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Pathways to abscisic acid-regulated gene expression

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CONTENTS

SUMMARY

Recent progress in ABA signalling is summarized from the perspectives gained by genetic (mutant) analysis, 'reverse genetics' (starting from unknown ABA-inducible sequences and working backwards) and biochemical studies. What emerges is a cell-biological model of overlapping tissue-specific stress (e.g. drought, salt and cold) and developmental (e.g. sugars and other hormones) response pathways that integrate into responses mediated by ABA, including but not limited to seed maturation, dormancy, inhibition of cell division and germination, stomatal closure and changes in gene expression leading to stress adaptation. ABA signalling involves putative ABA receptors (extracellular or intracellular), cell-surface membrane proteins including ion channels, glycoproteins and membrane trafficking components, secondary messengers such as phosphatidic acid, inositol 1,4,5-trisphosphate, cyclic ADP-ribose and calcium, and protein phosphorylation/dephosphorylation cascades leading to chromatin remodelling and binding of transcriptional complexes to ABA-responsive promoter elements. The large gaps in our understanding of complex regulatory networks such as ABA signalling can be best addressed by multidisciplinary, integrated approaches such as those discussed.

Key words: ABA, regulation of gene expression, hormone, plasma membrane, receptor, secondary messenger, signal transduction, stress.

Abbreviations: AAPK, ABA-activated protein kinase; ABF, ABRE binding factor; ABRE, ABA-responsive element; *ADH*, alcohol dehydrogenase gene, *BASI*, bifunctional α-amylase/subtilisin inhibitor (gene); bp, base pair; BR, brassinosteroid; bZIP, basic leucine zipper transcription factor; cADPR, cyclic ADP-ribose; CBF, cold binding factor; CDPK, Ca^{2+}-dependent protein kinase; *COR*, cold-responsive (gene); *DHN*, dehydrin (gene); DPBF, *Dc3*-promoter binding factor; DRE, dehydration-responsive element; DREB, DRE binding factor; *Em*, early methionine-labelled *LEA* (gene); EmBP-1, Em-promoter-binding protein 1; GFP, *Aequoria victoria* green fluorescent protein; *GUS*, gene for bacterial *uidA* gene (β-glucuronidase); IP_3, inositol 1,4,5-trisphosphate; JA, jasmonic acid; *LEA*, late-embryogenesis-abundant (gene); MAPK, mitogen-activated protein kinase; MBPK, myelin-basic-protein kinase; OKA, okadaic acid; PA, phosphatidic acid; PLC, PLD, phospholipases C and D; PP2C, protein phosphatase 2C; S, slow; TRAB1, transcription factor responsible for ABA regulation.

I. INTRODUCTION

One of the central paradoxes of plant biology is that there exist only a handful of low-molecular-mass compounds (phytohormones) that mediate many complex processes in plant growth. A related conundrum is that despite the elucidation of hormone structures decades ago, in many cases the characterization of plant hormone receptors and their downstream targets is still largely lacking. The pleiotropic effects of plant hormones, the interactions of hormones with other pathways and developmental programs, and the plasticity of plant cell identity make it difficult to apply a reductionist experimental approach to elucidate hormone signalling. The paradigm of conserved linear pathways from stimulus to response, borrowed from animal systems, might not be correct.

ABA is a small, lipophilic plant hormone that modulates plant development, seed dormancy, germination, cell division and cellular responses to environmental stresses such as drought, cold, salt, pathogen attack and UV radiation (Addicott & Carns, 1983; Zeevaart & Creelman, 1988; Sánchez-Serrano *et al*., 1991; McCarty, 1995; Rock & Quatrano, 1995; Ueno, 1998; Albinsky *et al*., 1999). It is ubiquitous in lower and higher plants and its biosynthetic and catabolic pathways have been elucidated (Hirai, 1986; Zeevaart, 1999). ABA acts to effect changes on multiple physiological processes, for example inducing the rapid closure of stomatal pores by ion efflux from guard cells, thereby limiting water loss through transpiration (Hetherington *et al*., 1998; MacRobbie, 1998; Assmann & Shimazaki, 1999), and by triggering slower changes in gene expression, which is thought to reprogram the cell to withstand dehydration stresses (Chandler & Robertson, 1994; Ingram & Bartels, 1996; Bray, 1997; Himmelbach *et al*., 1998; Leung & Giraudat, 1998). In developing seeds, ABA levels peak during late embryogenesis when storage proteins and nutritive reserves accumulate, and thereafter decline during desiccation (Rock & Quatrano, 1995). In stressed vegetative tissues, ABA levels rise severalfold to 40-fold within hours, and decrease after rehydration (Zeevaart, 1999). The available evidence suggests this modulation is due at least in part to the transcriptional regulation of the genes for the biosynthesis and catabolism of ABA (Cutler *et al*., 1997; Qin & Zeevaart, 1999).

Despite the complex multitude of physiological, molecular, genetic, biochemical and pharmacological data that implicate ABA in stress responses and developmental programs, the adaptive responses of plants to ABA and stresses, and the pathways that trigger them, are largely unknown. The nature of the cellular responses to ABA is a function of the cell type, and there might be separate ABA signalling pathways in the same and/or different cells. A comprehensive understanding of 'sensitivity' to ABA at the cellular and molecular levels will provide fundamental insights into growth and development and should lead to cogent strategies for genetic engineering applications including, but not limited to, increased crop productivity under stress conditions and value-added seed qualities.

The advent of the genomics era has spawned rapid advances in plant sciences, but the emerging picture of multiple and overlapping regulatory networks linking ABA, developmental programs and stress responses is still fragmentary. To use the analogy of Gelbart (1998), if the genome is an instruction book, then the information needed to deploy a given protein or polypeptide in the correct set of cells at the proper developmental times and in the requisite quantities would be equal to one sentence. A reasonable current assessment of our understanding is that:

> 'We have partial but still quite incomplete knowledge of how to identify and read certain nouns (the structures of the nascent polypeptides and protein-coding exons of mRNAs). Our ability to identify the verbs and adjectives and other components of these genomic sentences (e.g. regulatory elements that drive expression patterns or structural elements within chromosomes) is vanishingly low. Further, we do not understand the grammar at all– how to read a sentence, how to weave the different sentences together to form sensible paragraphs describing how to build multi-component proteins and other complexes, how to elaborate physiological or developmental pathways.'

It is becoming increasingly important to take a multidisciplinary approach to complex biological problems such as hormone signal transduction. Recent reviews have covered ABA signal transduction from various perspectives and are a testament to the spectacular progress being made (Bray, 1997; Shinozaki & Yamaguchi-Shinozaki, 1997; Bonetta & McCourt, 1998; Grill & Himmelbach, 1998; Koornneef *et al*., 1998; Leung & Giraudat, 1998). Detailed reviews on ABA regulation of stomatal closure (Hetherington *et al*., 1998; MacRobbie, 1998; Assmann & Shimazaki, 1999), transcription (Busk & Pagès, 1998), adaptive responses to drought (Chandler & Robertson, 1994; Ingram & Bartels, 1996; Thomashow, 1999) have also appeared. The aim of this review is to present the field of ABA signalling, especially the regulation of gene expression, from the perspective of the bench scientist. There are, broadly speaking, three complementary experimental approaches to the study of fundamental biological questions, each with its advantages and disadvantages: (1) classical genetics, (2) the broad and loosely termed 'reverse genetics' (starting with gene sequences and working backwards to establish function), and (3) biochemical (including pharmacological and biophysical) methods. All three together can be construed as contributing to the field of cell biology. Integration

of the emerging molecular models from diverse studies facilitates the formulation of hypotheses to test the molecular mechanisms of signalling at the cellular and whole-plant levels. This type of synthesis for ABA signalling was the subject of a previous Tansley Review (Hetherington & Quatrano, 1991).

II. GENETIC ANALYSIS OF ABA RESPONSES

The problem of reconciling the complexity of plant responses with the simplicity of the associated hormones has yielded in recent years to molecular genetic analysis, especially in the model organism *Arabidopsis* (Somerville & Meyerowitz, 1994; Meinke *et al*., 1998; Somerville & Somerville, 1999). There have been numerous recent reviews (e.g. Himmelbach *et al*., 1998; Koornneef *et al*., 1998; Leung & Giraudat, 1998; McCourt, 1999; Zeevaart, 1999) on the genetics of ABA signalling and biosynthesis; the reader is referred to those articles for a more thorough treatment. Reviewed here are the different types of mutant screen that have been used and the insights into the role of ABA in seed development and responses to environmental stress that have resulted from the characterization of mutants. More sophisticated 'second-generation' genetic screens for ABA responses that have used novel criteria are also covered. The intrinsic value of genetics over other experimental (namely correlative) studies is that pleiotropic mutant phenotypes can reveal genes involved in fundamental processes that are otherwise hidden, and thereby open new vistas of understanding, insight and experimentation.

1. *Seed development*

(a) *Maturation*. The first report of a mutant with defects in seed maturation and dormancy was *viviparous*1 (*vp*1) of maize (Eyster, 1931). Robichaud *et al*. (1980) demonstrated that excised immature *vp1* mutant embryos could develop normally *in vitro* when exposed to 10-fold higher exogenous concentrations of ABA than required for maturation by wild-type or other viviparous mutants (which are ABA-deficient owing to biosynthetic blocks in the formation of carotenoids, precursors to ABA (Zeevaart & Creelman, 1988; Zeevaart, 1999)). Neill *et al*. (1987) showed that *vp1* embryos had normal levels of ABA, thereby establishing that the *Vp1* locus is involved in ABA 'sensitivity' rather than ABA biosynthesis. Recently, another ABA-insensitive viviparous maize mutant, *rea* (*red embryonic axis*), has been described (Sturaro *et al*., 1996). McCarty *et al*. (1989) cloned the *Vp1* locus by transposon tagging and showed that it is expressed exclusively in developing seeds and precedes ABA-inducible storage protein and late-embryogenesis-abundant (*LEA*) marker gene expression (McCarty *et al*., 1991). Protoplasts prepared from embryonic callus that transiently overexpress the *Vp1* cDNA can transactivate ABA-inducible promoters (McCarty *et al*., 1991; Kao *et al*., 1996; Hagenbeek *et al*., 2000), suggesting that *Vp1* is a transcriptional activator. Similar transactivation results have been obtained in transient gene expression systems with the *Arabidopsis*, rice, *Phaseolus vulgaris* and *Craterostigma Vp*1 orthologues (Hattori *et al*., 1995; Bobb *et al*., 1997; Chandler & Bartels, 1997; Crowe *et al*., 2000).

Mutations in *Vp1* have pleiotropic effects on a number of developmental markers for seed maturation and germination that have different degrees of responsiveness to ABA (Pla *et al*., 1991; Hattori *et al*., 1992; Thomann *et al*., 1992; Guan & Scandalios, 1998), suggesting that VP1 does not regulate solely ABA sensitivity but might also interact with seed developmental factors. This model is supported by the observation that the *cis*-promoter elements sufficient for activation by ABA and VP1 are partly separable (Vasil *et al*., 1995; Kao *et al*., 1996; Carson *et al*., 1997). Remarkably, VP1 also has repressor activity towards expression of the germination-specific α-amylase genes, but repression is not cell-autonomous and requires embryo-specific factors other than ABA and VP1 (Hoecker *et al*., 1999).

The *ABA-INSENSITIVE3* (*ABI3*) gene of *Arabidopsis* is the genetic equivalent of maize *Vp1* and was identified in a genetic screen for mutants insensitive to the inhibitory effects of exogenous ABA on seed germination (Koornneef *et al*., 1984) (see later). The hypothesis that *ABI3*/*Vp1* interacts with seed developmental pathways other than ABA responses is supported by marker gene studies (Parcy *et al*., 1994), transactivation of the *Helianthus* non-ABA-inducible heat shock gene (*Ha hsp* 17.7*G*4) promoter in transient assays (Rojas *et al*., 1999), as well as double-mutant analysis with the *leafy cotyledon*1 (*lec1*), *fusca*3 (*fus3*), *de-etiolated*1 (*det1*) and *constans* (*co*) mutants (Parcy *et al*., 1997; Kurup *et al*., 2000; Rohde *et al*., 2000). The *lec1*, *fus3* and *det1* mutants affect different aspects of embryogenesis, seed maturation and photomorphogenesis (e.g. timing of dormancy compared with germination programs (Wobus & Weber, 1999) (Fig. 1)). However, these mutants are not altered in sensitivity to ABA (Bäumlein *et al*., 1994; Keith *et al*., 1994; Meinke *et al*., 1994; Lotan *et al*., 1998; Rohde *et al*., 2000). Mutants of *fus3* and *lec1* have cotyledons that bear trichomes and their ultrastructure is similar to that of leaf primordia. Immature *fus3* and *lec1* embryos enter germinative development, and the shoot apical meristems develop leaf primordia before seed desiccation begins. *DET1*, *FUS3* and *LEC1* positively regulate expression of the *ABI3* gene and its protein product (Parcy *et al*., 1997; Rohde *et al*.,

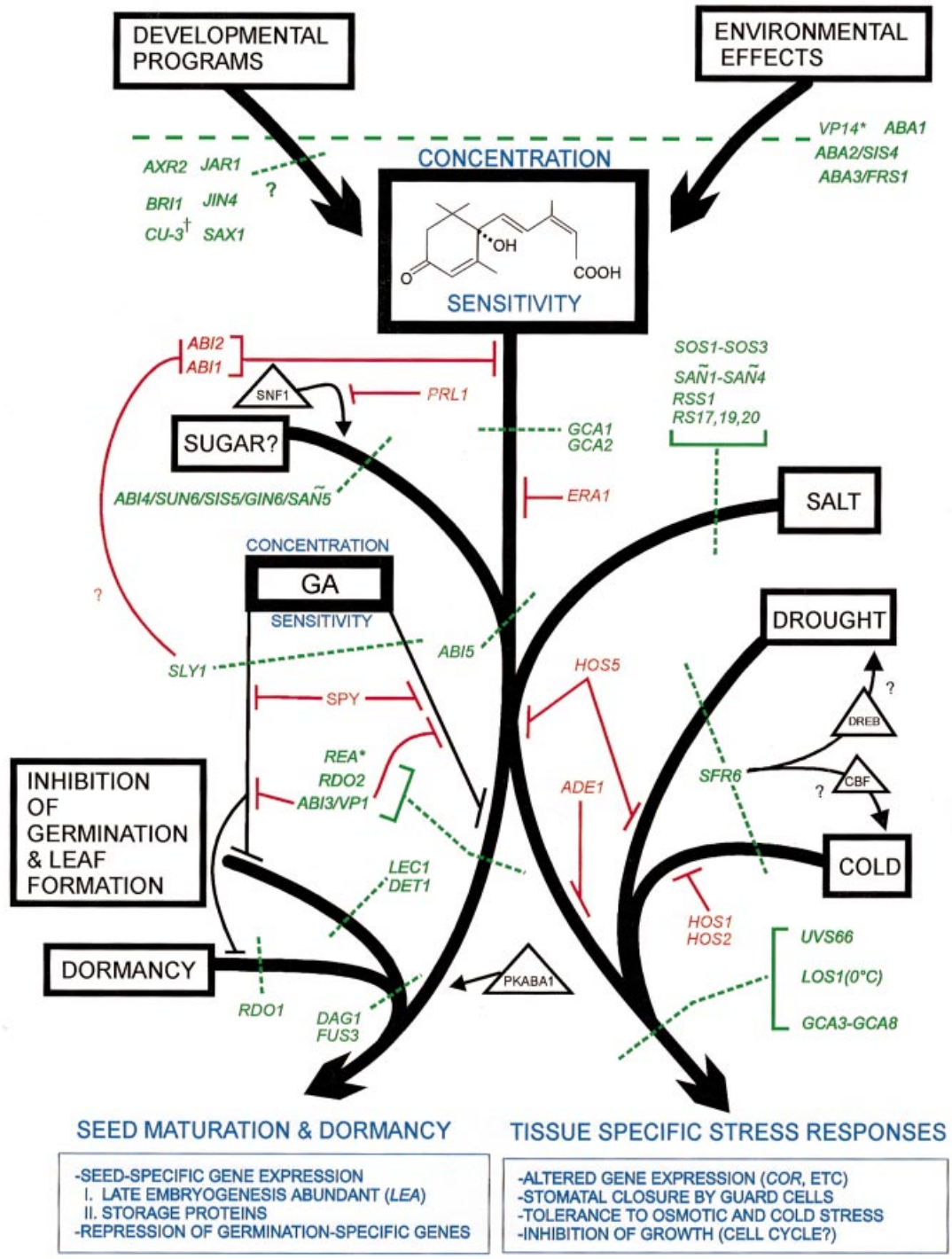

Fig. 1. *Arabidopsis* genes identified and positioned by mutant and genetic analyses define separate yet converging pathways affecting ABA physiology. Genes coloured green have a positive effect on a pathway; red genes signify a negative regulatory effect. Black triangles signify gene products shown functionally but not genetically to be involved in the pathway. *Maize mutants; †tomato mutant.

2000), lending further support to the notion that *ABI3*, *FUS3*, *LEC1* and *DET1* are key members of an integrated genetic regulatory network controlling complementary aspects of seed and vegetative development.

The molecular mechanisms of *ABI3/Vp1*, *FUS3*, *LEC1*, *DET1* and *CO* action are not known, but the genes have been cloned by chromosome walking (Giraudat *et al.*, 1992; Pepper *et al.*, 1994; Putterill *et al.*, 1995; Lotan *et al.*, 1998; Luerssen *et al.*, 1998), which provides some structural clues to function as well as permitting the direct testing of hypotheses. All five genes are expressed in developing seeds, and *DET1*, *CO* and *ABI3* are also

expressed in some vegetative parts of the plant (Pepper *et al*., 1994; Rohde *et al*., 1999; Kurup *et al*., 2000). *DET1* encodes a nuclear-localized protein that when mutated results in severe defects in the temporal and spatial regulation of light-regulated and developmental (e.g. *ABI3*) genes. The *CO* gene encodes a protein with homology to zinc-finger transcription factors that promotes flowering (Putterill *et al*., 1995). The *LEC1* gene encodes a transcription-factor homologue of a CCAAT-box-binding factor subunit (*HAP3*, involved in the regulation of carbon and nitrogen metabolism in yeast (Lotan *et al*., 1998)).

The *ABI3* gene is highly conserved at the sequence level to *Vp1* and orthologues from various species (Table 1). There are four highly conserved domains: an acidic amino-terminal stretch (A1) of 112 amino acid residues, and three basic domains (B1–B3) of 137, 36 and 128 residues, respectively. Interestingly, the predicted *FUS3* gene product has a continuous stretch of >100 residues with significant sequence similarity to the conserved B3 domains of ABI3 and VP1 (Luerssen *et al*., 1998) (Table 1). This suggests that ABI3 and FUS3 might act in partly redundant pathways, analogously to the regulatory networks controlling shoot and flower development (Hake & Meyerowitz, 1998). Indeed, it has recently been shown that FUS3 binds and transactivates maturation-specific gene promoters (Reidt *et al*., 2000). Recent evidence shows that *ABI3* also functions in the vegetative developmental programs of plastid differentiation and flowering; double mutants of *abi3/co* and *abi3/det1* flower early, and the B2 and B3 domains of ABI3 bind to the carboxy-terminus of CONSTANS (Kurup *et al*., 2000; Rohde *et al*., 2000). A recently isolated allele of *abi3* with an intermediate phenotype of dormancy reduction and insensitivity to ABA has a point mutation in the absolutely conserved B2 domain (Bies-Etheve *et al*., 1999). The conservation of VP1/ABI3 domains indicates that these motifs are fundamental to plant development and are a suitable model for a better comprehension of plant plasticity and functional redundancy.

Structure–function studies with *Vp1*, *ABI3* and the *Phaseolus* orthologue *PvALF* in transient gene expression assays demonstrate that the amino-terminal acidic domain functions as a transcriptional activator and acts in synergy with ABA (McCarty *et al*., 1991; Bobb *et al*., 1995; Carson *et al*., 1997; Rojas *et al*., 1999). The acidic domain of the protein is not required for repression of the gene encoding α-amylase during germination (Hoecker *et al*., 1995). The conserved basic B2 region is required for transactivation of the ABA-inducible *Em* (early methionine-labelled *LEA* gene) promoter and for enhancing the binding of various transcription factors to their cognate targets *in vitro* (Hill *et al*., 1996), but not for α-amylase gene repression (Hoecker *et al*., 1995). The B3 domain of VP1 and the *FUS3* gene product bind specifically to promoter sequences required for transactivation in seeds but not to ABA-responsive *cis*-elements (Suzuki *et al*., 1997; Reidt *et al*., 2000). The B3 domain is *c*. 35% similar to the amino-termini of ARF1, a transcription factor that binds to auxin response elements (Ulmasov *et al*., 1997), the ARF1-like *MONOPTEROS* gene involved in embryo axis formation and vascular development (Table 1) (Hardtke & Berleth, 1998), and several *Arabidopsis* DNA-binding proteins of unknown function (Kagaya *et al*., 1999). The VP1 B3 domain is not required for synergistic effects of transactivation with ABA or for α-amylase gene repression (Hoecker *et al*., 1995). Because VP1 acts in part through ABA-responsive *cis*-elements but does not bind them, it must interact indirectly through protein-protein interactions. Recently, a genuine transcription factor responsible for ABA regulation (TRAB1) has been identified that binds ABA-responsive *cis*-elements and VP1 (Hobo *et al*., 1999b) (see section IV.2b). It remains to be seen whether VP1 acts via multiple or conserved mechanisms in gene activation and repression.

(b) *Dormancy and germination*. Seed germination and dormancy lend themselves to facile genetic screens because of high throughput and low cost. Dormancy is the active repression of the germination developmental program under environmental conditions that would otherwise promote it (Rock & Quatrano, 1995). Seed dormancy and germination in higher plants are partly controlled by ABA and GA, respectively (Debeaujon & Koornneef, 2000). ABA promotes quiescence and establishes dormancy during embryo maturation, whereas GA breaks dormancy and induces germination (Rock & Quatrano, 1995; Grappin *et al*., 2000). The ratio, rather than the absolute amounts, of GA and ABA is an important factor in determining the developmental state of maturing seeds (White & Rivin, 2000). This phenomenon was elegantly exploited by Koornneef *et al*. (1982) to isolate an ABA-deficient mutant (*aba1*) that suppressed the nongerminating phenotype of a GA-deficient *Arabidopsis* mutant (*ga1*). Similar strategies have been employed with spectacular results by screening for germination in the presence of GA biosynthesis inhibitors. A strong allele of *abi3* (Nambara *et al*., 1995), two ABA biosynthesis mutants (*aba2*, *aba3*) (Léon-Kloosterziel *et al*., 1996a) and a constitutive 'slender' GA response mutant *spindly* (*spy*) (Jacobsen & Olszewski, 1993) have been isolated by such screens. When the barley orthologue of *SPY*, which encodes an O-glucosyl transferase, was transiently expressed in barley aleurone protoplasts, it abolished the GA-induced activity of an α-amylase promoter, which is consistent with its proposed role as a negative regulator of GA signalling (Robertson *et al*., 1998). Surprisingly, *HvSPY* coexpression also trans-

Table 1. *Conservation of structural domains between* Zea mays *VP1 and orthologues from monocots, dicots and FUS3 of* Arabidopsis thaliana

Species/Protein (GenBank accession no.)*	Domain				
	Acidic 1	Basic 1	Basic 2	Basic 3	Overall†
Zea mays/VP1 (*gi*: *138603*)	(residues 26–118)‡	(residues 120–234)	(residues 379–421)	(residues 496–619)	—
Oryza/OsVP1 (gi: 391885)					
Identity	77	73	84	91	
Similarity	94	93	98	98	90
Avena/AfVP1 (gi: 2924300)					
Identity	76	67	79	89	
Similarity	92	90	98	95	72
Daucus/DcABI3 (gi: 5578746)					
Identity	28	38	40	77	
Similarity	64	70	74	94	62
Phaseolus/PvALF (gi: 1046278)					
Identity	24	38	46	83	
Similarity	76	76	77	96	67
Craterostigma/CpVP1 (gi: 2288899)					
Identity	34	39	49	82	
Similarity	74	77	79	92	67
Populus/PtABI3 (gi: 2661461)					
Identity	25	20	14	14	
Similarity	60	39	42	37	50
Arabidopsis/ABI3 (gi: 584707)					
Identity	39	41	46	84	
Similarity	75	80	79	97	69
Arabidopsis/FUS3 (gi: 3582520)					
Identity	—	—	26	51	
Similarity	—	—	46	77	28
Arabidopsis/ARF1 (gi: 2245377)					
Identity	—	—	—	11	
Similarity	—	—	—	35	na§
Arabidopsis/MP (gi: 2982221)					
Identity	—	—	—	8	
Similarity	—	—	—	32	na§

*http://www.ncbi.nlm.nih.gov
†Similarity to full-length *Zea mays* VP1, except for FUS3, which was full length.
‡Amino acid numbering according to VP1 (McCarty *et al.*, 1991).
§na, not analysed.

activated the ABA-inducible *LEA* dehydrin (*DHN*) gene promoter, reminiscent of (but diametrically opposed to) the dual activator-repressor functions of *Vp1*. The mechanism of action of SPY is not known, but it might modify GA and ABA signalling molecules post-translationally.

Seed dormancy is the last stage of seed development. The *fus3* and *rdo* (*reduced dormancy*) mutants of *Arabidopsis* were isolated by plating freshly harvested (dormant) M2 seeds and selecting those that germinated (Keith *et al.*, 1994; Léon-Kloosterziel *et al.*, 1996b). The *rdo1* and *rdo2* mutants have normal ABA levels and ABA sensitivity to inhibition of germination, but *rdo2* is insensitive to GA biosynthesis inhibitors (Léon-Kloosterziel *et al.*, 1996b), whereas *fus3* and *rdo1* are not (Keith *et al.*, 1994; Léon-Kloosterziel *et al.*, 1996b). A double-mutant analysis of seed dormancy between *rdo1*/*rdo2*, *rdo1*/*abi3* and *rdo2*/*abi3* homozygotes showed that *RDO1* and *RDO2* act in different pathways, with *RDO2* acting in the *ABI3* pathway. The most parsimonious interpretation of these results is that *RDO2* positively regulates ABA-dependent dormancy and is in turn negatively

regulated by GA. *RDO1* and *FUS3* might act in an ABA-independent, yet integrated, dormancy pathway (Fig. 1). Alternative models are possible, of course. Dormancy, like flowering, might be a quantitative trait with many genes that affect it, and a number of dormancy mutants that have normal ABA levels and responsiveness have been isolated (M. Koornneef & G. Galau, pers. comm.) A model in which GA interacts with an ABA signalling pathway is consistent with the nondormant phenotype of *spy* mutants (Jacobsen & Olszewski, 1993) and transient assays showing that *HvSPY* (a negative regulator of GA action) activates the ABA-inducible *DHN* gene (Robertson *et al.*, 1998). It will be useful to know whether *SPY* interacts genetically with *ABI3*/*Vp1* or *RDO2* genes to affect ABA phenotypes.

The inhibitory effect of exogenous ABA on germination is the basis of genetic screens in *Arabidopsis* for altered sensitivity to ABA. The *ABA-insensitive* (*abi1–abi5*) mutants were isolated by germination on medium containing ABA at concentrations that normally inhibit germination (Koornneef *et al.*, 1984; Finkelstein, 1994). Double-mutant studies suggest that *ABI3*, *ABI4* and *ABI5* act in a seed-specific ABA response pathway (Finkelstein, 1994). The *ABI4* gene has been cloned by map-based methods and the predicted protein product shows homology to the *APETELA2* family of transcriptional regulators (Finkelstein *et al.*, 1998). *ABI4* is a member of a gene family and is expressed in both vegetative and seed tissues, despite the seed-specific nature of the mutant phenotype. The *ABI5* gene has recently been positionally cloned and encodes a member of the basic Leu zipper transcription factor (bZIP) family of transcriptional regulators; the mutant *abi5-1* allele lacks the DNA-binding and dimerization domains required for normal function (Finkelstein & Lynch, 2000b). This recent discovery finally provides genetic evidence for the involvement of bZIPs in ABA signalling; for more than 10 yr there has been suggestive, but not conclusive, biochemical evidence for this mechanism (Guiltinan *et al.*, 1990) (see section III.3). In addition, functional evidence for the involvement of TRAB1 in ABA-regulated gene expression has emerged recently from protein-protein interaction studies with VP1 (see section IV.2b). Similarly to *ABI4*, *ABI5* is expressed in vegetative as well as seed tissues, albeit at much lower levels. Furthermore, *ABI5* expression is regulated by ABA and other *ABI* genes and is required for expression of some ABA-inducible marker genes in vegetative tissue (Finkelstein & Lynch, 2000b). *ABI5* function is essential for the ABA hypersensitivity conferred by ectopically expressed *ABI3* (R. Finkelstein, pers. comm.). Because ABI3/Vp1, ABI4 and ABI5 are all transcription factors, they might regulate their own and each other's expression. *ABI5* is highly homologous to rice *TRAB1* and to the genes for the sunflower ABA-inducible *Dc3*-promoter binding factor *DPBF1* and the *Arabidopsis* ABA-responsive-element binding factors *ABF1–ABF4* (Kim *et al.*, 1997; Hobo *et al.*, 1999b; Choi *et al.*, 2000) (see section IV.2b). The conservation of Ser and Thr residues between these bZIPs suggests that they might be functionally important as targets of protein kinases and/or phosphatases (Schwechheimer *et al.*, 1998; Finkelstein & Lynch, 2000b).

