The Plant Cell, Vol. 13, 219, February 2001, www.plantcell.org © 2001 American Society of Plant Physiologists

IN THIS ISSUE

From Darkness into Light: Factors Controlling Photomorphogenesis

A seedling that emerges in darkness follows a developmental program known as skotomorphogenesis (dark development), which is characterized by etiolation, that is, a long, spindly hypocotyl and pale cotyledons and inhibition of chlorophyll and anthocyanin biosynthesis and true leaf development. Upon exposure to light, the seedling switches rapidly to photomorphogenesis (light development); the hypocotyl ceases rapid elongation and becomes thicker, the shoot apical meristem is activated, chlorophyll and anthocyanin biosynthesis is initiated, and true leaves begin to develop. It has long been known that this process is triggered directly by light perception and signal transduction, because developmental changes in the hypocotyl and cotyledons can be detected within minutes of exposure to a light source. Plants have an array of light-sensing molecules that function in association with numerous downstream targets to allow for the precise determination of light quality, quantity, and duration. This issue of *The Plant Cell* includes two papers related to light perception and photomorphogenesis. **On pages 425–436, Kohchi et al.** report the isolation of Arabidopsis *HY2* and its identification as the gene encoding phytochromobilin synthase. The HY2 protein catalyzes the last step in the production of the chromophore (phytochromobilin) entity of phytochromes, the red light photoreceptors that are essential for photomorphogenesis and many other light responses of plants. Meanwhile, **Yamamoto et al. (pages 399–411)** report on the characterization of a novel protein, CIP4, which acts downstream of the photoreceptors and is required for the promotion of photomorphogenesis via interactions with COP1 (a repressor of photomorphogenesis).

LIGHT PERCEPTION

There are two general classes of photomorphogenic mutants: those that display an etiolated, skotomorphogenic phenotype in the light and those that show aspects of a photomorphogenic phenotype when grown in the dark. Mutants that display an etiolated phenotype in the light identify positive regulatory components of photomorphogenesis. Koornneef et al. (1980) isolated a number of this type of mutant, called *long hypocotyl* or *hy* mutants, that fail to perceive light and produce long hypocotyls whether they are grown in light or darkness. Most of these are photoreceptor mutations, which collectively have shown that plants integrate signals perceived by numerous photoreceptors for normal photomorphogenic development. For example, the *hy8* mutant is insensitive to far-red light, and the gene was found to encode the apoprotein of phytochrome A (renamed *PHYA*); the *hy3* mutant is insensitive to red light, and the gene encodes the PHYB apoprotein (*PHYB* gene); and *HY4*, the mutant of which is insensitive to blue light, encodes the apoprotein of the blue light receptor CRY1. *HY5* is the only gene in this group that is not directly related to photoreception; rather, it encodes a transcription factor that acts downstream of the other *HY* genes (see below).

It has long been suspected that *hy1*, *hy2*, and *hy6* mutants are defective in chromophore biosynthesis or attachment (Parks and Quail, 1991; Chory, 1992). Phytochromes consist of an apoprotein with a thioether-linked chromophore called phytochromobilin. Apoproteins are encoded by five different genes in Arabidopsis (*PHYA* to *PHYE*), each of which has distinct spectral qualities. Phytochromobilin appears to be the chromophore partner of all of the phytochromes; thus, the *hy1*, *hy2*, and *hy6* mutants are deficient in all of the phytochromes and in both red and far-red light responses. Genetic analysis suggests that the *hy6* mutation lies at a locus distinct from *hy1* and *hy2* (Chory et al., 1989). The *hy6* mutation has not been well studied since this initial report, perhaps because the mutant seed currently available are reported to carry a mutation in the *HY1* gene (Muramoto et al., 1999). The *HY1* gene was found to encode a heme oxygenase that localizes to chloroplasts and catalyzes the conversion of heme to a biliverdin precursor of phytochromobilin (Muramoto et al., 1999). Kohchi et al. (2001) show that the *HY2* gene encodes phytochromobilin synthase, which is responsible for the next step in this pathway, the conversion of biliverdin to phytochromobilin. They demonstrate the catalytic activity of the recombinant protein and the chloroplast localization of a fusion protein consisting of the green fluorescent protein reporter fused to the putative phytochromobilin synthase chloroplast transit peptide.

