INVITED REVIEW

Plant hormone interactions during seed dormancy release and germination

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Abstract

This review focuses mainly on eudicot seeds, and on the interactions between abscisic acid (ABA), gibberellins (GA), ethylene, brassinosteroids (BR), auxin and cytokinins in regulating the interconnected molecular processes that control dormancy release and germination. Signal transduction pathways, mediated by environmental and hormonal signals, regulate gene expression in seeds. Seed dormancy release and germination of species with coat dormancy is determined by the balance of forces between the growth potential of the embryo and the constraint exerted by the covering layers, e.g. testa and endosperm. Recent progress in the field of seed biology has been greatly aided by molecular approaches utilizing mutant and transgenic seeds of Arabidopsis thaliana and the Solanaceae model systems, tomato and tobacco, which are altered in hormone biology. ABA is a positive regulator of dormancy induction and most likely also maintenance, while it is a negative regulator of germination. GA releases dormancy, promotes germination and counteracts ABA effects. Ethylene and BR promote seed germination and also counteract ABA effects. We present an integrated view of the molecular genetics, physiology and biochemistry used to unravel how hormones control seed dormancy release and germination.

Keywords: abscisic acid, after-ripening, *Arabidopsis*, auxin, brassinosteroid, coat dormancy, cytokinin,

endosperm-limited germination, ethylene, gibberellin, hormone mutants, *Nicotiana*, seed dormancy, seed germination, signal transduction pathways, transcription factors

Introduction

The German botanist Julius von Sachs (1832-1897) proposed that plants produce, transport and perceive 'organ-forming substances' responsible for the formation and growth of different plant organs. The plant hormones abscisic acid (ABA), gibberellins (GA), ethylene, brassinosteroids (BR), auxins, cytokinins and other signalling molecules have profound effects on plant development at vanishingly low concentrations. They are chemical messengers for the communication among cells, tissues and organs of higher plants. The seeds of higher plants contain an embryo surrounded by covering layers and function to ensure the establishment of a new plant generation. Plant hormones are extremely important for the regulation of seed dormancy and germination (Fig. 1, Koornneef et al., 2002; Finkelstein, 2004). Little is known about the interconnected key molecular processes controlling dormancy and germination in response to hormonal and environmental cues.

Seed dormancy is a temporary failure or block of a viable seed to complete germination under physical conditions that normally favour this (reviewed by Hilhorst, 1995; Bewley, 1997b; Li and Foley, 1997; Baskin and Baskin, 2004). Two major forms of physiological seed dormancy have been described, namely embryo and coat dormancy (sometimes termed coat-enhanced dormancy). Germination commences with the uptake of water by imbibition of the dry seed, followed by embryo expansion. This usually

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Figure 1. Hormonal interactions during tobacco seed dormancy release and germination and their effects on testa and endosperm rupture. Dormancy release involves the light/gibberellin (GA) pathway and results in testa rupture. The light/GA pathway also promotes endosperm rupture. Class I β -1,3-glucanase (β Glu I) and other hydrolases are induced by the light/gibberellin (GA) pathway in the micropylar endosperm and facilitate endosperm rupture and radicle protrusion. Endosperm-specific β Glu I expression and endosperm rupture are inhibited by abscisic acid (ABA). The light/GA pathway also counteracts ABA effects by promoting ABA degradation. Ethylene and brassinosteroids (BR) counteract ABA effects and promote endosperm rupture, but do not affect testa rupture. EREBPs (ethylene responsive element binding proteins) are transcription factors that mediate hormonal regulation of β Glu I expression and endosperm rupture. BR and light/GA promote tobacco endosperm rupture by distinct signal transduction pathways. In addition, seed after-ripening is associated with a decrease in ABA content and sensitivity, and an increase in GA sensitivity or loss of GA requirement. A 'plus' sign means promotion and a 'minus' sign inhibition.

culminates in rupture of the covering layers and emergence of the radicle, generally considered as the completion of the germination process. Radicle protrusion at the completion of seed germination depends on embryo growth driven by water uptake. Uptake of water by a seed is triphasic, with a rapid initial uptake (phase I, i.e. imbibition) followed by a plateau phase (phase II). A further increase in water uptake (phase III) occurs only when germination is completed, as the embryo axis elongates and breaks through its covering structures (Schopfer and Plachy, 1984; Bewley, 1997b; Manz *et al.*, 2005). Cell elongation is necessary, and is generally accepted to be sufficient, for the completion of radicle protrusion; cell division is not essential (Barroco *et al.*, 2005).

In many plant species the seed-covering layers impose a physical constraint to radicle protrusion, which has to be overcome by the growth potential of the embryo. In non-endospermic seeds and in *Arabidopsis*, with only one cell layer of endosperm, the testa (seed coat, diploid maternal tissue) characteristics are mainly responsible for this coat dormancy (Debeaujon and Koornneef, 2000; Debeaujon *et al.*, 2000). In many mature non-endospermic seeds, the cotyledons are the sole storage organs, complete endosperm assimilation occurs during seed development, and the embryo is enclosed by the testa as the only covering layer. The testa imposes a restraint to