Plant sugar sensing and signalling pathways are mediated by multiple sensors and linked to plant growth and development (Sheen *et al.*, 1999). Genetic screens for sugar sensing/response (germination on inhibitory concentrations of sucrose or glucose) and osmotic response mutants (germination on NaCl or mannitol) have recently revealed links between response pathways for ABA, soluble sugars and osmotic stress. It is difficult to distinguish between the role of sugars in signalling and that in metabolism, or the role of salt in stress (ionic) and that in osmotic pressure phenomena. Low concentrations of exogenous sugars permit germination on medium containing inhibitory concentrations of ABA (Garciarrubio *et al.*, 1997; Finkelstein & Lynch, 2000a). It has recently been shown that the *sis5* (*sugar insensitive* (Laby *et al.*, 2000)), *sun6* (*sucrose uncoupled* (Huijser *et al.*, 2000)), *gin6* (*glucose insensitive* (Arenas-Huertero *et al.*, 2000)) and *salobreño5* (salt-tolerant, *sañ5* (Quesada *et al.*, 2000)) mutants are allelic to *ABI4*, and *sis4* is allelic to *ABA2* (Laby *et al.*, 2000). The *aba1*, *aba3* and *abi5* mutants have a sucrose-insensitive phenotype, but *abi1*, *abi2* and *abi3* mutations confer only slight increases in sugar insensitivity (Arenas-Huertero *et al.*, 2000; Laby *et al.*, 2000; Huijser *et al.*, 2000), suggesting that *abi4* and *abi5* might act in ABA and/or sugar-sensing pathways upstream of *abi3* that also affect vegetative ABA responses (Fig. 1). Altogether, it is fair to say that tissue-specific gene expression regulated by ABA and other developmental and environmental pathways relies on the combinatorial action of a large number and variety of transcription factors. This fact points to the likelihood of genetic redundancy that would in turn permit subtle variations in gene expression during response to developmental and environmental cues.

Mutant *sañ1–sañ4* plants display incomplete penetrance of resistance to both NaCl and mannitol (ionic stress and osmotic pressure, respectively), but are sensitive to the inhibition of germination by KCl and Na_2SO_4. Similar mutants, *resistant to salt* (*rs17*, *rs19* and *rs20*) (Saleki *et al.*, 1993) and *reduced salt-sensitive* (*rss*) (Werner & Finkelstein, 1995), have been described previously but not linked directly to ABA signalling. A screen for seedling hypersensitivity to growth inhibition by low-K^+ culture medium resulted in the isolation of *salt-overly-sensitive* (*sos1–sos3*) mutants (Zhu *et al.*, 1998). The

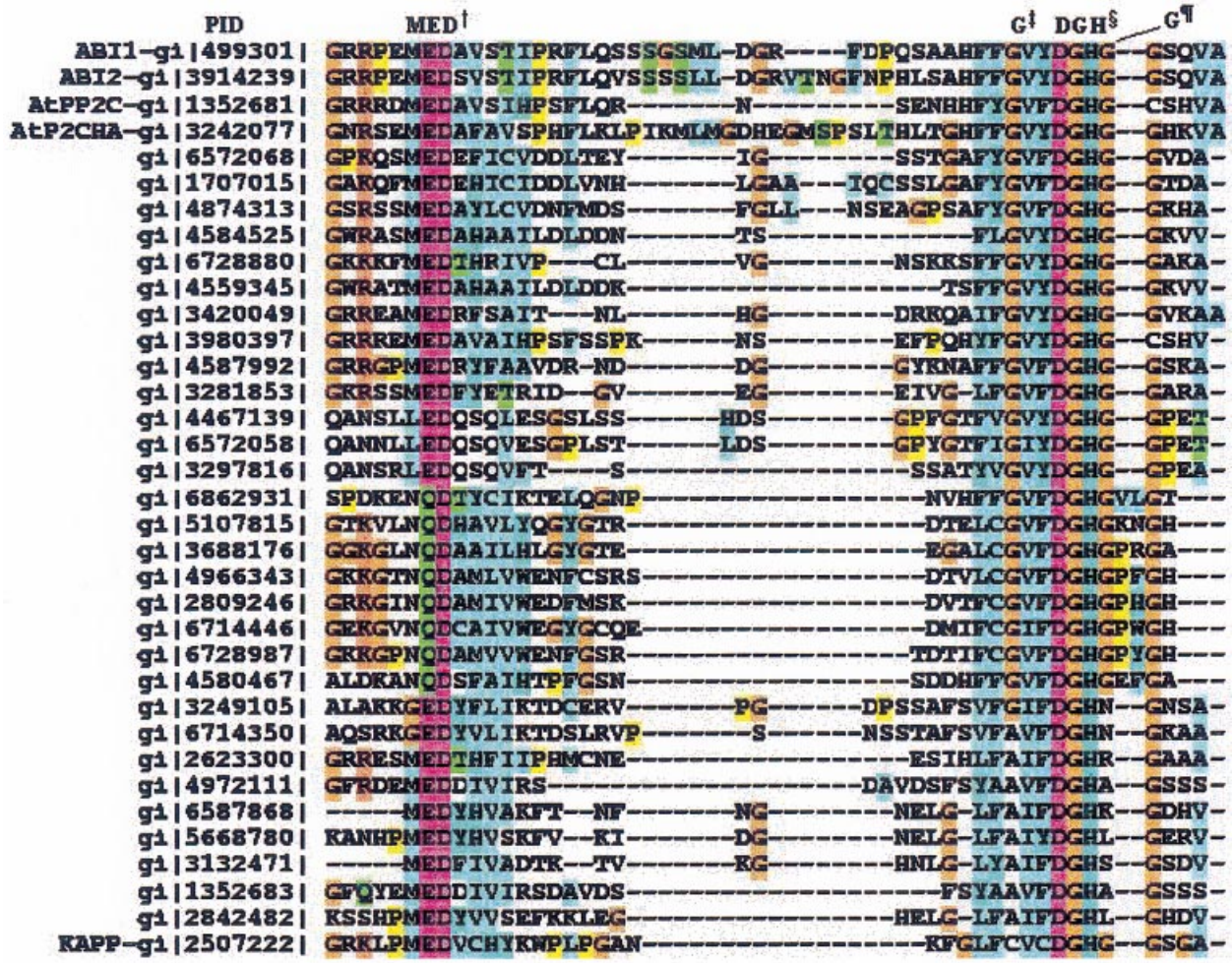

Fig. 2. *ABI1* homologues in *Arabidopsis*, identified from a BLAST search with full-length ABI1 as input for conserved amino acid residues known to be required for ABA signalling. MED† (residues 141–143) is implicated in the active site of PP2Cs; G‡ (residue 174), DGH§ (residues 177–179), and G¶ (residue 180) are required for phosphatase activity and ABA signalling activity, whereas kinase-associated protein phosphatase (KAPP) has been shown not to function in ABA signalling (Sheen, 1998). PID, GenBank protein identification numbers (http://www.ncbi.nlm.nih.gov). Sequences were aligned with Clustal X (Thompson *et al.*, 1997).

sos2 mutant overexpresses ABA- and drought-inducible reporter genes (Zhu *et al.*, 1998) and the *SOS3* gene encodes a Ca^{2+} sensor homologous to a calcineurin B subunit and important for potassium nutrition (Liu & Zhu, 1998). It is too early to speculate about mechanisms of sugar and salt cross-talk with ABA pathways; the action of GA in ABA responses has long been known, yet the mechanisms of interaction between these hormones are still far from understood. From the pleiotropic nature of mutants, it seems that sugar and salt signalling pathways affect both seed and vegetative ABA responses (Fig. 1).

The semi-dominant *abi1* and *abi2* mutations are the most pleiotropic in terms of physiological and tissue-specific ABA processes, affecting gene expression in seeds and vegetative tissues as well as rapid stomatal movements, seed dormancy and germination, and adaptive growth (Rock & Quatrano, 1994; Leung & Giraudat, 1998). The *ABI1* and *ABI2* genes have been cloned by map-based methods (Leung *et al.*, 1994, 1997; Meyer *et al.*, 1994; Rodriguez *et al.*, 1998a) and encode homologous type 2C Ser/Thr protein phosphatases (PP2Cs) with partly redundant but distinct tissue-specific functions in the regulation of genes and ion channels inducible by ABA, cold or drought (Gilmour & Thomashow, 1991; Yamaguchi-Shinozaki & Shinozaki, 1993; Mäntylä *et al.*, 1995; de Bruxelles *et al.*, 1996; Pei *et al.*, 1997; Chak *et al.*, 2000). Remarkably, the sole mutant alleles, *abi1-1* and *abi2-1*, are both mis-sense mutations of a conserved Gly to Asp mutation (G180D in *abi1-1*, G168D in *abi2-1*) that result in a dominant phenotype *in vivo* and decreased phosphatase activity *in vitro* (Leung *et al.*, 1997; Rodriguez *et al.*, 1998a; Sheen, 1998). The mutations can therefore be described as dominant-negative alleles that probably disrupt the metal coordination in the catalytic site (Sheen, 1998), although the molecular mechanism of action of the *abi1* and *abi2* mutants in ABA signalling is not understood.

Intragenic suppressor alleles of *abi1* have been described recently (Gosti *et al*., 1999). These alleles are all mis-sense mutations and null with respect to protein phosphatase activity. Interestingly, no true molecular null alleles (e.g. nonsense (stop codon)) were isolated in this screen, suggesting that some novel phenotype might occur when ABI1 is knocked out. The suppressor-allele plants exhibit higher seed dormancy and enhanced ABA sensitivity to inhibition of germination and stomatal movements. The ABA-hypersensitivity phenotype of *ABI1*-null mutants demonstrates that *ABI1* (and probably *ABI2*) act as negative regulators of ABA signalling (Gosti *et al*., 1999). Consistent with this conclusion is the finding that the overexpression of *abi1* or a constitutively active PP2C in transformed maize protoplasts can antagonize both the up-regulation and down-regulation of ABA-responsive promoters (Sheen, 1998). However, because the true targets of ABI1 and ABI2 are not known, the negative regulator model remains to be proved.

At least 25 *Arabidopsis* PP2C homologues have been sequenced so far by the Multinational Coordinated *Arabidopsis* genome project which have conserved amino acid residues critical for ABA signalling (Sheen, 1998) (Fig. 2). This suggests further genetic redundancy in ABA signalling through protein phosphorylation. The expression of *AtP2C-HA*, like that of *ABI1* and *ABI2* (Leung *et al*., 1997), is up-regulated by ABA (Rodriguez *et al*., 1998b), and overexpression of *AtPP2C* can antagonize ABA-inducible reporter gene expression in maize protoplasts (Sheen, 1998). The *ABI1* and *ABI2* genes are expressed in all tissues examined so far (Leung *et al*., 1997). In the ice plant *Mesembryanthemum crystallinum*, ten different PP2Cs whose genes have homology to *ABI1* are differentially expressed both temporally and spatially and in response to stress (Miyazaki *et al*., 1999). Clearly there is much more to be learned about the roles of PP2Cs in ABA signalling and physiology. See section IV.1 for a discussion of PP2C regulation.

A germination screen for extragenic suppressors of *abi1* resulted in the isolation of *sleepy1* (*sly1*), a GA-insensitive mutant that reflects the full spectrum of GA-associated phenotypes including the failure to germinate in the absence of the *abi1* lesion (Steber *et al*., 1998). It is possible that SLY1 protein interacts physically with ABI1, or (more probably) it might act early in GA signalling because it displays a dwarf phenotype. It will be interesting to identify, by yeast two-hybrid assay or other assays, proteins that interact with PP2Cs.

A screen for mutants that do not germinate in the presence of low concentrations of ABA (0.3 μM) that are not inhibitory to wild-type seeds resulted in the isolation of *enhanced response to ABA* (*era1–era3*) mutants (Cutler *et al*., 1996). Mutant *era1* seeds are hyperdormant, suggesting that *ERA1* encodes a negative regulator of ABA signalling. The *ERA1* gene is also expressed in vegetative tissues and regulates ion fluxes in guard cells (Pei *et al*., 1998). Genetic analysis indicates that *ERA1* is epistatic (downstream) to *ABI1* and *ABI2* and upstream of *ABI3* (Fig. 1). The *ERA1* gene encodes the β subunit of farnesyl transferase (Cutler *et al*., 1996) and is allelic to the *WIGGUM* gene involved in meristem cell division control (Running *et al*., 1998; Ziegelhoffer *et al*., 2000). The relationship between ABA-regulated protein phosphorylation, protein farnesylation and the cell cycle is not understood, but the structures of the *ABI1*, *ABI2* and *ERA1* genes compel tantalizing comparisons between ABA signalling and the mitogenic stress responses involving *RAS*, *RAF* and mitogen-activated protein kinases (MAPKs) in mammalian cells (see section IV.2a).

Lipidation of plant signalling molecules might be essential for their function (Nambara & McCourt, 1999). Given the large number of putative targets for ERA1, it is perhaps surprising that *era1* mutants are not more pleiotropic. It might be that *ERA1* does not have a direct role in ABA signalling but instead conditions elements of cellular machinery involved in ABA responses, or that farnesylation in plants is genetically redundant. Numerous plant transcription factors have putative farnesylation sites, which could function to coordinate transcription to the cell cycle, for example through association with the nuclear envelope (Nambara & McCourt, 1999). ABA promotes quiescence and blocks germination (cell division) in embryos: in this context it is interesting to note that ABA negatively regulates a nuclease involved in DNA degradation during GA-stimulated cell death of barley aleurone protoplasts (Fath *et al*., 1999), a process similar to apoptosis, which in animals is tightly coupled to cell cycle regulation.

2. *Vegetative responses*

There is a single report of a mutant of barley, *cool*, that has excessive transpiration and ABA-insensitive guard cells (Raskin & Ladyman, 1988); however, no further work has appeared and this mutant might have been lost. Similar screens for excessive transpiration (and hence 'cooler' leaves) in *Arabidopsis* should identify mutants involved in guard-cell-specific physiology. Ectopic expression of *ABI3* in guard cells suppresses the inhibitory effect of the *abi1* gene on stomatal closure, suggesting that transcriptional events might have a role in the ABA regulation of ion channels or that *ABI3* has other or different functions in ABA signalling besides seed development (Parcy & Giraudat, 1997). The recent reports of tissue-specific expression of *ABI3* in vegetative tissues (Rohde *et al*., 1999) and pleiotropic effects of *ABI3* on plastid development and flowering (Kurup *et al*., 2000; Rohde *et al*., 2000) support

this hypothesis. A carrot *ABI3/Vp1* orthologue can also direct embryo-specific ABA-inducible marker gene expression in leaves when expressed ectopically (Shiota *et al*., 1998). These results showing genetic interaction of *ABI1* and *ABI3* suggest that these genes act via a conserved ABA signalling pathway in seeds and vegetative tissues. Because both *ABI1* and *ABI2* genes are up-regulated by ABA (Leung *et al*., 1997), it is plausible that ABI3 overcomes the PP2C deficiency by effecting *ABI1* and *ABI2* transcription when expressed ectopically. However, not all ABA-inducible genes interact with or require *Vp1/ABI3* for expression (Pla *et al*., 1991; Thomann *et al*., 1992; Furini *et al*., 1996; Guan & Scandalios, 1998). The recent availability of *abi1*-null and *abi3*-null alleles will permit a definitive genetic test of whether they lie in the same pathway (Finkelstein & Somerville, 1990; Ooms *et al*., 1993; Gosti *et al*., 1999).

(a) *Root growth*. It has long been known that ABA arrests root mitotic activity (van Overbeek *et al*., 1967; Leung *et al*., 1994; Himmelbach *et al*., 1998). One possible mechanism is by reduced transcription of the mitotic cyclin *CDC2a* and up-regulation of the cyclin-dependent kinase inhibitor gene *ICK1* by ABA (Hemerly *et al*., 1993; Wang *et al*., 1998). The *abi1* and *abi2* mutants are less sensitive than the wild type to inhibition of root cell division by ABA (Leung *et al*., 1994). The *abi1* and *abi2* mutants differentially affect root hair growth and a morphogenetic drought stress response (rhizogenesis) (Schnall & Quatrano, 1992; Vartanian *et al*., 1994).

A genetic screen for seedling roots with unaltered growth in the presence of inhibitory ABA concentrations resulted in isolation of *growth control by ABA* (*gca1–gca8*) mutants (Himmelbach *et al*., 1998) (Fig. 1). The *gca1* and *gca2* mutants are pleiotropic in their effects and resemble the *abi1* and *abi2* mutants in that regard (Himmelbach *et al*., 1998). Physiological and molecular characterization of these mutants will shed much light on ABA sensitivity and responses. The *uvs66* mutant, which is hypersensitive to UV radiation and DNA-damaging chemicals, is hypersensitive to root growth inhibition by ABA (Albinsky *et al*., 1999) and therefore has the opposite effect to the *gca* mutants. The *uvs66* mutant defines a novel link between ABA and genomic responses, possibly through the involvement of cell cycle machinery.

Mutant screens similar to those used for the inhibition of root growth by ABA have been performed on *Arabidopsis* seedlings grown on inhibitory concentrations of auxin, brassinosteroid (BR) and jasmonic acid (JA). Interestingly, each of these hormone-resistance screens has resulted in the isolation of ABA-hypersensitive mutants (Staswick *et al*., 1992; Timpte *et al*., 1994; Berger *et al*., 1996; Clouse *et al*., 1996; Staswick *et al*., 1998; Ephritikhine *et al*., 1999). The *pleiotropic regulatory locus* (*prl1*) mutant has increased sensitivity to sugar, ethylene, ABA, auxin, cytokinin and cold stress and encodes a protein that interacts with an *SNF1*-like kinase (Németh *et al*., 1998; Bhalerao *et al*., 1999) shown to have an important role in plant sugar response pathways (Halford & Hardie, 1998). Again, a tantalizing link can be drawn between SNF1-like kinases and the integration of sugar and ABA signalling pathways. PRL is tentatively assigned as a negative regulator of an SNF1-dependent sugar-sensing pathway that feeds into ABA and stress signalling responses (Fig. 1), but it might interact with many components because it encodes a WD-domain protein, defined by amino acid motifs found in a large variety of plant and animal proteins that do not share any obvious functional properties except protein-protein interactions.

JA is a plant signalling molecule that is derived from lipids through the action of lipoxygenase; it regulates gene expression during plant development and in response to water deficit, wounding and pathogen elicitors. The induction of vegetative storage proteins by methyl-JA in the recessive *jasmonate resistant* (*jar1*) mutant is at most 25% that of the wild type, but seeds of *jar1* plants are more sensitive than the wild type to inhibition of germination by ABA (Staswick *et al*., 1992). Not one of the *jar1* alleles shows an altered sensitivity to auxin, cytokinin or the ethylene precursor 1-aminocyclopropane-1-carboxylic acid, indicating that the lesion does not affect the general uptake or transport of hormones. A soil fungus, *Pythium irregulare*, blights *jar1* mutants but not the wild type (Staswick *et al*., 1998). One of two recessive methyl *jasmonate-insensitive* (*jin*) mutants, which show reduced sensitivity to methyl-JA-mediated root growth inhibition and reduced induction of vegetative storage proteins in leaves, exhibits increased sensitivity to ABA in seed germination assays (Berger *et al*., 1996). ABA is thought to potentiate some wound responses in the Solanaceae (Sánchez-Serrano *et al*., 1991; Birkenmeier & Ryan, 1998; Carrera & Prat, 1998), but the relationship between JA and ABA signalling is poorly understood and is an important area for further study.

Plants carrying the dominant *auxin resistant* (*axr2-1*) mutation are severe dwarfs, lack root hairs and display defects in growth orientation of both the shoot and root, suggesting that the mutation affects some aspect of gravitropic growth. Growth inhibition experiments indicate that the roots of *axr2* plants are resistant to ethylene and ABA as well as auxin (Timpte *et al*., 1994). Interestingly, it has been shown recently that ABA effects growth at low water potentials by restricting ethylene production (Spollen *et al*., 2000). It might be that as yet undefined developmental programs involve other hormones that require ABA to potentiate them. Mutants provide crucial insights that might help to explain the longstanding complexity of hormone

interactions affecting morphogenesis and development (Fig. 1).

Arabidopsis sax1 (*sensitive to abscisic acid and auxin*) mutants show a short curled primary root and small, round, dark-green cotyledons, delayed development, dwarf stature and reduced fertility (Ephritikhine *et al*., 1999). Seedlings of *sax1* mutants are 40-fold more sensitive than the wild type to ABA inhibition of root elongation and, to a smaller extent, to ABA-induced stomatal closure. Treatment of mutant seedlings with BR partly restores wild-type growth, suggesting that BR biosynthesis might be affected in *sax1* plants. Wild-type sensitivities to ABA, auxin and GAs were also restored in *sax1* plants by the exogenous application of BR, illustrating the pivotal importance of the BR-related *SAX1* gene in hormone homeostasis (Ephritikhine *et al*., 1999). BRs might target a shared hypothetical signalling component of hormone pathways, or they might act downstream in some aspect of the cell elongation process. It is speculated that ABA and BR might affect the cell cycle through similar mechanisms. Another possibility is that genes controlling morphogenesis (*DET*, *FUSCA* and *CO*), ABA (*ABI* and *ERA*), BR (*DET* and *BRI*), GA (*SPY* and *SLY*) and other pathways (flowering, JA, auxin and sugars) are interacting in a complex regulatory network! Double-mutant genetic analyses and detailed tissue-specific gene expression studies can help to resolve these possibilities.

BRs are widely distributed plant compounds, with structural similarities to animal steroid hormones, that modulate cell elongation and division. However, little is known about the mechanism of action of these plant growth regulators (Schumacher & Chory, 2000). A *brassinosteroid-insensitive* mutant, *bri1*, and a phenotypically similar tomato mutant, *curl-3* (*cu-3*), do not respond to BR in hypocotyl elongation and primary root inhibition assays; they show pleiotropic developmental phenotypes, including a severely dwarfed stature, dark-green thickened leaves, male sterility, reduced apical dominance and de-etiolation of dark-grown seedlings, but retain sensitivity to auxins, cytokinins, ethylene, ABA and GAs (Clouse *et al*., 1996; Koka *et al*., 2000). The *BRI1* gene encodes a putative leucine-rich receptor kinase that is likely to be involved in BR signalling (Li & Chory, 1997).

(b) *Overlapping stress response pathways*. The *abi1* and *abi2* mutants provided the first conclusive evidence for separate yet overlapping ABA and stress signalling pathways (Gilmour & Thomashow, 1991; Nordin *et al*., 1991). The *COR* genes are responsive to cold, drought, salt and ABA; their protein products are heat stable and hydrophilic. Some *COR* genes have structural similarities to the *LEA* genes (Dure, 1993; Ingram & Bartels, 1996; Shinozaki & Yamaguchi-Shinozaki, 1997; Thomashow, 1999). The roles of *COR* and *LEA* genes in cold and desiccation tolerance are not yet known, but there is strong biochemical and genetic evidence that they have an adaptive function in tolerance to desiccation, freezing and salt, possibly by interaction with membranes and/or proteins (Chandler & Robertson, 1994; Ingram & Bartels, 1996; Ismail *et al*., 1999; Thomashow, 1999). The *abi1* mutant affects an ABA signalling pathway differentially over a drought- or cold signalling pathway leading to *COR* gene expression (Gilmour & Thomashow, 1991; Yamaguchi-Shinozaki & Shinozaki, 1993; Mäntylä *et al*., 1995). On the basis of Northern blot analysis of alcohol dehydrogenase (*ADH*) gene expression in mutant genotypes, de Bruxelles *et al*. (1996) proposed that *ABI2* specifically controls an *ABI1*-independent drought- and ABA response pathway. By contrast, Jarillo *et al*. (1993) concluded that *ABI1*, not *ABI2*, controls the expression in leaves of the same *ADH* gene induced by cold, ABA and, to a smaller extent, drought. To complicate the story further, Chak *et al*. (2000) showed that expression of the *COR47* and *COR78* (also called *RD29A*) genes inducible by ABA and mannitol is more impaired in *Arabidopsis abi2* mutants than in *abi1* mutants. ABA plus mannitol treatments were additive towards *COR47* gene expression. These results support the notion that drought and ABA signalling pathways are separate yet overlapping.

In transgenic tomato, expression of the *Arabidopsis abi1* mutant allele inhibits the wound induction of proteinase inhibitor *PINII* transcripts (Carrera & Prat, 1998), clearly demonstrating the link between wounding or pathogen signalling and ABA signalling pathways in solanaceous species. However, this link is restricted to specific organs (Peña-Cortez *et al*., 1991), and there are other wounding pathways in tomato that are ABA-independent (Birkenmeier & Ryan, 1998; Chao *et al*., 1999). Taken together, these diverse gene expression patterns suggest the existence of tissue-specific regulatory factors that modulate ABI1 and ABI2 activities and stress-inducible gene expression.

The *sensitivity to freezing* (*sfr*) and *freezing sensitive* (*frs*) mutants define genes that are required for tolerance to freezing (Knight *et al*., 1999; Llorente *et al*., 2000). The *frs1* mutant is allelic to the ABA-biosynthesis mutant *aba3* (Llorente *et al*., 2000) and has attenuated expression of marker genes inducible by cold and drought. These results demonstrate clearly that ABA is required for the full development of cold acclimation and freezing tolerance, and that cold and drought signalling pathways are linked. Mutant *sfr6* plants are impaired in the cold-inducible, ABA-inducible and drought-inducible expression of a subset of *COR* genes that are regulated by drought-responsive-element binding factors and cold-promoter-element binding factors (CBFs and DREBs; see section III.3). However, the

Table 2. *Mutants that affect ABA sensitivity or related physiological responses*

Species	Mutation/alleles	Phenotype	Gene product	References
Arabidopsis thaliana	*abi1*	Pleiotropic ABA insensitivity	Protein phosphatase 2C	Koornneef *et al.* (1984); Leube *et al.* (1998); Gosti *et al.* (1999)
	abi2	Pleiotropic ABA insensitivity	Protein phosphatase 2C	Koornneef *et al.* (1984); Leung *et al.* (1997); Rodriguez *et al.* (1998a)
	abi3	ABA insensitivity in seeds; altered plastid development and flowering	*Vp1*-like transcription factor	Koornneef *et al.* (1984); Giraudat *et al.* (1992); Kurup *et al.* (2000); Rohde *et al.* (1999, 2000)
	abi4 *sis5* *sun6* *sañ5*	ABA insensitivity in seeds; sugar- and salt-insensitive	APETELA2-like transcription factor	Finkelstein (1994); Finkelstein *et al.* (1998); Laby *et al.* (2000); Huijser *et al.* (2000); Quesada *et al.* (2000)
	abi5	ABA insensitivity in seeds	bZIP transcription factor	Finkelstein (1994); Finkelstein & Lynch (2000b)
	era1 *wiggum*	ABA hypersensitivity	β-subunit of farnesyl transferase	Cutler *et al.* (1996); Pei *et al.* (1998); Ziegelhoffer *et al.* (2000)
	era2/3	ABA hypersensitivity in seed		Cutler *et al.* (1996)
	gca1/2	Pleiotropic ABA insensitivity		Himmelbach *et al.* (1998)
	gca3/8	Insensitive to ABA inhibition of root growth		Himmelbach *et al.* (1998)
	axr2	Insensitive to ABA inhibition of root growth		Timpte *et al.* (1994)
	uvs66	Hypersensitivity to root growth inhibition by ABA		Albinsky *et al.* (1999)
	rdo1/2	Reduced seed dormancy		Léon-Kloosterziel *et al.* (1996b)
	fus3	Reduced seed dormancy	ABI3-like transcription factor?	Bäumlein *et al.* (1994); Keith *et al.* (1994); Luerssen *et al.* (1998)
	dag1	Seeds non-dormant; accelerated dark-germination	Zinc-finger transcription factor	Papi *et al.* (2000)
	lec1	Accelerated germination program	CCAAT-box binding transcription factor	Meinke *et al.* (1994); Lotan *et al.* (1998)
	det1	Accelerated germination and plastid differentiation	Nuclear-localized protein	Pepper *et al.* (1994); Rohde *et al.* (2000)
	prl1	ABA hypersensitivity	WD-40 domain protein; interacts with SNF1-like protein kinase	Németh *et al.* (1998); Bhalerao *et al.* (1999)
	bri1	Hypersensitivity to ABA inhibition of root growth	Leucine-rich repeat receptor kinase-like	Clouse *et al.* (1996); Li & Chory (1997)
	sax1	Hypersensitive to ABA inhibition of root growth		Ephritikhine *et al.* (1999)
	jar1	Hypersensitivity to ABA inhibition of germination		Staswick *et al.* (1992)
	jin4	Hypersensitivity to ABA inhibition of germination		Berger *et al.* (1996)
	sañ1/4	Resistant to salt inhibition of germination		Quesada *et al.* (2000)

	rss1	Resistant to salt inhibition of germination		Werner & Finkelstein (1995)
	rs17/19/20	Resistant to salt inhibition of germination		Saleki *et al.* (1993)
	sfr6	Impaired expression of ABA-inducible marker genes		Knight *et al.* (1999)
	los1	Impaired expression of ABA-inducible marker genes		Ishitani *et al.* (1997); Xiong *et al.* (1999b)
	hos1/2/5	Over-expression of ABA-inducible marker genes		Ishitani *et al.* (1997, 1998); Lee *et al.* (1999); Xiong *et al.* (1999a)
	ade1	Over-expression of ABA-inducible marker genes		Foster & Chua (1999)
Craterostigma plantagineum	*cdt-1*	Constitutive ABA response in callus	Regulatory RNA or small polypeptide	Furini *et al.* (1997)
Hordeum vulgare	*cool*	ABA insensitivity in guard cells		Raskin & Ladyman (1998)
Zea mays	*vp1*	ABA insensitivity in seeds	Transcriptional activator/repressor	Eyster (1931); McCarty *et al.* (1989, 1991); Hoecker *et al.* (1995, 1999)
	rea	ABA insensitivity in seeds		Sturaro *et al.* (1996)

CBF genes are normally expressed in *sfr6* mutants (Knight *et al.*, 1999). This result suggests that the *sfr6* lesion affects stress response pathways by potentiating pathway-specific transcription factors (Fig. 1).