Interestingly, the *hy1* mutant phenotype is severe only at the seedling stage; later in development, the mutant plants appear relatively healthy. One explanation is that the plants have an alternate pathway for chromophore biosynthesis that is activated later in development. Consistent with this view, the Arabidopsis genome has other genes

IN THIS ISSUE

with similarities to *HY1* (Muramoto et al., 1999; Kohchi et al., 2001). However, the *hy2* mutant phenotype is less severe than that of *hy1*, even at the seedling stage, and no genes with similarities to *HY2* have been found in the Arabidopsis genome (Kohchi et al., 2001). These observations might simply reflect differences in allele strength, and a definitive study of the phenotypic effects of null versus less severe mutant alleles is needed. Another interesting possibility is that the phytochrome signal transduction pathway overlaps with other developmental signal transduction pathways and the phytochrome pathway becomes relatively less critical to certain aspects of growth later in development, when other signals begin to act on many of the same downstream effectors. The identification of the *HY1* and *HY2* genes should help to resolve some of these questions.

LIGHT SIGNAL TRANSDUCTION

Mutants that exhibit aspects of a photomorphogenic phenotype when grown in the dark identify negative regulators of light signal transduction. These include the *constitutive photomorphogenic/de-etiolated/fusca* (*cop/det/fus*) group of mutants (reviewed in Chory, 1992; Howell, 1998; Hardtke and Deng, 2000). *cop* and *det* mutants are similar and highly pleiotropic; that is, the mutations cause a number of different phenotypic effects (in this case, phenotypic effects associated with photomorphogenesis, such as a shortened hypocotyl, opened and expanded cotyledons, and increased expression of certain light-regulated genes). The *fus* mutants, seedling-lethal mutants that accumulate anthocyanins in the cotyledons (a photomorphogenic characteristic), have been found to be null or severe alleles of various *cop/det* mutants. All of these mutations are recessive loss-of-function mutations that promote photomorphogenesis, suggesting that their normal function is to suppress photomorphogenesis in the dark.

The *COP1* gene product occupies a central position in the regulation of photomorphogenesis. COP1 appears to suppress the activity of downstream factors that promote photomorphogenesis by tagging them for proteolytic degradation. Interaction with COP1 has been definitively shown for HY5, a bZIP DNA binding transcriptional activator that promotes photomorphogenesis in the light (Osterlund et al., 2000). Consistent with a primary role in promoting photomorphogenesis, all of the photoreceptor *hy* mutants have abnormally low levels of HY5 in the light, and in wild-type seedlings, HY5 levels are positively correlated with light intensity and degree of hypocotyl elongation. COP1 is localized to the nucleus in the dark, where it appears to target HY5 for proteasome-mediated degradation; it is excluded from the nucleus and routed to the cytoplasm in the light, thus allowing the nuclear accumulation of HY5 and the consequent promotion of photomorphogenesis (Osterlund et al., 2000).

Most of the *COP/DET/FUS* genes have been found to encode components of what has been named the *COP9 signalosome*, or CSN (Wei and Deng, 1999). The CSN is a complex of eight subunits that are similar to the subunits that make up the lid of the 26S proteasome, a protein degradation complex responsible for the degradation of ubiquitinated proteins that are present in all eukaryotes. The CSN itself also is present in animal as well as plant cells, and the CSN and the 26S proteasome lid are probably derived from a common ancestral complex (Wei and Deng, 1999). At least eight of the *cop/det/fus* mutants are "CSN" mutants, which fail to accumulate the complex and likely encode CSN structural components. The exact function of the CSN is unknown, but evidence suggests that it may be related to protein degradation. Deshaies and Meyerowitz (2000) propose a model for CSN function wherein the CSN recognizes and delivers COP1-targeted proteins to the proteasome for degradation, either functioning in place of the proteasome lid for COP1-targeted proteins or perhaps delivering COP1-targeted proteins to the lid of the intact proteasome. COP1 contains a RING finger domain and WD-40 repeats, both of which have been shown to be involved in the activity of ubiquitin ligases, thus offering the attractive possibility that COP1 is a ubiquitin ligase that targets proteins for interaction with the CSN and the proteasome via ubiquitination (Deshaies and Meyerowitz, 2000).

