Journal Club

First plant circadian phase mutant identified

Circadian rhythms exhibited by plants include leaf movements, CO_2 fixation, stomatal conductance and gene expression. They have evolved in response to, and are entrained by, environmental periodicities (zeitgeber), primarily daily changes in light intensity, such that the endogenous oscillation adopts the 24 h periodicity of the zeitgeber and a particular phase relationship to it. The phytochrome (PHY) and cryptochrome (CRY) photoreceptor families mediate light input to the clock and the importance of several of these proteins in establishing period length has recently become apparent. However, how the phase of plant rhythms is determined is unknown. Indeed, the *Arabidopsis* Genome Initiative revealed no plant orthologues to clock proteins involved in phase determination in animal and prokaryote circadian systems.

'…this study identifies PHYB as an important regulator of white light-mediated phase determination…'

In a new study, Patrice A. Salomé and colleagues [1] describe a new allele of *PHYB, out of phase 1* (*oop1*), which confers an altered circadian phase phenotype. The *Arabidopsis thaliana* mutant was isolated by exploiting a rhythm of sensitivity to the air pollutant sulfur dioxide, selecting plants exhibiting damaged leaves at a time when wild-type plants were resistant. After 7 days entrainment in a 12-h:12-h (white) light–dark regime, the peaks of leaf movement and CO_2 assimilation rhythms in *oop1* plants occurred 3.6 and 3.4 h earlier, respectively, than in wild-type plants. Period lengths were unaltered. Sequencing revealed two mutations in $PHYB^{oop1}$; one introducing an early stop codon such that a truncated protein lacking most of the C-terminal kinase domain is expressed. Because *oop1* seedlings displayed light-induced inhibition of hypocotyl elongation under blue or far-red light but not red light, the authors propose that the altered phase phenotype results primarily from impaired red-light photoreception through PHYB. Indeed, when *phyB-9*, a previously identified complete loss-of-function allele, was crossed into plants harbouring a transcriptional fusion of the *LIGHT-HARVESTING CHLOROPHYLL a/b BINDING PROTEIN* promoter and luciferase (*LHCB::LUC*), *LHCB::LUC* rhythms were coincident with those exhibited by the corresponding *oop1* cross. However, the data suggest that the *oop1* mutation also interferes with other light-signalling pathways because the elongated hypocotyl phenotype was also observed when *oop1* seedlings were illuminated by white light or combinations of red light with high fluence blue light. This implies a red light-dependent alteration of blue-light signalling, presumably through *CRY1*, the primary blue-light photoreceptor at high fluence rates.

In characterizing *oop1*, the first circadian phase mutant of a plant, this study identifies PHYB as an important regulator of white light-mediated phase determination, and, as such, represents an important milestone in circadian biology. Observations that *oop1* plants exhibit wild-type period length and do not show the altered phase phenotype when entrained to temperature cycles indicate that oscillator function itself is not affected. However, whether impaired PHYB signalling affects light input to the clock or components of the output pathway downstream of the oscillator remains to be determined. Future studies on PHY INTERACTING FACTOR 3, a target of PHYB signalling, which activates transcription of two known clock components in plants should help resolve this question and provide further insight into the molecular basis of phase determination in plants.

1 Salomé, P.A. *et al.* (2002). The *out of phase 1* mutant defines a role for PHYB in circadian phase control in *Arabidopsis*. *Plant Physiol.* 129, 1674–1685

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Revealing a savior of manly vigor in plants

The superior performance of heterozygous hybrid progeny over its homozygous parents is widely exploited in crop production. Hybrid seed production is facilitated by the maternally inherited absence of viable pollen in cytoplasmic male-sterile plants, thereby eliminating the need of hand emasculation. Cytoplasmic male-sterility (CMS) occurs in many plant species, and is usually ascribed to mitochondrial defects. If seeds are the harvested products, the use of restorer genes suppressing the male-sterile phenotype in the hybrids is essential. These opposite players, called restorers of fertility (Rf), are able to avoid or overcome the mitochondrial defects and are encoded in the nuclear genome. However, the nature of these components and the mechanisms underlying the restoration of fertility through changing the expression of CMS-associated mitochondrial genes were unknown.

Now, Stéphane Bentolila and colleagues [1] describe the identification of the first *Rf* gene that reduces the expression of an aberrant mitochondrial protein. The authors analyzed a petunia CMS system, composed of the male sterility-conferring chimeric mitochondrial open reading frame *PCF* (petunia CMS-associated fused) and the nuclear restorer locus *Rf* which, if present, reduces *PCF* expression and the amount of protein back to non-harming levels. Previous genetic delimitation of the *Rf* locus to a small genomic region facilitated its identification. The *Rf* gene was found to encode a protein of 592 amino acids that has a functional mitochondrial transit peptide and is almost entirely composed of a degenerate 35-amino acid pentatricopeptide-repeat (PPR) motif, and thus named *Rf-PPR592*. Importantly, Bentolila and colleagues [1] demonstrate that transformation of CMS petunia lines with a construct encoding Rf-PPR592 restores fertility, concomitant

 1360-1385/02/$ – see front matter

with a reduction of the PCF protein level. Interestingly, the non-restoring *rf-PPR592* allele in a CMS plant has a deletion in its promoter region that prevents its expression in CMS floral buds.