radicle protrusion in radish (Schopfer and Plachy, 1993) and Arabidopsis (Debeaujon and Koornneef, 2000; Debeaujon et al., 2000; Rajjou et al., 2004), but is no hindrance to radicle protrusion in rape (Schopfer and Plachy, 1984) and pea (Petruzzelli et al., 2000). In addition to the testa, the covering layers of endospermic seeds include the endosperm, which is usually triploid in angiosperms, with two-thirds of its genome of maternal origin. In endospermic seeds the contributions of both the testa and the endosperm layers to the extent of coat dormancy have to be considered (reviewed by Hilhorst, 1995; Bewley, 1997a; Leubner-Metzger, 2003b). Endosperm rupture is the main germination-limiting process in seeds of the Asteraceae (e.g. lettuce), Solanaceae (e.g. tomato and tobacco) and Rubiaceae (e.g. coffee). In these cases of endosperm-limited germination, weakening of the micropylar endosperm surrounding the radicle tip appears to be required for radicle protrusion, and is likely to involve cell-wall hydrolysis by hydrolytic enzymes. Major recent contributions to our understanding of endosperm-limited germination have come from research on tomato and Nicotiana species (reviewed by Hilhorst, 1995; Bewley, 1997a; Koornneef *et al.*, 2002; Leubner-Metzger, 2003b).

However, recent progress in wider aspects of seed biology has been enhanced greatly by molecular genetic studies with *Arabidopsis thaliana* hormone mutants. This review focuses mainly on eudicot seeds, and on the interactions between ABA, GA, ethylene and BR in controlling the interconnected molecular processes of dormancy release and germination (Figs 1 and 2). We present an overview on how molecular genetics, physiology and biochemistry are helping to unravel the basis of germination and dormancy in seed biology.

Abscisic acid (ABA): a positive regulator of dormancy induction, a negative regulator of germination

Development of 'orthodox seeds' is completed by maturation and desiccation, when storage compounds have accumulated, water content decreases, ABA accumulates, and desiccation tolerance and primary dormancy are established. No receptor for the perception of ABA has been identified conclusively (Finkelstein *et al.*, 2002). However, possible candidates are known (Razem *et al.*, 2004; Osakabe *et al.*, 2005). RPK1, a leucine-rich repeat receptor-like protein kinase, may be a key membrane-bound regulator of early ABA signalling in seeds and seedlings; RPK1 knockout mutant seeds exhibit decreased ABA sensitivity of germination (Table 1). In many plant species endogenous ABA is involved in the induction, and perhaps in the maintenance, of the dormant state (reviewed by Hilhorst, 1995; Bewley, 1997b; Koornneef et al., 2002; Leubner-Metzger, 2003b; Nambara and Marion-Poll, 2003). Overexpression of genes for ABA biosynthesis can increase seed ABA content and enhance seed dormancy or delay germination (Frey et al., 1999; Thompson et al., 2000; Lindgren et al., 2003; Nambara and Marion-Poll, 2003). Enhanced dormancy is also evident in Arabidopsis cyp707a2 mutants with increased ABA content due to a block of seed ABA catabolism (ABA 8' hydroxylase, Table 1, Kushiro et al., 2004). The abscisic aldehyde oxidase 3 (AAO3) protein of Arabidopsis catalyses the final step in ABA biosynthesis and is a target of a selfregulatory loop regulating ABA biosynthesis (Xiong



Figure 2. Schematic representation of the interactions between the gibberellin (GA), abscisic acid (ABA) and ethylene signalling pathways in the regulation of seed dormancy and germination. The model is mainly based on *Arabidopsis* hormone mutant analyses, the positions of some components are speculative, and details are explained in the text. Promotion or inhibition is indicated by thick arrows and blocks, respectively. Interactions based on extragenic suppressor or enhancer screens are indicated by thin grey lines. Small black arrows indicate enhancement (up-arrow) or reduction (down-arrow) of seed dormancy, and small blue arrows indicate enhancement or reduction of seed ABA sensitivity upon mutation of the corresponding protein. Corresponding hormone mutants *of Arabidopsis thaliana: aba1, aba2* = *ABA-deficient1,2; abi1* to *abi5* = *ABA-insensitive1* to *ABA-insensitive5; ctr1* = *constitutive triple response1; ein2, ein3* = *ethylene insensitive2, 3; era3* = *enhanced response to ABA3; gai* = *GA-insensitive; sly1* = *sleepy1; spy* = *spindly; rga* = *repressor-of-ga1-3; rgl 1, rgl2* = *rga-like1, 2.* Other abbreviations: ACO = ACC oxidase; ACS = ACC synthase; EREBP = ethylene responsive element binding protein; ERF = ethylene responsive factor; GA30x = GA 3-oxidase; Man = mannanase; β Glu I = class I β -1,3-glucanase; *vp1* = *viviparous1* (maize mutant). (From Basra, 2005a with permission)