On the basis of the hypothesis that COP1 is a key negative regulator of photomorphogenesis with multiple protein–protein interaction domains, Yamamoto et al. (1998) set out to identify COP1-interacting proteins (CIPs) by screening an Arabidopsis cDNA expression library using ^{32}P-labeled COP1 protein as a probe. They identified CIP7 as a positive regulator of anthocyanin biosynthesis and chlorophyll accumulation during photomorphogenesis and as a potential target for COP1-mediated repression. In this issue, Yamamoto et al. (2001) characterize CIP4, another potential target of COP1 and a positive regulator of chlorophyll accumulation and hypocotyl elongation during photomorphogenesis. Thus, a clearer picture of COP1 emerges, one of a protein that acts in the nucleus and interacts specifically with transcriptional activators of photomorphogenesis to target them for degradation. Consistent with this view, Cao et al. (2000) identified CR88 as another possible target of COP1. The *cr88* mutant is a chlorate-resistant mutant that displays a long-hypocotyl phenotype in red light and reduced expression of several light-induced genes. Although a direct interaction between COP1 and CR88 has not been shown, Cao et al. (2000) demonstrated that *CR88* acts downstream of *COP1*, in a branch separate from *HY5*, to pro-

mote the greening process during photomorphogenesis.

The approach used by Yamamoto et al. (1998, 2001) may also work to identify upstream factors that interact with COP1 and/or factors involved directly with COP1 function. For example, CIP1 is a cytosolic protein associated with the cytoskeleton; thus, it might be involved in the localization of COP1 to the cytoplasm in the light (Matsui et al., 1995; Hardtke and Deng, 2000). Another CIP that has been described is CIP8, which is a RING finger protein that interacts with the COP1 RING finger domain (Torii et al., 1999). RING finger interactions are a feature of E3 ubiquitin ligase complexes; thus, CIP8 offers another tantalizing piece of evidence for a similar role for COP1. Further characterization of these and other CIPs should help to define COP1 function and its mechanism of action more clearly.

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Plant Development: From Cell Fate to Organ Formation

An excellent workshop under this title was held on the island of Capri from October 21 to 24, 2000. It was organized by Chris Bowler and Roberto Defez, as the 13th Meeting of the International Institute of Genetics and Biophysics (IIGB, Naples, Italy), and brought together plant developmental biologists covering a diverse set of topics, which were actually much broader than betrayed by the title. This report summarizes the presentations at the workshop, which was made possible through financial support from the European Union.

HORMONE SIGNALING

Hormones control not only many aspects of development, but also responses of plants to the environment. One of the emerging themes is the convergence and interaction of several

MEETING REPORT

hormone pathways. Jérôme Giraudat (Gif-sur-Yvette) discussed several genetic screens to identify new Arabidopsis loci mediating the responses to the hormone abscisic acid (ABA). Screens for enhancers and suppressors of ABA insensitive mutants led to the isolation of ethylene response mutants. Incorporating recent work from several laboratories, Giraudat presented a model in which ABA acts downstream of hexose sensing to affect seed germination, and the effects of ABA are modulated both by gibberellins and ethylene.

The effects of gibberellins on Arabidopsis development are mediated by a subgroup of GRAS transcription factors, including GAI and RGA, which are distinguished from other family members by an N-terminal extension (Nick Harberd, Norwich). Partial deletion of this N-terminal extension in GAI causes constitutive repression of gibberellin responses, resulting in dwarf plants. Similar deletions are found in the *GAI* ortholog of wheat, appropriately called *Reduced height* (*Rht*). The dwarfism caused by the dominant *Rht* alleles was the basis for the green revolution. While the agronomic utility of *GAI* and its orthologs has been aptly demonstrated, it is still unclear how GAI affects gibberellin responses at the molecular level. GAI has some similarity to the mammalian family of STAT transcription factors, many members of which reside in their inactive form in the cytoplasm, and are translocated into the nucleus when activated. Experiments to determine if gibberellin treatment similarly affects the subcellular localization of GAI are in progress. It has already been shown that gibberellins are required for nuclear localization of PHOR1, a potato protein with armadillo repeats (Jaime Martínez-García, Barcelona). Interestingly, gibberellins regulate *PHOR1* at several levels, since its RNA is upregulated upon gibberellin treatment, which in turn is dependent on photoperiod. Gibberellins inhibit tuberization in potato, and *PHOR1* is apparently like GAI and RGA a negative regulator of gibberellin response, since *PHOR1* antisense plants are semidwarves that tuberize early. Another gene expressed differentially during tuberization was described by Christian Bachem (Wageningen). Upregulation of this gene, *CB12*, which encodes a protein with similarity to short chain alcohol dehydrogenases, closely correlates with tuber formation, and overexpression experiments confirmed that *CBI2* has a regulatory role in tuber formation. The overexpressing plants are also spindly, mimicking the effects of exogenous gibberellin application, which provides a further possible link between gibberellin signaling and tuberization.