Recently, genetic screens in transgenic *Arabidopsis* for altered expression of a 'bait' reporter gene (luciferase) driven by ABA-inducible and stress-inducible promoters (*KIN2* and *RD29A*) have been performed with great success (Ishitani *et al.*, 1997; Foster & Chua, 1999). The complexity of overlapping osmotic (cold, salt and ABA) signalling pathways has been teased apart by the isolation of mutants affected in each of the pathways. Most of the mutants recovered, termed *hos* (*high osmotic stress* response), *los* (*low osmotic stress*), and *cos* (*constitutive osmotic stress*) lack specificity for any one stimulus, which suggests that the ABA, salt and cold response pathways interact before converging to activate gene expression (Ishitani *et al.*, 1997). Synergy between the pathways is also affected by temperature, and the *los1* mutant has a temperature-conditional phenotype (Xiong *et al.*, 1999b).

Mutants of *hos1* and *hos2* show super-induction of cold-responsive genes at higher temperatures than the wild type, suggesting that *HOS1* and *HOS2* are negative regulators of cold signal transduction (Ishitani *et al.*, 1998; Lee *et al.*, 1999) (Fig. 1). Gene expression in response to osmotic stress or ABA is not affected in the *hos1* and *hos2* mutants. Mutant *hos2* plants are less capable of developing freezing tolerance when treated with low nonfreezing temperatures, demonstrating the importance of *HOS2* in the acclimation of plants to cold (Lee *et al.*, 1999).

The expression of stress genes is enhanced by the *hos5* mutation (Xiong *et al.*, 1999a) and is specific to ABA and osmotic stress pathways; low temperature regulation is not altered in the mutant. Double-mutant analysis of *hos5* and the ABA-deficient *aba1* and ABA-insensitive *abi1* mutants indicates that the hypersensitivity of *hos5* to osmotic stress is independent of sensitivity to ABA. Synergy experiments with ABA and osmotic stress also support the conclusion that *hos5* hypersensitivity to osmotic stress is ABA-independent. Interestingly, seed sensitivity to ABA was increased in *hos5* mutants, but not the sensitivity of stomatal closure, proline accumulation or growth responses to ABA (Xiong *et al.*, 1999a). *HOS5* is therefore a negative regulator of osmotic stress-responsive gene expression shared by ABA-dependent and ABA-independent osmotic stress signalling pathways in a subset of tissues (Fig. 1).

Foster & Chua (1999) used the *KIN2* promoter in transgenic seedlings in their search for *trans*-acting mutations that alter *KIN2-LUC* reporter activity (*LUC* being the reporter gene for luciferase) in response to ABA. The *ade1* mutation (*ABA-deregulated gene expression*) has sustained and enhanced levels of both transgenic and endogenous ABA-responsive marker genes. Cold-inducible *KIN2* expression is normal, suggesting that *ADE1* is a specific negative regulator of ABA signalling (Foster & Chua, 1999). An intrinsic value of these gene expression screens is that they can identify mutations that otherwise have no visible phenotype, which might be true in redundant genetic systems such as ABA and stress signalling. Fig. 1 presents a montage of the genetic pathways affecting ABA-inducible gene expression that have been defined by mutation in *Arabidopsis*. Table 2 is a description of well documented mutants affecting ABA perception or physiology.

The great strides made by genetic analysis of ABA responses in *Arabidopsis* and maize represent only the first generations of clever screens. It is becoming clear that hormone responses in plants involve redundant negative regulators, such as the *ETHYLENE RESISTANT1*-related ethylene receptors (Hua & Meyerowitz, 1998) and putative GA transcription factors *GA-INSENSITIVE* and *REPRESSOR OF GA* (Silverstone *et al.*, 1998; Peng *et al.*, 1999). ABA response mutants described until now are a harbinger of exciting discoveries yet to come from more sophisticated genetic approaches such as enhancer, suppressor or conditional screens, tissue-specific screens, screens for altered expression of genes normally down-regulated by ABA (e.g. *Chl a-binding protein* (*CAB*), *light-harvesting Chl b-binding protein* (*LHCP*)) and physiological screens for 'cool' (excessive transpiration) mutants.

Despite the awesome power of genetics, it has limitations: in yeast only about half of all 6000 genes were identified by 40 yr of exhaustive genetic screens, and many genes are turning out to give no detectable phenotype when inactivated (Cherry *et al.*, 1997; Ross-Macdonald *et al.*, 1999). With ethylene, auxin and ABA signal transduction, breakthroughs came from the fortuitous isolation of dominant 'gain-of-function' alleles of *ETR1*, *AXR2* (Wilson *et al.*, 1990), *ABI1* and *ABI2* (Koornneef *et al.*, 1984). It is not understood how genetic redundancy such as that with genes encoding PP2C affects a pathway's function, or how different pathways might interact genetically. The retrofitting of regulatory pathways involving ABA, GA, auxins, sugars, BR and cell division is a testament to the power of genetics, but it also forces one to realize that the screens performed so far were somehow inadequate. The question of cross-talk between pathways, which is the hallmark of plant hormone action and growth plasticity, is only starting to be addressed. The emerging complexity of overlapping environmental responses and developmental programs in comparison with the small number of isolated mutants leaves no doubt that many more genes will be identified by carefully designed and executed genetic screens. The upshot for neophytes interested in contributing to this

'golden age' of gene discovery (Somerville & Meyerowitz, 1994) is that mutant phenotypes are often subtle, and a thorough understanding of plant physiology is necessary to interpret complex phenotypes.

III. 'REVERSE GENETIC' ANALYSIS OF ABA-REGULATED GENE EXPRESSION

The rubric 'reverse genetics' is jargon and might annoy readers, but it serves to make an important point on methodology. The English philosopher Francis Bacon (1561–1626) proposed 'induction' as the logic of scientific discovery and 'deduction' as the logic of argumentation. The process of scientific inquiry is a combination of inductive reasoning (from a particular observation to the general case) and deductive reasoning (from the general principle to the particular case). In this context, genetic analysis (characterization of phenotypic mutants) can be considered as primarily an inductive process: the mutants point to a *de facto* underlying process. Reverse genetics relies largely on deductive reasoning and is therefore couched in an argument that can be rejected a priori. Because ABA-regulated sequences of unknown function are operationally defined in the laboratory, it is assumed that their characterization will result in the deduction of fundamental processes. (Ironically, these genes are 'induced' by ABA.) Carl Sagan (1977) aptly summed up the limitations of deductive reasoning: 'absence of evidence is not evidence of absence.' In other words, when an experiment does not work, it does not necessarily mean that the hypothesis is wrong! Nevertheless, working backwards from ABA-regulated sequences by using established techniques and principles has proved extremely productive and is an important complementary approach to genetics.

1. *ABA-regulated gene products*

About 50% of plant genes are homologous to genes of known function (Somerville & Somerville, 1999). There are hundreds of reports in the literature of genes that are ABA-inducible (some are ABA-repressible), and naturally many of them have predicted structures that fuel speculation about their role in ABA physiology or signalling. It should be kept in mind that the accumulation of endogenous ABA during stress or development and the manipulation of cellular ABA levels by the application of ABA are not equivalent states: there are cases in which genes regulated by applied ABA are not markedly induced after stress treatments that elevate ABA levels (Cohen *et al.*, 1991; de Bruxelles *et al.*, 1996). Table 3 is a list of some recently described ABA-regulated genes that might be involved in stress physiology because they encode proteins associated with stress, such as water channels, chaperonins, key enzymes for osmolyte and cell wall biosynthesis, proteinases and detoxifying enzymes. There are similar numbers of novel genes whose incomprehensibility makes them more intriguing (Ingram & Bartels, 1996). Table 4 is a list of putative 'orphan' ABA-regulated genes that have predicted structures suggesting a role in signalling. It should be pointed out that the expression of many of these genes is also regulated by other stresses. It remains difficult, without integrated information about cellular and whole-plant responses, to resolve functions against the backdrop of multiple stress responses. In only a few cases have functional links been forged between an ABA-regulated gene product and ABA or stress signalling, and these are discussed in section III.3.

The advent of the genomics age and massively parallel profiling technologies such as gene chips and microarrays, which can quantify the expression of all genes represented in a complex mRNA sample, is revolutionizing the study of gene activity and function (Schena *et al.*, 1995; Somerville & Somerville, 1999). For example, temporal clusters of expressed genes could be identified in ABA-treated plants or in ABA response mutants and then assigned a hypothetical function on the basis of relational criteria for a complex response (e.g. metabolic pathways, supramolecular architecture and regulatory networks).

There is a more restsrictive definition of 'reverse genetics' that has evolved from the field of functional genomics: efficient screening by PCR of large collections of DNA insertion mutants for inactivation of any one of the approx. 25000 genes in plants (Winkler *et al.*, 1998; Tissier *et al.*, 1999). Function can then be ascribed to the inactivated gene by characterization of the mutant plant phenotype. Application of this technique to ABA-regulated (indeed all) genes and gene families (e.g. PP2Cs) will eventually provide a comprehensive understanding of gene function.

The *Arabidopsis* gene *DAG1* encodes a zinc-finger transcription factor that is involved in the control of seed germination, as demonstrated by the phenotype of mutants isolated by reverse genetics (Papi *et al.*, 2000). Seeds homozygous for the *dag1-1* knock-out allele, generated by a random T-DNA insertion, do not develop dormancy and also germinate in the absence of light. Segregation analysis indicates that the effect of the mutation is maternal. Accordingly, *in situ* mRNA hybridizations revealed the expression of *DAG1* in the vascular tissue of the flower and maturing fruit but not in the seed (Papi *et al.*, 2000).

A specialized application of this technique called 'activation tagging' (using a strong constitutive promoter next to the inserted T-DNA border to overexpress sequences flanking the insertion) has resulted in the cloning of a gene (*CDT-1*), the overexpression of which can transactivate ABA-inducible

Table 3. *Some recently described ABA-regulated genes that might function in stress physiology*

Genus	Hypothetical gene function	References
Abies	Metallothionein	Chatthai *et al.* (1997)
Amaranthus	Betaine aldehyde dehydrogenase	Legaria *et al.* (1998)
Arabidopsis	Alcohol dehydrogenase	de Bruxelles *et al.* (1996); Conley *et al.* (1999)
	Stelar K^+ outward rectifying channel*	Gaymard *et al.* (1998)
	Cytosolic O-acetylserine(thiol)lyase	Barroso *et al.* (1999)
	Polyubiquitin, endoxyloglucan transferase	Park *et al.* (1998)
	L-Isoaspartylmethyltransferase	Mudgett & Clarke (1996)
	δ(1)-Pyrroline-5-carboxylate synthetase 1	Yoshiba *et al.* (1999)
	Light-harvesting Chl *b*-binding protein (LHCP)*; Chl *a*-binding protein (CAB)*	Chang & Walling (1991); Capel *et al.* (1998)
	Type 5 acid phosphatase	Del Pozo *et al.* (1999)
Brassica	3-Ketoacyl-coenzyme A synthase	Qi *et al.* (1998)
Helianthus	Early light-induced protein	Ouvrard *et al.* (1996)
	Heat shock proteins	Coca *et al.* (1996)
Hordeum	Nuclease I	Muramoto *et al.* (1999)
	α-Amylase/subtilisin inhibitor	Liu & Hill (1995)
	Aldose reductase	Roncarati *et al.* (1995)
	α-Amylase*, Cys proteinase*	Cercós *et al.* (1999)
Lilium	Pathogenesis-related PR-10 protein	C. S. Wang *et al.* (1999)
Lupinus	Protochlorophyllide-oxidoreductase*	Kusnetsov *et al.* (1998)
Lycopersicon	Leu aminopeptidase	Chao *et al.* (1999)
	Ripening-related (ASR) protein	Rossi *et al.* (1998)
Mesembryanthemum	Enolase	Forsthoefel *et al.* (1995)
Nicotiana	Osmotin, endo-β-1,3-glucanase?*	Grenier *et al.* (1999); Raghothama *et al.* (1997); Rezzonico *et al.* (1998)
	Isocitrate lyase*	Bustos *et al.* (1998)
Oryza	Lipid transfer protein	Garcia-Garrido *et al.* (1998); Trevino & O'Connell (1998)
	Tubulin*	Giani *et al.* (1998)
	Pyruvate orthophosphate dikinase	Moons *et al.* (1998)
	Glutathione reductase	Kaminaka *et al.* (1998)
Phaseolus	Lipoxygenase	Porta *et al.* (1999)
Physcomitrella	Selenium-binding protein	Machuka *et al.* (1999)
Solanum	Fibrillin, chromoplast protein C	Gillet *et al.* (1998)
	Metallocarboxypeptidase inhibitor	Villanueva *et al.* (1998)
Spirodela	Anionic peroxidase	Chaloupkova & Smart (1994)
Zea	Cytosolic copper/zinc-superoxide dismutase	Guan & Scandalios (1998); Machuka *et al.* (1999); Kaminaka *et al.* (2000)
	Ribulose-bisphosphate carboxylase*	Sheen (1998)
	Ferritin	Fobis-Loisy *et al.* (1995)

*Down-regulation by ABA.

genes and confer drought tolerance on callus of the resurrection plant *Craterostigma* (Furini *et al.*, 1997). The *CDT-1* gene resembles in several features SINE retrotransposons (noncoding Short Interspersed Nuclear Elements, widespread in most eukaryote genomes) and might activate ABA signalling via a regulatory RNA or via a short polypeptide. This intriguing discovery of a novel signalling mechanism raises many fundamental questions; future structure-function studies with transgenic *CDT-1* mutants might provide clues to its role in ABA responses.

2. Cis-*acting elements*

The gene for bacterial *uidA* (β-glucuronidase (GUS)) has been spectacularly successful as a sensitive plant reporter gene for structure-function analysis of *cis*-acting sequences in a wide range of response systems, including ABA-inducible transcription. The transient transformation experiments that have defined promoter sequences that are necessary and sufficient for ABA-inducible transcription have recently been reviewed (Ingram & Bartels, 1996; Shen & Ho, 1997; Busk & Pagès,

Table 4. *Some ABA-regulated genes that might function in signalling*

Genus	Hypothetical gene function	References
Arabidopsis	GSK3/shaggy-like protein kinase	Piao *et al.* (1999)
	Receptor-like protein kinase	Hong *et al.* (1997)
	Ribosomal S6 kinase-like; MAPK kinases	Mizoguchi *et al.* (1996)
	Phospholipase C	Hirayama *et al.* (1995)
	His kinase osmosensor	Urao *et al.* (1999)
	Cyclin-dependent kinase (cdc2A)*	Hemerly *et al.* (1993)
	Cyclin-dependent protein kinase inhibitor (*ICK1*)	Wang *et al.* (1998)
	Root-specific Ser/Thr kinase	Hwang & Goodman (1995)
	Membrane intrinsic channel proteins (MIPs) from various spp.	Kaldenhoff *et al.* (1993); Malz & Sauter (1999); Gao *et al.* (1999); Pih *et al.* (1999)
	A cluster of genes on chromosome 2	M. L. Wang *et al.* (1999)
	Nematode feeding structure transcription factor	Puzio *et al.* (1999)
	Phosphatidylinositol-4-phosphate 5-kinase	Mikami *et al.* (1998)
	G-box factor 3	Lu *et al.* (1996)
	ABRE-binding factors (*ABF1–4*)	Choi *et al.* (2000)
Craterostigma	Homeodomain-Leu zipper transcription factors	Söderman *et al.* (1999); Lee & Chun (1998); Frank *et al.* (1998)
	Phospholipase D-2	Frank *et al.* (2000)
	Myb-like transcription factors	Iturriaga *et al.* (1996); Kirik *et al.* (1998)
Fagus	Gly-rich RNA-binding protein	Nicolas *et al.* (1997)
	GTP-binding proteins	Nicolas *et al.* (1998); O'Mahony & Oliver (1999)
Hordeum	Binds to anti-anti-ABA antibodies	Liu *et al.* (1999)
Lycopersicon	Histone H1	Wei & O'Connell (1996)
Medicago	Annexin-like protein	Kovacs *et al.* (1998)
Mesembryanthemum	Tonoplast H^+-translocating ATPase	Barkla *et al.* (1999)
Nicotiana	Ser/Thr protein kinases	Yoon *et al.* (1999)
	Syntaxin (Nt-SYR1)	Leyman *et al.* (1999)
Oryza	Ca^{2+}-binding EF-hand protein	Frandsen *et al.* (1996)
	Basic Leu zipper transcription factor	Nakagawa *et al.* (1996)
Ricinus	Phospholipase Dα	Xu *et al.* (1997)
Spirodela	D-*myo*-Inositol-3-phosphate synthase	Smart & Fleming (1993)
	ATP-binding cassette transporter	Smart & Fleming (1996)
Triticum	Plasma membrane protein	Koike *et al.* (1997)
	Prolyl isomerase FK506-binding protein (cyclophilin)	Godoy *et al.* (2000); Kurek *et al.* (2000)
Zea mays	Basic Leu zipper transcription factor	Kusano *et al.* (1995)

*Down-regulation by ABA.

1998; Leung & Giraudat, 1998; Singh, 1998), and the reader is referred to those articles for details. Table 5 shows a consolidated list of ABA-regulated *cis*-elements. An important consideration that limits the interpretation of such transient assay results is the extent to which different hormonal, developmental, environmental and tissue-specific response pathways or factors can also contribute to the expression of ABA-responsive promoters. For example, transient gene expression is an artificial system: the quantities of input DNA and output transcription/translation might alter the kinetics of regulatory processes sensitive to small changes in substrate concentrations. Results should be validated by independent criteria that indicate that the experimental system reflects the state *in planta*.

Promoters containing ACGT-containing 'G-boxes' can bind bZIP transcription factors and have been shown to function as ABA-responsive elements (ABREs); however, many G-box-containing promoters are not ABA-regulated (Menkens *et al.*, 1995; Schwechheimer *et al.*, 1998). Single copies of ABREs require a *cis*-acting coupling element to achieve ABA induction (Shen & Ho, 1997; Busk & Pagès, 1998; Singh, 1998). Recently, Hobo *et al.* (1999a) have shown that the CE3 sequence that was

Table 5. Cis-*acting promoter elements functionally defined in the regulation of ABA-regulated and stress-regulated genes*

Gene	Element	Sequence†	Binding factors	Reference
Em	Em1a	AC<u>ACGT</u>GGC	BZIPs,	Busk & Pagès (1997); Busk *et al.*
Em, *Dc3*	Em1b	AC<u>ACGT</u>GCC	TRAB1,	(1999); Chern *et al.* (1996); Choi
OsEm	Motif A	GT<u>ACGT</u>GTC	ABFs?,	*et al.* (2000); de Bruxelles *et al.*
Rab16	Motif I	GT<u>ACGT</u>GGC	DPBFs?,	(1996); Ezcurra *et al.* (1999);
Adh	G-box-1	CC<u>ACGT</u>GGA	ABI5	Guiltinan *et al.* (1990); Hattori
HVA1	ABRE2	CT<u>ACGT</u>GGC		*et al.* (1995); Hobo *et al.*
Rab28	ABRE A,B	Y<u>ACGT</u>GGC		(1999a); Kim *et al.* (1997); Kim
HVA22	ABRE3	CC<u>ACGT</u>ACA		& Thomas (1998); Lam & Chua
AR-H	(−57)	CC<u>ACGT</u>ACT		(1991); Marcotte *et al.* (1988);
DLEC2, *PHS*β	sites A, D	C<u>ACGT</u>CA		Ono *et al.* (1996); Roncarati *et*
OsEm, *HVA1*	CE3	ACG<u>CGT</u>GTCCTC		*al.* (1995); Shen & Ho (1997);
Rab28		ACG<u>CG</u>CCTCCTC		Skriver *et al.* (1991); Su *et al.*
Rab16B	Motif III	GCCG<u>CGT</u>GGC		(1998); Vasil *et al.* (1995);
(synthetic)	Hex3	GACG<u>CGT</u>GGC		Weatherwax *et al.* (1998)
NPR1	LS5	CGACG<u>CGT</u>GTC		
napA	distB	GCC<u>ACTTG</u>TC	MYC?	
rd22	MYC	AC<u>AC</u>A<u>T</u>GT	MYC rd22BP1 TRAB1?	Abe *et al.* (1997)
rd22	MYB	YAAC(G/T)G	AtMYB2	Abe *et al.* (1997)
HVA22	CE1	TGCCACCGG	?	Shen & Ho (1997)
Rab16A	Motif IIa	CCGCCGCGCCTG	?	Mundy *et al.* (1990)
	Motif IIb	CCGCCGCGCTG		
C1	Sph/RY	CGTGTCGTC*CATGCATG*	VP1	Bobb *et al.* (1997); Hill *et al.*
Em		*GCATGCATG*C		(1996); Hattori *et al.* (1992,
DLEC2		G/C*CATG*CNNG/C	PvALF	1995); Kao *et al.* (1996)
CdeT27-45		AAGCCCAAATTTCACA GCCCGATAACCG	?	Nelson *et al.* (1994)
Rab17	GRA	CACTGGCCGCCC	?	Busk *et al.* (1997); Busk & Pagès
Rab28		CATGCCGCC		(1997)
COR,*rd22*	DRE	TA**CCGAC**AT	DREB	Thomashow (1999); Liu *et al.*
Rab17	DRE1	AA**CCGAG**A	DREB?	(1998); Busk *et al.* (1997)
Rab17	DRE2	CA**CCGAC**GC	DREB?	
α-*amylase*	GA-response*	GGCCGATAACAAACTC	?	Skriver *et al.* (1991)
Amy1/6-4		CGGCC		

*Down-regulated by ABA.
†The invariant <u>ACGT</u> core in G-box-like ABREs and <u>CG</u> cores of the functionally equivalent CE3-like elements are underlined; the SphI *CATG* core is italicized; the DRE-like **CCGAC** element is in bold.

originally identified in the barley *HVA1* promoter (Shen & Ho, 1997) and was found approx. 30 base pairs (bp) downstream of motif A (a ACGT-containing ABRE) in the *OsEm* promoter are functionally equivalent. Linker scan analyses of a 55-bp *OsEm* promoter fragment revealed that both motif A and the CE3 sequence are required not only for responsiveness to ABA but also for trans-activation by overexpressed *Vp1* in transient assays. Because the sequences of motif A and CE3 are similar, motif-exchange experiments were performed and the results show that motif A and CE3 are interchangeable with each other with respect to both ABA and *Vp1* regulation. In addition, both sequences are bound by a VP1-interacting, ABA-responsive bZIP factor, TRAB1 (Hobo *et al.*, 1999a,b) (see section IV.2b). Furthermore, TRAB1 binds to two other non-ACGT ABREs, Motif III and Hex3 (Hobo *et al.*, 1999a), which are strongly conserved second 'half sites' of the G-box palindrome GCCA<u>CGTGGC</u> (the 'half site' is underlined). These results indicate that ACGT-containing ABREs, non-ACGT ABREs, and CE3 are functionally equivalent *cis*-acting elements of essentially the same nature, and the grouping of ABREs and CE3-like elements from numerous genes in Table 5 reflects this concept.

As already mentioned, *cis*-acting elements defined by a reductionist approach should be validated in the context of the whole plant. Chak *et al.* (2000) have exploited a well characterized ABA-inducible and drought-inducible promoter from the carrot *LEA Dc3* gene (Seffens *et al.*, 1990; Vivekananda *et al.*, 1992; Siddiqui *et al.*, 1998) in transgenic *Arabidopsis* to study gene expression inducible by ABA and drought. One immediate question that arises about such a heterologous system is: can the *Dc3* promoter be properly integrated into the underlying ABA and stress signalling pathways of *Arabidopsis*? Circumstantial evidence in support of this hypothesis is that

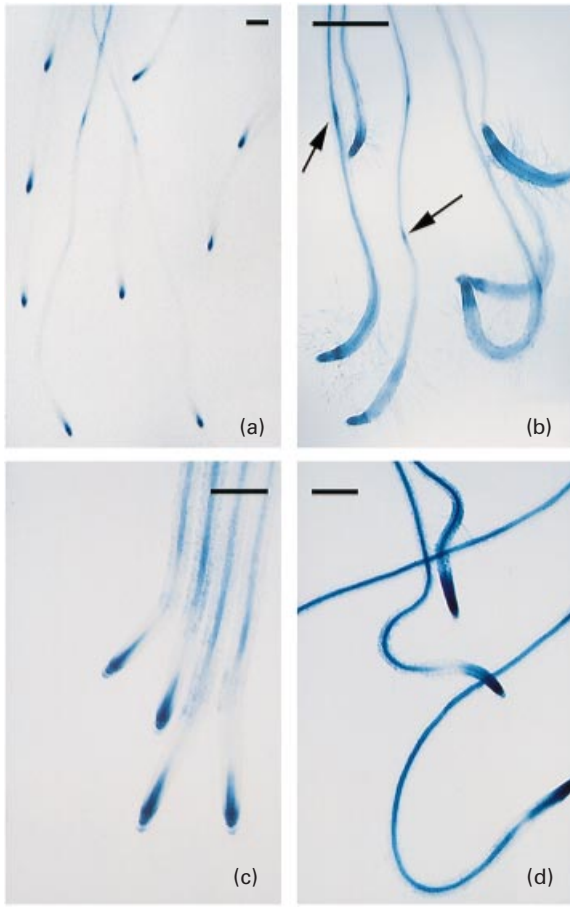

Fig. 3. Tissue-specific expression of *Dc3-GUS* in 6-d-old transgenic *Arabidopsis* roots in response to 24 h of treatment with water (a), 15% (m/v) mannitol (b), 100 μM ABA (c) or 100 μM ABA plus 15% (m/v) mannitol treatments (d), revealed by 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-gluc) staining for 18 h. Bars, 500 μm. Arrows in (b) point to lateral root primordia.

a bZIP (DPBF1) from sunflower binds the *Dc3* promoter (Kim *et al*., 1997), and its orthologous gene from *Arabidopsis* (*AtDPBF1*) is identical to *ABI5* (T. Thomas, pers. comm.). To address this question, Chak *et al*. (2000) analysed ABA-inducible and mannitol-inducible expression of *Dc3–GUS* in transgenic wild-type, ABA-deficient *aba1* and ABA-insensitive *abi1* and *abi2* mutant leaves. (Mannitol is a nonpenetrating solute that mimics drought stress.) The *Dc3* promoter directs ABA-inducible and mannitol-inducible *GUS* expression in *Arabidopsis* guard cells and the two treatments are additive. The *aba1*, *abi1* and *abi2* mutant genotypes have lower *GUS* expression in guard cells of cotyledons in response to mannitol, whereas *abi1* and *abi2* mutants are lower in ABA-inducible *GUS* expression, which is consistent with known overlapping pathways responsive to ABA and drought. Quantitative fluorimetric *GUS* assays showed that *abi2* mutants respond less to exogenous ABA than *abi1* mutants, and *abi2* mutants respond more to mannitol than *abi1* mutants. On the basis of these results, one can argue that *Dc3–GUS* transgenic *Arabidopsis* is a tractable system in which to study tissue-specific ABA and drought signalling, especially in guard cells, which are the best-characterized single cell system (see section IV).

