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Control of cell elongation and stress responses by steroid hormones and carbon catabolic repression in plants

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Molecular analysis of *Arabidopsis* mutants displaying hypocotyl elongation defects in both the dark and light revealed recently that steroids play an essential role as hormones in plants. Deficiencies in brassinosteroid biosynthesis and signalling permit photomorphogenic development and light-regulated gene expression in the dark, and result in severe dwarfism, male sterility and de-repression of stress-induced genes in the light. A cytochrome P450 steroid hydroxylase (CYP90) controls a rate limiting step in brassinosteroid biosynthesis and appears to function as a signalling factor in stress responses. Another key step in steroid biosynthesis is controlled by the *Arabidopsis* SNF1 kinases that phosphorylate the 3-hydroxy-3methylglutaryl-CoA reductase. The activity of SNF1 kinases is regulated by PRL1, an evolutionarily conserved α-importin-binding nuclear WD-protein. The *prl1* mutation results in cell elongation defects, de-repression of numerous stress-induced genes, and augments the sensitivity of plants to glucose, cold stress and several hormones, including cytokinin, ethylene, auxin, and abscisic acid.

Keywords: brassinosteroids; glucose repression; CYP90; PLR1; SNF1 kinase; control of transcription

1. REGULATION OF CELL ELONGATION BY LIGHT AND BRASSINOSTEROIDS

Hypocotyl elongation of seedlings during skotomorphogenesis in the dark is inhibited by light signals perceived by the photoreceptor phytochrome A (phyA) controlling the onset of photomorphogenesis and de-etiolation (Mustilli & Bowler 1997). As for light signals, ethylene also inhibits hypocotyl elongation in the dark, but prevents the opening of apical hook of cotyledons (Ecker 1995). Induction of photomorphogenesis by phyA-activation is repressed by glucose signalling (Barnes *et al.* 1996) involving the functions of, as yet, unknown *SUN* genes (Dijkwel *et al.* 1997). Mutations in the *DET* (de-etiolated) and *COP* (constitutive photomorphogenesis) genes induce photomorphogenesis in the dark (Chory *et al.* 1996). The DET and COP functions act downstream of the photoreceptors and their absence results in the activation of light-induced genes, as well as genes involved in general stress responses (von Armin & Deng 1996; Mayer *et al.* 1996). The DET–COP functions also seem to affect cytokinin signalling. For example, cytokinin signalling enhances, synergistically, the light-induced onset of photomorphogenesis, and cytokinins phenocopy the *det1* mutation (Chory *et al.* 1994). In the dark, the COP1 WD-protein and DET1 are located in the nucleus and probably function as general repressors of light-regulated genes. COP1 and DET1 act in concert with elements of the COP9 complex that shows a similarity to chromatin remodelling modulator complexes of RNA polymerase II (Chamovitz *et al.* 1996; Wilson *et al.* 1996).

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Whereas the DET1 and COP functions are needed for safe-guarding the light- and stress-regulated pathways in the dark, the *DET2*, *CPD1* (constitutive photomorphogenic dwarf), *DIM1* (diminuto), and *BRI1* (brassinosteroid insensitivity) genes are required for positive control of skotomorphogenesis. *DET2*, *DIM1*, and *CPD1* code for enzymes involved in the biosynthesis of plant steroid hormones, termed brassinosteroids (BRs) (Sakurai & Fujioka 1997; Yokota 1997), whereas BRI1 is required for BR-perception (Li & Chory 1997). Mutations affecting BR biosynthesis and signalling result in the inhibition of hypocotyl elongation in the dark, and cause severe dwarfism in light-grown plants. DET2 is a 5α-steroid reductase that is required for the production of campestanol by catalysing the synthesis of (*24R*)-24-methyl-5α-cholestan-3-one from (*24R*)-24-methylcholestan-4-en-3-one. DET2 mediates also the conversion of progesterone, teasterone, and androstendione to their 5α-reduced forms in animal cells. Similarly, animal 5α-steroid reductases can complement the *Arabidopsis det2* mutation, indicating a remarkable conservation of functions in plant and animal steroid biosynthesis (Li *et al.* 1997). Our studies show that the *CPD* gene codes for a cytochrome P450 enzyme (CYP90) involved in C23-hydroxylation of cathasterone to teasterone, whereas DIM1 is probably involved in the biosynthesis of typhasterol (Szekeres *et al.* 1996).