The gibberellin antagonist ABA acts also antagonistically with brassinosteroids, as discussed by Joanne Chory (La Jolla), whose laboratory has identified two Arabidopsis transcription factor genes that integrate brassinosteroid and ABA signals. These factors act downstream of the BRI1 leucine-rich receptor kinase, a candidate receptor for brassinosteroids. Chory described a series of elegant experiments proving that BRI is indeed the brassinosteroid receptor. First, *bri1* mutants have reduced brassinosteroid binding, while overexpression of a BRI1:GFP fusion protein increases binding. Second, radioactively labeled brassinosteroid can be co-immunoprecipitated with the BRI1:GFP protein from plants. Third, brassinosteroid treatment induces BRI1 autophosphorylation. How the changes in BRI1 phosphorylation ultimately regulate downstream targets is not known, but candidates for signaling components have been identified through mutants that are resistant to the effects of the brassinosteroid biosynthesis inhibitor brassinazole.

In contrast to brassinosteroids, receptors for most other major plant hormones have not been identified. A candidate for a hormone receptor is GCR1, an Arabidopsis protein similar to G-protein coupled receptors from fungi and mammals (Richard Hooley, Long Ashton). Initial results suggested that this receptor has a role in cytokinin signaling, but this seems less likely now, as a knockout mutant has normal cytokinin sensitivity. However, GCR1 can couple to chimeric G protein alpha subunits in yeast, confirming that it plays a role in transducing a—yet to be identified—signal.

Most plant hormones act systemically, but are not necessarily synthesized throughout the plant. This is especially true for auxins, in which transport involves efflux carriers of the PIN family. Two of these have been shown before to be required for auxin-dependent processes in the shoot and root, and Klaus Palme (Cologne) presented work showing that knockouts of two other family members, PIN3 and PIN4, also cause auxin-related phenotypes. He showed beautiful images of PIN protein localization, including predominantly lateral localization of PIN3 in endodermal cells. The practical value of being able to manipulate auxins was discussed by Angelo Spena (Verona), who has been able to improve fruit quality and productivity in several vegetable and fruit species by inducing parthenocarpy, using ovule-specific expression of the bacterial *iaaM* auxin biosynthetic gene in transgenic plants. Parthenocarpy has several applications, including increasing the size of tasty fruits from wild plants; inducing fruit development during seasons in which plants do not easily self-fertilize; and producing seed-less fruits. Contrary to conventional wisdom, these results were achieved in the absence of mutations that cause male or female sterility.

LIGHT SIGNALING

One the most important external cues for plants is light. While the phytochrome family of red-light photoreceptors has been known for a long time, it has only been very recently that the

MEETING REPORT

molecular mechanisms of phytochrome signaling have been elucidated. Some of these signaling pathways have turned out to be surprisingly short (Peter Quail, Berkeley). Phytochrome B can interact with a basic helix-loop-helix protein, PIF3, both on and off DNA. Importantly, phytochrome B binds only to PIF3 in its biologically active Pfr form, but not in the inactive Pr form. In contrast to PIF3, the related HFR1 protein does not bind to either phytochrome A or B, even though HFR1 also acts downstream of phytochromes in mediating light response. Phytochromes are likely to modulate HFR1 activity through PIF3, which can heterodimerize with HFR1. Given the large number of basic helix-loop-helix proteins encoded in the Arabidopsis genome, there is an enormous potential for interactions of hetero- and homodimers of these proteins with the various phytochromes, providing a possible mechanism through which phytochromes can relatively directly affect a large number of target genes. Support for a role of phytochromes in transcriptional regulation comes from recent reports on dynamic, light-regulated nuclear localization of phytochromes (Eberhard Schäfer, Freiburg). However, phytochromes interact not only with DNA binding proteins. Schäfer reported that ARR4, a member of the response regulator-like family of proteins, interacts with phytochrome B both in vitro and in vivo, and that ARR4 stabilizes phytochrome B in its active, Pfr form. Another nuclear effector of phytochrome A was identified through mutations in the *EID1* gene, which has just been cloned.

An important readout of light signaling is elongation growth, and Monica Carabelli (Rome) described the role of homeodomain-leucine zipper proteins in this process. The gene encoding one of these proteins, ATHB-2, is rapidly induced under light with a low red to far-red ratio, which mimics shading by neighboring plants. As part of the phytochrome-dependent shade avoidance response, plants elongate in the shade, and overexpression of ATHB-2 was shown to be sufficient to induce elongation growth. As with hypocotyl elongation that is induced as part of the shade avoidance response, hypocotyl elongation induced by ATHB-2 requires polar auxin transport. Based on transgenic phenotypes and expression analyses, Carabelli proposed that other members of this family regulate similar processes as ATHB-2, but in response to other signals.