Table 1. St plumbaginij	elected plant hormone folia and Zea mays are	e mutants and their grouped in defined	seed phenotyp classes for abs	es. Hormone mutants cisic acid (ABA), gibb	s of Arabidopsi erellin (GA), e	s thaliana, Lyco ethylene (C ₂ H ₄)	<i>persicon esculen</i>) and brassinos	tum, Nicotiana taba steroids (BR)	icum, Nicotiana
Hormone	Mutant class ^a	Species	Gene/locus ^b	Protein function ^c	Mutant ^d or transgenic line	Dominance ^e	Mutant seed dormancy	Select. other mutant seed effects	References ^f
ABA	Deficiency	A. thaliana	ABA1	ABA biosynthesis	aba1-1	R	Reduced	Vivipary	13, 24, 26, 27
	2	L. esculentum	NOT	ABA biosynthesis	not	R	Reduced	Vivipary	13, 19, 54
			SIT^{W}	ABA biosynthesis	$_{sit}{}^{W}$	R	Reduced	Vivipary	
		Z. mays	VP14	Carotinoid	vp14	R	Reduced	Vivipary	31, 55
				cleavage, ABA					
				biosynthesis					
		N. tabacum		Anti-ABA antibodv			Reduced		42
		N. plumbaginifolia	ABA2	ABA biosynthesis	aba2	R	Reduced	Vivipary	14, 15, 34
				ABA biosynthesis	antisense- ABA2	D	Reduced		
	Overproduction	A. thaliana	CYP707A	ABA degradation	cyp707a2	R	Enhanced		27
	4	N. plumbaginifolia	ABA2	ABA biosynthesis	sense-ABA2	D	Enhanced		14
	Insensitivity	A. thaliana	ABI1	Ser/Thr protein	abi1-1	SD	Reduced	Reduced ABA	2, 26, 30, 33,
				phosphatases				sensitivity of	38, 51
			ABI2	ZC (FTZC) PP2C	abi2-1	SD	Reduced	germuation	
		A. thaliana	ABI3	VP1 / A BI3-type	ahi3	К	Severely	Reduced ABA	1.21.30.
				B3-domain TF		ł	reduced	sensitivity of	33, 36, 38,
								germination;	43, 45
								reduced seed	
		-				¢	-	longevity	
		A. thattana	ABI4	Arz/ekedr if	<i>ab</i> 14	К	INOTIMAL	keaucea AbA sensitivitv	3, 12, 39, 48
								of germination	
		A. thaliana	ABI5	bZIP TF	abi5	R	Normal	Reduced ABA	3, 11, 12,
								sensitivity of	32, 33
		A thaliana	4 R18	~	ahis	~	6	germination Reduced ARA	Ľ
		× ×• ••••••••	01017		0122			sensitivity	0
		A thelicano	1700	Tourism T	1-	Q	Doducod (2)	of germination	202
		т	IN IN	reneat recentor-like	TVdi	4	menarca (:)	sensitivity	07 a
				kinase				of germination	
		Z. mays	VP1	VP1/ABI3-type B3-domain TF	vp1	R	Reduced	Vivipary	1, 21, 35, 47

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Hormone	Mutant class ^a	Species	Gene/locus ^b	Protein function ^c	Mutant ^d or transgenic line	Dominance ^e	Mutant seed dormancy	Select. other mutant seed effects	References ^f
	Hypersensitivity or constitutive response	A. thaliana	ERA1	Farnesyl transferase	era1	R	Enhanced		2, 8, 16, 46
			ERA3	EIN2 allele	era3	R	Enhanced		
		A. thaliana	SAD1	Sm-like snRNP	sad1	R	Enhanced	ABA hyper- sensitive	56
		A. thaliana	ABH1	protein mRNA	abh1	R	د:	germination ABA hyper-	22
				cap-binding protein				sensitive germination	
GA	Deficiency	A. thaliana	GA1	GA biosynthesis	ga1	R	Enhanced	D	9, 25, 26, 41 44 51
		L. esculentum	GIB-1	GA biosynthesis	gib-1	R	Enhanced		71, 77, 91 20, 25, 26, 44
	Insensitivity	A. thaliana	GAI	DELLA-type GRAS TF	<i>gai</i> gain-of function	SD	Enhanced		10, 26, 41, 44
		A. thaliana	SLY1	F-box protein	sly1	R	Enhanced		41, 50, 51, 57
	Hypersensitivity or	A. thaliana	GAI	DELLA-type	gai-t6,	R	Reduced		6, 10,
	constitutive response			GRAS TF	loss-of function				26, 41, 44
	-		RGL2	DELLA-type GRAS TF	rgl2, loss-of function	R	Reduced		
		A. thaliana	SPY	O-GlcNAc transferase	ĥds	R	Reduced		18, 23, 41, 52, 54, 57
C_2H_4	Insensitivity	A. thaliana	ETR1	Ethylene	etr1-1	D	Enhanced	ABA hyper-	2, 4, 16, 17, 60, 40
				receptor				sensitive germination	17, 29, 40
			EIN2	Metal transmer?	ein2	R	Enhanced	ABA hyper-	2, 17
								germination	
			EIN3	TF	ein3	R	¢.		49
	Hypersensitivity or constitutive response	A. thaliana	CTR1	Raf-like protein kinase (MAPKKK)	ctr1	Я	Slightly reduced	Slightly reduced ABA sensitivity of germination	2, 7, 17, 40

Hormone interactions during dormancy release and germination

Table 1. Continued

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Table	Tante

	germination								
	sensitive				4			`	
28, 50, 53	germination ABA hyper-		R	bri1-1	BR receptor	BRI1	A. thaliana	Insensitivity	
	sensitive								
50	ABA hyper-		R	det2-1	BR biosynthesis	DET2	A. thaliana	Deficiency	BR
References ^f	effects	dormancy	Dominance ^e	line	Protein function ^c	Gene/locus ^b	Species	Mutant class ^a	Hormone
	Select. other mutant seed	Mutant seed		Mutant ^a or transcenic					

^a Four mutant classes either altered in hormone biosynthesis (hormone-deficient mutants, hormone-overproducing mutants) or in hormone response/signal transduction (hormone-insensitive mutants, constitutive response/hormone-hypersensitive mutants).

ctr1 = constitutive triple response1; det2-1 = de-etiolated2; ein2, ein3 = ethylene insensitive2, 3; era1, era3 = enhanced response to ABA1, ABA3; etr1 = ethylene resistant1; ^b Mutant abbreviations: aba1, aba2 = ABA-deficient1, 2; abh1 = ABA-lupersensitive1; abi1 to abi8 = ABA-insensitive1 to ABA-insensitive8; bri1 = Brassinosteriod-insensitive1; ga1 = GA-deficient1; gai = GA-insensitive; gib-1 = GA-deficient-1; not = notabilis; $sit^W = sitiens^W$; sly1 = sleepy1; spy = spindly; rga = repressor-of-ga1-3; rgl2 = rga-like2; pk1 = receptor-like protein kinase1; sad1 = supersensitive to ABA and drought; spy = spindly; vp1 = viviparous1^c TF = transcription factor.