2. SIGNALLING FUNCTION OF CYP90 IN BRASSINOSTEROID BIOSYNTHESIS

Complete loss of the CPD function results in the de-repression of light regulated-genes (e.g. *RBCS* and

CAB) in the dark, as well as activation of a set of stress-regulated genes (e.g. chalcone synthase, lipoxygenase, alcohol dehydrogenase and so on) in the light. This indicates that brassinosteroids are not only required for stimulation of cell elongation by controlling the activity of genes involved in cell wall biosynthesis (Clouse 1996), but also exert a negative control over several pathways regulated by stress and hormonal stimuli. Overexpression of the CYP90 steroid hydroxylase in transgenic plants induces the expression of pathogenesis-related genes independently of BR production. This suggests that CYP90 may also perform a signalling function. Recent data demonstrate that expression of the *CPD* gene is feedback regulated by BRs at the level of transcription. The fact that protein synthesis inhibitors, such as cycloheximide, prevent down-regulation of the *CPD* gene by BRs indicates that BR-mediated signalling requires *de novo* synthesis of a repressor (Mathur *et al.* 1998). Feedback regulation of the *CPD* gene by BRs shows a remarkable analogy to oxysterol signalling in mammals. Oxysterols are hydroxylated cholesterol derivatives that, by analogy to BRs, down-regulate the transcription of steroid hydroxylase genes in mammals (Honda *et al.* 1993; Janowski *et al.* 1996; Lala *et al.* 1997). Oxysterols inhibit the proteolytic processing of membrane-bound sterol regulatory element-binding factors (SREBPs) controlling the transcription of genes involved in steroid biosynthesis, including the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) (Brown & Goldstein 1997). It is therefore intriguing that CYP90 has been found to interact in the yeast two-hybrid system with a sterol-binding protein, as well as with RING-finger factors showing homology to protease inhibitors (Z. Koncz-Kálmán, unpublished data).

3. THE *CPD* GENE IS REGULATED BY *PRL1* IN *ARABIDOPSIS*

By characterizing the regulation of the *CPD* gene in diverse *Arabidopsis* mutants, we have found that, as in BRs, a mutation in *pleiotropic regulatory locus-1* (*PRL1*) down-regulates transcription of the *CPD* gene in light-grown *Arabidopsis* plants. The *PRL1* gene codes for a WD-protein that, by analogy to COP1, functions as a negative regulator of a set of light-, hormone-, and stress-regulated genes (Németh *et al.* 1998). Mutation of *PLR1* results in complex phenotypic defects, including the inhibition of hypocotyl elongation in the dark. Intriguingly, whereas the *det1* mutation is phenocopied by cytokinin in the dark, the phenotype of the *prl1* mutation is mimicked by cytokinin treatment of wild-type plants only in the light. The *prl1* mutation results in hypersensitivity to glucose and sucrose, as well as transcriptional de-repression of genes that are positively or negatively regulated by glucose, or cytokinin, or both. The *prl1* mutation also augments the sensitivity of seedlings to auxin, ethylene, abscisic acid, and cold temperature. As a result of these regulatory alterations, root elongation of the *prl1* mutant is inhibited in both the dark and light. In addition, *prl1* roots produce ectopic root hairs and show de-repressed initiation of side-root development.