Further analysis of tissue-specific expression of *Dc3–GUS* transgenic *Arabidopsis* plants presented here provides evidence that 'separate but overlapping' ABA and stress response pathways might be due in part to differential tissue-specific gene expression in response to separate stresses. Fig. 3 shows the root-specific expression of *GUS* activity in 4–6-d-old transgenic *Dc3–GUS Arabidopsis* in response to various treatments. In the absence of ABA or mannitol induction treatments, the primary and lateral root meristems exhibited constitutive *GUS* expression (Fig. 3a; note the staining of lateral root primordia in Fig. 3b). In response to treatment with 100 μM ABA, primary roots swelled at the distal end of the zone of differentiation (Fig. 3b), presumably owing to the inhibitory effects of ABA on root cell growth (Leung *et al*., 1994; Himmelbach *et al*., 1998). There was also moderate induction of *GUS* expression in the cortex and trichoblast (root hair) cells of the distal zone of differentiation and in the vascular tissue of the root (Fig. 3b). It is interesting to note that there is another stelar-specific ABA-regulated gene *SKOR* (for 'stelar K^+ outward rectifier'), which is involved in ABA-regulated long-distance K^+ transport (Gaymard *et al*., 1998). Thus the vascular tissue is particularly sensitive to ABA, and *Dc3–GUS* expression is correlated with this sensitivity. In contrast with ABA, treatment with 15% (m/v) mannitol did not result in root swelling or *GUS* expression in the cortex or trichoblast cells; however, there was moderate induction of *GUS* in the root vasculature (Fig. 3c), analogous to that caused by ABA treatments. When seedlings were treated with ABA plus mannitol, an additive effect on *GUS* expression in the vascular tissue and distal root zone of elongation was observed (Fig. 3d). These differential tissue-specific expression patterns in response to ABA compared with mannitol suggest a plausible mechanism that can partly account for additive ABA and desiccation response pathways observed in whole plants. Work in my laboratory is currently focused on the characterization of tissue-specific effects of the *abi1* and *abi2* mutations on overlapping stress pathways affecting *Dc3–GUS* expression, as well as the characterization of mutants (e.g. *harlequin*, *hlq*; *short blue root*, *sbr*) identified in a mutant screen for the ectopic expression of *Dc3–GUS* in roots (B. Rajagopal *et al*., unpublished).

3. Trans-*acting factors*

The first identified ABRE-binding proteins, *Em*-binding protein (EmBP-1) and activating transcrip-

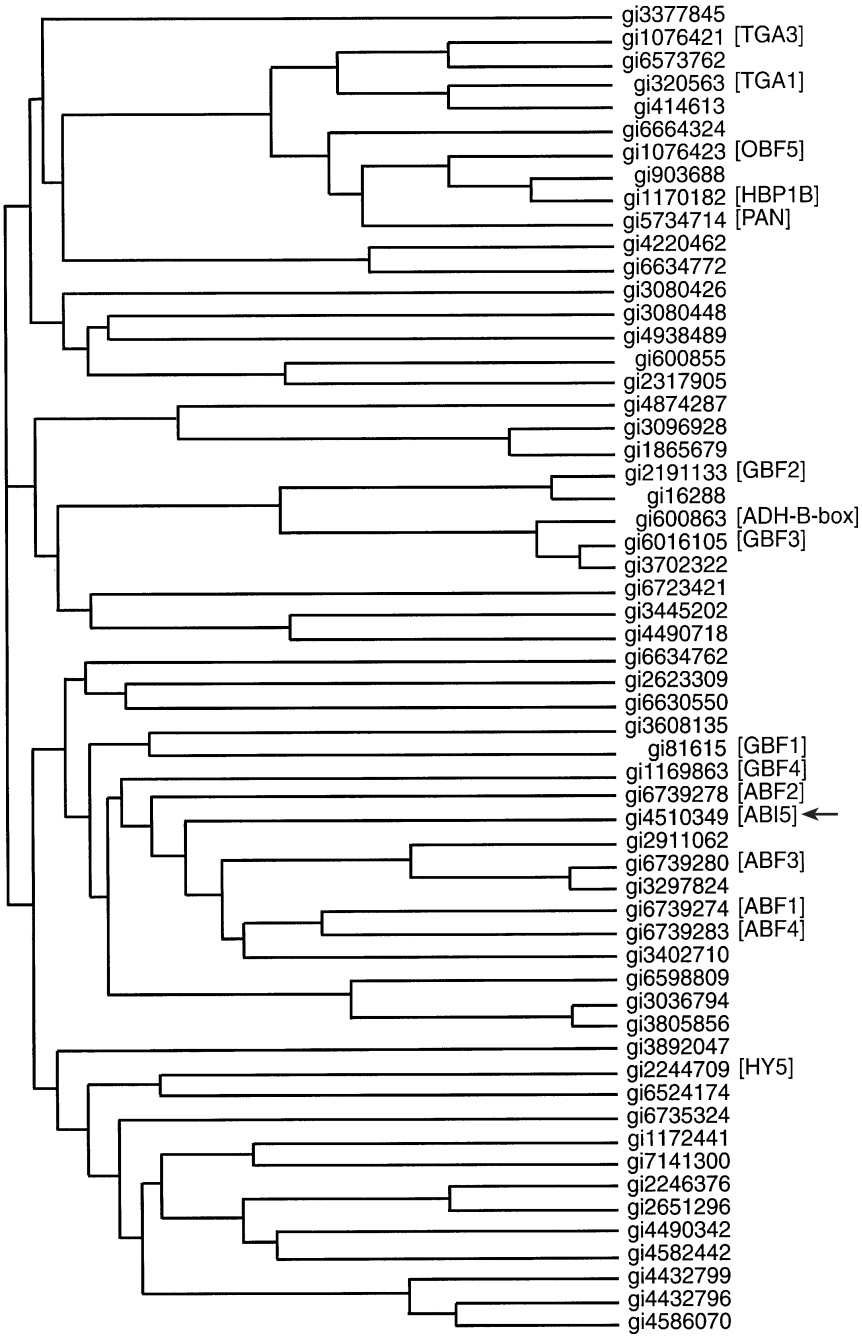

Fig. 4. Phylogenetic analysis of 58 *Arabidopsis* bZIP factors. Sequences were obtained by searching GenBank (http://www.ncbi.nlm.nih.gov/) for the string 'bZIP' and performing a BLAST search with the ABI5 (arrowed) protein sequence as the query. A tree was calculated from full-length amino acid sequences aligned by ClustalX (Thompson *et al.*, 1997). Sequences are represented as GenBank ID number and gene symbol, if known.

tion factor (ATF-1), were cloned by probing cDNA expression libraries with labelled ABREs (Guiltinan *et al.*, 1990; Oeda *et al.*, 1991). These genes are homologous to a large family of dimeric bZIP transcription factors, and other members (G-box factor, *GBF3*; *OSBZ8*) have been cloned by homology and degenerate oligonucleotide probes (Lu *et al.*, 1996; Nakagawa *et al.*, 1996). Nantel & Quatrano (1996) probed a rice cDNA expression library with labelled EmBP-1 protein and isolated three bZIPs (osZIP1a, 2a and 2b) that can form heterodimers with EmBP-1 and each other, suggesting a mechanism for both positive and negative regulation of gene expression. Indeed, a bZIP protein from french bean that binds G-box elements (regulator of *MAT2,* ROM2) accumulates during seed maturation when transcription of the seed storage proteins *DLEC2* and *PHS*β declines (Chern *et al.*, 1996). When ROM2 is co-expressed in transient assays it can antagonize transactivation of the ABA-inducible *DLEC2* and *PHS*β promoters by PvALF (Chern *et al.*, 1996). Several bZIPs have been cloned from

sunflower and *Arabidopsis* (ABRE binding factors, ABFs; *Dc3*-promoter-binding factors, DPBFs) with ABREs or the *Dc3* promoter as 'baits' in yeast one-hybrid screens (Kim *et al.*, 1997; Kim & Thomas, 1998; Choi *et al.*, 2000). These bZIPs are structurally more related to the *ABI5* gene than any characterized previously (Fig. 4), suggesting that functional redundancy in ABA signalling also applies to bZIPs. Two uncharacterized bZIPs (gi:3402710 and gi:3297824) are highly homologous to ABI5 (Fig. 4). Reverse genetic studies of these family members to generate bZIP gene knock-outs and ectopic bZIP 'overexpression' lines, and characterization of tissue-specific marker and bZIP gene expression patterns, should help to clarify the functions of these gene family members.

Protein synthesis *de novo* is required for some, but not all, ABA-inducible gene expression (Nakagawa *et al.*, 1996; Abe *et al.*, 1997), suggesting that some *trans*-acting ABA factors must be induced, possibly by ABA. Expression of some of the ABRE-binding bZIPs is indeed induced by ABA (Lu *et al.*, 1996; Kusano *et al.*, 1995; Nakagawa *et al.*, 1996). Although some slight *trans*-activation of ABA-inducible reporter genes by transient overexpression of bZIP factors has been reported (Oeda *et al.*, 1991; Izawa *et al.*, 1994; Nantel & Quatrano, 1996), none of these proteins has been demonstrated conclusively to function as transcription factors in ABA-responsive gene expression. Recently, two true functional bZIP genes involved in ABA-inducible expression have been cloned: *TRAB1* from a yeast two-hybrid screen with VP1 as bait (Hobo *et al.*, 1999b) (see section IV.2b), and *ABI5* by map-based cloning of an ABA-insensitive mutant (see section II.1b).

The genetic and functional relationships between *COR* and *LEA* gene expression and freezing and drought responses have recently been elucidated with the cloning of genes for *APETELA2*-domain-like transcription factors (cold binding factor (*CBF*) and dehydration-responsive-element binding factor (*DREB*)) (Jaglo-Ottosen *et al.*, 1998; Liu *et al.*, 1998; Medina *et al.*, 1999) that transactivate cold-inducible and drought-inducible promoters. The *cis*-acting core target sequence CCGAC, designated the C-repeat/dehydration-responsive element (DRE) (Table 5), is found in the promoters of *COR* genes and imparts cold-regulated and dehydration-regulated gene expression through overlapping ABA-independent pathways (Shinozaki & Yamaguchi-Shinozaki, 1997; Thomashow, 1999) (Fig. 1). The *DREB2A* transcription factor has a conserved Ser/Thr-rich region adjacent to the DNA binding domain that might be a target for protein kinases (Liu *et al.*, 1998). It is possible that protein kinases and/or phosphatases, such as ABI1 and ABI2, might act in environmental or cellular response modules that include targets such as DREBs, ABI3/Vp1, ABI4 and ABI5. Consistent with this notion is the fact that *DREB*, *ABI1*, *ABI2* and *ABI5* expression are induced by ABA and dehydration (Leung *et al.*, 1997; Liu *et al.*, 1998; Finkelstein & Lynch, 2000b). Differential cellular localization and/or substrate specificities of the *ABI1* and *ABI2* phosphatases or other factors might integrate environmental (such as drought) and cellular (such as ABA) signals, respectively. Although the mechanisms of tolerance to cold and drought are not known, transgenic plants that overexpress CBFs and DREBs have altered endogenous gene expression patterns and are tolerant to desiccation and cold (Thomashow, 1999; Liu *et al.*, 1998). This exciting practical advance sets the stage for agronomic applications that could increase yields under suboptimal environmental conditions.

Sheen (1996) took a bold reverse genetics approach to address the role of protein kinases in ABA signalling: given the limited availability of eight sequenced protein kinases from the literature, she systematically overexpressed each one in maize protoplasts and assayed their ability to affect ABA-inducible gene expression. The constitutively active mutants of two related Ca^{2+}-dependent protein kinases (CDPK1 and CDPK1a, which are drought-inducible and salt-inducible (Urao *et al.*, 1994)) *trans*-activated the *HVA1* promoter, bypassing stress signals. The other six plant protein kinases, including two distinct CDPKs, failed to mimic this stress signalling (Sheen, 1996). Coexpression of PP2Cs, including *abi1*, that are capable of blocking responses to ABA partly blocked the CDPK *trans*-activation of gene expression, suggesting that PP2Cs act downstream of CDPK in an ABA signalling pathway and that stresses other than ABA also transduce signals through CDPK (Sheen, 1996, 1998). A variety of cellular functions are activated by CDPKs, for example the activation of Cl^- channels in the tonoplast of guard cells involved in stomatal closure (Pei *et al.*, 1996), which is a contradictory activity to ABA-inducible gene expression. CDPKs might therefore be positive and negative regulators controlling stress signal transduction in plants.

The GA response element (Table 5) from the barley α-amylase gene *Amy1/6–4* is repressed by ABA (Skriver *et al.*, 1991), as is the Cys proteinase gene *EPB-1*, presumably through the GA-responsive elements (Cercós *et al.*, 1999). The constitutive transient expression in barley aleurone of a unique Ser/Thr protein kinase, *PKABA1*, which is up-regulated by ABA in seeds, drastically suppressed the expression of α-amylase and protease genes induced by GA (Gómez-Cadenas *et al.*, 1999). However, the presence of PKABA1 had only a small effect on the induction of the *HVA1* promoter by ABA. These results indicate that PKABA1 acts as a key intermediate in the ABA signal transduction pathway leading to the suppression of GA-inducible gene expression in cereal aleurone layers (Gómez-

Cadenas *et al*., 1999) (Fig. 1). It will be interesting to learn whether the *PKABA1* gene product interacts with the PP2C-dependent ABA response pathway, because Sheen (1998) showed that PP2C can antagonize the repression of the (*CAB*) gene by ABA.

In *Arabidopsis*, the induction of a dehydration-responsive gene, *rd22*, is mediated by ABA (Abe *et al*., 1997). A 67-bp DNA fragment of the *rd22* promoter is sufficient for dehydration-induced and ABA-induced gene expression, and two recognition sites for the basic helix-loop-helix protein MYC and one recognition site for MYB *Chl a*-binding protein can function as *cis*-acting elements in the dehydration-induced expression of the *rd22* gene in transgenic tobacco (Table 5). A cDNA encoding a MYC-related DNA-binding protein, *rd22BP1*, was isolated by screening an expression library with the 67-bp region (Abe *et al*., 1997). RNA gel blot analysis revealed that dehydration stress and treatment with ABA induce the transcription of *rd22BP1*, and its induction precedes that of *rd22* (Abe *et al*., 1997). Furthermore, overexpression in transient transactivation experiments demonstrated that both the rd22BP1 and ATMYB2 proteins activate (*c*. twofold each) transcription of a tandem hexamer of the 67-bp element. These results suggest that MYC and MYB proteins might be involved in expression of the *rd22* gene (Abe *et al*., 1997). In this context, it is interesting to note that the ABA-inducible *Em* and *rab28* promoters are efficiently and accurately transcribed in the well characterized cell-free transcription system prepared from human HeLa nuclei (Razik & Quatrano, 1997). USF, a MYC transcription factor in HeLa nuclear extracts, activates transcription of the *Em* promoter by binding to the Em1b *cis*-ABRE element (Razik & Quatrano, 1997). It is unclear whether the functional equivalences of MYC and EmBP-1 proteins *in vitro* and the ABREs and CE3 elements *in vivo* and *in vitro* (Hobo *et al*., 1999a) are related. However, because the *rd22* MYC element has similarities to CE3 and ABREs (Table 5), it is speculated that TRAB1 or other bZIP factors might bind to the *rd22* MYC site. Alternatively, MYC factors might bind CE3-like elements.

ABA has been implicated in the post-transcriptional and translational regulation of gene expression. The steady-state levels of bifunctional α-amylase/subtilisin inhibitor (*BASI*) mRNA and protein are increased by applied ABA, but nuclear run-on assays demonstrate that ABA has no effect on *BASI* transcriptional activity (Liu & Hill, 1995). *BASI* mRNA is not detectable in barley embryos treated with the protein synthesis inhibitor cycloheximide, which has no inhibitory effect on the transcription rate of *BASI*, suggesting that ABA increases the stability of *BASI* mRNA through the synthesis of a short-lived protein that protects the message (Liu & Hill, 1995). Gillet *et al*. (1998) showed, by Northern and Western analyses of *Solanum* plants, that both transcript and protein of chloroplastic drought-induced stress protein (CDSP34) accumulated from early stages of water deficit and in response to ABA. In water-stressed ABA-deficient *flacca* mutants of tomato, similar increases in the *CDSP34*-related transcript amount were observed in comparison with the wild type, but protein accumulation was decreased in *flacca*, suggesting a post-transcriptional role for ABA in CDSP34 synthesis. In this context it is interesting to note the ABA-inducible expression of two ribosomal S6 kinase genes of *Arabidopsis* (Mizoguchi *et al*., 1996). Ribosomal S6 kinases have a key role in the regulation of cell growth by controlling the biosynthesis of translational components (Dufner & Thomas, 1999).

IV. BIOCHEMICAL AND CELLULAR ANALYSES OF ABA SIGNALLING

Single-cell studies offer the technical advantage of a simple, integrated system for the systematic testing of hypotheses about ABA signalling. Cell types that have received considerable attention are the guard cells, and protoplasts of embryo-derived callus or aleurone. Guard cells are one of the best-characterized plant cells and an excellent experimental system for the study of rapid ABA signalling phenomena such as intracellular and intercellular ion fluxes and secondary messengers involved in stomatal movements. This is because they have no plasmodesmata (Ding *et al*., 1997) and their cytoplasm is therefore isolated, and electrophysiology is feasible both *in planta* and with isolated protoplasts. Because ABA effects on guard cells are seen on the order of seconds, it is generally believed that changes in gene expression are not required and that separate ABA signal transduction cascades control stomatal movements and gene expression. However, Parcy & Giraudat's (1997) demonstration that ectopic expression of the *ABI3* transcription factor overcomes *abi1*-mediated defects in stomatal closure and induces seed-specific ABA pathways raises the possibility that ABA signalling pathway(s) are conservative. Several recent reviews are available on the role of ion channels as terminal effectors of ABA signalling in guard cells (Hetherington *et al*., 1998; MacRobbie, 1998; Assmann & Shimazaki, 1999), and readers are referred to them for details.

Because the regulation of guard cell ion channels probably shares at least some conserved mechanisms with the regulation of gene expression by ABA (see later), an attempt is made to integrate all known pathways into a hypothetical cell (which of course does not exist). Other single-cell systems for studying gene expression, such as the microinjection of sub-epidermal cells and the transient transformation of ABA-responsive mesophyll protoplasts (see below), have also led to advances in understanding

ABA-regulated gene expression and are integrated into the model (Fig. 5). It is not known to what extent there exist species-specific or tissue-specific differences in ABA signalling, so such a model is speculative at best. The emerging theme is that it is likely that there are multiple pathways of ABA signalling, with some elements that are analogous to animal paradigms (such as linear cascades) and others that are unique to plants (such as nonlinear cascades).

1. *Secondary messengers*

ABA induces the rapid depolarization of the plasma membrane potential, which in turn triggers a massive redistribution of ions and solutes from the tonoplast and cytoplasm to the apoplast, resulting in stomatal closure by loss of turgor and cell volume. ABA also inhibits stomatal opening. Cytosolic free [Ca^{2+}] and pH are thought to be intracellular second messengers of these responses (Irving *et al.*, 1992). ABA activates the vacuolar H^+ ATPase (Barkla *et al.*, 1999). Influx of Ca^{2+} across the plasma membrane occurs through ABA-activated channels (Schroeder & Hagiwara, 1990; Hamilton *et al.*, 2000; J. Schroeder, pers. comm.). ABA affects membrane potential in barley aleurone protoplasts (Heimovaara-Dijkstra *et al.*, 1994). Membrane voltage oscillations also initiate [Ca^{2+}] waves and potentiate ABA-activated [Ca^{2+}] increases (Grabov & Blatt, 1998). There is circumstantial evidence for the involvement of inositol 1,4,5-trisphosphate (IP_3) and cyclic ADP-ribose (cADPR) in ABA-regulated intracellular Ca^{2+} release from the tonoplast or other intracellular stores (Blatt *et al.*, 1990; Gilroy *et al.*, 1990; Allen *et al.*, 1995). Ca^{2+} has opposite effects on the inward-rectifying (Ca^{2+} inhibits) and outward-rectifying (Ca^{2+} activates) K^+ channels that contribute to stomatal opening and closing, respectively. Ca^{2+} regulation of the outward K^+ channel is indirect: the channel is voltage-gated and is thus activated by membrane depolarization, caused largely by the activity of an ABA-activated and Ca^{2+}-activated outward-rectifying slow (S) anion channel that extrudes Cl^- and malate (Grabov *et al.*, 1997; Pei *et al.*, 1997, 1998). The S-anion channel is probably a cystic-fibrosis-transmembrane-regulator-like ATP binding cassette protein (Leonhardt *et al.*, 1999) whose expression is up-regulated by ABA in *Spirodela* (Smart & Fleming, 1996). Other channels in the plasma membrane and tonoplast of guard cells as well as other tissues (such as the stele) (Gaymard *et al.*, 1998; Roberts, 1998) are also likely to be effectors involved in ABA-regulated physiology (Hetherington *et al.*, 1998; MacRobbie, 1998; Assmann & Shimazaki, 1999).

Allen *et al.* (1999) recently demonstrated that the *abi1* and *abi2* mutants have decreased, but not abolished, intracellular [Ca^{2+}] transients and are blocked in the ABA-induced S-anion currents involved in stomatal closure. However, transgenic tobacco expressing the *abi1* mutant phosphatase is not affected in the S-anion channel (Grabov *et al.*, 1997). Experimental modulation of intracellular free [Ca^{2+}] bypasses the effects of the *abi1* and *abi2* mutations on the S-anion channel and restores stomatal function (Allen *et al.*, 1999), suggesting that *ABI1* and *ABI2* act upstream of or near the Ca^{2+} messenger. However, results of similar experiments prompted Webb & Hetherington (1997) to conclude that the *ABI1* and *ABI2* phosphatases act near, or downstream of, Ca^{2+}. A Ca^{2+}-independent, temperature-sensitive pathway for ABA-induced stomatal closure has been described (Allan *et al.*, 1994). These and other results suggest the existence of multiple pools of ABA-induced Ca^{2+}, or Ca^{2+} 'signatures' (e.g. extracellular and intracellular?; dependent on cADPR, IP_3, voltage, temperature or time period/amplitude?) that specify the magnitude and direction of ABA responses. Different Ca^{2+} signatures might be involved in distinct ABA signalling mechanisms or control points (as well as in other signalling pathways). A calcineurin (PP2B)-like Ca^{2+}-binding protein is induced by drought and might modulate guard cell movements (Kudla *et al.*, 1999). Because Ca^{2+} is an intermediate in many signalling cascades, its promiscuity makes it difficult to pinpoint its role in ABA signalling or to demonstrate its importance as a 'cross-talk' element in plant behavioural plasticity (Bush, 1995; Sanders *et al.*, 1999; Trewavas, 1999).

In barley aleurone protoplasts, ABA triggers an increase in intracellular pH and a decrease in free intracellular [Ca^{2+}] (Gilroy & Jones, 1992; Heimovaara-Dijkstra *et al.*, 1995) that precedes ABA-inducible gene expression. Ca^{2+} is required for ABA-inducible gene expression in transiently transformed protoplasts, embryos and sub-epidermal cells of tomato (Rock & Quatrano, 1996; Wu *et al.*, 1997; Bustos *et al.*, 1998), and Ca^{2+} perfusion is sufficient to trigger ABA-inducible gene expression in protoplasts and microinjected sub-epidermal cells (Sheen, 1996; Wu *et al.*, 1997).

Several recent discoveries have resulted in the linkage of cADPR and phospholipase C (PLC) (which produces IP_3) to Ca^{2+} fluxes, ABA-regulated gene expression and stomatal movements, lending credence to the idea that stomatal and nuclear ABA signalling might share at least some elements, if not entire signalling pathways. Wu *et al.* (1997) microinjected tomato hypocotyl sub-epidermal cells with chimaeric *GUS* reporter constructs driven by the ABA-inducible *rd29A* and *kin2* promoters and showed that added cADPR and Ca^{2+} significantly and specifically induced *GUS* expression. 8-Amino-cADPR, an antagonist of cADPR, inhibited the induction. Furthermore, by using a sensitive sea-urchin microsome bioassay they showed elegantly that the level of cADPR increased after tissue

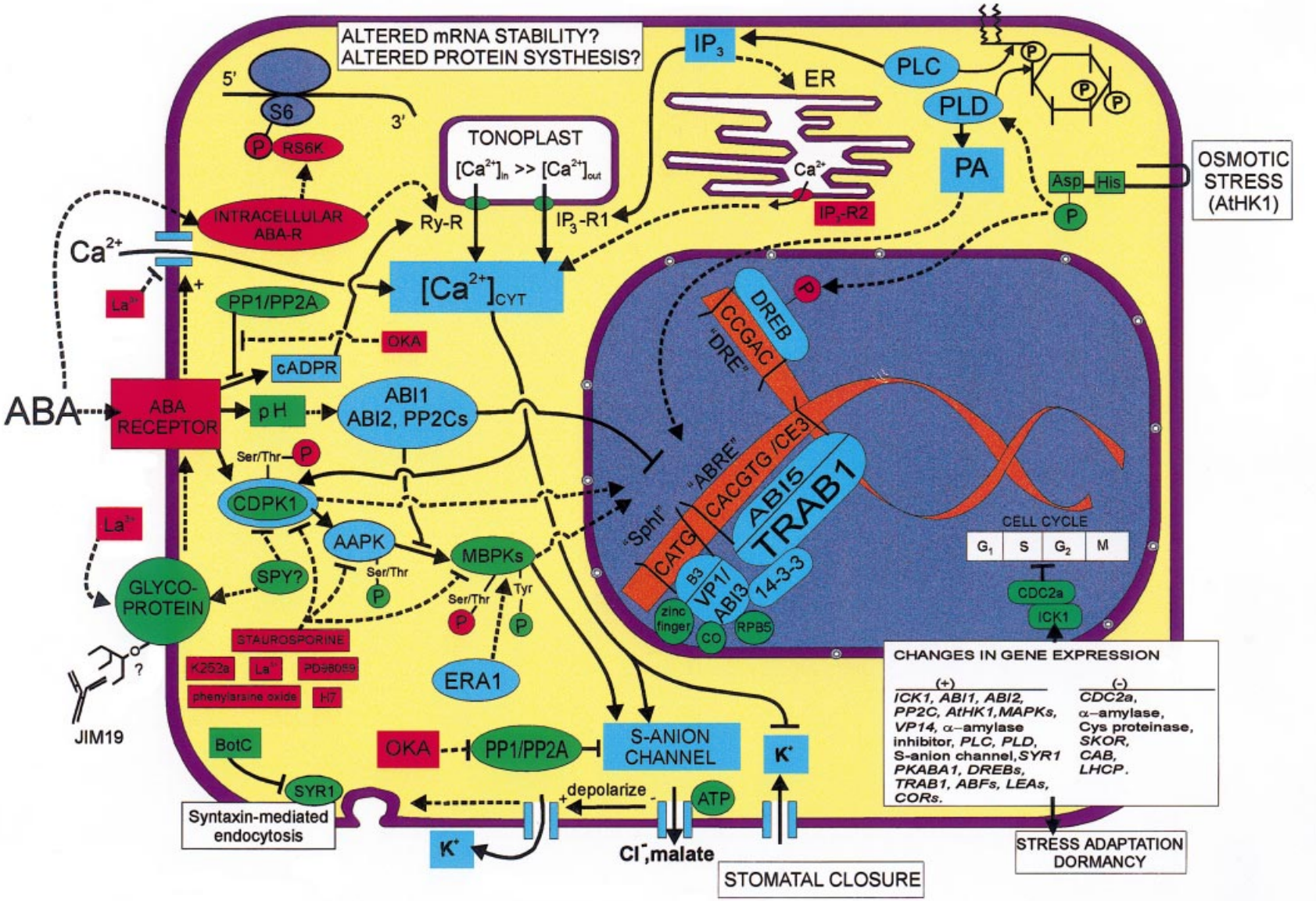

Fig. 5. Montage of known and speculative components of ABA signalling pathways to the nucleus, drawn from numerous systems. Factors shown in blue are well established experimentally; those in green are backed by some evidence; those in red are speculative. Dashed black pathway lines have not been established experimentally. Ry-R, tonoplast ryanodine-sensitive IP_3 receptor.

treatment with ABA and was dependent on NAD^+, the substrate for ADPR cyclase. These results provide convincing evidence that plants produce and respond to cADPR to effect ABA-regulated gene expression. Leckie *et al*. (1998) showed that cADPR microinjection into guard cells caused cytosolic [Ca^{2+}] transients and stomatal closure, and that 8-amino-cADPR or nicotinamide, a product and inhibitor of ADPR cyclase activity, could likewise slow or block (respectively) ABA-induced stomatal closure. Consistent with the role of Ca^{2+} in numerous responses, cADPR is also implicated in plant stress responses other than ABA (Durner *et al*., 1998). Two recent reviews focus on the central role of cyclic nucleotides in signalling (Allen & Schroeder, 1998; Walden, 1998).