^d In some cases several alleles, in some cases specific alleles, are given.

^e Dominance over wild-type locus: D = dominant, SD = semi-dominant, R = recessive.

Selected references: 1, Baumbusch et al. (2004); 2, Beaudoin et al. (2000); 3, Bensmihen et al. (2005); 4, Bleecker et al. (1988); 5, Brocard-Gifford et al. (2004); 6, Cao et al. (2005); 7, Chang (2003); 8, Cutler *et al.* (1996); 9, Debeaujon and Koornneef (2000); 10, Derkx and Karssen (1993); 11, Finkelstein and Lynch (2000); 12, Finkelstein *et al.* (2002); 13, Finkelstein et al. (1998); 14, Frey et al. (1999); 15, Frey et al. (2004); 16, Ghassemian et al. (2000); 17, Hall et al. (2001); 18, Hartweck et al. (2002); 19, Hilhorst (1995); 20, Hilhorst Ohta *et al.* (2000); 39a, Osakabe *et al.* (2005); 40, Ouaked *et al.* (2003); 41, Peng and Harberd (2002); 42, Phillips *et al.* (1997); 43, Raz *et al.* (2001); 44, Richards *et al.* (2001); 45, Phillips *et al.* (2000); 39a, Osakabe *et al.* (2001); 44, Richards *et al.* (2001); 45, Phillips *et al.* (2000); 39a, Osakabe *et al.* (2001); 44, Richards *et al.* (2001); 45, Phillips *et al.* and Karssen, 1992; 21, Holdsworth et al. (2001); 22, Hugouvieux et al. (2002); 23, Izhaki et al. (2001); 24, Karssen et al. (1983); 25, Karssen et al. (1989); 26, Koornneef and Karssen (1994); 27, Kushiro et al. (2004); 28, Leubner-Metzger (2001); 29, Leubner-Metzger et al. (1998); 30, Leung and Giraudat (1998); 31, Liotenberg et al. (1999); 32, Lopez-Molina *et al.* (2001); 33, Lopez-Molina *et al.* (2003); 34, Marin *et al.* (1996); 35, McCarty (1995); 36, Nambara *et al.* (1992); 37, Nambara *et al.* (1998); 38, Nambara *et al.* (2002); 39, Molina *et al.* (2003); 39, Molina *et al.* (2003); 34, Marin *et al.* (2003); 35, Molina *et al.* (2001); 33, Molina *et al.* (2003); 34, Marin *et al.* (2003); 35, Molina *et al.* (2001); 35, Molina *et al.* (2003); 34, Molina *et al.* (2003); 34, Molina *et al.* (2003); 34, Molina *et al.* (2003); 35, Molina *et al.* (2003); 34, Molina *et al.* (2003); 34, Molina *et al.* (2003); 35, Molina *et al.* (2003); 34, Molina *et al.* (2003); 34, Molina *et al.* (2003); 35, Molina *et al.* (2003); 34, Molina *et al.* (2003); 35, Molina *et al.* (2003); 34, Molina *et al.* (2003); 35, Molina *et al.* (2003); 36, Molina *et al.* (2003); 36, Molina *et al.* (2003); 36, Molina *et al.* (2003); 37, Molina *et al.* (2003); 37, Molina *et al.* (2003); 37, Molina *et al.* (2003); 38, Molina *et al.* (2003); 37, Moli Rohde et al. (2000); 46, Ross and O'Neill (2001); 47, Schwechheimer and Bevan (1998); 48, Söderman et al. (2000); 49, Solano et al. (1998); 50, Steber and McCourt (2001); 51. Steber et al. (1998); 52, Swain et al. (2001); 53, Szekeres (2003); 54, Thompson et al. (2000); 55, White et al. (2000); White and Rivin (2000); 56, Xiong et al. (2001); 57, Yamaguchi and Kamiva (2002) *et al.*, 2001). The *aao3* mutants with reduced seed ABA biosynthesis exhibit reduced dormancy (Gonzalez-Guzman *et al.*, 2004).

In contrast to ABA overproduction, ABA deficiency during seed development is associated with the absence of primary dormancy of the mature seed (Table 1). ABA production during seed development can be of dual origin, the embryo and/or the maternal tissues. Reciprocal crosses and/or grafting experiments between wild-type and ABA-deficient mutants of Arabidopsis (Karssen et al., 1983; Koornneef and Karssen, 1994; Nambara and Marion-Poll, 2003), tomato (Groot and Karssen, 1992; Hilhorst, 1995) and Nicotiana plumbaginifolia (Frey et al., 1999) have shown that only ABA produced by the embryo itself during seed development is necessary to impose a lasting dormancy. Maternal ABA produced by the seedcovering layers, or ABA application (resembling maternal ABA) during seed development, both fail to induce seed dormancy. It is not known if maternal ABA penetrates the embryonic axis, but it is known that it affects aspects of seed development other than dormancy (Finkelstein, 1994; Koornneef and Karssen, 1994). For example, it increases both seed yield and rate of embryo growth in N. plumbaginifolia (Frey et al., 2004) and promotes carrot embryo growth (Homrichhausen et al., 2003). This suggests either that the covering layers control embryo growth, or that maternal ABA penetrates the zygotic embryonic axis. Why it does not affect dormancy is unknown.