4. PRL1 IS A CONSERVED α-IMPORTIN-BINDING NUCLEAR PROTEIN THAT FUNCTIONS AS A REGULATOR OF *ARABIDOPSIS* SNF1 KINASES

PRL1 encodes a basically charged protein of 54 kDa that is found in both a free cytoplasmic form and in association with microsomal (endoplasmatic reticulum and nuclear) membranes. Immunolocalization studies show that a proportion of PRL1 protein is located in the nucleus in *Arabidopsis*. PRL1 carries seven WD-40 repeats that share over 60% identity with PRL1 orthologues found in yeast, *Caenorhabditis*, *Drosophila*, and mouse. Recently, a PRL1 homologue (GenBank AF044333) was located on human chromosome 4q31.2. Functional conservation of PRL1 orthologues in mammals is indicated by the observation that PRL1 is also imported into the nucleus when expressed in human cells. PRL1 functions as a receptor for activated human protein kinase C *in vitro* (i.e. RACK, Ron & Mochly-Rosen 1995). Specific binding of PRL1 to activated protein kinase C-βII, but not to PKC-βI, suggests that PRL1 orthologues may regulate glucose signalling in mammals where differential splicing, expression, and nuclear transport of PKC-βII are induced by insulin signalling (Chalfant *et al.* 1995).

Genetic screening for PRL1 interacting proteins in the yeast two-hybrid system revealed that PRL1 specifically binds a novel α-importin, ATHKAP2 (Németh *et al.* 1998). ATHKAP2 does not bind to proteins containing monopartite and bipartite nuclear localization signals (NLS). Thus, although a variant of the SV40-type NLS is carried by the C-terminus of PLR1, binding of ATHKAP2 to PRL1 in the yeast two-hybrid system is probably not owing to a recognition of PRL1-NLS, but rather reflects a regulatory interaction. PRL1-binding to human PKC-βII also suggests that PRL1 may in fact serve as a protein kinase-targeting subunit involved in the control of nuclear import by either phosphorylation of NLS sequences or α-importin.

Homologues of the yeast Ser–Thr protein kinase SNF1p have also been identified as PRL1-binding proteins in the yeast two-hybrid system. In yeast, SNF1p is a master kinase in glucose signalling and its function is required for de-repression of glucose-repressible genes (reviewed by Ronne (1995)). Yeast SNF1p controls the phosphorylation, and thereby nuclear localization, of MIG1p that acts as a repressor of glucose-regulated genes by recruiting the general transcriptional repressor complex TUP1-SSN6 (Tzamarias & Struhl 1995). Although TUP1 is a prototype of WD-protein repressors showing functional analogy to PRL1, the *tup1* mutations cannot be complemented by the *Arabidopsis PRL1* cDNA. Binding of PRL1 to yeast and *Arabidopsis* SNF1 kinases is regulated by glucose. PRL1, as the yeast SNF1-activator SNF4, shows an enhanced binding to SNF1p in the absence of glucose, whereas the PRL1–SNF1 interaction is inhibited when yeast is grown in the presence of glucose. The fact that *Arabidopsis* SNF1 homologues can functionally complement the yeast *snf1* mutation and suppress the *snf4* deficiency demonstrates the conservation of SNF1 and PRL1 functions between yeast and plants.

Arabidopsis SNF1 homologues Akin10 and Akin11, similarly to other plant SNF1-kinases, are capable of phosphorylating proteins carrying the SAMS peptide motif,