Another group of genes acting downstream of photoreceptors comprises the *COP*/*DET*/*FUS* genes, which suppress light-dependent development (Xing-Wang Deng, New Haven). Many of the COP/FUS proteins form a multimeric protein complex, known as the COP9 signalosome (originally called the COP9 complex). The similarity of the COP9 complex with the lid of the 26S proteasome suggests a role in ubiquitin-mediated degradation, and another protein, COP1, may act as a ubiquitin ligase with a regulatory role in this process. One substrate of COP1-targeted degradation is the HY5 transcription factor, which is phosphorylated by a light-regulated kinase. In the light, HY5 is predominantly unphosphorylated, which increases both its affinity for target promoters and for the interacting COP1 protein. It is also the unphosphorylated form of HY5 that is preferentially degraded. However, because COP1 is predominantly found in the cytoplasm in light, nuclearly localized HY5 accumulates in the light. COP1 can interact with several distinct substrates through its WD-40 repeats, and Deng discussed specific point mutations in the WD-40 repeats that either enhance or reduce interaction with subsets of COP1 partners. The relevance of these differential interactions was confirmed in vivo, where overexpression of the point mutants induced different, specific gain-of-function or loss-of-function phenotypes.

Another member of the COP/DET/FUS class is encoded by the tomato ortholog of the Arabidopsis DET1 gene (Chris Bowler, Naples). Although DET1 homologs exist in humans and flies, their biochemical function has remained enigmatic. A possible role in chromatin modification has been revealed through the ability of tDET1 to bind the N-terminal domain of one of the histones. Given that endogenous DET1 levels are very low, it is possible that the interaction with histones points to an enzymatic role of DET1 in chromatin modification. Bowler also described his very interesting research on marine diatoms, which provide about one quarter of all fixed carbon on the planet. In contrast to land plants, diatoms live in an environment that has only very little red light. Interestingly, although red light is rapidly extinguished as sun light travels through the water column of the ocean, red light intensities at depths of up to 100 meters are similar to those inducing low and very low-fluence responses in land plants. This red light is likely due to fluorescence of photosynthetic pigment, and might be exploited by diatoms in a neighbor-sensing quorum mechanism. It will be exciting to find out whether diatoms use phytochrome-like molecules for such responses.

STRESS SIGNALING

Because plants cannot move, they have to respond to environmental stress by modifying cellular and developmental parameters. One class of transcription factors that plays an important role in adjusting metabolic pathways in response to environmental stress is the Myb family (Chiara Tonelli, Milan). Because few Myb factors have been identified in mutant screens and because only a minority of knockout mutants has obvious phenotypes, a European consortium aims to characterize all Arabidopsis Myb genes at several different levels. Most Myb

MEETING REPORT

genes are under exquisite transcriptional control themselves, and by comparing their RNA expression profiles under different conditions with that of known response genes, it has been possible to assign several Myb genes to specific stress response pathways. A large-scale genomic approach to stress response is also taken by a US consortium, whose efforts were discussed by Ray Bressan (West Lafayette). In one experimental approach, several hundreds of thousands of transgenic plants have been generated, to isolate tagged loss- and gain-of-function mutants with altered stress responses. Perhaps the most impressive slide of the meeting showed one of Bressan's coworkers holding a flask containing more than 10 liters of seeds from *Agrobacterium*-treated Arabidopsis plants. This batch, which comprised hundreds of millions of seeds, contained more than a million transformed seeds, aptly demonstrating the power of Arabidopsis transformation.

A surprising route to salt stress tolerance was discussed by Dirk Inzé (Ghent). Plants contain homologs of many cell cycle regulators that were first described in yeast and mammals, including several families of cyclins and cyclin dependent kinases (CDKs). To test the role of CDK Inhibitors (CKIs) in arresting cell cycle progression in response to abiotic stress, a CDK A variant that can no longer be phosphorylated by the CKI WEE1 was introduced into plants. These plants showed increased resistance of salt stress compared with wild-type plants. Another class of CKIs comprises the $p27^{KIP}$-related proteins (KRPs), one of which shows a particularly interesting expression pattern in the epidermis of young leaf primordia, but not in the shoot apical meristem. Overexpression of this KRP isoform caused a remarkable leaf phenotype, with larger cells and reduced ploidy levels.

A ubiquitous response to heat stress is the transcriptional activation of heat shock protein (HSP) genes encoding chaperones. However, these proteins play also important roles under other conditions. For example, HSP90 buffers against morphogenetic variation in Drosophila, and mutating HSP90 reveals phenotypic variation caused by small genetic differences that on their own do not cause phenotypic differences in wild-type animals. Christine Queitsch (Chicago) discussed that a similar phenomenon exists in plants, as treatment with the HSP90 inhibitor geldanamycin can induce many developmental abnormalities in Arabidopsis. Different effects were observed in different accessions and in different recombinant inbred lines, revealing a genetic component in Arabidopsis as well.