The formation of non-dormant seeds and precocious germination on the mother plant (vivipary) can occur in ABA-deficient biosynthesis mutants (Table 1), e.g. in aba1 and aba2 of Arabidopsis (Karssen et al., 1983; Koornneef and Karssen, 1994), sitiens (sit^w) and notabilis (not) of tomato (Hilhorst, 1995; Thompson et al., 2000), aba2 of N. plumbaginifolia (Marin et al., 1996), and in several viviparous (vp) mutants of maize (Liotenberg et al., 1999; White et al., 2000). In the aba2 mutant of N. plumbaginifolia, ABA deficiency is due to a mutation in the ABA2 gene, encoding zeaxanthin epoxidase, a key step in ABA biosynthesis (Marin et al., 1996). Antisense and sense ABA2 transformation of N. plumbaginifolia results in decreased and increased ABA biosynthesis and seed dormancy, respectively (Table 1, Frey et al., 1999). The onset of dormancy induction in Nicotiana tabacum is associated with a peak in ABA at approximately 15-20 d after pollination (DAP), followed by a rapid decline in ABA during later seed maturation. Tobacco dormancy is present in seeds harvested 25 DAP (Yamaguchi-Shinozaki et al., 1990; Jiang et al., 1996; Phillips et al., 1997; Leubner-Metzger, 2005b). Seed dormancy is not established in transgenic tobacco expressing an anti-ABA antibody that causes a deficiency in free ABA (Phillips et al., 1997).

The Arabidopsis ABA-insensitive (abi) response mutants, abi1 to abi5 and abi8, have been identified

by selecting for seeds capable of germination and good seedling growth in the presence of ABA concentrations that are inhibitory to the wild type (Koornneef and Karssen, 1994; Leung and Giraudat, 1998; Brocard-Gifford et al., 2004). Like the ABAdeficient mutants, several of these ABA-insensitive mutants also exhibit a marked reduction in seed dormancy (Table 1). The original abi1 mutant seed phenotype was described as having: (1) reduced dormancy, which can be broken by chilling or dry storage; (2) reduced ABA sensitivity of germination; and (3) no precocious germination (Karssen et al., 1990). The ABI1 and ABI2 genes encode paralogous serine/threonine protein phosphatases 2C (PP2C) (Leung and Giraudat, 1998; Beaudoin et al., 2000; Schweighofer et al., 2004). The abi1-1 and abi2-1 mutations are dominant and lead to the ABAinsensitive seed phenotype and to vegetative responses. In contrast to the gain of function mutant allele abi1-1 response, loss-of-function alleles of ABI1 lead to an ABA-hypersensitive response, indicating that the ABI1 PP2C is a negative regulator of ABA responses. However, results from overexpression and microinjection experiments are not compatible with a negative regulatory role of ABI1 (reviewed in Schweighofer et al., 2004). Transcripts of a PP2C are synergistically up-regulated by ABA and calcium in dormant seeds of Fagus sylvatica (Lorenzo et al., 2002).

The seed responses of strong alleles of the Arabidopsis ABI3 gene (Table 1) are severe compared to the abi1, abi2 and the ABA-deficient mutant alleles (Leung and Giraudat, 1998; Schwechheimer and Bevan, 1998; Raz et al., 2001; Finkelstein, 2004). ABI3 gene promoter activity has been detected in seeds and in vegetative meristems (Rohde et al., 1999; Ng et al., 2004). ABI3 might play a major role in seed and bud dormancy (Rohde et al., 2000). The ABA-insensitive *viviparous1 (vp1)* mutant of maize is characterized by severe seed responses, including reduced sensitivity of germination to exogenous ABA and vivipary (e.g. McCarty, 1995; Li and Foley, 1997; Schwechheimer and Bevan, 1998). The Arabidopsis ABI3 and the maize VP1 are orthologous genes that encode transcription factors of the B3 domain class that are essential for ABA action. Other members of this class influencing germination include the wild oat AfVP1 (Jones et al., 2000), and the Arabidopsis FUS3 and LEC2 (Rohde et al., 2000; Mönke et al., 2004). Binding of ABI3 and FUS3 to the RY motif located in promoter elements has been shown (Mönke et al., 2004); the B3 motif is both necessary and sufficient for DNA binding of both transcription factors. VP1/ABI3-like proteins can function as activators and repressors of ABAdependent and -independent gene expression in seeds (e.g. Ezcurra et al., 2000; Holdsworth et al., 2001; Suzuki et al., 2001; Clerkx et al., 2003; Zeng et al., 2003). Ectopic expression of ABI3 or VP1 in Arabidopsis

confers ABA- and seed-specific responses to vegetative tissues (Leung and Giraudat, 1998; Suzuki et al., 2001; Ng et al., 2004), suggesting that the VP1/ABI3like proteins are multifunctional transcription factors that integrate ABA and other regulatory signals of seed maturation and developmental arrest. However, expression studies in severely and moderately dormant ecotypes of Arabidopsis suggest that ABI3 is not the major immediate regulator required for the establishment of dormancy (Baumbusch et al., 2004). Factors that interact with VP1/ABI3 have been identified, and it has been proposed that the VP1/ ABI3-like transcription factors not only regulate seed responses, but function as general regulators for the timing of developmental transitions throughout the life cycle of plants (Hobo et al., 1999; Rohde et al., 2000; Holdsworth et al., 2001). Post-translational targeting of ABI3 for protein degradation (Zhang et al., 2005) and perhaps also farnesylation of ABI3 (Brady et al., 2003) are mechanisms to regulate ABI3-mediated ABA signalling. ABA induction of ABI1 and ABI2, possible negative regulators of ABA signaling, is inhibited by VP1/ABI3 in a feed-forward pathway (Suzuki et al., 2003). Mutation alleles like *abi3-4* show reduced seed longevity and reduced chlorophyll breakdown, which may be partially related to the reduced seed dormancy phenotype (Clerkx et al., 2003).