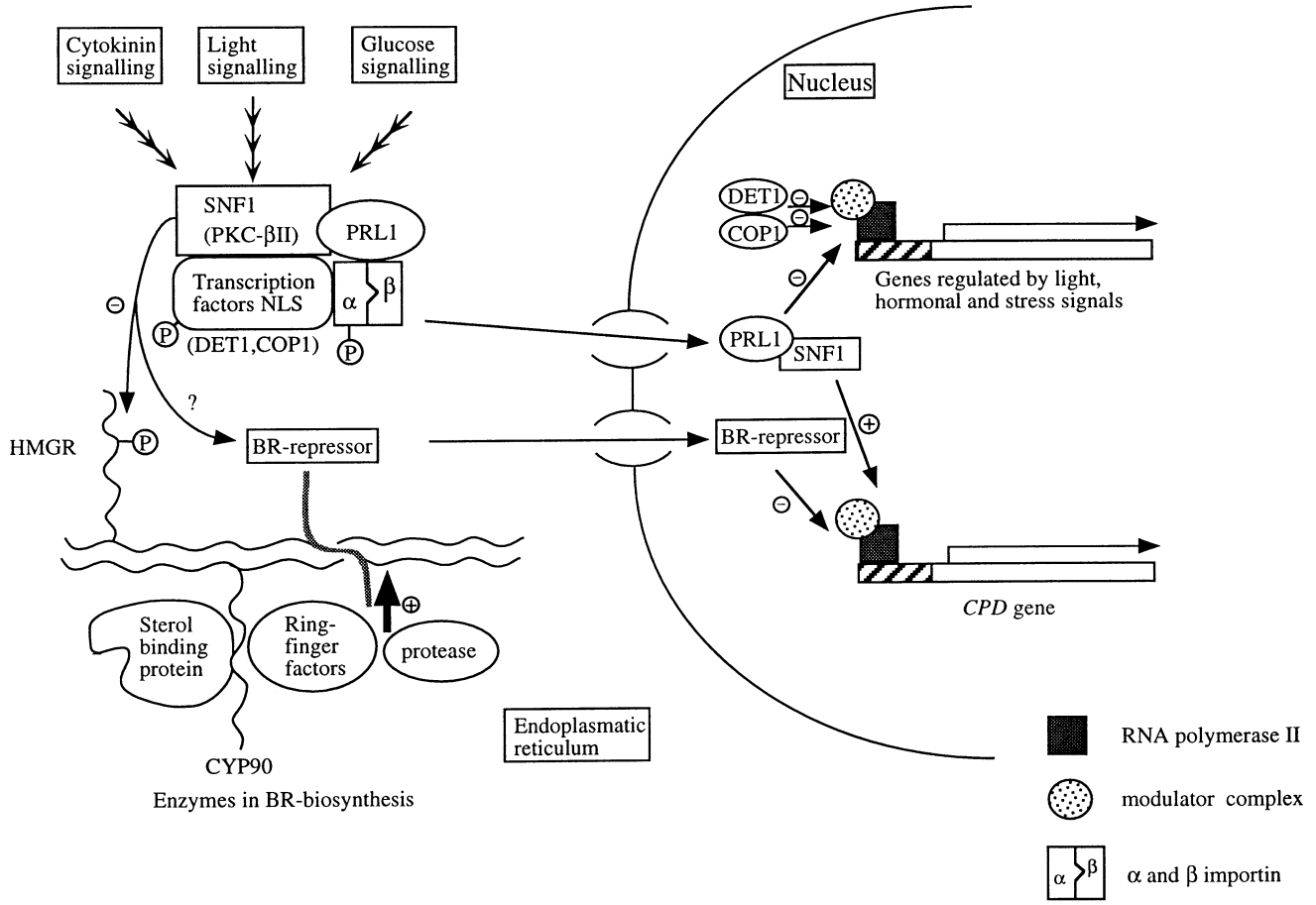

Figure 1. Regulatory connections between glucose and steroid signalling: a model. As outlined in the text, the α-importin-binding PRL1 protein is a regulator of the SNF1 kinase, and functions as a specific receptor for human protein kinase C-βII *in vitro*. PRL1 may target protein kinases to complexes of α and β importins and affect the regulation of nuclear import by phosphorylation of nuclear localization sequences (NLS) of transcription regulatory proteins or α-importins. The *CPD* gene controls a rate-limiting step in BR biosynthesis. Negative feedback regulation of *CPD* expression by BRs requires the synthesis of a BR-repressor. The *CPD* gene is down-regulated in the *prl1* mutant, suggesting that PRL1 controls the transcription of *CPD*. The PRL1 controlled SNF1 kinase down-regulates the activity of HMGR by phosphorylation and thereby may modulate the first committed step in steroid biosynthesis. The *CPD* gene product, CYP90, interacts in the yeast two-hybrid system with an oxysterol-binding protein and signalling factors carrying RING-finger motives of protease inhibitors. CYP90 may thus be part of a mechanism that, by analogy to oxysterol signalling in mammals (Brown & Goldstein 1997), controls the activity of a membrane-bound steroid regulatory factor by, for example, proteolytic processing or phosphorylation.