PLANT–MICROBE INTERACTIONS

Plants respond not only to abiotic, but also to biotic components of their environment. For example, many plants can form root symbioses either with fungi or bacteria, which requires complex signaling between microbes and plants. *Rhizobium* bacteria produce Nod factors, which induce a number of sequential responses in the host plant that are required for productive infection by *Rhizobium*, beginning with root hair curling tip growth and culminating in nodule formation (Ton Bisseling, Wageningen). Use of Nod factors with a fluorescent tag showed that Nod factors became predominantly associated with the cell wall, whereas very little Nod factor was associated with the plasma membrane of host cells. By applying a new single-molecule detection technique, fluorescence correlation microscopy, it could furthermore be demonstrated that Nod factors become up to fifty-fold concentrated in the cell wall of root hairs and are markedly immobilized. In this way, local secretion of Nod factors might provide positional information to direct root hair curling. Biochemical approaches to identifying Nod-factor receptors have so far not been successful, but genetic approaches to dissect the downstream events of Nod factor binding to the plant cell wall are very promising. Natural variants of pea that respond differently to different Nod factors have been identified and the responsible loci placed on the genetic map. Positional cloning of such alleles is possible by exploiting synteny with the model legume *Medicago truncatula*, for which many genomic resources are available.

Genes involved in the early events of nodulation have also been isolated from the semi-aquatic tropical legume *Sesbania rostrata*, in which stem nodulation occurs from dormant lateral root meristems, which are present at predetermined positions (Marcelle Holsters, Ghent). Among the genes identified because of their differential expression upon nodulation is an acidic chitinase that can degrade specific Nod factors in vitro. Another gene, which encodes a chitinase homolog that has lost its hydrolytic activity, is activated during early stages of the infection. Experiments to test whether this protein is involved in Nod factor binding are under way.

The formation of nodules with a longer life time can increase plant productivity, and one promising avenue is by increasing the delivery of auxin from endosymbiontic bacteria (Roberto Defez, Naples). This can be achieved by expressing auxin biosynthetic genes from *Agrobacterium rhizogenes* in *Rhizobium* under control of the *rolA* prokaryotic promoter. Infecting both temperate and tropical legumes with *Rhizobium* carrying the appropriate constructs led to an increase in nitrogen fixation, plant dry weight and seed production.

Apart from symbionts, plants have to respond to a large number of pathogenic bacteria. Nitric oxide is an important signaling molecule in this process (Francesca Fenzi, Freiburg). Using pharmacological experiments and plant cells expressing the calcium indicator aequorin, it was shown that calcium fluxes

from both extra- and intracellular pools function downstream of nitric oxide.

EMBRYO AND SEED DEVELOPMENT

In contrast to similar genetic screens in animals, genetic screens for patterning mutants in Arabidopsis have turned up only a limited number of genes that are directly involved in early patterning events in the embryo. One possible reason for this is that many patterning mutants never develop to the seedling stage, at which mutants in previous screens were scored. As an alternative, the group of Chris Somerville (Stanford) has conducted an extensive screen for embryonic lethal mutants in Arabidopsis, collecting more than 7000 different lines, each of which was examined for early patterning defects by differential interference contrast microscopy. Some 300 lines showed detectable defects at the heart stage, and were examined further. For most loci, many alleles were recovered, indicating that the screen has been largely saturated. Somerville described several classes of mutants. One of the mutants with the clearest patterning defect was *monopole*, with early basal defects. Surprisingly, some mutant individuals can recover and form a relatively normal seedling from the upper tier of the octant-stage embryo, underscoring the plasticity of embryo development in plants. The gene has been cloned and found to encode a transcription factor. Relatively normal adult plants can stochastically produced by two other mutants, *yoda* and *grave2*, both of which show abnormal patterns of cell division during very early stages. Finally, a large class of mutants produced radially swollen embryos, resulting from direct or indirect effects on cellulose synthesis and thus an aberrant cell wall.