In another feed-forward pathway, VP1/ABI3 activates ABI5 (Table 1), a positive regulator of ABA signalling (Nambara and Marion-Poll, 2003; Suzuki et al., 2003; Nakabayashi et al., 2005). The Arabidopsis ABI5 gene encodes a basic leucine zipper (bZIP) transcription factor that binds to the ABA responsive cis-acting element (ABRE) of ABA-induced gene promoters. Physical interaction of ABI3 and ABI5 has been demonstrated and appears to be important for the transcriptional activation of genes. Recently, a nuclear protein, Imbibition-inducible 1 (IBM1) was identified that promotes germination of Arabidopsis seeds by negatively regulating the ABA transduction pathway (Duque and Chua, 2003). Loss-of-function imb1 mutants have higher ABI5 contents than wild type. ABI5 is important in determining ABA responsiveness during embryogenesis, seed maturation and regulating the transition after germination to vegetative growth (Finkelstein and Lynch, 2000; Lopez-Molina et al., 2001, 2002; Bensmihen et al., 2002, 2005; Lu et al., 2002). A post-germination developmental arrest checkpoint is mediated by ABA and requires the ABI5 transcription factor. Degradation of ABI5 is regulated by interaction with the ABI five binding protein (AFP), which itself is induced by ABA. In Arabidopsis, two mutant alleles, *afp-1* and *afp-2*, have been identified that confer hypersensitivity to ABA (Lopez-Molina et al., 2003).

The transition from water uptake (phases 1 and 2) during germination to water uptake during

post-germination growth (phase 3) is inhibited by ABA. ABA inhibits phase 3 water uptake, endosperm rupture, further embryo extension and seedling growth after radicle emergence. However, ABA does not inhibit initial imbibition of water, initial embryo extension growth or testa rupture, e.g. of rape (Schopfer and Plachy, 1984), N. plumbaginifolia (Frey et al., 2004), N. tabacum (Manz et al., 2005), carrot (Homrichhausen et al., 2003), and Arabidopsis (Müller et al., 2005). ABA may inhibit embryo growth by regulating ion-channel activities, aquaporin abundance or other tissue-specific alterations of water uptake (Gao et al., 1999; Manz et al., 2005). The inhibition by ABA of Arabidopsis and tobacco seed germination is not a consequence of inhibition of storage lipid mobilization (Pritchard et al., 2002; Penfield et al., 2004; Manz et al., 2005). Two independent programmes appear to operate; one is inhibited by ABA and governs developmental growth resulting in germination, and a second, largely ABAindependent, programme governs storage lipid mobilization.

Arabidopsis ABI4 (Table 1) shows the greatest sequence homology with ethylene-responsive element binding protein (EREBP)-type transcription factors, and contains an APETALA2 (AP2)-like DNA binding domain characteristic of AP2/EREBP family transcriptional regulators (Finkelstein et al., 1998; Ohta et al., 2000; Söderman et al., 2000). ABI4 is likely to be seed-specific and may act downstream of ABI3 or in a parallel pathway. ABA signal transduction via ABI4 is also involved in responses of seeds to sugars (Wobus and Weber, 1999; Huijser et al., 2000; Finkelstein and Gibson, 2002; Finkelstein, 2004). High sugar induces both ABA biosynthesis and expression of ABI3, ABI4 and ABI5. Germination of the abi4 mutant is insensitive to ABA and sugar; their signalling occurs in either parallel or intersecting pathways. ABI3, ABI4 and ABI5 interact, and it is proposed that these transcription factors are key factors in a signalling network that regulates ABA-related seed responses (Brocard-Gifford et al., 2003; Finkelstein, 2004; Katagiri et al., 2005). Another component of this signaling pathway is MARD1 (Mediator of ABA-regulated dormancy 1). Mard1 mutant seeds are insensitive to external ABA, are less dormant than wild-type seeds and are able to germinate in complete darkness (He and Gan, 2004).

Several *Arabidopsis* ABA-hypersensitive response mutants (Table 1), including *enhanced response to ABA1* (*era1*) and *supersensitive to ABA and drought* (*sad1*), exhibit enhanced seed dormancy (Cutler *et al.*, 1996; Ghassemian *et al.*, 2000; Xiong *et al.*, 2001; Brady *et al.*, 2003). Their germination is inhibited by low ABA concentrations that do not affect the wild type. The *ERA1* gene encodes the β -subunit of a farnesyl transferase, and it is proposed that a negative

regulator of ABA sensitivity is modulated by protein farnesylation. The SAD1 gene encodes a Sm-like U6 small nuclear riboprotein and appears to be involved in the self-regulatory loop that regulates ABA biosynthesis by affecting the AAO3 transcript content. Germination of the ABA-hypersensitive1 (abh1) mutant is also hypersensitive to ABA, and ABH1 encodes an mRNA cap-binding protein (Hugouvieux et al., 2002). Further proteins that are associated with RNAdependent processes in germinating seeds are two ribosomal proteins (Toorop et al., 2005). These findings are in agreement with the view that translation of stored mRNAs is required for seed germination and that de novo mRNA synthesis controls germination rate and onset (Rajjou et al., 2004). Two other ABA hypersensitive response mutants, atpirin1 and gpa1 are impaired in a G-protein-coupled signal transduction pathway (Lapik and Kaufman, 2003), and the ABAhypersensitive germination (ahg) mutants exhibit an altered ABI5 expression pattern (Nishimura et al., 2004). However, not all Arabidopsis mutants showing reduced dormancy are altered in ABA biosynthesis or sensitivity (e.g. Leon-Kloosterziel et al., 1996; Papi et al., 2000; Peeters et al., 2002).