Phospholipases hydrolyse phospholipids, and they have been proposed to have roles in mediating a wide range of cellular processes in plants, including hormone action, membrane trafficking, cell proliferation, cytoskeletal organization, defence responses, differentiation and reproduction (Wang, 1999). After years of uncertainty, recent biochemical evidence has provided a strong link in plants between the hydrolysis of phospholipids by the specific PLC, which catalyses the production of IP_3, and diacylglycerol, and ABA signalling. A proven inhibitor of plant PLC, U-73122, partly inhibited ABA-specific [Ca^{2+}] oscillations and stomatal closure, whereas a biologically inactive structural analogue did not (Staxén *et al*., 1999). The plant vacuole has receptors for both cADPR and IP_3 (Allen *et al*., 1995); the tonoplast IP_3 receptor, Ry-R, is sensitive to ryanodine. Cauliflower possesses at least two distinct membrane populations that are sensitive to IP_3; one of these membrane populations is nonvacuolar in origin and relies on a Ca^{2+}-ATPase to accumulate Ca^{2+} (Muir & Sanders, 1997). Wu *et al*. (1997) showed that microinjected IP_3 acts in synergy with ABA to induce ABA-regulated gene expression. Taken together, these results suggest that PLC and IP_3 are important for establishing an intracellular Ca^{2+} signature required for ABA-regulated stomatal movement and gene expression, possibly derived from the tonoplast and other membranes or organelles such as the plasma membrane or endoplasmic reticulum.

Simon Gilroy's group has recently established an apparently conserved mechanism of ABA-regulated gene expression and stomatal movements, namely phospholipase D (PLD) activity, which produces phosphatidic acid (PA) and a head group (e.g. choline or inositol bisphosphate) from phospholipids. Application of ABA to barley aleurone protoplasts increases the activity of PLD within 10 min and the subsequent accumulation of PA. Exogenous PA induces ABA-regulated gene expression (Richie & Gilroy, 1998). The inhibition of PLD by butan-1-ol inhibits ABA-inducible, and promotes GA-inducible, marker gene expression (Richie & Gilroy, 1998). Jacob *et al*. (1999) went on to show that in guard cells, ABA activates PLD and that the application of PA blocks the inward-rectifying K^+ channel and promotes stomatal closure; diacylglycerol has no such effect. The application of butan-1-ol prevents PA production and ABA-induced stomatal closure; furthermore, butan-1-ol acts synergistically with nicotinamide (a cADPR biosynthesis inhibitor), demonstrating that PA and cADPR act in parallel pathways to elicit ABA responses (Jacob *et al*., 1999) (Fig. 5). In the resurrection plant *Craterostigma*, drought (but not ABA) induces PLD activity (Frank *et al*., 2000), suggesting that PLD is also involved in other stress signalling pathways. However, not all ABA-inducible genes can be expressed in guard cells (Taylor *et al*., 1995), suggesting that there are multiple ABA stimulus-response coupling pathways and that guard cells might not be able to provide a complete picture of ABA signalling.

The upstream and downstream elements involved in PLC, PLD and cADPR regulation of ABA responses remain to be determined. PA binds to and activates many kinases and enzymes in animals, and the head group released by PLD might also have regulatory functions (Wang, 1999). It is also possible that membrane properties are affected by PLD activities, because PA destabilizes and increases the net charge of membranes, which can affect membrane protein functions. *PLC* and *PLD* gene expression is induced by ABA and stress (Hirayama *et al*., 1995; Xu *et al*., 1997; Wang, 1999), providing circumstantial evidence in support of their roles in ABA and stress signalling. The identity or regulation of the ADPR cyclase in plants has not been established.

2. *Biochemical approaches to ABA signalling mechanisms*

(a) *Biochemistry of protein kinases and phosphatases*. A similar problem of complexity arises when interpreting the pharmacological evidence for the role of protein kinases, phosphatases, or other signalling pathways in ABA signalling. Benzophenanthridine alkaloid biosynthesis in suspension-cell cultures of *Sanguinaria canadensis* is mediated by ABA and might involve Ca^{2+}, protein kinases and G-proteins, on the basis of pharmacological evidence (Mahady *et al*., 1998). The general Ser/Thr kinase inhibitors K252a and H7 block the S-anion channel and stomatal closure in *Commelina* and *Vicia*, and the block can be relieved by okadaic acid (OKA), an inhibitor of PP1/PP2A phosphatases (Schmidt *et al*., 1995) (Fig. 5). However, the opposite effects were observed with these and similar inhibitors in guard cell experiments with *Arabidopsis* and tobacco (Armstrong *et al*., 1995; Grabov *et al*., 1997; Pei *et al*.,

1997). In pea, kinase inhibitors and activators, and the PP2B (calcineurin) inhibitor cyclosporin A, blocks ABA-induced stomatal closure and guard-cell-specific *DHN* gene expression, whereas OKA promotes guard cell closure (Hey *et al*., 1997). In microinjected sub-epidermal cells of tomato, OKA stimulated the expression of ABA-inducible reporter genes and activity was blocked by EGTA or 8-amino-cADPR, placing the action of the putative PP1/PP2A upstream of Ca^{2+} (Wu *et al*., 1997) (Fig. 5). In this same system, K252a blocked ABA-regulated gene expression downstream of Ca^{2+}. OKA inhibit the induction of *PHAV1* mRNA by ABA in barley aleurone (Kuo *et al*., 1996). It might be that differences in ABA signalling between species or cell types, or different rate-limiting steps of conserved pathways, or different experimental conditions, contribute to the confusion in the quest for a unified model of ABA responses. Caution should always be exercised when interpreting inhibitor studies; they might have nonspecific effects and the targets of well characterized animal drugs in many cases have not been directly demonstrated in plants.

The molecular mechanisms of ABI1, ABI2 and related PP2Cs are not known; for example, the phosphatase activities might be regulated (negatively) via ABA or independently of it. The dominant-negative *abi1-1* and *abi2-1* mutant gene products might bind to cognate substrates and titrate them out in a 'poison' complex, or the proteins might have lost the ability to be regulated by ABA (Gosti *et al*., 1999). ABI1 and ABI2 phosphatase activities are activated by increasing $[Mg^{2+}]$ and are highly sensitive to pH *in vitro* (Leube *et al*., 1998). The alkalization of 0.2–0.4 pH units triggered by ABA would be sufficient to increase ABI1 and ABI2 activities twofold (Leube *et al*., 1998). Guard cells of tobacco plants expressing the mutant *abi1-1* phosphatase, although ABA-insensitive, do still respond to ABA by a pH shift in the cytosol (Armstrong *et al*., 1995), suggesting that pH lies upstream of PP2Cs (Fig. 5). A pH-driven increase of PP2C activity would result in decreased ABA sensitivity, on the basis of the genetic evidence that ABI1 is a negative regulator of ABA responses (Gosti *et al*., 1999). Therefore, for pH to be a positive regulator of ABA signalling as generally believed, a second negative regulator downstream of ABI1 and ABI2 would need to be invoked. Perhaps cytosolic alkalization acts to 'desensitize' the cell to ABA; this phenomenon is the hallmark of regulatory systems and has been observed for ABA-induced volume changes (MacRobbie, 1998). Alternatively, the induction of *ABI1*, *ABI2* and *AtPP2C-HA* mRNAs by ABA (Leung *et al*., 1997; Rodriguez *et al*., 1998b) could serve as an autoregulatory feedback loop that could reset the cell to monitor ABA levels continuously. ABI1 and ABI2 might have integrative functions at different steps of a complex, nonlinear network of cellular (developmental) and environmental (stress) ABA response pathways that include Ca^{2+} pools, pH, protein kinases and phosphatases. Substrates of these PP2Cs are not known; however, another PP2C (MP2C) negatively regulates a stress-activated MAPK in alfalfa (Meskiene *et al*., 1998), suggesting that MAPKs might be targets of ABI1 and ABI2 (Jonak *et al*., 1999; Heimovaara-Dijkstra *et al*., 2000).

MAPKs are involved in the signal transduction pathways associated with growth-factor-dependent cell proliferation and with stress responses in yeast, animals and plants, and evidence is mounting that they are involved in ABA responses. ABA rapidly stimulates the activity of a myelin-basic-protein kinase (MBPK; MBP is an artificial substrate for plants) in barley aleurone protoplasts and pea epidermal peels, and MBPK activity is correlated with the induction of *rab16* and *DHN* mRNA, antagonism of GA action in aleurone, and stomatal closure (Knetsch *et al*., 1996; Burnett *et al*., 2000). Protoplasts are well suited for short time-course experiments because populations of individual cells can be manipulated simultaneously, for example by effector solutions. MBPK activity peaks within 5 min and decreases to basal levels after treatment with ABA *in vivo*. Antibodies recognizing phosphorylated tyrosine residues or a mammalian MAPK (ERK1) precipitate with the MBPK kinase activity (Burnett *et al*., 2000; Heimovaara-Dijkstra *et al*., 2000). The Tyr phosphatase inhibitor phenylarsine oxide and the MAPK inhibitor PD98059 partly block ABA-induced MBPK activation and *rab16* and *DHN* gene expression, which supports the conclusion that Tyr phosphorylation of a MBPK or MAPK occurs during activation by ABA and is necessary for the induction of gene expression by ABA (Knetsch *et al*., 1996; Burnett *et al*., 2000) (Fig. 5). These results are also consistent with multiple ABA signalling pathways, with MAPKs having a role in some of them (Shinozaki & Yamaguchi-Shinozaki, 1997). Systematic gene knock-out studies of PP2Cs, MAPKs and candidate upstream- and downstream effectors will, it is hoped, be informative and help to unravel the complexities of these intracellular networks.

Two groups independently reported characterization of a 48 kDa ABA-activated Ca^{2+}-independent protein kinase (AAPK) in *Vicia* guard cell protoplasts by an in-gel protein kinase assay with either MBP or histone type III-S as substrates (Li & Assmann, 1996; Mori & Muto, 1997). One group described ABA-activated autophosphorylation on Ser but not on Tyr residues (Li & Assmann, 1996), whereas the other group reported that general kinase inhibitors and EGTA, a Ca^{2+} chelator, suppressed the activation of AAPK and stomatal closure, suggesting that an influx of extracellular Ca^{2+} is required for the activation of AAPK by a

CDPK (Mori & Muto, 1997). Taken together, these results suggest that AAPK might have a role in both the Ca^{2+}-independent and Ca^{2+}-dependent ABA signalling pathways of guard cells. Mass-spectrometry-generated peptide sequence information was used to clone the *AAPK* cDNA (Li *et al*., 2000). *AAPK* is expressed only in guard cells. The expression of *AAPK* altered by one amino acid (Lys 43 to Ala 43 at the ATP-binding site) in transformed guard cells renders stomata insensitive to ABA-induced closure by eliminating the activation of plasma membrane S-anion channels by ABA (Li *et al*., 2000).

(b) *Protein-protein interactions with VP1/ABI3.* The use of established *cis*-acting DNA elements as probes to identify *trans*-acting factors has been only partly successful (see section III.2), which is not surprising given the limited complexity of ABA-regulated *cis* elements (Table 5) and the fact that approx. 13% of *Arabidopsis* genes (more than 3000!) are likely to be involved in transcription (Somerville & Somerville, 1999). Because it is likely that transcription factors will interact with each other as well as with DNA, a complementary and more specific approach has been to use established *trans*-acting ABA regulatory proteins as probes for protein-protein interactions in yeast functional two-hybrid assays. Because VP1/ABI3 can interact with many transcription factors (Hill *et al*., 1996) and functions as both a transcriptional activator and a repressor (Hoecker *et al*., 1995), it is a prime 'bait' to use for interaction cloning experiments. Several groups have demonstrated biologically relevant interactions in yeast between VP1/ABI3 and plant proteins that might be involved in ABA-regulated transcriptional activity. A host of proteins bind to VP1 or ABI3 or both; perhaps more importantly, functional relevance in many cases can also be demonstrated or inferred. Proteins that bind to VP1/ABI3 include the following: novel expressed sequence tags in rice and maize (Jones *et al*., 2000), a bZIP protein that transactivates ABA-inducible promoters (Hobo *et al*., 1999b), a 14-3-3 chaperone-like protein that binds a bZIP (Schultz *et al*., 1998), a novel protein with limited homology to *CONSTANS* (a gene encoding a nuclear zinc-finger protein involved in flowering that genetically interacts with *ABI3*) (Kurup *et al*., 2000), zinc-finger transcription factors with homology to *Drosophila GOLIATH* and *Arabidopsis CONSTITUTIVELY PHOTOMORPHOGENIC-1* (*COP1*), a subunit (*RPB5*) of RNA polymerase II, and a plant homologue of a human helix–loop–helix transcription factor (*C1*) involved in G_1–S phase transition during the cell cycle (Jones *et al*., 2000; Kurup *et al*., 2000). It is tempting to speculate that the mechanism of cell cycle regulation and quiescence-related processes in seed development by ABA is through this VP1/ABI3-interacting transcription factor. Structure–function studies of mutated forms of these proteins in transgenic plants, and analysis of the corresponding ABA-related phenotypes, will help to establish the importance of these gene products in tissue-specific and combinatorial interactions between proteins and DNA elements during growth and development.

Using an amino-terminal fragment of the rice VP1 orthologue (OsVP1) containing A1, B1 and B2 domains as 'bait', Hobo *et al*. (1999b) have recently cloned a bZIP transcription factor (TRAB1) that specifically binds to OsVP1 and to *cis*-ABREs containing the ACGT core sequence. Transcription from a chimaeric promoter with yeast *GAL4*-binding sites is ABA-inducible if protoplasts express a GAL4 DNA-binding domain::TRAB1 fusion protein, and OsVP1 expression enhances this activation. Furthermore, transient expression of *TRAB1* is sufficient for transactivation of the native 55-bp ABA-responsive complex of the *OsEm* gene, and *TRAB1* acts synergistically with ABA and transiently overexpressed *OsVP1* to increase *OsEm* promoter activity. *TRAB1* is expressed in embryos, roots and leaves, and its mRNA levels are increased by ABA (Hobo *et al*., 1999b). It is most homologous (82% identical over four conserved domains) to *DPBF1* from *Helianthus* (Kim *et al*., 1997), and least homologous (56% identical over the DNA-binding domain) to EmBP-1 (Hobo *et al*., 1999a). These results provide the first compelling evidence for a genuine *trans*-acting factor involved in ABA-regulated transcription, and reveal a molecular mechanism for the VP1-dependent, ABA-inducible transcription that controls maturation and dormancy in plant embryos. Together with previous work (Bobb *et al*., 1997; Carson *et al*., 1997; Suzuki *et al*., 1997), it resolves a long-standing question of how the VP1 and PvALF proteins can act as transcriptional activators by two distinct mechanisms depending on target *cis*-elements: direct binding to Sph*I*/RY elements through the B3 domain, and indirect interaction with ABREs via TRAB1 (Fig. 5). The observed synergistic effect of ABA on VP1 and TRAB1 functions suggests that the VP1–TRAB1 interaction might be regulated by ABA (Hobo *et al*., 1999b). Furthermore, the ability of ABA to activate transcription through a heterologous (*GAL4* chimaeric) DNA-binding domain and target sequence suggests that the regulation of TRAB1 transactivation by ABA is not at the level of DNA binding. This is consistent with evidence from footprinting of ABA-regulated promoters *in vivo* in the presence or absence of ABA (Busk *et al*., 1999; Li *et al*., 1999). On the basis of its tissue-specific expression (Hobo *et al*., 1999b) and homology to *ABI5* (Finkelstein & Lynch, 2000b), *TRAB1* is likely to be involved in both seed and vegetative ABA response pathways. Choi *et al*. (2000) have recently isolated a family of bZIP genes (*ABF1*–

ABF4) from *Arabidopsis* by a yeast one-hybrid screen with a prototypical ABRE as bait. All the *ABF*s are induced by ABA, are highly homologous to *DPBF1* and *TRAB1* in their bZIP domain, and might be functional homologues of *TRAB1*, *DPBF1* and *ABI5* (Fig. 4).

14-3-3 proteins are acidic, dimeric proteins that mediate protein–protein interactions involved in plant-kinase-mediated signalling, transcription, interorganellar transport and enzyme functions (Finnie *et al*., 1999; Pan *et al*., 1999; May & Soll, 2000; Sehnke *et al*., 2000). A yeast two-hybrid experiment with the B1–B2–B3 region of maize VP1 recently provided evidence that 14-3-3 proteins interact with VP1 (Schultz *et al*., 1998). Chemical cross-linking also demonstrated that 14-3-3 proteins interact with purified VP1 and EmBP-1 (Schultz *et al*., 1998) (Fig. 5). Originally, 14-3-3 proteins were identified in plants by using a monoclonal antibody screening approach coupled with electrophoretic mobility-shift assays with a G-box element probe (Lu *et al*., 1992). ABA can antagonize the phosphorylation and activation of phosphoenolpyruvate carboxylase by fusiococcin (Du *et al*., 1997), which binds a 14-3-3 protein as part of a receptor complex (Finnie *et al*., 1999), suggesting yet another possible interaction of ABA signalling with 14-3-3 protein complexes.

Electrophoretic mobility-shift assays with *cis*-promoter elements sufficient for ABA-inducible expression show that nuclear extracts from suspension cells treated with ABA have enhanced binding activity compared with untreated cells (Guiltinan *et al*., 1990; Nelson *et al*., 1994; Nakagawa *et al*., 1996). These results suggest that ABA-regulated transcription could be due to increased DNA binding activity. 14-3-3 epitopes are present in a transcriptional complex in rice nuclear extracts, but their concentrations do not change after treatment of cells with ABA (Schultz *et al*., 1998). VP1, histone H1 and the high-mobility-group protein HMGb can enhance the DNA-binding activity of EmBP-1 to the *Em* ABRE *in vitro*, but the VP1 activity is not specific: it enhances the binding of diverse transcription factors to cognate targets (Hill *et al*., 1996; Schultz *et al*., 1996; Razik & Quatrano, 1997). It is interesting to note that PvALF (the French bean orthologue of VP1) has been shown to remodel chromatin *in vivo* in an ABA-independent manner (Li *et al*., 1999), which is consistent with the activity of histones and HMG proteins in promoting protein–ABRE interactions. Histone H1 expression is up-regulated by ABA (Wei & O'Connell, 1996). It is tempting to speculate that chromatin-altering activities such as histone acetylation or phosphorylation might have a role in ABA signalling. Because the expression of histone H1 and TRAB1 is ABA-inducible, they could be responsible for increased DNA-binding activity in nuclear extracts. Because 14-3-3 proteins can function as chaperones (Finnie *et al*., 1999), they might act to facilitate the formation of an ABA-transactivation complex containing TRAB1, VP1 and chromatin.

3. *ABA receptors*

With the exception of an unconfirmed report (Hornberg & Weiler, 1984), no ABA receptors have been described. Although ABA-binding proteins (Hocking *et al*., 1978; Curvetto *et al*., 1988; Wan & Hasenstein, 1996; Pédron *et al*., 1998) and carrier-mediated uptake of ABA (Rubery & Astle, 1982; Bianco-Colomas *et al*., 1991; Hartung & Slovik, 1991; Windsor *et al*., 1992) have been reported, there is no evidence to link these proteins to the physiological effects of ABA. To confound the situation further, ABA has direct effects on membrane fluidity and thermal behaviour (Parasassi *et al*., 1990; Burner *et al*., 1993; Shripathi *et al*., 1997), raising the possibility that ABA activity does not require interaction with a receptor. Indeed, it is entirely plausible that ABA might be analogous to lipophilic vitamins such as α-tocopherol (vitamin E) or vitamin K needed by animals. These fat-soluble, low-molecular-mass compounds are required in animals for fertility and blood clotting, respectively; vitamin E can modulate transcription, yet its molecular mechanism of action is not known (Carlberg, 1999). By contrast, the similarities between ABA in plants and retinoic acid in animals, which binds to an intracellular receptor, cannot be ignored. Both are synthesized from carotenoids (also known as pro-vitamin A) by oxidative cleavage (Carlberg, 1999; Qin & Zeevaart, 1999).

ABA is known to regulate plasma membrane and tonoplast ion channel activities very rapidly (Hetherington *et al*., 1998; MacRobbie, 1998; Assmann & Shimazaki, 1999); it is plausible that ABA interacts directly with transport proteins or other metabolic factors. In other words, enzymes or complexes such as ABI1 might have allosteric sites for ABA binding. It is quite within the realm of possibility that there are multiple ABA response mechanisms operating simultaneously.

It has been suggested, on the basis of indirect evidence, that both intracellular and extracellular ABA receptors exist. Schwartz *et al*. (1994) tested by three different methods whether ABA can act from within guard cells. They first observed a correlation of the extent to which ABA inhibits stomatal opening and promotes stomatal closure in *Commelina* in proportion to the uptake of radioactive ABA. They then showed that direct microinjection of ABA into the cytoplasm of *Commelina* guard cells precipitates stomatal closure. Finally, they showed that the application of ABA to the cytosol of *Vicia* guard-cell protoplasts by means of patch-clamp techniques inhibits inward K^+ currents, an effect sufficient to inhibit stomatal opening. These results are con-

sistent with, but do not prove, an intracellular site of phytohormone action. Anderson *et al*. (1994) obtained contradictory results to those of Schwartz *et al*. (1994) after microinjecting various quantities of ABA into *Commelina* guard cells, yet an extracellular application of 10 μM ABA inhibited stomatal opening by 98% at pH 6.15 and by 57% at pH 8.0. As a weak acid that permeates membranes only in the protonated form, ABA is trapped in the alkaline compartments such as the chloroplast in the light, and in the apoplast during stress (Hartung & Slovik, 1991; Zeevaart, 1999). MacRobbie (1995) also observed a correlation between high external pH and attenuation of ABA-induced ion efflux. Furthermore, Allan *et al*. (1994) showed that stomatal closure followed the intracellular release of microinjected 'caged' ABA after photolysis. Taken together, the microinjection results, the uptake and patch clamping experiments and the pH dependence of ABA action are consistent with a contribution of both extracellular and intracellular ABA receptors to stomatal regulation. However, other interpretations are possible, for example direct ABA action on plasma and tonoplast membranes (or ion channels) from the cytoplasmic side, the higher affinity of an ABA receptor for the protonated form, or pH-dependent pathways.

Extracellular ABA perception leading to gene expression has also been observed, but these results also constitute circumstantial evidence for a genuine ABA receptor. Two studies used ABA-protein conjugates that could not enter the cell yet were biologically active in inducing ion channel activity (Jeannette *et al*., 1998) and gene expression (Schultz & Quatrano, 1997; Jeannette *et al*., 1998). The use of ABA analogues in germination and gene expression bioassays has permitted the inference of multiple ABA receptors with different structural requirements for activity in different response pathways (Walker-Simmons *et al*., 1997; Kim *et al*., 1999). Given the lack of conclusive information, the search for ABA receptors should include intracellular compartments and nonproteinaceous molecules. It is critically important for any receptor studies to correlate the specificity of interaction with ABA analogues possessing different degrees of biological activity.

Screening of cDNA expression libraries with novel polyclonal antisera against ABA-related antigens has resulted in reports that approach the question of a putative ABA receptor. Liu *et al*. (1999) described a novel barley cDNA (*aba45*) whose product binds a polyclonal antiserum raised against an anti-ABA monoclonal antibody. In theory, this means that the polyclonal antibodies might have epitopes (anti-idiotypic) that mimic the structure of ABA and could therefore bind to ABA-binding proteins, including an ABA receptor. Even more interesting is that *aba45* is ABA-inducible in embryos (Liu *et al*., 1999). However, there is yet no functional evidence, for example specific and saturable binding of ABA to the gene product, to indicate that this gene encodes an ABA receptor.

Another antibody-screening approach resulted in the isolation of two classes of clones from a maize cDNA expression library probed with antibodies against ABA-binding proteins (Zheng *et al*., 1998). A single clone showed 60% homology to nucleic acid binding proteins, and the other class of clones encoded the 17S rRNA gene. It was then shown that the affinity-purified ABA-binding complex contained rRNA; the biological significance of the binding of ABA to a ribonucleoprotein complex is not known; however, two S6 protein-kinase homologues in *Arabidopsis* are induced by stress and ABA (Mizoguchi *et al*., 1996), also providing circumstantial evidence that these kinases might be involved in ABA regulation of translation (Table 4).

How plants perceive stresses that modulate water status and affect ABA levels is not known. A crucial link between ABA signalling and osmotic stress perception was recently elucidated with the demonstration that an *Arabidopsis* transmembrane two-component histidine kinase (*AtHK1*) is a functional osmosensor (Urao *et al*., 1999). The *AtHK1* gene is up-regulated by ABA, drought, hypotonic solutions, cold and salt stress. Perhaps the osmosensor is the mechanism for initiating ABA biosynthesis and other stress responses, thereby integrating ABA signalling with other overlapping stress pathways (Fig. 5).

4. *Cell biology*

Protoplasts are a heterogeneous population with potentially different characteristics that might complicate the analysis of signalling pathways (Ritchie *et al*., 1999). For example, (transgenic) manipulation (e.g. the overexpression of regulatory molecules) might distort the intracellular dynamics of the regulatory components studied (such as transcriptional, post-transcriptional, translational, post-translational; the 'uncertainty principle') and the system should be validated by comparison with the state *in planta*, if possible.

