On the Inside

Blue Light Photoreception by Chlamydomonas

Cryptochromes are flavin-binding proteins that act as blue light receptors in bacteria, fungi, plants, and insects and are components of the circadian oscillator in mammals. Animal and plant cryptochromes are evolutionarily divergent, although the unicellular alga Chlamydomonas reinhardtii has both an animal-like cryptochrome and a plant cryptochrome (pCRY; formerly CPH1). Müller et al. (pp. 185–201) present a functional characterization of pCRY based on an insertional mutant that expresses only 11% of the wild-type pCRY level. The pcry mutant is defective for central properties of the circadian clock. In the mutant, the period is significantly lengthened, ultimately resulting in arrhythmicity, while blue light-based phase shifts show large deviations from what is observed in wild-type cells. Gamete formation by vegetative Chlamydomonas cells is known to be triggered mainly by blue light. Thus, it is of interest that pCRY also is involved in gametogenesis in Chlamydomonas. pCRY is down-regulated in pregametes and gametes, and in the pcry mutant, there is altered transcript accumulation under blue light of the strictly light-dependent, gamete-specific gene GAS28. pCRY acts as a negative regulator for the induction of mating ability in the light and for the loss of mating ability in the dark (during which it accumulates). Moreover, pCRY is necessary for light-dependent germination, during which the zygote undergoes meiosis that gives rise to four vegetative cells. In summary, pCRY is a key blue light receptor in Chlamydomonas that is involved in both circadian timing and life cycle progression.

Suberin and Seed Dormancy

Environmental signals during seed production are important determinants of seed properties, including seed dormancy and seed longevity. The mother plant plays an important role in this signaling process, collecting signals throughout her life history and modulating dormancy by providing hormones to maturing seeds and by plastic development of the tissues surrounding the embryo. This process is especially important in seeds with physiological dormancy that is coat imposed, which requires the presence and activity of the seed coat and endosperm structures that form a barrier between the embryo and the external environment. To further understand the mechanisms by which the control of coat-induced dormancy takes place in Arabidopsis (Arabidopsis thaliana), Fedi et al. (pp. 276–283) conducted a forward genetic screen to isolate mutants that fail to enter dormancy in response to variation in temperature during seed set. They show that in one of these mutants, designated awake1, the maternal allele is required for entry into strongly dormant states. awake1 mutants show seed phenotypes shown previously to be associated with the loss of suberin in the seed. The authors identify awake1 as an allele of ABCG20, an ATP-binding cassette transporter-encoding gene required for the transport of fatty acids during suberin deposition, and show that further suberin-deficient mutants have seed dormancy defects. It has been previously been established that the suberin composition of seed coats is affected by temperature during seed maturation, but this response appears to be independent of ABCG20. The authors conclude that seed coat suberin is essential for seed dormancy imposition by low temperature and that the exclusion of oxygen and water from the seed by the suberin and tanin layers is important for dormancy imposition.

Thapsigargin Formation in Thapsia

The Mediterranean plant Thapsia garganica (Apiaceae), also known as deadly carrot, produces the highly toxic compound thapsigargin. This compound is a potent inhibitor of the sarcoplasmic-endooplasmic reticulum Ca2+-ATPase calcium pump in mammals and is of industrial importance as the active moiety of the anticancer drug mipsagargin, currently in clinical trials. Thapsigargin is found in most parts of the plant T. garganica. Ripe fruits contain the highest amount of thapsigargin, with 0.7% to 1.5% of the dry weight, followed by roots (0.2%–1.2% of dry weight) and leaves (0.1% of dry weight). It is well established that many Apiaceae species store lipophilic compounds such as phenyl propanoids and terpenoids in secretory ducts, and this appears to be the case with T. garganica as well. Andersen et al. (pp. 56–72) show that transcripts for two key enzymes in thapsigargin biosynthesis are found only in the epithelial cells lining these secretory ducts. This emphasizes the involvement of these cells in the biosynthesis of thapsigargin. This study paves the way for further studies of thapsigargin biosynthesis.