ABA is not only a positive regulator of dormancy induction; it also inhibits seed germination, has a role during after-ripening and has been proposed to be a positive regulator of dormancy maintenance. Afterripening, i.e. a period of dry storage at room temperature of freshly harvested, mature seeds, is a common method to facilitate dormancy release (Bewley, 1997b; Leubner-Metzger, 2003b). A decline in ABA content, decreased sensitivity to ABA and increased sensitivity to gibberellins (GA) are associated with the after-ripening-mediated transition from the dormant to the non-dormant state of many species (e.g. Hilhorst, 1995; Benech-Arnold et al., 1999; Beaudoin et al., 2000; Grappin et al., 2000; Romagosa et al., 2001; Koornneef et al., 2002; Leubner-Metzger, 2002; Schmitz et al., 2002; Ali-Rachedi et al., 2004; Feurtado et al., 2004). An exception is the seed of Avena fatua, in which ABA and after-ripening, but not GA, are the primary regulators of seed dormancy (Fennimore and Foley, 1998). High ABA contents are present in the strongly dormant seeds of an Arabidopsis ecotype (Cape Verde Island, Cvi), but not in afterripened, non-dormant ones (Ali-Rachedi et al., 2004). The carotenoid biosynthesis inhibitors, norflurazon and fluridone, have been used in experiments to inhibit ABA biosynthesis (e.g. Grappin et al., 2000; Ali-Rachedi et al., 2004; Chae et al., 2004; da Silva et al., 2004). Grappin et al. (2000) demonstrated that freshly harvested N. plumbaginifolia seeds have higher ABA content and sensitivity. Moreover, de novo ABA biosynthesis occurs in imbibed fresh, but not imbibed after-ripened seeds. This de novo ABA biosynthesis occurs very early upon imbibition of fresh seeds. Grappin et al. (2000) also added gibberellins (GA) or fluridone to the seeds, both of which prevented ABA biosynthesis and accelerated the rate of germination. Simultaneous addition of GA + ABA or fluridone + ABA negated the germination-promoting effects. Expression studies of ABA-regulated genes in N. tabacum seeds are in agreement with decreased ABA content and sensitivity in after-ripened seeds, and suggest a common role for ABA during the afterripening-mediated release from dormancy and control of germination rate of Nicotiana species (Leubner-Metzger and Meins, 2000; Leubner-Metzger, 2002, 2005; Bove et al., 2005). ABA inhibits embryo growth potential and endosperm cap weakening during coffee seed germination (da Silva et al., 2004). A transient rise in ABA content in the embryo was evident early during imbibition. ABA treatment inhibits, and fluridone treatment accelerates, radicle protrusion of coffee seeds. Vegetation-derived ABA is also of ecological importance in the regulation of seed dormancy and germination. ABA leached from plant litter plays an important role in the germination control of the post-fire annual Nicotiana attenuata (Krock *et al.*, 2002; Preston *et al.*, 2002; Schwachtje and Baldwin, 2004).

Rupture of the testa and the endosperm are distinct and temporally separate events during the germination of Nicotiana seeds (Fig. 1; Leubner-Metzger, 2003b; Web site: 'The Seed Biology Place' http://www. seedbiology.de). Such two-step germination, with testa rupture subsequently followed by endosperm rupture, has also been demonstrated recently for Arabidopsis (Liu et al., 2005; Müller et al., 2005). The after-ripening-mediated promotion of tobacco germination is due to the promotion of both testa and subsequent endosperm rupture (Leubner-Metzger, 2005b). The addition of ABA to the medium during imbibition has effects resembling those of maternal ABA during seed development and residual ABA in mature seeds. It does not appreciably affect the kinetics of testa rupture, but it delays endosperm rupture and results in the formation of a novel structure, consisting of the enlarged radicle with a sheath of greatly elongated endosperm tissue (Leubner-Metzger, 2003b). The visible distinction between testa and endosperm rupture, and the finding that ABA inhibits endosperm rupture, but not testa rupture, is typical for the seed germination of the Cestroideae (Nicotiana, Petunia) subgroup of the Solanaceae (Krock et al., 2002; Petruzzelli et al., 2003a). A visible distinction between testa rupture and endosperm rupture is not possible for the seed germination of the *Solanoideae* (*Capsicum*, *Lycopersicon*) subgroup of the Solanaceae (Wu et al., 2000; Leubner-Metzger, 2003b; Petruzzelli et al., 2003a), nor the Asteraceae (Lactuca). However, ABA also inhibits the germination of Solanoideae-type seeds, and

