory region, and a calmodulin-like domain. CDPKs are activated upon binding calcium to their calmodulin-like domain, which makes them effective switches for the transduction of calcium signals in plant cells. In this issue, Dammann et al. (pp. 1840–1848) identify the subcellular targeting potentials of 9 of the 34 CDPK isoforms in Arabidopsis. Two of the 9 isoforms were primarily soluble, whereas the other 7 isoforms were associated with membranes. Membrane association was correlated with the presence of potential N-terminal acylation sites. All but one of the membrane-associated isoforms targeted exclusively to the plasma membrane. The exception (AtCPK1-GFP) targeted to peroxisomes. Targeting to the peroxisome was disrupted by a deletion of two potential N-terminal acylation sites. The observation of a peroxisome-located CDPK suggests a mechanism for calcium regulation of peroxisomal functions involved in oxidative stress and lipid metabolism. These results demonstrate that in Arabidopsis, different CDPKs are targeted to specific subcellular locations, providing the potential for isoform-specific differences in regulating various cellular functions.

Cytokinin Overproduction Delays Corolla Senescence in Petunia x hybrida

Floral senescence is accompanied by changes in endogenous ethylene, abscisic acid (ABA), and cytokinins. In contrast to the actions of ethylene and ABA, cytokinins delay floral senescence. Plants with altered cytokinin content have previously been generated by transformation with the Agrobacterium tumefaciens cytokinin biosynthetic gene ipt that encodes isopentenyl transferase. The specific targeting of ipt expression to senescing tissues can be achieved by means of the promoter from SAG12, a senescence-associated gene from Arabidopsis. In this issue, Chang et al. (pp. 2174–2183) used transgenic petunias (Fig 1) expressing the P\textsubscript{SAG12}::ipt chimeric gene to determine the effects of increased cytokinin levels in petals on flower senescence, and to investigate the interactions between cytokinin accumulation and ethylene production, ethylene sensitivity, and ABA accumulation. Two transformed lines that had flowers that lived 6 to 10 d longer than normal were studied. Ipt transcripts increased in abundance following pollination and were accompanied by increased cytokinin accumulation. Endogenous ethylene production was induced by pollination in both wild-type (WT) and ipt corollas, but this increase was delayed in IPT flowers, and flowers from IPT plants were less sensitive to exogenous ethylene. Accumulation of ABA was significantly greater in WT corollas, confirming that floral senescence was delayed in IPT plants. Thus, floral senescence is regulated by complex interactions between ethylene, cytokinins, and ABA. Extending these studies to include signal transduction mutants such as ethylene-insensitive petunias should aid in elucidating these hormonal interactions.

Microarray Study of UV Effects in Maize (Zea mays)

Because of the destruction of the ozone layer by pollutants such as chlorofluorocarbons, terrestrial levels of UV-B are increasing. Because of its absorption spectrum, DNA is a major target of UV-B damage and, thus, these increases in UV-B could potentially have deleterious consequences for all living organisms, including plants. In flowering plants, flavonoids, including anthocyanins, accumulate in the vacuoles of epidermal cells where they attenuate the UV component of sunlight with minimal absorption of photosynthetically active radiation. In this issue, Casati and Walbot (pp. 1739–1754) evaluate how UV absorbing pigments modulate plant response to UV-B radiation. For this purpose, they used microarray hybridization to examine the acclima-
tion responses of four nearly isogenic maize (Zea mays) lines varying in flavonoid content to four ecologically relevant UV regimes. Microarray analysis confirmed literature reports on the impact of UV-B on photosynthetic genes and on flavonoid biosynthesis. Lines lacking UV absorbing pigments had more dramatic responses than did lines with these pigments, confirming the shielding role of these compounds. UV-B also increased the expression of stress response and ribosomal protein genes. Since there is evidence that UV-B radiation may inhibit protein synthesis in maize leaves by inducing crosslinks between RNA and ribosomal proteins, an important finding is the significant activation of genes supporting translation following UV irradiation.

**PAUSED Encodes an Exportin-t Ortholog**

Structurally related proteins known as importins and exportins mediate nucleocytoplasmic transport by binding a variety of protein and RNA cargoes and guiding their movement through the nuclear pore. Many fundamental cellular processes, such as the nuclear import of ribosomal proteins and the export of tRNA molecules, require importin or exportin activity. In this issue, Hunter et al. (pp. 2135–2143) show that **PAUSED** (PSD) is the Arabidopsis ortholog of **LOS1/exportin-t**. The protein Los1p/exportin-t mediates the nuclear export of tRNAs in Saccharomyces cerevisiae and mammals. Their demonstration that PSD is capable of rescuing the tRNA export defect of los1 in S. cerevisiae suggests that its function has been conserved. PSD was originally identified in a screen for mutations that affect meristem initiation during embryogenesis. An initial characterization of the **psd-1** mutant phenotype demonstrated that psd transiently disrupts the organization of the shoot apical meristem and delays leaf production, but does not have a significant effect on the timing of the transition to the adult phase of vegetative development. As a result, mutant plants produce their first adult leaves at inappropriate basal positions. Here, the authors show that plants doubly mutant for **paused** and **hasty**, the Arabidopsis ortholog of exportin 5, are viable but have a more severe phenotype than either single mutant. These results suggest that PAUSED plays a role in tRNA export in Arabidopsis, but that at least one, and perhaps several, additional tRNA export pathways also exist. The PAUSED transcript in all its alternative forms is broadly expressed during development. The authors propose that the mutant phenotype of **paused** reflects defects in developmental events and cell/tissue types that require elevated levels of protein synthesis and are therefore acutely sensitive to a reduction in tRNA export.

**Evolution and Function of Tocopherol Synthesis**

Tocopherols (vitamin E) are lipophilic antioxidants that are an essential nutrient for both humans and animals. Tocopherol synthesis has only been observed in photosynthetic organisms (plants, algae, and some cyanobacteria), a distribution that suggests the pathway evolved in cyanobacteria, possibly to protect cells from the reactive oxygen species generated by photosynthesis. Plant tocopherol biosynthetic enzymes are nuclear encoded and were presumably acquired from the endosymbiotic cyanobacteria that gave rise to plastids. The localization of tocopherols and most of the tocopherol biosynthetic enzymes in plastid membranes supports the cyanobacterial origins of the pathway in plants. Tocopherol cyclase catalyzes the conversion of various phytyl quinol intermediates to their corresponding tocopherols through the formation of the chromanol ring. In this issue, Sattler et al. (pp. 2184–2195) characterize tocopherol cyclase-deficient mutants from Arabidopsis, maize (Zea mays), and the cyanobacterium *Synechocystis* sp. PCC6803. Mutations in the genes that encode tocopherol cyclase result in both tocopherol deficiency and the accumulation of 2,3-dimethyl-6-phytyl-1,4-benzoquinone, a tocopherol cyclase substrate. The three tocopherol cyclases studied share significant amino acid similarity with each other and define an evolutionarily conserved gene family. The expression of the wild-type tocopherol gene from maize in a *Synechocystis* knockout mutant restored tocopherol synthesis, indicating that tocopherol cyclase activity is evolutionarily conserved between plants and cyanobacteria. The maize tocopherol biosynthesis mutant, which was originally isolated in a screen for Suc export mutants, is especially interesting because its phenotype includes aberrant plasmodesmata at the interface between the bundle sheath cells and the vascular parenchyma cells surrounding the minor veins. These defective plasmodesmata block symplastic transport of Suc to the phloem and hence cause the Suc export defect. The link between Vit E and plasmodesmatal function remains uncertain, but the authors discuss a number of fascinating possibilities.

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