This is an important step towards understanding the mechanism of fertility restoration and nuclear impact on mitochondrial gene expression in plants. It would be interesting to determine the exact mode of action of Rf-PPR592. PPR proteins comprise a large gene family in plants with >200 family members in the *Arabidopsis* genome. Current data on the molecular function of PPR-motif-containing proteins in different organisms indicate RNA binding and involvement in post-transcriptional processing of particular organellar transcripts – this fits well with the function now identified for at least one of its members as an *Rf* gene. The work of Bentolila *et al.* [1] provides insight into the nuclear regulation of mitochondrial functions and opens the way to a more systematic analysis of the involvement of PPR-motif-containing proteins in restoring male-fertility in plants.

1 Bentolila, S. *et al.* (2002) A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Natl. Acad. Sci. U. S. A.* 99, 10887–10892

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Regulatory gene networks involved in the cold transduction pathway

Arabidopsis can increase its freezing tolerance in response to low, non-freezing temperature. This process, called cold acclimation, involves several biochemical and physiological changes that seem to be regulated through changes in gene expression. Genetic analyses have revealed that multiple genes are involved in cold acclimation, and many genes whose transcript levels accumulate in response to low temperature have been isolated and characterized. CBF/DREB are ap2 transcriptional factors whose expression is induced rapidly and transiently by low temperature, binding the low temperature *cis*-acting element CCGAC. Different studies have established that CBF/DREB can function as transcriptional activators controlling the level of low temperature gene expression of their target genes (CBF regulon) and promoting freezing tolerance through an abscisic acid-independent pathway.

Now Sarah Fowler and Michael Thomashow [1] report an important insight into the cold-regulatory networks in *Arabidopsis*. The group performed a transcriptome analysis with ~8000 *Arabidopsis* genes and studied the level of gene expression in response to low temperatures in plants that were grown in low temperature and in untreated transgenic plants that constitutively expressed *CBF1*, *CBF2* or *CBF3*. Among the genes analysed, they found that 306 were cold responsive; 218 of the 306 were up-regulated and the remaining 88 were down-regulated by low temperature. These results could indicate that unexpected general down-regulation occurs during exposure to low temperature. Furthermore, among the cold-responsive genes, up to 48 encode transcription factors. Surprisingly, the expression levels of two of the transcription factors, RAP2.1 and RAP2.6, are turned on by the action of *CBF* genes. These data suggest that these transcription factors control a 'subregulon inside a CBF regulon'. Further analysis of the cold-responsive genes indicated that only 12% of the cold-responsive genes are positive members of the CBF regulon. In addition, analysis of the CBF over-expressing plants shows that 28% of the cold-responsive genes are not under the control of the CBF transcription factors, as well as 15 cold-responsive genes encoding putative transcription factors not known to be involved before in the cold-acclimation process. These data suggest that these genes belong to a separate low-temperature regulon.

'...data suggest a new scenario where, in addition to the previously described CBF cold-response pathway, other multiple regulatory pathways exist...'

The current research has identified >250 newly described cold-responsive genes, prompting new ideas about the different biochemical and physiological changes that happen during cold acclimation. Furthermore, this study demonstrates the highly multifaceted nature of plant adaptation to low temperature. The results indicate that expression of many *Arabidopsis* genes is affected by low temperature. These genes connect the action of multiple cold-regulatory pathways, including the activation of regulons inside regulons. These data suggest a new scenario where, in addition to the previously described CBF cold-response pathway, other multiple regulatory pathways exist that are likely to function in a cold signal transduction cascade.

Now that the multiplicity of cold-dependent stress signalling pathways has been demonstrated in *Arabidopsis*, we need to determine whether other low-temperature gene networks contribute significantly to the freezing tolerance of other features of growth and development at low temperature. Furthermore, it would be meaningful to reveal the range of stress responses involving *CBF/DREB1* genes. How does low temperature activate a specific set of transcription factors? Fowler and Thomashow have studied the mRNA profiles of many genes that have the potential to modulate plant adaptation to low temperature, an important step in understanding how plants integrate environmental responses.

1 Fowler, S. and Thomashow, M.F. (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell* 14, 1675–1690

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 1360-1385/02/$ – see front matter