Nematode Cysts and DNA Methylation

Plant-parasitic cyst nematodes (Heterodera species) are among the most devastating pathogens of plant roots. These obligate parasites initiate an extended biotic interaction with their host plants involving formation of an operative feeding structure, the syncytium, that is vital for nematode survival and development. The nematode provokes differentially terminated cells in the vascular root tissues to redifferentiate into a syncytium cell type, a switch that involves simultaneous changes in the expression of thousands of genes. Though the mechanisms controlling gene expression changes in the syncytium remain ill defined, recent studies indicate that epigenetic mechanisms including noncoding small RNAs and DNA methylation may play fundamental roles. DNA methylation can regulate the expression of protein-coding genes and the activity of transposable elements. Hewezı et al. (pp. 405–420) have generated mRNA and small RNA transcriptomes of Arabidopsis roots infected with the beet cyst nematode Heterodera schachtii as well as methylome maps of single-base resolution. They report extensive differences in the methylomes of Arabidopsis roots during the nematode infective stages corresponding to syncytium formation and maintenance phases. H. schachtii-induced methylome changes are characterized by substantial increases in hypomethylation patterns...
that occurred predominantly in gene 

bodies and transposable elements in 
a context-specific fashion. Collectively, 

their data suggest that differential DNA 
methylation associated with gene ex-

pression changes in the syncytium 
may determine the compatibility of 
the interaction between Arabidopsis and 

H. schachtii.

Coproporphyrinogen III 

Oxidase and Gametophyte 

Development

All organisms including plants share 

the tetrapyrrole biosynthesis pathway 

that is critical for the production of 

compounds such as heme and chloro-

phyll. During tetrapyrrole biosynthesis, 
coproporphyrinogen III oxidase (CPO) 
catalyzes the conversion of copropor-

phyrinogen III into protoporphyrino-

gen IX. Pratibha et al. (pp. 258–275) 
report the results of the characterization 
of a mutation in the Arabidopsis gene 

At5g63290 that is orthologous to bacte-

rial and mammalian CPO. As this gene 

shows greater homology with HemN-

like CPO, they named it AtHEMN1. 
Loss of AtHEMN1 function increased 
coproporphyrinogen III level and re-

duced protoporphyrinogen IX level, 
suggesting the impairment of tetrapyr-

role biosynthesis. Mutations that dis-

rupted AtHEMN1 adversely affected 
silique length, ovule number, and seed 
set. ATHEMN1 mutant alleles could be 
transmitted via both male and female 
gametes, but homozygous mutants 
were never recovered. Embryo devel-

opment in AtHEMN1 was arrested at 
the globular stage, but the mutant 
phenotype was completely rescued by 
transgenic expression of AtHEMN1. 
Promoter and transcript analyses in-

dicated that AtHEMN1 is expressed 

mainly in floral tissues and developing 

seeds. AtHEMN1-GFP fusion protein 

was found targeted to mitochondria. 

Athemn1 mutant alleles also showed 
defects in gametophyte development, 
including nonviable pollen and em-

bryo sacs with unfused polar nuclei. 
Improper differentiation of the central 
cell led to defects in endosperm devel-

opment. Inactivation of any of the en-

zymes of the tetrapyrrole biosynthetic 
pathway leads to the accumulation 
of porphyrin compounds and causes 
cell death in plants through reactive 

oxygen species (ROS) production. As 
expected, the blockage of tetrapyrrole 
biosynthesis in the AtHEMN1 mutant 
also led to increased ROS accumula-

tion in anthers and embryo sacs, as 
evidenced by nitroblue tetrazolium 
staining. Thus, it appears that that 
the tetrapyrrole/heme biosynthesis 
pathway operates in mitochondria 

and its impairment disturbs ROS 
homeostasis in flower buds, thereby 
adversely affecting male and fe-

male gametophyte development in 

Arabidopsis.