A different set of cell wall mutants was discussed by Herman Höfte (Versailles). A central enzyme in cell wall formation is cellulose synthase, and different isoforms are encoded by a moderately large family of genes in Arabidopsis. Interestingly, the different isoforms have distinct functions, and mutations at several cellulose synthase loci have been isolated in forward genetic screens. Some loci are required for primary cell wall formation, while others are required for secondary wall formation in the vasculature. Mutations in one of these loci, *PROCUSTE*, were originally identified because they cause a dark-specific effect in hypocotyl cell elongation. *PROCUSTE* RNA is expressed in both the dark and the light, indicating that bypass of the *procuste* phenotype in the light is mediated by a light-dependent activity. Experiments with light of different wavelengths and with double mutants showed that this light-dependent activity requires *PHYA* function.

Sacco de Vries (Wageningen) reported on a leucine-rich repeat transmembrane kinase, SERK, which can homodimerize in the plant plasma membrane, as shown by fluorescence-resonance energy transfer (FRET) with fusions of SERK to different fluorescent proteins. SERK was originally identified in carrot, where its expression marks embryogenic competence of cells in culture. The Arabidopsis SERK gene is expressed from before fertilization in the ovule primordium and in the female gametophyte, and expression persists throughout the embryo and suspensor until the heart stage. Ectopic overexpression of SERK increases embryogenic potential, demonstrating that SERK not only is a marker of embryogenic competence, but that it also has an instructive role in this process. Another gene expressed in ovules was identified by molecular means in tobacco and found to encode a carpel-specific ACC oxidase isoform (Domenico De Martinis, Rome/Nijmegen). Reducing expression of this gene causes an arrest of ovule development. The specificity of this phenotype was confirmed by rescuing the phenotype using an exogenous ethylene source, indicating that ethylene is important for ovule development at least in some species.

In mammals, certain genes are imprinted, such that only the paternally or maternally inherited allele is expressed in the embryo. Imprinting exists also in Arabidopsis, and one of the imprinted genes is *MEDEA* (*MEA*), which encodes a SET-domain protein of the *Polycomb* group (Ueli Grossniklaus, Zürich). During early stages of seed development, *MEDEA* is expressed only from the maternal allele in both endosperm and embryo, where it represses growth. Because imprinting-based silencing of the paternal allele may involve DNA methylation, the effect of several mutants differentially affecting methylation and transcriptional gene silencing phenomena were examined. For instance, experiments with *DDM1*, a chromatin remodeling factor gene required for DNA methylation, showed that embryos inheriting a maternally inherited mutant *mea* allele can survive when they are zygotically homozygous for *ddm1*. The interpretation is that, in the absence of *DDM1*, the paternally inherited non-expressed wild-type *MEA* allele becomes reactivated. *ddm1*, however, does not interfere with subsequent inactivation, as the paternal allele is initially silent again in the next generation. It is known that demethylated cytosines do not seem to be remethylated in *ddm1* mutants, and experiments with methylation-sensitive restriction enzymes did not reveal changes in the methylation pattern across the *MEA* locus. Thus, it is likely that the observed reactivation and inactivation of the paternal *MEA* allele is independent of methylation and related to chromatin remodeling.

Maternal contribution to seed development was also discussed by Paolo Costantino (Rome). His group identified a zinc finger transcription factor of the Dof family, DAG1, which acts in maternal tissue to repress dormancy. *dag1* mutant seeds are still sensitive to the

MEETING REPORT

inhibitory effects of ABA on germination, and require gibberellin biosynthesis for germination, confirming that *DAG1* acts relatively far upstream in controlling germination. Interestingly, *DAG1* is expressed in the vascular tissue (of the mother plant), suggesting that DAG1 controls the transport of a substance required to confer dormancy. Moreover, that *dag1* mutant seeds do not require light for germination suggests that the activity of this substance is light regulated. Another pair of transcription factors that control seed maturation, ABI3 and FUS3, was discussed by Vered Raz (Wageningen). Raz showed that growth arrest and cessation of cell division in the embryo and endosperm are regulated by FUS3 and precede the induction of dormancy, which is regulated by ABI3 and ABA. Embryo growth arrest can be uncoupled from seed dormancy genetically, and aberration in these two sequential pathways causes vivipary.

POSTEMBRYONIC DEVELOPMENT

A developmental mechanism that has been intensively studied in animals is lateral inhibition, which allows specialized cells to emerge from a uniform field of undifferentiated cells at more or less even spatial intervals. The epidermis of dicot leaves contains two types of specialized structures, trichomes and stomata, whose distribution is likely to be at least partially regulated by lateral inhibition. A candidate for a protein involved in lateral inhibition of stomata was identified through Arabidopsis mutants with increased stomatal density (Thomas Altmann, Golm). This gene, *SDD1*, is expressed at low levels in the mesophyll of very young leaf primordia, and more strongly in guard mother cells and young, developing guard cells, consistent with *SDD1* being involved in generating a negative signal emanating from these cells. The similarity of the SDD1 protein to subtilisin-like serine proteases suggests that SDD1 acts by activating a peptide ligand.