My group has applied the technique of flow cytometry to measurement of the activity of the ABA-inducible *Em* promoter fused to the gene encoding the *Aequoria* green fluorescent protein (GFP) in transiently transformed rice protoplasts (Fig. 6). It is apparent that in response to ABA, the population of protoplasts increases expression of *Em–GFP* uniformly; this conclusion could not be drawn from results obtained by grinding up millions of cells for reporter enzyme assays. Flow cytometry permits the quantification of gene expression (and other correlative cell biology parameters) in potentially complex populations on a per cell basis. On the basis of scoring an equal number of transiently

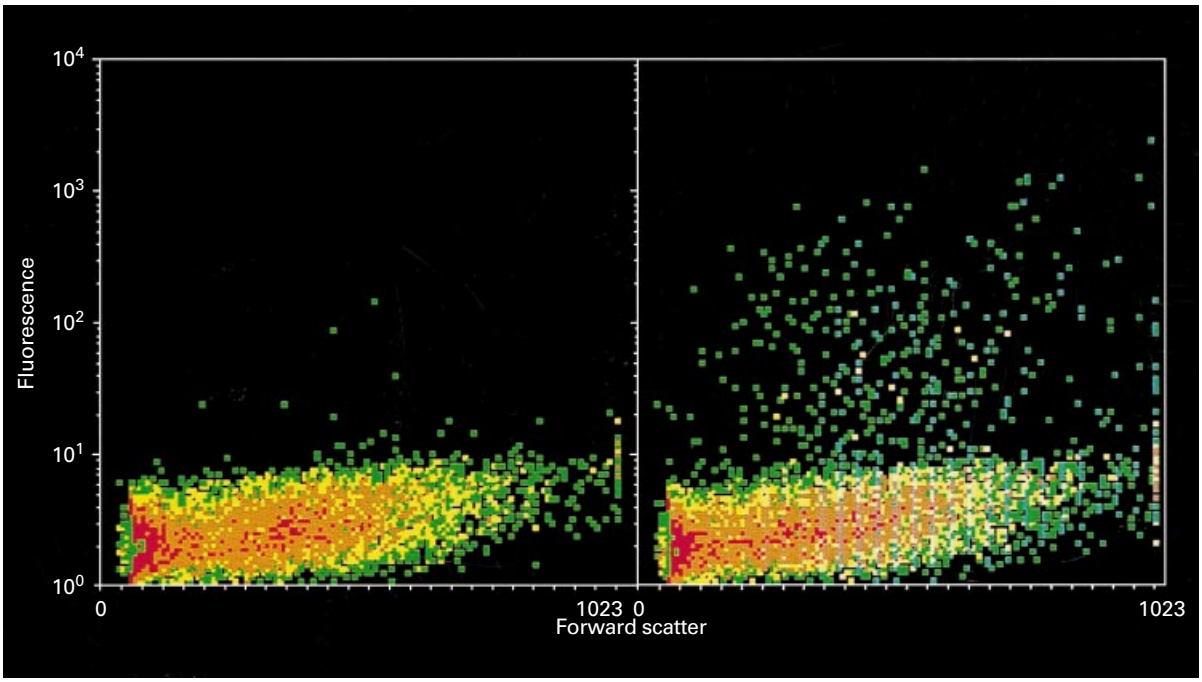

Fig. 6. ABA-inducible *Em* promoter expression in transiently transformed rice protoplasts detected by flow-cytometric measurement of GFP demonstrates a homogeneous population response. False-colour scatter plot (red indicates more cells) of *Em-GFP*-transformed rice embryonic protoplasts treated for 20 h without (left panel) or with (right panel) 100 μM ABA. A total of 10 000 protoplasts were measured for each treatment.

transformed cells, it was shown that cells expressing *Em–GFP* respond uniformly as a population (both in terms of cell numbers and fluorescence intensities) to treatments of ABA and/or lanthanum chloride (Hagenbeek *et al.*, 2000). Lanthanide ions act in synergy with ABA on *Em* gene expression (Rock & Quatrano, 1996), but the mechanism of action is not known. Lanthanides are well known Ca^{2+} channel blockers (Bush, 1995) but they have other effects such as the inhibition of CDPKs (Polya *et al.*, 1987) and nonspecific inhibition of ion channels (Lewis & Spalding, 1998). Because overexpression of the *abi1-1* dominant-negative PP2C allele antagonized the lanthanum effect, it was concluded that lanthanum acts upstream of *ABI1* in ABA-signalling (Hagenbeek *et al.*, 2000). In animal systems, lanthanide ions bind to and activate cell-surface glycoproteins (integrins) involved in numerous signalling pathways (D'Souza *et al.*, 1994; Obsil *et al.*, 1999). In this context it is interesting to note that *SPY*, which can effect the expression of an ABA-inducible *DHN* gene when overexpressed (Robertson *et al.*, 1998), encodes an O-glucosyl transferase that could theoretically modify cell-surface glycoproteins.

A surface plasmon resonance biosensor was used in conjunction with flow cytometry of protoplasts to provide indirect, correlative *in vitro* evidence for an ABA receptor complex that interacts with a cell-surface glycoprotein (Desikan *et al.*, 1999). JIM19 is one of a panel of monoclonal antibodies previously generated against pea guard cell protoplasts that can modulate ABA responses in barley aleurone and rice protoplasts (Wang *et al.*, 1995; Desikan *et al.*, 1999). Using surface plasmon resonance biosensor technology, Desikan *et al.* (1999) observed specific binding of plasma membranes to JIM19; the binding was antagonized significantly by ABA but not by the biologically inactive ABA catabolite phaseic acid. The interactions of plasma membranes, JIM19 and ABA *in vitro* are correlated with the biological activities of JIM19, ABA and phaseic acid on the activation of *Em–GFP* measured by flow cytometry. Taken together, these results suggest that JIM19 interacts with a functional complex involved in ABA signalling. It will be interesting to determine whether lanthanide ions (including terbium, which is fluorescent and can therefore be easily measured) can also interact with this complex. For example, two-colour or three-colour flow cytometry of protoplasts expressing ABA-inducible GFP could be performed to provide multiparameter-correlated analysis of the relationships between JIM19 epitope abundance, terbium binding and ABA sensitivity (Desikan *et al.*, 1999).

Disrupting syntaxin function (involved in intracellular vesicle trafficking, fusion and secretion) in tobacco by cleavage with *Clostridium botulinum* type C toxin or by competition with a soluble fragment of the tobacco syntaxin SYR1 prevented ion-channel responses to ABA in guard cells (Leyman *et al.*, 1999). Because the ABA-induced changes in the solute content of guard cells result in a 50% decrease in membrane surface area by endocytosis (Homann, 1998), it is likely that the

regulation of membrane vesicle trafficking by ABA is one of the early events in ABA signalling and might be linked to ion channel activities. It would be interesting to know whether inhibitors of post-translational processing (glycosylation) or vesicular trafficking have effects on ABA-inducible gene expression. An annexin-like gene, possibly involved in plasma membrane endocytosis or exocytosis, is induced by ABA (Kovács *et al*., 1998), which is consistent with a role of vesicular trafficking in ABA response.

V. CONCLUSIONS AND PERSPECTIVES

It is apparent that our understanding of ABA action at the molecular level is quite incomplete, although the three experimental approaches (forward and reverse genetics, and biochemistry) have contributed to spectacular progress in recent years. It is still assumed that tissue-specific 'sensitivity' (Trewavas, 1992) is a fundamental basis of ABA action, but the term is mostly theoretical in the absence of established molecular mechanisms. Subcellular compartmentation or intracellular gradients might contribute to the presence of 'active' ABA pools, but little is known about ABA compartmentation. Is 'sensitivity' a function of receptor density, alteration of enzyme kinetics on ABA binding, and/or subcellular distribution of ABA? Are there multiple ABA response pathways, for example for stomatal versus nuclear responses or extracellular and intracellular receptors, or is there underlying conservation of one or more shared processes? Is there 'cross-talk' between pathways, including other hormones? These are some of the questions yet to be addressed. Cell biological studies that establish the subcellular dynamics of known ABA signalling factors should be informative in the quest to link events at the cell surface to those in the nucleus.

We can synthesize a cell-biological model of ABA signalling from a putative receptor, through ion channels, secondary messengers, cell-surface glycoproteins, protein kinases and phosphatases, to transcription factors in the nucleus, but the evidence that these linear paradigms are correct is not yet definitive. It is important to consider that ABA signalling might be part of a complex web of stress pathways that have feedback loops (autoregulation) and nodes (e.g. $[Ca^{2+}]$, pH, MAPKs, PP2Cs); any tripping of one response might have ripple effects on the activities of others. In the future, even more exciting revelations are in store as the gaps are filled in and paradoxes are resolved in the integrative phase of plant biology.

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REFERENCES

Abe H, Yamaguchi-Shinozaki K, Urao T, Iwasaki T, Hosokawa D, Shinozaki K. 1997. Role of *Arabidopsis* MYC and MYB homologs in drought- and abscisic acid-regulated gene expression. *Plant Cell* **9**: 1859–1868.

Addicott FT, Carns HR. 1983. History and introduction. In: Addicott FT, ed. *Abscisic acid*. New York, USA: Praeger Scientific, 1–21.

Albinsky D, Masson JE, Bogucki A, Afsar K, Vass I, Nagy F, Paszkowski J. 1999. Plant responses to genotoxic stress are linked to an ABA/salinity signalling pathway. *Plant Journal* **17**: 73–82.

Allan AC, Fricker MD, Ward JL, Beale MH, Trewavas AJ. 1994. Two transduction pathways mediate rapid effects of abscisic acid in *Commelina* guard cells. *Plant Cell* **6**: 1319–1328.

Allen GJ, Kuchitsu K, Chu SP, Murata Y, Schroeder JI. 1999. *Arabidopsis abi1-1* and *abi2-1* phosphatase mutations reduce abscisic acid-induced cytoplasmic calcium rises in guard cells. *Plant Cell* **11**: 1785–1798.

Allen GJ, Muir SR, Sanders D. 1995. Release of Ca^{2+} from individual plant vacuoles by both InsP(3) and cyclic ADP-ribose. *Science* **268**: 735–737.

Allen GJ, Schroeder JI. 1998. Cyclic ADP-ribose and ABA signal transduction. *Trends in Plant Science* **3**: 123–125.

Anderson BE, Ward JM, Schroeder JI. 1994. Evidence for an extracellular reception site for abscisic acid in *Commelina* guard cells. *Plant Physiology* **104**: 1177–1183.

Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, León P. 2000. Analysis of *Arabidopsis* glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. *Genes and Development*. **14**: 2085–2096.

Armstrong F, Leung J, Grabov A, Brearley J, Giraudat J, Blatt MR. 1995. Sensitivity to abscisic acid of guard-cell K^+ channels is suppressed by *abi1-1*, a mutant *Arabidopsis* gene encoding a putative protein phosphatase. *Proceedings of the National Academy of Sciences, USA* **92**: 9520–9524.

Assmann S, Shimazaki K. 1999. The multisensory guard-cell: stomatal responses to blue-light and abscisic acid. *Plant Physiology* **119**: 809–815.

Barkla BJ, Vera-Estrella R, Maldonado-Gama M, Pantoja O. 1999. Abscisic acid induction of vacuolar H^+-ATPase activity in *Mesembryanthemum crystallinum* is developmentally regulated. *Plant Physiology* **120**: 811–820.

Barroso C, Romero LC, Cejudo FJ, Vega JM, Gotor C. 1999. Salt-specific regulation of the cytosolic O-acetylserine(thiol)-lyase gene from *Arabidopsis thaliana* is dependent on abscisic acid. *Plant Molecular Biology* **40**: 729–736.

Bäumlein H, Miséra S, Luerssen H, Kölle K, Horstmann C, Wobus U, Müller AJ. 1994. The *FUS3* gene of *Arabidopsis thaliana* is a regulator of gene expression during late embryogenesis. *Plant Journal* **6**: 379–387.

Berger S, Bell E, Mullet JE. 1996. Two methyl jasmonate-insensitive mutants show altered expressionof *AtVsp* in response to methyl jasmonate and wounding. *Plant Physiology* **111**: 525–531.

Bhalerao RP, Salchert K, Bakó L, Ökrész L, Szabados L, Muranaka T, Machida Y, Schell J, Koncz C. 1999. Regulatory interaction of PRL1 WD protein with *Arabidopsis* SNF1-like protein kinases. *Proceedings of the National Academy of Sciences, USA* **96**: 5322–5327.

Bianco-Colomas J, Barthe P, Orlandini M, Le Page-Degivry MT. 1991. Carrier-mediated uptake of abscisic acid by

suspension-cultured *Amaranthus tricolor* cells. *Plant Physiology* **95**: 990–996.

Bies-Etheve N, da Silva Conceicao A, Giraudat J, Koornneef M, Léon-Kloosterziel K, Valon C, Delseny M. 1999. Importance of the B2 domain of the *Arabidopsis* ABI3 protein for *Em* and *2S* albumin gene regulation. *Plant Molecular Biology* **40**: 1045–1054.

Birkenmeier GF, Ryan CA. 1998. Wound signaling in tomato plants: evidence that ABA is not a primary signal for defense gene activation. *Plant Physiology* **117**: 687–693.

Blatt MR, Thiel G, Trentham DR. 1990. Reversible inactivation of K^+ channels of *Vicia* stomatal guard cells following the photolysis of caged inositol 1,4,5-trisphosphate. *Nature* **346**: 766–769.

Bobb AJ, Chern MS, Bustos MM. 1997. Conserved RY-repeats mediate transactivation of seed-specific promoters by the developmental regulator PvALF. *Nucleic Acids Research* **25**: 641–647.

Bobb AJ, Eiben HG, Bustos MM. 1995. PvALF, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters. *Plant Journal* **8**: 331–343.

Bonetta D, McCourt P. 1998. Genetic analysis of ABA signal transduction pathways. *Trends in Plant Science* **3**: 231–235.

Bray EA. 1997. Plant responses to water deficit. *Trends in Plant Science* **2**: 48–54.

Burner H, Benz R, Gimmler H, Hartung W, Stillwell W. 1993. Abscisic acid-lipid interactions: a phospholipid monolayer study. *Biochimica et Biophysica Acta* **1150**: 165–172.

Burnett EC, Desikan R, Moser RC, Neill SJ. 2000. ABA activation of an MBP kinase in *Pisum sativum* epidermal peels correlates with stomatal responses to ABA. *Journal of Experimental Botany* **51**: 197–205.

Bush DS. 1995. Calcium regulation in plant cells and its role in signalling. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**: 95–122.

Busk PK, Jensen AB, Pagès M. 1997. Regulatory elements *in vivo* in the promoter of the abscisic acid responsive gene *rab*17 from maize. *Plant Journal* **11**: 1285–1295.

Busk PK, Pagès M. 1997. Protein binding to the abscisic acid-responsive element is independent of VIVIPAROUS1 *in vivo*. *Plant Cell* **9**: 2261–2270.

Busk PK, Pagès M. 1998. Regulation of abscisic acid-induced transcription. *Plant Molecular Biology* **37**: 425–435.

Busk PK, Pujal J, Jessop A, Lumbreras V, Pagès M. 1999. Constitutive protein-DNA interactions on the abscisic acid-responsive element before and after developmental activation of the *rab28* gene. *Plant Molecular Biology* **41**: 529–536.

Bustos MM, Iyer M, Gagliardi SJ. 1998. Induction of a beta-phaseolin promoter by exogenous abscisic acid in tobacco: developmental regulation and modulation by external sucrose and Ca^{2+} ions. *Plant Molecular Biology* **37**: 265–274.

Capel J, Jarillo JA, Madueno F, Jorquera MJ, Martinez-Zapater JM, Salinas J. 1998. Low temperature regulates *Arabidopsis Lhcb* gene expression in a light-independent manner. *Plant Journal* **13**: 411–418.

Carlberg C. 1999. Lipid soluble vitamins in gene regulation. *Biofactors* **10**: 91–97.

Carrera E, Prat S. 1998. Expression of the *Arabidopsis Abi1-1* mutant allele inhibits proteinase inhibitor wound induction in tomato. *Plant Journal* **15**: 765–771.

Carson CB, Hattori T, Rosenkrans L, Vasil V, Vasil IK, Peterson PA, McCarty DR. 1997. The quiescent/colorless alleles of *Viviparous1* show that the conserved B3 domain of *Vp1* is not essential for ABA-regulated gene expression in the seed. *Plant Journal* **12**: 1231–1240.

Cercós M, Gómez-Cadenas A, Ho TH. 1999. Hormonal regulation of a cysteine proteinase gene, *EPB-1*, in barley aleurone layers: *cis*- and *trans*-acting elements involved in the co-ordinated gene expression regulated by gibberellins and abscisic acid. *Plant Journal* **19**: 107–118.

Chak RKF, Thomas TL, Quatrano RS, Rock CD. 2000. The genes *ABI1* and *ABI2* are involved in abscisic acid- and drought-inducible expression of the *Daucus carota* L. *Dc3* promoter in guard cells of transgenic *Arabidopsis thaliana* L. Heynh. *Planta* **210**: 875–883.

Chaloupkova K, Smart CC. 1994. The abscisic acid induction of a novel peroxidase is antagonized by cytokinin in *Spirodela polyrrhiza* L. *Plant Physiology* **105**: 497–507.

Chandler JW, Bartels D. 1997. Structure and function of the *Vp1* gene homologue from the resurrection plant *Craterostigma plantagineum* Hochst. *Molecular and General Genetics* **256**: 539–546.

Chandler PM, Robertson M. 1994. Gene expression regulated by abscisic acid and its relation to stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**: 113–141.

Chang YC, Walling LL. 1991. Abscisic acid negatively regulates expression of chlorophyll A/B binding protein genes during soybean embryogeny. *Plant Physiology* **97**: 1260–1264.

Chao WS, Gu YQ, Pautot V, Bray EA, Walling LL. 1999. Leucine aminopeptidase RNAs, proteins, and activities increase in response to water deficit, salinity, and the wound signals systemin, methyl jasmonate, and abscisic acid. *Plant Physiology* **120**: 979–992.

Chatthai M, Kaukinen KH, Tranbarger TJ, Gupta PK, Misra S. 1997. The isolation of a novel metallothionein-related cDNA expressed in somatic and zygotic embryos of Douglas-fir: regulation by ABA, osmoticum, and metal ions. *Plant Molecular Biology* **34**: 243–254.

Chern MS, Bobb AJ, Bustos MM. 1996. The regulator of *MAT2* (ROM2) protein binds to early maturation promoters and represses PvALF-activated transcription. *Plant Cell* **8**: 305–321.

Cherry JM, Ball C, Weng S, Juvik G, Schmidt R, Adler C, Dunn B, Dwight S, Riles L, Mortimer RK, Botstein D. 1997. Genetic and physical maps of *Saccharomyces cerevisiae*. *Nature* **387**: 67S–73S.

Choi Hi, Hong Jh, Ha Jo, Kang Jy, Kim SY. 2000. ABFs, a family of ABA-responsive element binding factors. *Journal of Biological Chemistry* **275**: 1723–1730.

Clouse SD, Langford M, McMorris TC. 1996. A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiology* **111**: 671–678.

Coca MA, Almoguera C, Thomas TL, Jordano J. 1996. Differential regulation of small heat-shock genes in plants: analysis of a water-stress-inducible and developmentally activated sunflower promoter. *Plant Molecular Biology* **31**: 863–876.

Cohen A, Plant AL, Moses MS, Bray EA. 1991. Organ-specific and environmentally regulated expression of two abscisic acid-induced genes of tomato. *Plant Physiology* **97**: 1367–1374.

Conley TR, Peng HP, Shih MC. 1999. Mutations affecting induction of glycolytic and fermentative genes during germination and environmental stresses in *Arabidopsis*. *Plant Physiology* **119**: 599–607.

Crowe AJ, Abenes M, Plant A, Moloney MM. 2000. The seed-specific transactivator, *ABI3*, induces oleosin gene expression. *Plant Science* **151**: 171–181.

Curvetto NR, Delmastro SE, Aguero MS. 1988. An abscisic acid binding protein from abaxial epidermis of *Vicia faba* isolated by affinity chromatography. *Phyton* **48**: 7–11.

Cutler AJ, Squires TM, Loewen MK, Balsevich JJ. 1997. Induction of (+)-abscisic acid 8′-hydroxylase by (+)-abscisic acid in cultured maize cells. *Journal of Experimental Botany* **48**: 1787–1795.

Cutler S, Ghassemian M, Bonetta D, Cooney S, McCourt P. 1996. A protein farnesyl transferase involved in abscisic acid signal transduction in *Arabidopsis*. *Science* **273**: 1239–1241.

Debeaujon I, Koornneef M. 2000. Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiology* **122**: 415–424.

de Bruxelles GL, Peacock WJ, Dennis ES, Dolferus R. 1996. Abscisic acid induces the alcohol dehydrogenase gene in *Arabidopsis*. *Plant Physiology* **111**: 381–391.

Del Pozo JC, Allona I, Rubio V, Leyva A, De La Pena A, Aragoncillo C, Paz-Ares J. 1999. A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilising/oxidative stress conditions. *Plant Journal* **19**: 579–589.

Desikan R, Hagenbeek D, Neill SJ, Rock CD. 1999. Flow cytometry and surface plasmon resonance analyses demonstrate that the monoclonal antibody JIM19 interacts with a rice cell

surface component involved in abscisic acid signalling in protoplasts. *FEBS Letters* **456**: 257–262.

Ding B, Kwon MO, Hammond R, Owens R. 1997. Cell-to-cell movement of potato spindle tuber viroid. *Plant Journal* **12**: 931–936.

D'Souza SE, Haas TA, Piotrowicz RS, Byers-Ward V, McGrath DE, Soule HR, Cierniewski C, Plow EF, Smith JW. 1994. Ligand and cation binding are dual functions of a discrete segment of the integrin beta 3 subunit: cation displacement is involved in ligand binding. *Cell* **79**: 659–667.

Du Z, Aghoram K, Outlaw WH Jr. 1997. *In vivo* phosphorylation of phosphoenolpyruvate carboxylase in guard cells of *Vicia faba* L. is enhanced by fusicoccin and suppressed by abscisic acid. *Archives of Biochemistry and Biophysics* **337**: 345–350.

Dufner A, Thomas G. 1999. Ribosomal S6 kinase signaling and the control of translation. *Experimental Cell Research* **253**: 100–109.

Dure L III. 1993. A repeating 11-mer amino acid motif and plant desiccation. *Plant Journal* **3**: 363–369.

Durner J, Wendehenne D, Klessig DF. 1998. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. *Proceedings of the National Academy of Sciences, USA* **95**: 10328–10333.

Ephritikhine G, Fellner M, Vannini C, Lapous D, Barbier-Brygoo H. 1999. The *sax1* dwarf mutant of *Arabidopsis thaliana* shows altered sensitivity of growth responses to abscisic acid, auxin, gibberellins and ethylene and is partially rescued by exogenous brassinosteroid. *Plant Journal* **18**: 303–314.

Eyster WH. 1931. Vivipary in maize. *Genetics* **16**: 574–590.

Ezcurra I, Ellerström M, Wycliffe P, Stålberg K, Rask L. 1999. Interaction between composite elements in the *napA* promoter: both the B-box ABA-responsive complex and the RY/G complex are necessary for seed-specific expression. *Plant Molecular Biology* **40**: 699–709.

Fath A, Bethke PC, Jones RL. 1999. Barley aleurone cell death is not apoptotic: characterization of nuclease activities and DNA degradation. *Plant Journal* **20**: 305–315.

Finkelstein R. 1994. Mutations at two new *Arabidopsis* ABA response loci are similar to the *abi3* mutations. *Plant Journal* **5**: 765–771.

Finkelstein R, Somerville C. 1990. Three classes of abscisic acid (ABA)-insensitive mutations of *Arabidopsis* define genes that control overlapping subsets of ABA responses. *Plant Physiology* **94**: 1172–1179.

Finkelstein RR, Lynch TJ. 2000a. Abscisic acid inhibition of radicle emergence, but not seedling growth, is suppressed by sugars. *Plant Physiology* **122**: 1179–1186.

Finkelstein RR, Lynch TJ. 2000b. The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* **12**: 599–609.

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM. 1998. The *Arabidopsis* abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. *Plant Cell* **10**: 1043–1054.

Finnie C, Borch J, Collinge DB. 1999. 14-3-3 proteins: eukaryotic regulatory proteins with many functions. *Plant Molecular Biology* **40**: 545–554.

Fobis-Loisy I, Loridon K, Lobreaux S, Lebrun M, Briat JF. 1995. Structure and differential expression of two maize ferritin genes in response to iron and abscisic acid. *European Journal of Biochemistry* **231**: 609–619.

Forsthoefel NR, Cushman MA, Cushman JC. 1995. Post-transcriptional and posttranslational control of enolase expression in the facultative Crassulacean acid metabolism plant *Mesembryanthemum crystallinum* L. *Plant Physiology* **108**: 1185–1195.

Foster R, Chua NH. 1999. An *Arabidopsis* mutant with deregulated ABA gene expression: implications for negative regulator function. *Plant Journal* **17**: 363–372.

Frandsen G, Müller-Uri F, Nielsen M, Mundy J, Skriver K. 1996. Novel plant Ca^{2+}-binding protein expressed in response to abscisic acid and osmotic stress. *Journal of Biological Chemistry* **271**: 343–348.

Frank W, Munnik T, Kerkmann K, Salamini F, Bartels D. 2000. Water deficit triggers phospholipase D activity in the resurrection plant *Craterostigma plantagineum*. *Plant Cell* **12**: 111–124.

Frank W, Phillips J, Salamini F, Bartels D. 1998. Two dehydration-inducible transcripts from the resurrection plant *Craterostigma plantagineum* encode interacting homeodomain-leucine zipper proteins. *Plant Journal* **15**: 413–421.

Furini A, Koncz C, Salamini F, Bartels D. 1997. High level transcription of a member of a repeated gene family confers dehydration tolerance to callus tissue of *Craterostigma plantagineum*. *EMBO Journal* **16**: 3599–3608.

Furini A, Parcy F, Salamini F, Bartels D. 1996. Differential regulation of two ABA-inducible genes from *Craterostigma plantagineum* in transgenic *Arabidopsis* plants. *Plant Molecular Biology* **30**: 343–349.

Gao YP, Young L, Bonham-Smith P, Gusta LV. 1999. Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of *Brassica napus* during germination under stress conditions. *Plant Molecular Biology* **40**: 635–644.

Garcia-Garrido JM, Menossi M, Puigdomenech P, Martinez-Izquierdo JA, Delseny M. 1998. Characterization of a gene encoding an abscisic acid-inducible type-2 lipid transfer protein from rice. *FEBS Letters* **428**: 193–199.

Garciarrubio A, Legaria J, Covarrubias A. 1997. Abscisic acid inhibits germination of mature *Arabidopsis* seeds by limiting the availability of energy and nutrients. *Planta* **203**: 182–187.

Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferriere N, Thibaud JB, Sentenac H. 1998. Identification and disruption of a plant Shaker-like outward channel involved in K^+ release into the xylem sap. *Cell* **94**: 647–655.

Gelbart WM. 1998. Databases in genomic research. *Science* **282**: 659–661.

Giani S, Qin X, Faoro F, Breviario D. 1998. In rice, oryzalin and abscisic acid differentially affect tubulin mRNA and protein levels. *Planta* **205**: 334–341.

Gillet B, Beyly A, Peltier G, Rey P. 1998. Molecular characterization of CDSP34, a chloroplastic protein induced by water deficit in *Solanum tuberosum* L. plants, and regulation of CDSP34 expression by ABA and high illumination. *Plant Journal* **16**: 257–262.

Gilmour SJ, Thomashow MF. 1991. Cold acclimation and cold-regulated gene expression in ABA mutants of *Arabidopsis thaliana*. *Plant Molecular Biology* **17**: 1233–1240.

Gilroy S, Jones RL. 1992. Gibberellic acid and abscisic acid coordinately regulate cytoplasmic calcium and secretory activity in barley aleurone protoplasts. *Proceedings of the National Academy of Sciences, USA* **89**: 3591–3595.

Gilroy S, Read ND, Trewavas AJ. 1990. Elevation of cytoplasmic calcium by caged calcium or caged inositol trisphosphate initiates stomatal closure. *Nature* **346**: 769–771.

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM. 1992. Isolation of the *Arabidopsis ABI3* gene by positional cloning. *Plant Cell* **4**: 1251–1261.

Godoy AV, Lazzaro AS, Casalongue CA, San Segundo B. 2000. Expression of a *Solanum tuberosum* cyclophilin gene is regulated by fungal infection and abiotic stress conditions. *Plant Science* **152**: 123–134.

Gómez-Cadenas A, Verhey SD, Holappa LD, Shen Q, Ho THD, Walker-Simmons MK. 1999. An abscisic acid-induced PKABA1 mediates abscisic acid-suppressed gene expression in aleurone layers. *Proceedings of the National Academy of Sciences, USA* **96**: 1767–1772.

Gosti F, Beaudoin N, Serizet C, Webb AAR, Vartanian N, Giraudat J. 1999. The *ABI1* protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell* **11**: 1897–1909.

Grabov A, Blatt MR. 1998. Membrane voltage inititates Ca^{2+} waves and potentiates Ca^{2+} increases with abscisic acid in stomatal guard cells. *Proceedings of the National Academy of Sciences, USA* **95**: 4778–4783.

Grabov A, Leung J, Giraudat J, Blatt MR. 1997. Alteration of anion channel kinetics in wild-type and *abi1–1* transgenic *Nicotiana benthamiana* guard cells by abscisic acid. *Plant Journal* **12**: 203–213.

Grappin P, Bouinot D, Sotta B, Miginiac E, Jullien M. 2000. Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposes dormancy maintenance. *Planta* **210**: 279–285.

Grenier J, Potvin C, Trudel J, Asselin A. 1999. Some

thaumatin-like proteins hydrolyse polymeric beta-1,3-glucans. *Plant Journal* **19**: 473–480.

Grill E, Himmelbach A. 1998. ABA signal transduction. *Current Opinion in Plant Biology* **1**: 412–418.