including HMGR (Dale *et al.* 1995). Phosphorylation by SNF1 inhibits the activity of HMGR catalysing the first committed step in steroid biosynthesis. As in yeast, the activity of Akin10 and Akin11 SNF1-kinases is negatively regulated in *Arabidopsis* by glucose in the dark. In contrast, glucose increases the activity of SNF1 kinases in light-grown *Arabidopsis* seedlings, indicating a connection with light signalling. In the light, the activity of SNF1 kinases is three- to fivefold higher in *prl1* than in wild-type plants, showing that PRL1 is a negative regulator of SNF1 kinases in light-grown plants. Abnormal activation of the SNF1 kinases thus correlates well with cell elongation defects and down-regulation of the *CPD* gene observed in the *prl1* mutant. In the dark, the SNF1 kinase activity is lower in *prl1* than in wild-type plants indicating that PRL1 is a positive modulator of SNF1 kinase in dark-grown plants (Bhalerao *et al.* 1998). PRL1 inhibits *in vitro* the phosphorylation of SAMS peptide by both *Arabidopsis* Akin10 and Akin11 SNF1 kinases. Therefore, it is likely that *in vivo* regulation of SNF1 kinases by PRL1 involves light-, and glucose-dependent post-translational modifications, such as phosphorylation by activating kinases, and dephosphorylation by protein phosphatases. This conclusion is supported by data showing that the levels of protein phosphatase type 1 and 2a are significantly reduced in the light-grown *prl1* mutant, as well as that PRL1 has been found to interact also with a specific Ca^{2+}-dependent kinase.

5. CONCLUSIONS

In this review, we aimed to give a brief insight into recent studies of novel signalling functions coordinating plant responses to metabolic, hormonal and environmental stimuli. In particular, regulatory relations (see figure 1) between two seemingly unrelated signalling proteins, CYP90 in steroid biosynthesis and PRL1 in the control of glucose repression, have been discussed. Research in progress shows that the fragmentary relations described here will be soon supplemented by many more

partners and regulatory connections. Thus, genetic analyses suggest that the *prl1* mutation is exacerbated by the *amp1* function regulating cytokinin production and cell division of meristems (Chaudhury *et al.* 1993). In addition, the *ein2* mutation that confers cytokinin resistance and ethylene insensitivity (Ecker 1995) appears to be epistatic to *prl1* in the dark, but this epistatic relation is reversed in the light (Németh *et al.* 1998). Biochemical and genetic data demonstrate that PRL1 is a regulator of many diverse genes, including *CPD* in BR biosynthesis. *CPD* may also specify a signalling function, because its overexpression activates a set of pathogenesis-related genes, and because its product, the CYP90 C23-steroid hydroxylase, interacts with novel signalling proteins. Thus, CYP90 may, in fact, control a pathway of stress signalling in addition to being essential for the biosynthesis of BR hormones. PRL1 is a regulator of SNF1 kinases that control the expression of glucose-, and cytokinin-responsive genes by overcoming the regulation of these genes by other signals. Analysis of the PRL1 function not only supports the notion that light, glucose, and cytokinin signalling are tightly cross-connected (Chory *et al.* 1996; von Armin & Deng 1996), but also suggests a considerable conservation of eukaryotic regulatory mechanisms modulating nuclear import by protein kinases and their targeting subunits, and cross-talk between glucose and steroid signalling.

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