The question of whether plants make cells or cell make plants, was addressed by Andrew Fleming (Zürich). His laboratory has combined a tetracycline-inducible system for overexpression of cyclin A with local induction using tetracycline-loaded beads. Surprisingly, spatially restricted induction of cyclin A in the shoot meristem of tobacco had little effect, but placement of the tetracycline-loaded beads on young leaf primordia caused ectopic tissue to form, indicating that the role of cell division in morphogenesis is context dependent.

One of the most studied events in postembryonic development is the switch from the vegetative to the reproductive phase. The two most important environmental cues controlling flowering are vernalization and daylength. A requirement for vernalization, or exposure to a prolonged period of cold temperature, ensures that plants growing in Northern latitudes overwinter vegetatively and flower only in spring (Caroline Dean, Norwich). A locus determining vernalization requirement in wild accessions of Arabidopsis the *FRIGIDA* (*FRI*) gene. Accessions with a functional *FRI* allele are more common at Northern latitudes, suggesting that loss of *FRI* function was a major event in the loss of vernalization requirement in Southern accessions. Comparison of sequence polymorphisms at the *FRI* locus confirmed that the ancestral form of Arabidopsis likely had a functional *FRI* allele, and that early-flowering forms arose independently at least twice. It is not yet known how plants sense the cold temperature signal, but two loci required for the vernalization response, *VRN1* and *VRN2*, have recently been cloned and found to encode apparent transcription factors.

Remarkable progress has also been made in understanding how Arabidopsis senses long days, which promote flowering. A central factor in this process is the *CONSTANS* gene (George Coupland, Norwich). *CONSTANS* encodes a protein with zinc fingers of the B-box type, which are known to mediate protein-protein interactions among mammalian transcription factors. *CONSTANS* RNA accumulation is circadian clock-regulated, with peak levels during the night. The *CONSTANS* peak is broader under long-day conditions than under short-day conditions, such that high *CONSTANS* RNA levels are still present at dawn in long-day grown plants, but not in short-day grown plants. Excitingly, CONSTANS protein function seems to be light regulated, as CONSTANS overexpression is lethal, when plants are grown in blue light, which is known to have a strong promoting effect on flowering. A simple model derived from these observations is that long-day dependent flowering occurs only when CONSTANS protein is present during the light phase of the day. Which photoreceptors regulate the circadian expression of *CONSTANS* RNA is not known, but their effects are mediated by several genes such as *GI*, *LHY*, *CCA1* and *ELF3*. These genes are part of the general clock machinery, as they affect circadian expression not only of *CONSTANS*, but also of other genes not involved in flowering.

In Arabidopsis, the action of flowering-time genes including *FRI* and *CONSTANS* ultimately results in the upregulation of floral meristem-identity genes such as *LEAFY*. Detlef Weigel (La Jolla) discussed the use of activated versions that make a transcription factor independent of other coregulators, which presents a powerful method to probe the interaction of a transcription factor with its targets in vivo. This approach was used to demonstrate that homeotic genes such as *APETALA1* and *AGAMOUS* are direct targets of LEAFY, which was subsequently confirmed by several other methods. Interestingly, the LEAFY transcription factor can move between cells, which raises the question of how LEAFY activity is

MEETING REPORT

limited. One possible mechanism involves other, non-mobile transcription factors that repress LEAFY targets in the shoot apical meristem. Orthologs of *LEAFY* as well as its coregulator *UFO* have been isolated in several other species including petunia (Erik Souer, Amsterdam). However, in contrast to Arabidopsis, the loss-of-function phenotype of the *UFO* ortholog *DOT* is as severe as that of the *LEAFY* ortholog *ALF*. *DOT* is expressed only in a subset of cells that express *ALF*, pointing to non-autonomous effects of *DOT* on floral identity. A feature that makes petunia particularly interesting is its cymose inflorescence, in which the meristem bifurcates at each node, generating a floral meristem and a new inflorescence meristem. This bifurcation requires the activity of the *EXP* gene, which encodes a MADS domain protein.

In summary, the workshop presented an excellent overview of many important directions in contemporary plant developmental biology. Perhaps the most impressive aspect of the workshop was that the vast majority of presentations dealt with molecular mechanisms that went beyond the mere cloning of genes. This together with the beautiful location and the intimate format, which mixed both senior and junior scientists, made the meeting a particularly successful and memorable one.

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