Guan L, Scandalios JG. 1998. Two structurally similar maize cytosolic superoxide dismutase genes, *Sod4* and *Sod4A*, respond differentially to abscisic acid and high osmoticum. *Plant Physiology* **117**: 217–224.

Guiltinan MJ, Marcotte WR Jr, Quatrano RS. 1990. A plant leucine zipper protein that recognizes an abscisic acid response element. *Science* **250**: 267–271.

Hagenbeek D, Quatrano RS, Rock CD. 2000. Trivalent ions activate ABA-inducible promoters through an *ABI1*-dependent pathway in rice (*Oryza sativa* L.) protoplasts. *Plant Physiology* **123**: 1553–1560.

Hake S, Meyerowitz EM. 1998. Growth and development: growing up green. *Current Opinion in Plant Biology* **1**: 9–11.

Halford NG, Hardie DG. 1998. SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Molecular Biology* **37**: 735–748.

Hamilton DWA, Hills A, Köhler B, Blatt MR. 2000. Ca^{2+} channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. *Proceedings of the National Academy of Sciences, USA* **97**: 4967–4972.

Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO Journal* **17**: 1405–1411.

Hartung W, Slovik S. 1991. Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid in leaves. *New Phytologist* **119**: 361–382.

Hattori T, Terada T, Hamasuna S. 1995. Regulation of the *OsEm* gene by abscisic acid and the transcriptional activator *Vp1*: analysis of *cis*-acting promoter elements required for regulation by abscisic acid and *Vp1*. *Plant Journal* **7**: 913–925.

Hattori T, Vasil V, Rosenkrans L, Hannah LC, McCarty DR, Vasil IK. 1992. The *Viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. *Genes and Development* **6**: 609–618.

Heimovaara-Dijkstra S, Mundy J, Wang M. 1995. The effect of intracellular pH on the regulation of the *Rab16A* and the alpha-amylase *1/6-4* promoter by abscisic acid and gibberellin. *Plant Molecular Biology* **27**: 815–820.

Heimovaara-Dijkstra S, Testerink C, Wang M. 2000. Mitogen-activated protein kinase and abscisic acid signal transduction. *Results and Problems in Cell Differentiation* **27**: 131–144.

Heimovaara-Dijkstra S, Van Duijn B, Libbenga KR, Heidekamp F, Wang M. 1994. ABA-induced membrane potential changes in barley aleurone protoplasts – a possible relevance for the regulation of *RAB* gene expression. *Plant and Cell Physiology* **35**: 743–750.

Hemerly AS, Ferreira P, Engler JD, Van Montagu M, Engler G, Inze D. 1993. Cdc2A expression in *Arabidopsis* is linked with competence for cell division. *Plant Cell* **5**: 1711–1723.

Hetherington AM, Gray JE, Leckie CP, McAinsh MR, Ng C, Pical C, Priestly AJ, Saxén I, Webb AAR. 1998. The control of specificity in guard cell signal transduction. *Philosophical Transactions of the Royal Society of London* B **353**: 1489–1494.

Hetherington AM, Quatrano RS. 1991. Mechanisms of action of abscisic acid at the cellular level. *New Phytologist* **119**: 9–32.

Hey SJ, Bacon A, Burnett E, Neill SJ. 1997. Abscisic acid signal transduction in epidermal cells of *Pisum sativum* L. Argenteum: both dehydrin mRNA accumulation and stomatal responses require protein phosphorylation and dephosphorylation. *Planta* **202**: 85–92.

Hill A, Nantel A, Rock CD, Quatrano RS. 1996. A conserved domain of the *Viviparous-1* gene product enhances the DNA binding activity of the bZIP protein EmBP-1 and other transcription factors. *Journal of Biological Chemistry* **271**: 3366–3374.

Himmelbach A, Iten M, Grill E. 1998. Signalling of abscisic acid to regulate plant growth. *Philosophical Transactions of the Royal Society of London* B **353**: 1439–1444.

Hirai N. 1986. Abscisic acid. In: Takahashi N, ed. *Chemistry of plant hormones*. Boca Raton, FL, USA: CRC Press, 201–248.

Hirayama T, Ohto C, Mizoguchi T, Shinozaki K. 1995. A gene encoding a phosphatidylinositol-specific phospholipase C is induced by dehydration and salt stress in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **92**: 3903–3907.

Hobo T, Asada M, Kowyama Y, Hattori T. 1999a. ACGT-containing abscisic acid response element (ABRE) and coupling element 3 (CE3) are functionally equivalent. *Plant Journal* **19**: 679–689.

Hobo T, Kowyama Y, Hattori T. 1999b. A bZIP factor, TRAB1, interacts with VP1 and mediates abscisic acid-induced transcription. *Proceedings of the National Academy of Sciences, USA* **96**: 15348–15353.

Hocking TJ, Clapham J, Cattell KJ. 1978. Abscisic acid binding to subcellular fractions from leaves of *Vicia faba*. *Planta* **138**: 303–304.

Hoecker U, Vasil IK, McCarty DR. 1995. Integrated control of seed maturation and germination programs by activator and repressor functions of *Viviparous-1* of maize. *Genes and Development* **9**: 2459–2469.

Hoecker U, Vasil IK, McCarty DR. 1999. Signaling from the embryo conditions *Vp1*-mediated repression of alpha-amylase genes in the aleurone of developing maize seeds. *Plant Journal* **19**: 371–377.

Homann U. 1998. Fusion and fission of plasma membrane material accomodates for osmotically induced changes in the surface area of guard cell protoplasts. *Planta* **206**: 329–333.

Hong SW, Jon JH, Kwak JM, Nam HG. 1997. Identification of a receptor-like protein kinase gene rapidly induced by abscisic acid, dehydration, high salt, and cold treatments in *Arabidopsis thaliana*. *Plant Physiology* **113**: 1203–1212.

Hornberg C, Weiler E. 1984. High affinity binding sites for ABA on the plasmalemma of *Vicia* guard cells. *Nature* **310**: 321–324.

Hua J, Meyerowitz EM. 1998. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. *Cell* **94**: 261–271.

Huijser C, Kortstee A, Pego J, Weisbeek P, Wisman E, Smeekens S. 2000. The *Arabidopsis* SUCROSE UNCOUPLED-6 gene is identical to ABSCISIC ACID-4. Involvement of abscisic acid in sugar signaling. *Plant Journal* **23**: 577–586.

Hwang I, Goodman HM. 1995. An *Arabidopsis thaliana* root-specific kinase homolog is induced by dehydration, ABA, and NaCl. *Plant Journal* **8**: 37–43.

Ingram J, Bartels D. 1996. The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**: 377–403.

Irving HR, Gehring CA, Parish RW. 1992. Changes in cytosolic pH and calcium of guard cells precede stomatal movement. *Proceedings of the National Academy of Sciences, USA* **89**: 1790–1794.

Ishitani M, Xiong L, Lee H, Stevenson B, Zhu JK. 1998. *HOS1*, a genetic locus involved in cold-responsive gene expression in *Arabidopsis*. *Plant Cell* **10**: 1151–1161.

Ishitani M, Xiong L, Stevenson B, Zhu JK. 1997. Genetic analysis of osmotic and cold stress signal transduction in *Arabidopsis*: interactions and convergence of abscisic acid-dependent and abscisic acid-independent pathways. *Plant Cell* **9**: 1935–1949.

Ismail AM, Hall AE, Close TJ. 1999. Allelic variation of a dehydrin gene cosegregates with chilling tolerance during seedling emergence. *Proceedings of the National Academy of Sciences, USA* **96**: 13566–13570.

Iturriaga G, Leyns L, Villegas A, Gharaibeh R, Salamini F, Bartels D. 1996. A family of novel *myb*-related genes from the resurrection plant *Craterostigma plantagineum* are specifically expressed in callus and roots in response to ABA or desiccation. *Plant Molecular Biology* **32**: 707–716.

Izawa T, Foster R, Nakajima M, Shimamoto K, Chua NH. 1994. The rice bZIP transcriptional activator RITA-1 is highly expressed during seed development. *Plant Cell* **6**: 1277–1287.

Jacob T, Ritchie S, Assmann SM, Gilroy S. 1999. Abscisic acid signal transduction in guard cells is mediated by phospholipase D activity. *Proceedings of the National Academy of Sciences, USA* **96**: 12192–12197.

Jacobsen SE, Olszewski NE. 1993. Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. *Plant Cell* **5**: 887–896.

Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF. 1998. *Arabidopsis CBF1* overexpression induces *COR* genes and enhances freezing tolerance. *Science* **280**: 104–106.

Jarillo JA, Leyva A, Salinas J, Martinez-Zapater JM. 1993. Low temperature induces the accumulation of alcohol dehydrogenase mRNA in *Arabidopsis thaliana*, a chilling-tolerant plant. *Plant Physiology* **101**: 833–837.

Jeannette E, Rona JP, Bardat F, Cornel D, Sotta B, Miginiac E. 1998. Induction of *Rab18* gene expression and activation of K^+ outward rectifying channels depend on an extracellular perception of ABA in *Arabidopsis thaliana* suspension cells. *Plant Journal* **18**: 13–22.

Jonak C, Ligterink W, Hirt H. 1999. MAP kinases in plant signal transduction. *Cellular and Molecular Life Sciences* **55**: 204–213.

Jones HD, Kurup S, Peters NCB, Holdsworth MJ. 2000. Identification and analysis of proteins that interact with the *Avena fatua* homologue of the maize transcription factor VIVIPAROUS 1. *Plant Journal* **21**: 133–142.

Kagaya Y, Ohmiya K, Hattori T. 1999. RAV1, a novel DNA-binding protein, binds to bipartite recognition sequence through two distinct DNA-binding domains uniquely found in higher plants. *Nucleic Acids Research* **27**: 470–478.

Kaldenhoff R, Kolling A, Richter G. 1993. A novel blue light-inducible and abscisic acid-inducible gene of *Arabidopsis thaliana* encoding an intrinsic membrane protein. *Plant Molecular Biology* **23**: 1187–1198.

Kaminaka H, Morita S, Nakajima M, Masumura T, Tanaka K. 1998. Gene cloning and expression of cytosolic glutathione reductase in rice (*Oryza sativa* L.). *Plant and Cell Physiology* **39**: 1269–1280.

Kaminaka H, Morita S, Tokumoto M, Masumura T, Tanaka K. 2000. Differential gene expressions of rice superoxide dismutase isoforms to oxidative and environmental stresses. *Free Radical Research* **31**: S219-S225.

Kao CY, Cocciolone SM, Vasil IK, McCarty DR. 1996. Localization and interaction of the *cis*-acting elements for abscisic acid, *VIVIPAROUS1*, and light activation of the *C1* gene of maize. *Plant Cell* **8**: 1171–1179.

Keith K, Kraml M, Dengler NG, McCourt P. 1994. *Fusca3*: A heterochronic mutation affecting late embryo development in *Arabidopsis*. *Plant Cell* **6**: 589–600.

Kim BT, Min YK, Asami T, Park NK, Kwon OY, Cho KY, Yoshida S. 1999. 2-Fluoroabscisic acid analogs – their synthesis and biological activities. *Journal of Agricultural Food Chemistry* **47**: 313–317.

Kim SY, Chung HJ, Thomas TL. 1997. Isolation of a novel class of bZIP transcription factors that interact with ABA-responsive and embryo-specification elements in the *Dc3* promoter using a modified yeast one-hybrid system. *Plant Journal* **11**: 1237–1251.

Kim SY, Thomas TL. 1998. A family of novel basic leucine zipper proteins binds to seed-specification elements in the carrot *Dc3* gene promoter. *Journal of Plant Physiology* **152**: 607–613.

Kirik V, Kolle K, Misera S, Baumlein H. 1998. 2 Novel myb homologs with changed expression in late embryogenesis-defective *Arabidopsis* mutants. *Plant Molecular Biology* **37**: 819–827.

Knetsch MLW, Wang M, Snaar-Hagalska BE, Heimovaara-Dijkstra S. 1996. Abscisic acid induces mitogen-activated protein kinase activation in barley aleurone protoplasts. *Plant Cell* **8**: 1061–1067.

Knight H, Veale EL, Warren GJ, Knight MR. 1999. The *sfr6* mutation in *Arabidopsis* suppresses low-temperature induction of genes dependent on the CRT/DRE sequence motif. *Plant Cell* **11**: 875–886.

Koike M, Takezawa D, Arakawa K, Yoshida S. 1997. Accumulation of 19-kDa plasma membrane polypeptide during induction of freezing tolerance in wheat suspension-cultured cells by abscisic acid. *Plant and Cell Physiology* **38**: 707–716.

Koka CV, Cerny RE, Gardner RG, Noguchi T, Fujioka S, Takatsuto S, Yoshida S, Clouse SD. 2000. A putative role for the tomato genes *DUMPY* and *CURL-3* in brassinosteroid biosynthesis and response. *Plant Physiology* **122**: 85–98.

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM. 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics* **61**: 385–393.

Koornneef M, Léon-Kloosterziel KM, Schwartz SH, Zeevaart JAD. 1998. The genetic and molecular dissection of abscisic acid biosynthesis and signal transduction in *Arabidopsis*. *Plant Physiology and Biochemistry* **36**: 83–89.

Koornneef M, Reuling G, Karssen CM. 1984. The isolation and characterization of abscisic acid insensitive mutants of *Arabidopsis thaliana*. *Physiologia Plantarum* **61**: 377–383.

Kovács I, Ayaydin F, Oberschall A, Ipacs I, Bottka S, Pongor S, Dudits D, Tóth EC. 1998. Immunolocalization of a novel annexin-like protein encoded by a stress and abscisic acid responsive gene in alfalfa. *Plant Journal* **15**: 185–197.

Kudla J, Xu Q, Harter K, Gruissem W, Luan S. 1999. Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. *Proceedings of the National Academy of Sciences, USA* **96**: 4718–4723.

Kuo A, Cappelluti S, Cervantes-Cervantes M, Rodriguez M, Bush DS. 1996. Okadaic acid, a protein phosphatase inhibitor, blocks calcium changes, gene expression, and cell death induced by gibberellin in wheat aleurone cells. *Plant Cell* **8**: 259–269.

Kurek I, Harvey AJ, Lonsdale DM, Breiman A. 2000. Isolation and characterization of the wheat prolyl isomerase FK506-binding protein (FKBP) 73 promoter. *Plant Molecular Biology* **42**: 489–497.

Kurup S, Jones HD, Holdsworth MJ. 2000. Interactions of the developmental regulator ABI3 with proteins identified from developing *Arabidopsis* seeds. *Plant Journal* **21**: 143–156.

Kusano T, Berberich T, Harada M, Suzuki N, Sugawara K. 1995. A maize DNA-binding factor with a bZIP motif is induced by low temperature. *Molecular and General Genetics* **248**: 507–517.

Kusnetsov V, Herrmann RG, Kulaeva ON, Oelmuller R. 1998. Cytokinin stimulates and abscisic acid inhibits greening of etiolated *Lupinus luteus* cotyledons by affecting the expression of the light-sensitive protochlorophyllide oxidoreductase. *Molecular and General Genetics* **259**: 21–28.

Laby RJ, Kincaid MS, Kim D, Gibson SI. 2000. The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *Plant Journal* **23**: 587–596.

Lam E, Chua N-H. 1991. Tetramer of a 21 base pair synthetic element confers seed expression and transcriptional enhancement in response to water stress and abscisic acid. *Journal of Biological Chemistry* **266**: 7131–7135.

Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM. 1998. Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. *Proceedings of the National Academy of Sciences, USA* **95**: 15837–15842.

Lee H, Xiong L, Ishitani M, Stevenson B, Zhu JK. 1999. Cold-regulated gene expression and freezing tolerance in an *Arabidopsis thaliana* mutant. *Plant Journal* **17**: 301–308.

Lee YH, Chun JY. 1998. A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* induced by water stress and abscisic acid treatment. *Plant Molecular Biology* **37**: 377–384.

Legaria J, Rajsbaum R, Munoz-Clares RA, Villegas-Sepulveda N, Simpson J, Iturriaga G. 1998. Molecular characterization of two genes encoding betaine aldehyde dehydrogenase from amaranth. Expression in leaves under short-term exposure to osmotic stress or abscisic acid. *Gene* **218**: 69–76.

Leonhardt N, Vavasseur A, Forestier C. 1999. ATP binding cassette modulators control abscisic acid-regulated slow anion channels in guard cells. *Plant Cell* **11**: 1141–1151.

Léon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevaart JAD, Koornneef M. 1996a. Isolation and characterization of abscisic acid-deficient *Arabidopsis* mutants at two new loci. *Plant Journal* **10**: 655–661.

Léon-Kloosterziel KM, van de Bunt GA, Zeevaart JA, Koornneef M. 1996b. *Arabidopsis* mutants with a reduced seed dormancy. *Plant Physiology* **110**: 233–240.

Leube MP, Grill E, Amrhein N. 1998. ABI1 of *Arabidopsis* is a protein serine/threonine phosphatase highly regulated by the proton and magnesium ion concentration. *FEBS Letters* **424**: 100–104.

Leung J, Bouvier-Durand M, Morris PC, Guerrier D, Chefdor F, Giraudat J. 1994. *Arabidopsis* ABA response

gene ABI1: features of a calcium-modulated protein phosphatase. *Science* **264**: 1448–1452.

Leung J, Giraudat J. 1998. Abscisic acid signal transduction. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 199–222.

Leung J, Merlot S, Giraudat J. 1997. The *Arabidopsis Abscisic Acid-Insensitive2* (*ABI2*) and *ABI1* genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. *Plant Cell* **9**: 759–771.

Lewis BD, Spalding EP. 1998. Nonselective block by La^{3+} of *Arabidopsis* ion channels involved in signal transduction. *Journal of Membrane Biology* **162**: 81–90.

Leyman B, Geelen D, Quintero FJ, Blatt MR. 1999. A tobacco syntaxin with a role in hormonal control of guard cell ion channels. *Science* **283**: 537–540.

Li GF, Bishop KJ, Chandrasekharan MB, Hall TC. 1999. Beta-phaseolin gene activation is a 2-step process – PvALF-facilitated chromatin modification followed by abscisic acid-mediated gene activation. *Proceedings of the National Academy of Sciences, USA* **96**: 7104–7109.

Li J, Chory J. 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* **90**: 929–938.

Li J, Wang XQ, Watson MB, Assmann SM. 2000. Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. *Science* **287**: 300–303.

Li JX, Assmann SM. 1996. An abscisic acid-activated and calcium-independent protein kinase from guard cells of fava bean. *Plant Cell* **8**: 2359–2368.

Liu J, Zhu J-K. 1998. A calcium sensor homolog required for plant salt tolerance. *Science* **280**: 1943–1945.

Liu JH, Hill RD. 1995. Post-transcriptional regulation of bifunctional alpha-amylase/subtilisin inhibitor expression in barley embryos by abscisic acid. *Plant Molecular Biology* **29**: 1087–1091.

Liu JH, Luo M, Cheng KJ, Mohapatra SS, Hill RD. 1999. Identification and characterization of a novel barley gene that is ABA-inducible and expressed in embryo and aleurone. *Journal of Experimental Botany* **50**: 727–728.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* **10**: 1391–1406.

Llorente F, Oliveros JC, Martínez-Zapater JM, Salinas J. 2000. A freezing sensitive mutant of *Arabidopsis*, *frs1*, is a new *ABA3* allele. *Planta*. **211**: 648–655

Lotan T, Ohto M, Yee KM, West MAL, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ. 1998. *Arabidopsis Leafy Cotyledon1* is sufficient to induce embryo development in vegetative cells. *Cell* **93**: 1195–1205.

Lu G, DeLisle AJ, de Vetten NC, Ferl RJ. 1992. Brain proteins in plants: an *Arabidopsis* homolog to neurotransmitter pathway activators is part of a DNA binding complex. *Proceedings of the National Academy of Sciences, USA* **89**: 11490–11494.

Lu G, Paul AL, McCarty DR, Ferl RJ. 1996. Transcription factor veracity: is GBF3 responsible for ABA-regulated expression of *Arabidopsis Adh*? *Plant Cell* **8**: 847–857.

Luerssen K, Kirik V, Herrmann P, Misera S. 1998. *Fusca3* encodes a protein with a conserved *Vp1/Abi3*-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. *Plant Journal* **15**: 755–764.

Machuka J, Bashiardes S, Ruben E, Spooner K, Cuming A, Knight C, Cove D. 1999. Sequence analysis of expressed sequence tags from an ABA-treated cDNA library identifies stress response genes in the moss *Physcomitrella patens*. *Plant and Cell Physiology* **40**: 378–387.

MacRobbie EAC. 1995. ABA-induced ion efflux in stomatal guard cells: multiple actions of ABA inside and outside the cell. *Plant Journal* **7**: 565–576.

MacRobbie EAC. 1998. Signal transduction and ion channels in guard cells. *Philosophical Transactions of the Royal Society of London* B **353**: 1475–1488.

Mahady GB, Liu C, Beecher CW. 1998. Involvement of protein kinase and G proteins in the signal transduction of benzophenanthridine alkaloid biosynthesis. *Phytochemistry* **48**: 93–102.

Malz S, Sauter M. 1999. Expression of two *PIP* genes in rapidly growing internodes of rice is not primarily controlled by meristem activity or cell expansion. *Plant Molecular Biology* **40**: 985–995.

Mäntylä E, Lang V, Palva ET. 1995. Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LTI78 and RAB18 proteins in *Arabidopsis thaliana*. *Plant Physiology* **107**: 141–148.

Marcotte WR Jr, Bayley CC, Quatrano RS. 1988. Regulation of a wheat promoter by abscisic acid in rice protoplasts. *Nature* **335**: 454–457.

May T, Soll J. 2000. 14-3-3 proteins form a guidance complex with chloroplast precursor proteins in plants. *Plant Cell* **12**: 53–64.

McCarty DR. 1995. Genetic control and integration of maturation and germination pathways in seed development. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**: 71–93.

McCarty DR, Carson CB, Stinard PS, Robertson DS. 1989. Molecular analysis of *viviparous-1*: an abscisic acid insensitive mutant of maize. *Plant Cell* **1**: 523–532.

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK. 1991. The *viviparous-1* developmental gene of maize encodes a novel transcription activator. *Cell* **66**: 895–905.

McCourt P. 1999. Genetic analysis of hormone signaling. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 219–243.

Medina J, Bargues M, Terol J, Pérez-Alonso M, Salinas J. 1999. The *Arabidopsis CBF* gene family is composed of three genes encoding *AP2* domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiology* **119**: 463–469.

Meinke DW, Cherry JM, Dean C, Rounsley SD, Koornneef M. 1998. *Arabidopsis thaliana* – a model plant for genome analysis. *Science* **282**: 662–682.

Meinke DW, Franzmann LH, Nickle TC, Yeung EC. 1994. Leafy cotyledon mutants of *Arabidopsis*. *Plant Cell* **6**: 1049–1064.

Menkens AE, Schindler U, Cashmore AR. 1995. The G-box: a ubiquitous regulatory element in plants bound by the GBF family of bZIP proteins. *Trends in Biochemical Science* **20**: 506–510.

Meskiene I, Bögre L, Glaser W, Balog J, Brandstötter M, Zwerger K, Ammerer G, Hirt H. 1998. MP2C, a plant protein phosphatase 2C, functions as a negative regulator of mitogen-activated protein kinase pathways in yeast and plants. *Proceedings of the National Academy of Sciences, USA* **95**: 1938–1943.

Meyer K, Leube MP, Grill E. 1994. A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. *Science* **264**: 1452–1455.

Mikami K, Katagiri T, Iuchi S, Yamaguchi-Shinozaki K, Shinozaki K. 1998. A gene encoding phosphatidylinositol-4-phosphate 5-kinase is induced by water stress and abscisic acid in *Arabidopsis thaliana*. *Plant Journal* **15**: 563–568.

Miyazaki S, Koga R, Bohnert HJ, Fukuhara T. 1999. Tissue- and environmental response-specific expression of 10 PP2C transcripts in *Mesembryanthemum crystallinum*. *Molecular and General Genetics* **261**: 307–316.

Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K. 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **93**: 765–769.

Moons A, Valcke R, Van Montagu M. 1998. Low-oxygen stress and water deficit induce cytosolic pyruvate orthophosphate dikinase (PPDK) expression in roots of rice, a C3 plant. *Plant Journal* **15**: 89–98.

Mori IC, Muto S. 1997. Abscisic acid activates a 48-kilodalton protein kinase in guard cell protoplasts. *Plant Physiology* **113**: 833–839.

Mudgett MB, Clarke S. 1996. A distinctly regulated protein repair L-isoaspartylmethyltransferase from *Arabidopsis thaliana*. *Plant Molecular Biology* **30**: 723–737.

Muir SR, Sanders D. 1997. Inositol 1,4,5-trisphosphate-

sensitive Ca^{2+} release across nonvacuolar membranes in cauliflower. *Plant Physiology* **114**: 1511–1521.

Mundy J, Yamaguchi-Shinozaki K, Chua NH. 1990. Nuclear proteins bind conserved elements in the abscisic acid-responsive promoter of a rice *rab* gene. *Proceedings of the National Academy of Sciences, USA* **87**: 1406–1410.

Muramoto Y, Watanabe A, Nakamura T, Takabe T. 1999. Enhanced expression of a nuclease gene in leaves of barley plants under salt stress. *Gene* **234**: 315–321.

Nakagawa H, Ohmiya K, Hattori T. 1996. A rice bZIP protein, designated OSBZ8, is rapidly induced by abscisic acid. *Plant Journal* **9**: 217–227.

Nambara E, Keith K, McCourt P, Naito S. 1995. A regulatory role for the *ABI3* gene in the establishment of embryo maturation in *Arabidopsis thaliana*. *Development* **121**: 629–636.

Nambara E, McCourt P. 1999. Protein farnesylation in plants: a greasy tale. *Current Opinion in Plant Biology* **2**: 388–392.

Nantel A, Quatrano RS. 1996. Characterization of three rice basic/leucine zipper factors, including two inhibitors of EmBP-1 DNA binding activity. *Journal of Biological Chemistry* **271**: 31296–31305.

Neill SJ, Horgan R, Rees AF. 1987. Seed development and vivipary in *Zea mays* L. *Planta* **171**: 358–364.

Nelson D, Salamini F, Bartels D. 1994. Abscisic acid promotes novel DNA-binding activity to a desiccation-related promoter of *Craterostigma plantagineum*. *Plant Journal* **5**: 451–458.

Németh K, Salchert K, Putnoky P, Bhalerao R, Koncz-Kálmán Z, Stankovic-Stangeland B, Bakó L, Mathur J, Ökrész L, Stabel S, Geigenberger P, Stitt M, Redei GP, Schell J, Koncz Z. 1998. Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in *Arabidopsis*. *Genes and Development* **12**: 3059–3073.

Nicolas C, Nicolas G, Rodriguez D. 1998. Transcripts of a gene, encoding a small GTP-binding protein from *Fagus sylvatica*, are induced by ABA and accumulated in the embryonic axis of dormant seeds. *Plant Molecular Biology* **36**: 487–491.

Nicolas C, Rodriguez D, Poulsen F, Eriksen EN, Nicolas G. 1997. The expression of an abscisic acid-responsive glycine-rich protein coincides with the level of seed dormancy in *Fagus sylvatica*. *Plant and Cell Physiology* **38**: 1303–1310.

Nordin K, Heino P, Palva ET. 1991. Separate signal pathways regulate the expression of a low-temperature-induced gene in *Arabidopsis thaliana* (L.) Heynh. *Plant Molecular Biology* **16**: 1061–1071.

Obsil T, Hofbauerova K, Amler E, Teisinger J. 1999. Different cation binding to the I domains of alpha1 and alpha2 integrins: implication of the binding site structure. *FEBS Letters* **457**: 311–315.

Oeda K, Salinas J, Chua NH. 1991. A tobacco bZip transcription activator (TAF-1) binds to a G-box-like motif conserved in plant genes. *EMBO Journal* **10**: 1793–1802.

O'Mahony PJ, Oliver MJ. 1999. Characterization of a desiccation-responsive small GTP-binding protein (Rab2) from the desiccation-tolerant grass *Sporobolus stapfianus*. *Plant Molecular Biology* **39**: 809–821.

Ono A, Izawa T, Chua NH, Shimamoto K. 1996. The *rab16B* promoter of rice contains two distinct abscisic acid-responsive elements. *Plant Physiology* **112**: 483–491.

Ooms JJJ, Léon-Kloosterziel KM, Bartels D, Koornneef M, Karssen CM. 1993. Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana*. *Plant Physiology* **102**: 1185–1191.

Ouvrard O, Cellier F, Ferrare K, Tousch D, Lamaze T, Dupuis JM, Casse-Delbart F. 1996. Identification and expression of water stress- and abscisic acid-regulated genes in a drought-tolerant sunflower genotype. *Plant Molecular Biology* **31**: 819–829.

Pan S, Sehnke PC, Ferl RJ, Gurley WB. 1999. Specific interactions with TBP and TFIIB *in vitro* suggest that 14-3-3 proteins may participate in the regulation of transcription when part of a DNA binding complex. *Plant Cell* **11**: 1591–1602.

Papi M, Sabatini S, Bouchez D, Camilleri C, Costantino P, Vittorioso P. 2000. Identification and disruption of an *Arabidopsis* zinc finger gene controlling seed germination. *Genes and Development* **14**: 28–33.

Parasassi T, De Stasio G, Miccheli A, Bruno F, Conti F, Gratton E. 1990. Abscisic acid-induced microheterogeneity in phospholipid vesicles: a fluorescence study. *Biophysical Chemistry* **35**: 65–73.

Parcy F, Giraudat J. 1997. Interactions between the *ABI1* and the ectopically expressed *ABI3* genes in controlling abscisic acid responses in *Arabidopsis* vegetative tissues. *Plant Journal* **11**: 693–702.

Parcy F, Valon C, Kohara A, Misera S, Giraudat J, 1997. The *ABSCISIC ACID-INSENSITIVE3, FUSCA3,* and *LEAFY COTYLEDON1* loci act in concert to control multiple aspects of *Arabidopsis* seed development. *Plant Cell* **9**: 1265–1277.

Parcy F, Valon C, Raynal M, Gaubier-Comella P, Delseny M, Giraudat J. 1994. Regulation of gene expression programs during *Arabidopsis* seed development: roles of the *ABI3* locus and of endogenous abscisic acid. *Plant Cell* **6**: 1567–1582.

Park JH, Oh SA, Kim YH, Woo HR, Nam HG. 1998. Differential expression of senescence-associated mRNAs during leaf senescence induced by different senescence-inducing factors in *Arabidopsis*. *Plant Molecular Biology* **37**: 445–454.

Pédron J, Brault M, Näke C, Miginiac E. 1998. Detection of abscisic acid-binding proteins in the microsomal protein fraction of *Arabidopsis thaliana* with abscisic acid protein conjugates used as affinity probes. *European Journal of Biochemistry* **252**: 385–390.

Pei ZM, Ghassemian M, Kwak CM, McCourt P, Schroeder JI. 1998. Role of farnesyltransferase in ABA regulation of guard cell anion channels and plant water loss. *Science* **282**: 287–290.

Pei ZM, Kuchitsu K, Ward JM, Schwarz M, Schroeder JI. 1997. Differential abscisic acid regulation of guard cell slow anion channels in *Arabidopsis* wild-type and *abi1* and *abi2* mutants. *Plant Cell* **9**: 409–423.

Pei ZM, Ward JM, Harper JF, Schroeder JI. 1996. A novel chloride channel in *Vicia faba* guard cell vacuoles activated by the serine/threonine kinase, CDPK. *EMBO Journal* **15**: 6564–6574.

Peña-Cortez H, Willmitzer L, Sánchez-Serrano JJ. 1991. Abscisic acid mediates wound induction but not developmental-specific expression of the proteinase inhibitor II gene family. *Plant Cell* **3**: 963–972.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* **400**: 256–261.

Pepper A, Delaney T, Washburn T, Poole D, Chory J. 1994. *DET1*, a negative regulator of light-mediated development and gene expression in *Arabidopsis*, encodes a novel nuclear-localized protein. *Cell* **78**: 109–116.

Piao HL, Pih KT, Lim JH, Kang SG, Jin JB, Kim SH, Hwang I. 1999. An *Arabidopsis* GSK3/shaggy-like gene that complements yeast salt stress-sensitive mutants is induced by NaCl and abscisic acid. *Plant Physiology* **119**: 1527–1534.

Pih KT, Kabilan V, Lim JH, Kang SG, Piao HL, Jin JB, Hwang I. 1999. Characterization of two new channel protein genes in *Arabidopsis*. *Molecules and Cells* **9**: 84–90.

Pla M, Gómez J, Goday A, Pagès M. 1991. Regulation of the abscisic acid-responsive gene *rab28* in maize viviparous mutants. *Molecular and General Genetics* **230**: 394–400.

Polya GM, Klucis E, Haritou M. 1987. Resolution and characterization of two soluble calcium dependent protein kinases from silver beet leaves. *Biochimica et Biophysica Acta* **931**: 68–77.

Porta H, Rueda-Benitez P, Campos F, Colmenero-Flores JM, Colorado JM, Carmona MJ, Covarrubias AA, Rocha-Sosa M. 1999. Analysis of lipoxygenase mRNA accumulation in the common bean (*Phaseolus vulgaris* L.) during development and under stress conditions. *Plant and Cell Physiology* **40**: 850–858.

Putterill J, Robson F, Lee K, Simon R, Coupland G. 1995. The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc-finger transcription factors. *Cell* **80**: 847–857.

Puzio PS, Lausen J, Almeida-Engler J, Cai D, Gheysen G, Grundler FM. 1999. Isolation of a gene from *Arabidopsis thaliana* related to nematode feeding structures. *Gene* **239**: 163–172.

Qi Q, Rose PA, Abrams GD, Taylor DC, Abrams SR, Cutler AJ. 1998. (+)-Abscisic acid metabolism, 3-ketoacyl-coenzyme A synthase gene expression, and very-long-chain mono-

unsaturated fatty acid biosynthesis in *Brassica napus* embryos. *Plant Physiology* **117**: 979–987.

Qin X, Zeevaart JAD. 1999. The 9-*cis*-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proceedings of the National Academy of Sciences, USA* **96**: 15354–15361.

Quesada V, Ponce MR, Micol JL. 2000. Genetic analysis of salt-tolerant mutants in *Arabidopsis thaliana*. *Genetics* **154**: 421–436.

Raghothama KG, Maggio A, Narasimhan ML, Kononowicz AK, Wang G, D'Urzo MP, Hasegawa PM, Bressan RA. 1997. Tissue-specific activation of the osmotin gene by ABA, C_2H_4 and NaCl involves the same promoter region. *Plant Molecular Biology* **34**: 393–402.

Raskin I, Ladyman JAR. 1988. Isolation and characterization of a barley mutant with abscisic-acid insensitive stomata. *Planta* **173**: 73–78.

Razik MA, Quatrano RS. 1997. Effect of the nuclear factors EMBP1 and VIVIPAROUS1 on the transcription of the *Em* gene in HeLa nuclear extracts. *Plant Cell* **9**: 1791–1803.

Reidt W, Wohlfarth T, Ellerström M, Czihal A, Tewes A, Ezcurra I, Rask L, Bäumlein H. 2000. Gene regulation during late embryogenesis: the RY motif of maturation-specific gene promoters is a direct target of the FUS3 gene product. *Plant Journal* **21**: 401–408.

Rezzonico E, Flury N, Meins F Jr, Beffa R. 1998. Transcriptional down-regulation by abscisic acid of pathogenesis-related beta-1,3-glucanase genes in tobacco cell cultures. *Plant Physiology* **117**: 585–592.

Richie SM, Gilroy S. 1998. Abscisic acid signal transduction in the barley aleurone is mediated by phospholipase D activity. *Proceedings of the National Academy of Sciences, USA* **95**: 2697–2702.

Ritchie S, McCubbin A, Ambrose G, Kao T, Gilroy S. 1999. The sensitivity of barley aleurone tissue to gibberellin is heterogeneous and may be spatially determined. *Plant Physiology* **120**: 361–370.

Roberts SK. 1998. Regulation of K^+ channels in maize roots by water stress and abscisic acid. *Plant Physiology* **116**: 145.

Robertson M, Swain SM, Chandler PM, Olszewski NE. 1998. Identification of a negative regulator of gibberellin action, HvSPY, in barley. *Plant Cell* **10**: 995–1007.

Robichaud CS, Wong J, Sussex IM. 1980. Control of *in vitro* growth of viviparous embryo mutants of maize by abscisic acid. *Developmental Genetics* **1**: 325–330.

Rock CD, Quatrano RS. 1994. Plant signals: insensitivity is in the genes. *Current Biology* **4**: 1013–1015.

Rock CD, Quatrano RS. 1995. The role of hormones during seed development. In: Davies PJ, ed. *Plant hormones: physiology, biochemistry, and molecular biology*. Dordrecht, The Netherlands: Kluwer, 671–697.

Rock CD, Quatrano RS. 1996. Lanthanide ions are agonists of transient gene expression in rice protoplasts and act in synergy with ABA to increase *Em* gene expression. *Plant Cell Reports* **15**: 371–376.

Rodriguez PL, Benning G, Grill E. 1998a. *ABI2*, a second protein phosphatase 2C involved in abscisic acid signal transduction in *Arabidopsis*. *FEBS Letters* **421**: 185–190.

Rodriguez PL, Leube MP, Grill E. 1998b. Molecular cloning in *Arabidopsis thaliana* of a new protein phosphatase 2C (PP2C) with homology to *ABI1* and *ABI2*. *Plant Molecular Biology* **38**: 879–883.

Rohde A, De Rycke R, Beeckman T, Engler G, Van Montagu M, Boerjan W. 2000. *ABI3* affects plastid differentiation in dark-grown *Arabidopsis* seedlings. *Plant Cell* **12**: 35–52.

Rohde A, Van Montagu M, Boerjan W. 1999. The *ABSCISIC ACID-INSENSITIVE* 3 (*ABI3*) gene is expressed during vegetative quiescence processes in *Arabidopsis*. *Plant, Cell & Environment* **22**: 261–270.

Rojas A, Almoguera C, Jordano J. 1999. Transcriptional activation of a heat shock gene promoter in sunflower embryos: synergism between ABI3 and heat shock factors. *Plant Journal* **20**: 601–610.

Roncarati R, Salamini F, Bartels D. 1995. An aldose reductase homologous gene from barley: regulation and function. *Plant Journal* **7**: 809–822.

Ross-Macdonald P, Coelho PSR, Roemer T, Agarwal S, Kumar A, Jansen R, Cheung K-H, Sheehan A, Symoniatis D, Umansky L, Heidtman M, Nelson FK, Iwasaki H, Hager K, Gerstein M, Miller P, Roeder GS, Snyder M. 1999. Large-scale analysis of the yeast genome by transposon tagging and gene disruption. *Nature* **402**: 413–418.

Rossi M, Carrari F, Cabrera-Ponce JL, Vazquez-Rovere C, Herrera-Estrella L, Gudesblat G, Iusem ND. 1998. Analysis of an abscisic acid (ABA)-responsive gene promoter belonging to the *Asr* gene family from tomato in homologous and heterologous systems. *Molecular and General Genetics* **258**: 1–8.

Rubery PH, Astle M. 1982. The mechanism of transmembrane abscisic acid transport and some of its implications. In: Wareing PF, ed. *Plant growth substances*. Berlin, Germany: Springer-Verlag, 353–362.

Running MP, Fletcher JC, Meyerowitz EM. 1998. The *Wiggum* gene is required for proper regulation of floral meristem size in *Arabidopsis*. *Development* **125**: 2545–2553.

Sagan C. 1977. The dragons of Eden: speculations on the evolution of human intelligence. New York, USA: Ballantine.

Saleki R, Young PG, Lefebvre DD. 1993. Mutants of *Arabidopsis thaliana* capable of germination under saline conditions. *Plant Physiology* **101**: 839–845.

Sánchez-Serrano JJ, Amati S, Ebneth M, Hildmann T, Mertens R, Peña-Cortés H, Prat S, Willmitzer L. 1991. The involvement of ABA in wound responses of plants. In: Davies WJ, Jones HG, eds. *Abscisic acid: physiology and biochemistry*. Oxford, UK: BIOS Scientific, 201–216.

Sanders D, Brownlee C, Harper JF. 1999. Communicating with calcium. *Plant Cell* **11**: 691–706.

Schena M, Shalon D, Davis RW, Brown PO. 1995. Quantitative monitoring of gene expression patterns with a complementary-DNA microarray. *Science* **270**: 467–470.

Schmidt C, Schelle I, Liao Y-J, Schroeder JI. 1995. Strong regulation of slow anion channels and abscisic acid signaling in guard cells by phosphorylation and dephosphorylation events. *Proceedings of the National Academy of Sciences, USA* **92**: 9535–9539.

Schnall JA, Quatrano RS. 1992. Abscisic acid elicits the water-stress response in root hairs of *Arabidopsis thaliana*. *Plant Physiology* **100**: 216–218.

Schroeder JI, Hagiwara S. 1990. Repetitive increase in cytosolic Ca^{2+} of guard cells by abscisic acid activation of nonselective Ca^{2+} permeable channels. *Proceedings of the National Academy of Sciences, USA* **87**: 9305–9309.

Schultz TF, Medina J, Hill A, Quatrano RS. 1998. 14-3-3 proteins are part of an abscisic acid-*VIVIPAROUS1* (*Vp1*) response complex in the *Em* promoter and interact with VP1 and EMBP1. *Plant Cell* **10**: 837–847.

Schultz TF, Quatrano R. 1997. Surface perception of ABA by rice cells assayed by *Em* gene expression. *Plant Science* **130**: 63–71.

Schultz TF, Spiker S, Quatrano RS. 1996. Histone H1 enhances the DNA binding activity of the transcription factor EmBP-1. *Journal of Biological Chemistry* **271**: 25742–25745.

Schumacher K, Chory J. 2000. Brassinosteroid signal transduction: still casting the actors. *Current Opinion in Plant Biology* **3**: 79–84.

Schwartz A, Wu W-H, Tucker EB, Assmann SM. 1994. Inhibition of inward K^+ channels and stomatal response by abscisic acid: an intracellular locus of phytohormone action. *Proceedings of the National Academy of Sciences, USA* **91**: 4019–4023.

Schwechheimer C, Zourelidou M, Bevan MW. 1998. Plant transcription factor studies. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 127–150.

Seffens WS, Almoguera C, Wilde HD, Haar RAV, Thomas TL. 1990. Molecular analysis of a phylogenetically conserved carrot gene: developmental and environmental regulation. *Developmental Genetics* **11**: 65–76.

Sehnke PC, Henry R, Cline K, Ferl RJ. 2000. Interaction of a plant 14–3-3 protein with the signal peptide of a thylakoid-targeted chloroplast precursor protein and the presence of 14-3-3 isoforms in the chloroplast stroma. *Plant Physiology* **122**: 235–242.

Sheen J. 1996. Ca^{2+}-dependent protein kinases and stress signal transduction in plants. *Science* **274**: 1900–1902.

Sheen J. 1998. Mutational analysis of PP2C involved in ABA signaling in higher plants. *Proceedings of the National Academy of Sciences, USA* **95**: 975–980.

Sheen J, Zhou L, Jang J-C. 1999. Sugars as signalling molecules. *Current Opinion in Plant Biology* **2**: 410–418.

Shen Q, Ho TH. 1997. Promoter switches specific for abscisic acid (ABA)-induced gene expression in cereals. *Physiologia Plantarum* **101**: 653–664.

Shinozaki K, Yamaguchi-Shinozaki K. 1997. Gene expression and signal transduction in water-stress response. *Plant Physiology* **115**: 327–334.

Shiota H, Satoh R, Watabe K, Harada H, Kamada H. 1998. C-ABI3, the carrot homologue of the *Arabidopsis* ABI3, is expressed during both zygotic and somatic embryogenesis and functions in the regulation of embryo-specific ABA-inducible genes. *Plant and Cell Physiology* **39**: 1184–1193.

Shripathi V, Swamy GS, Chandrasekhar KS. 1997. Microviscosity of cucumber (*Cucumis sativus* L.) fruit protoplast membranes is altered by triacontanol and abscisic acid. *Biochimica et Biophysica Acta* **1323**: 263–271.

Siddiqui NU, Chung HJ, Thomas TL, Drew MC. 1998. Abscisic acid-dependent and -independent expression of the carrot late-embryogenesis-abundant class gene *Dc3* in transgenic tobacco seedlings. *Plant Physiology* **118**: 1181–1190.

Silverstone AL, Clampaglio CN, Sun TP. 1998. The *Arabidopsis RGA* gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. *Plant Cell* **10**: 155–169.

Singh KB. 1998. Transcriptional regulation in plants: the importance of combinatorial control. *Plant Physiology* **118**: 1111–1120.

Skriver K, Olsen FL, Rogers JC, Mundy J. 1991. *Cis*-acting DNA elements responsive to gibberellin and its antagonist abscisic acid. *Proceedings of the National Academy of Sciences, USA* **88**: 7266–7270.

Smart CC, Fleming AJ. 1993. A plant gene with homology to D-myo-inositol-3-phosphate synthase is rapidly and spatially up-regulated during an abscisic-acid-induced morphogenic response in *Spirodela polyrrhiza*. *Plant Journal* **4**: 279–293.

Smart CC, Fleming AJ. 1996. Hormonal and environmental regulation of a plant PDR5-like ABC transporter. *Journal of Biological Chemistry* **271**: 19351–19357.

Söderman E, Hjellström M, Fahleson J, Engström P. 1999. The HD-Zip gene *ATHB6* in *Arabidopsis* is expressed in developing leaves, roots and carpels and up-regulated by water deficit conditions. *Plant Molecular Biology* **40**: 1073–1083.

Somerville CR, Meyerowitz EM. 1994. Introduction. In: Meyerwitz EM, Somerville CR, eds. *Arabidopsis*. Plainview, New York, USA: Cold Spring Harbor Laboratory Press, 1–6.

Somerville C, Somerville S. 1999. Plant functional genomics. *Science* **285**: 380–383.

Spollen WG, LeNoble ME, Samuels TD, Bernstein N, Sharp RE. 2000. Abscisic acid accumulation maintains maize primary root elongation at low water potentials by restricting ethylene production. *Plant Physiology* **122**: 967–976.

Staswick PE, Su WP, Howell SH. 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proceedings of the National Academy of Sciences, USA* **89**: 6837–6840.

Staswick PE, Yuen GY, Lehman CC. 1998. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. *Plant Journal* **15**: 747–754.

Staxén I, Pical C, Montgomery LT, Gray JE, Hetherington AM, McAinsh MR. 1999. Abscisic acid induces oscillations in guard-cell cytosolic free calcium that involve phosphoinositide-specific phospholipase C. *Proceedings of the National Academy of Sciences, USA* **96**: 1779–1784.

Steber CM, Cooney SE, McCourt P. 1998. Isolation of the GA-response mutant *sly1* as a suppressor of *ABI1-1* in *Arabidopsis thaliana*. *Genetics* **149**: 509–521.

Sturaro M, Vernieri P, Castiglioni P, Binelli G, Gavazzi G. 1996. The *rea* (*r*ed *e*mbryonic *a*xis) phenotype describes a new mutation affecting the response of maize embryos to abscisic acid and osmotic stress. *Journal of Experimental Botany* **47**: 755–762.

Su J, Shen QX, Ho THD, Wu R. 1998. Dehydration-stress-regulated transgene expression in stably transformed rice plants. *Plant Physiology* **117**: 913–922.

Suzuki M, Kao CY, McCarty DR. 1997. The conserved B3 domain of *Viviparous1* has a cooperative DNA-binding activity. *Plant Cell* **9**: 799–807.

Taylor JE, Renwick KF, Webb AA, McAinsh MR, Furini A, Bartels D, Quatrano RS, Marcotte WR Jr, Hetherington AM. 1995. ABA-regulated promoter activity in stomatal guard cells. *Plant Journal* **7**: 129–134.

Thomann EB, Sollinger J, White C, Rivin CJ. 1992. Accumulation of group 3 late embryogenesis abundant proteins in *Zea mays* embryos: roles of abscisic acid and the *Viviparous-1* gene product. *Plant Physiology* **99**: 607–614.

Thomashow MF. 1999. Plant cold-acclimation genes and regulatory mechanisms. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**: 571–599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.

Timpte C, Wilson AK, Estelle M. 1994. The *axr2-1* mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. *Genetics* **138**: 1239–1249.

Tissier AF, Marillonnet S, Klimyuk V, Patel K, Torres MA, Murphy G, Jones JD. 1999. Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: a tool for functional genomics. *Plant Cell* **11**: 1841–1852.

Trevino MB, O'Connell MA. 1998. Three drought-responsive members of the nonspecific lipid-transfer protein gene family in *Lycopersicon pennellii* show different developmental patterns of expression. *Plant Physiology* **116**: 1461–1468.

Trewavas AJ. 1992. Growth substances in context: a decade of sensitivity. *Biochemical Society Transactions* **20**: 102–108.

Trewavas AJ. 1999. Le calcium, c'est la vie: calcium makes waves. *Plant Physiology* **120**: 1–6.

Ueno O. 1998. Induction of Kranz anatomy and C_4-like biochemical characteristics in a submerged amphibious plant by abscisic acid. *Plant Cell* **10**: 571–583.

Ulmasov T, Hagen G, Guilfoyle TJ. 1997. ARF1, a transcription factor that binds to auxin response elements. *Science* **276**: 1865–1868.

Urao T, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N, Shinozaki K. 1994. Two genes that encode Ca^{2+}-dependent protein kinases are induced by drought and high-salt stresses in *Arabidopsis thaliana*. *Molecular and General Genetics* **244**: 331–340.

Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K. 1999. A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor. *Plant Cell* **11**: 1743–1754.

van Overbeek J, Loeffler JE, Mason MIR. 1967. Dormin (abscisin II), inhibitor of plant DNA synthesis. *Science* **156**: 1497–1499.

Vartanian N, Marcotte L, Giraudat J. 1994. Drought rhizogenesis in *Arabidopsis thaliana*: differential responses of hormonal mutants. *Plant Physiology* **104**: 761–767.

Vasil V, Marcotte WR Jr, Rosenkrans L, Cocciolone SM, Vasil IK, Quatrano RS, McCarty DR. 1995. Overlap of Viviparous1 (Vp1) and abscisic acid response elements in the *Em* promoter: G-box elements are sufficient but not necessary for VP1 transactivation. *Plant Cell* **7**: 1511–1518.

Villanueva J, Canals F, Prat S, Ludevid D, Querol E, Aviles FX. 1998. Characterization of the wound-induced metallocarboxypeptidase inhibitor from potato. cDNA sequence, induction of gene expression, subcellular immunolocalization and potential roles of the C-terminal propeptide. *FEBS Letters* **440**: 175–182.

Vivekananda J, Drew MC, Thomas TL. 1992. Hormonal and environmental regulation of the carrot *lea*-class gene *Dc3*. *Plant Physiology* **100**: 576–581.

Walden R. 1998. The alphabet soup of plant intracellular signalling: enter cyclic nucleotides. *Current Opinion in Plant Biology* **1**: 419–423.

Walker-Simmons MK, Holappa LD, Abrams GD, Abrams SR. 1997. ABA metabolites induce *group 3 LEA* mRNA and inhibit germination in wheat. *Physiologia Plantarum* **100**: 474–480.

Wan Y, Hasenstein KH. 1996. Purification and identification of ABA-binding proteins and antibody preparation. *Journal of Molecular Recognition* **9**: 722–727.

Wang CS, Huang JC, Hu JH. 1999. Characterization of two subclasses of *PR-10* transcripts in lily anthers and induction of their genes through separate signal transduction pathways. *Plant Molecular Biology* **40**: 807–814.

Wang H, Qi Q, Schorr P, Cutler AJ, Crosby WL, Fowke LC. 1998. *ICK1*, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and CycD3, and its expression is induced by abscisic acid. *Plant Journal* **15**: 501–510.

Wang M, Heimovaara-Dijkstra S, Van der Meulen RM, Knox JP, Neill SJ. 1995. The monoclonal antibody JIM19 modulates abscisic acid action in barley aleurone protoplasts. *Planta* **196**: 271–276.

Wang ML, Belmonte S, Kim U, Dolan M, Morris JW, Goodman HM. 1999. A cluster of ABA-regulated genes on *Arabidopsis thaliana* BAC T07M07. *Genome Research* **9**: 325–333.

Wang X. 1999. The role of phospholipase D in signaling cascades. *Plant Physiology* **120**: 645–651.

Weatherwax SC, Williams SA, Tingay S, Tobin EM. 1998. The phytochrome response of the *Lemna gibba NPR1* gene is mediated primarily through changes in abscisic acid levels. *Plant Physiology* **116**: 1299–1305.

Webb AAR, Hetherington AM. 1997. Convergence of the abscisic acid, CO_2, and extracellular calcium signal transduction pathways in stomatal guard cells. *Plant Physiology* **114**: 1557–1560.

Wei T, O'Connell MA. 1996. Structure and characterization of a putative drought-inducible H1 histone gene. *Plant Molecular Biology* **30**: 255–268.

Werner JE, Finkelstein RR. 1995. *Arabidopsis* mutants with reduced response to NaCl and osmotic stress. *Physiologia Plantarum* **93**: 659–666.

White CN, Rivin CJ. 2000. Gibberellins and seed development in maize. II. Gibberellin synthesis inhibition enhances abscisic acid signaling in cultured embryos. *Plant Physiology* **122**: 1089–1097.

Wilson AK, Pickett FB, Turner JC, Estelle M. 1990. A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Molecular and General Genetics* **222**: 377–383.

Windsor M, Milborrow BV, McFarlane IJ. 1992. The uptake of (+)-S- and (−)-R abscisic acid by suspension culture cells of hopbush (*Dodonaea viscosa*). *Plant Physiology* **100**: 54–62.

Winkler RG, Frank MR, Galbraith DW, Feyereisen R, Feldmann KA. 1998. Systematic reverse genetics of transfer-DNA-tagged lines of *Arabidopsis*: isolation of mutations in the cytochrome P450 gene superfamily. *Plant Physiology* **118**: 743–750.

Wobus U, Weber H. 1999. Seed maturation: genetic programmes and control signals. *Current Opinion in Plant Biology* **2**: 33–38.

Wu Y, Kuzma J, Maréchal E, Graeff R, Lee HC, Foster R, Chua NH. 1997. Abscisic acid signaling through cyclic ADP-ribose in plants. *Science* **278**: 2126–2130.

Xiong L, Ishitani M, Lee H, Zhu JK. 1999a. *HOS5*-a negative regulator of osmotic stress-induced gene expression in *Arabidopsis thaliana*. *Plant Journal* **19**: 569–578.

Xiong L, Ishitani M, Zhu, JK. 1999b. Interaction of osmotic stress, temperature, and abscisic acid in the regulation of gene expression in *Arabidopsis*. *Plant Physiology* **119**: 205–211.

Xu L, Zheng S, Zheng L, Wang X. 1997. Promoter analysis and expression of a phospholipase D gene from castor bean. *Plant Physiology* **115**: 387–395.

Yamaguchi-Shinozaki K, Shinozaki K. 1993. Characterization of the expression of a desiccation-responsive *rd29* gene of *Arabidopsis thaliana* and analysis of its promoter in transgenic plants. *Molecular and General Genetics* **236**: 331–340.

Yoon GM, Cho HS, Ha HJ, Liu JR, Lee HS. 1999. Characterization of *NtCDPK1*, a calcium-dependent protein kinase gene in *Nicotiana tabacum*, and the activity of its encoded protein. *Plant Molecular Biology* **39**: 991–1001.

Yoshiba Y, Nanjo T, Miura S, Yamaguchi-Shinozaki K, Shinozaki K. 1999. Stress-responsive and developmental regulation of Δ(1)-pyrroline-5-carboxylate synthetase 1 (P5CS1) gene expression in *Arabidopsis thaliana*. *Biochemical and Biophysical Research Communications* **261**: 766–772.

Zeevaart JAD. 1999. Abscisic acid metabolism and its regulation. In: Hooykaas PJJ, Hall MA, Libbenga KR, eds. *Biochemistry and molecular biology of plant hormones*. Amsterdam, The Netherlands: Elsevier Science, 189–207.

Zeevaart JAD, Creelman RA. 1988. Metabolism and physiology of abscisic acid. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**: 439–473.

Zheng Z, Jin Z, Zhou X, Xia K, Ma C. 1998. An ABA-binding protein with nucleic acid-binding property. *Science in China* C **41**: 209–216.

Zhu J-K, Liu J, Xiong L. 1998. Genetic analysis of salt tolerance in *Arabidopsis*: evidence for a critical role of potassium nutrition. *Plant Cell* **10**: 1181–1191.

Ziegelhoffer EC, Medrano LJ, Meyerowitz EM. 2000. Cloning of the *Arabidopsis* WIGGUM gene identifies a role for farnesylation in meristem development. *Proceedings of the National Academy of Sciences, USA* **97**: 7633–7638.