experiments with the ABA-deficient sit^w mutant of tomato demonstrate that ABA deficiency is associated with a thinner testa (Hilhorst and Downie, 1995; Toorop et al., 2000). In Arabidopsis, an arabinogalactanprotein (APG30) regulates the timing of germination by modulating ABA perception and presumably by altering the force generated by the penetrating radicle (Van Hengel and Roberts, 2003). Class I β -1,3glucanase (BGlu I) is induced after testa rupture and just prior to endosperm rupture of Nicotiana seeds (Leubner-Metzger, 2003b). This induction is exclusively localized in the micropylar endosperm where the radicle will emerge. ABA inhibits the induction of βGlu I genes during tobacco seed germination and specifically delays endosperm rupture. Direct evidence for a causal role of β Glu I during endosperm rupture comes from sense-transformation of N. tabacum with a chimeric ABA-inducible BGlu I transgene (Leubner-Metzger and Meins, 2000). Sense-BGlu I transformation results in overexpression of BGlu I in seeds and promotes endosperm rupture of fresh, mature seeds and ABA-treated after-ripened seeds. BGlu I overexpression in the seed-covering layers can replace the promoting effect of afterripening on testa rupture (Leubner-Metzger, 2002, 2005). ABA inhibition of germination and βGlu accumulation in the micropylar endosperm appears to be a widespread event during the germination of Solanaceous species (Wu et al., 2000; Leubner-Metzger, 2003b; Petruzzelli et al., 2003a).

Gibberellins (GA) release dormancy, promote germination and counteract ABA effects

According to the revised hormone-balance hypothesis for seed dormancy proposed by Karssen and Lacka (1986), ABA and GA act at different times and sites during 'seed life'. ABA induces dormancy during maturation, and GAs play a key role in dormancy release and in the promotion of germination. GA biosynthesis in developing seeds of many species leads to the accumulation and storage of either bioinactive GA precursors or bioactive GA (e.g. Groot et al., 1987; Toyomasu et al., 1998; Kamiya and Garcia-Martinez, 1999; Yamaguchi et al., 2001). GA biosynthesis in developing seeds appears not to be involved in the establishment of primary dormancy per se (Karssen and Laçka, 1986; Groot et al., 1987; Koornneef and Karssen, 1994; Bewley, 1997b), but in other aspects of seed development, including fertilization, embryo growth, assimilate uptake, fruit growth and the prevention of seed abortion, in tomato, pea and several species of the Brassicaceae (e.g. Groot et al., 1987; Swain et al., 1997; Batge et al., 1999; Hays et al., 2002; Koornneef et al., 2002; Singh et al., 2002). However, experiments with ABAdeficient and -insensitive mutants of maize, and with

GA biosynthesis inhibitors, demonstrate that GA is a positive regulator of vivipary (White and Rivin, 2000; White *et al.*, 2000). Bioactive GAs accumulate prior to the ABA peak during embryo development of maize. Inhibition of GA biosynthesis mimics the effects of exogenous ABA, e.g. in suppressing vivipary. Interestingly, the GA/ABA ratio, and not the absolute hormone amounts, appears to control vivipary. Thus, it is possible that GA directly antagonizes ABA signalling during maize kernel development.

The temporal and spatial expression pattern of GA biosynthesis genes has been investigated during Arabidopsis seed germination (Yamaguchi et al., 2001; Ogawa et al., 2003; Yamauchi et al., 2004). Bioactive GAs accumulate just prior to radicle protrusion and appear to occur in two separate locations within the embryo: (1) the early biosynthetic pathway, including the geranylgeranyl diphosphate cyclization reaction catalysed by ent-copalyl diphosphate synthetase (CPS), in the provascular tissue where CPS1 gene promoter activity is localized, and (2) the late biosynthetic pathway, including the formation of bioactive GA by GA 3-oxidase, in the cortex and endodermis of the root, where GA3ox1 and GA3ox2 transcripts accumulate, and GA3ox2 gene promoter activity is localized. This implies that intercellular transport of an intermediate of the GA biosynthetic pathway (probably ent-kaurene) is required to produce bioactive GA. Two functions for GA during seed germination have been proposed (reviewed by Hilhorst, 1995; Bewley, 1997a, b; Koornneef et al., 2002; Leubner-Metzger, 2003b). First, GA increases the growth potential of the embryo. Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers, by weakening of the tissues surrounding the radicle. The localization of seed GA biosynthesis in the Arabidopsis radicle (Yamaguchi et al., 2001) is consistent with the hypothesis that embryonic GA is released and triggers the weakening of seed-covering layers. This is further supported by the finding that at least some GAresponsive genes are expressed in non-GA-producing seed tissues (Ogawa et al., 2003). Environmental cues like light and temperature can alter the tissue-specific localization of GA biosynthesis (Yamauchi et al., 2004). The temporal and spatial pattern of GA biosynthesis and sensitivity are both important for the GAmediated seed responses.

GA-deficient biosynthesis mutants (Table 1) of *Arabidopsis* (e.g. *ga1*) and tomato (e.g. *gib-1*) have been isolated (Karssen *et al.*, 1989; Hilhorst and Karssen, 1992; Koornneef and Karssen, 1994; Richards *et al.*, 2001). Seed germination of several of these GA-deficient mutants absolutely depends on the addition of GA to the medium during imbibition. The *Arabidopsis GA1* gene encodes a CPS, which catalyses a key cyclization step in early GA biosynthesis. It was

cloned by genomic subtraction based on a 5-kb deletion in the severe ga1-3 mutant allele (Sun and Kamiya, 1994). The mechanisms imposing a GA requirement to promote the germination of dormant and non-dormant Arabidopsis seeds have been analysed using the GA-deficient mutant ga1, the ABAdeficient mutant aba1, and several testa mutants, which exhibit reduced seed dormancy (Debeaujon and Koornneef, 2000; Debeaujon et al., 2000). Testa mutants are not resistant to GA biosynthesis inhibitors. However, in the presence of the inhibitors, or when transferred to a GA-deficient background, they are more sensitive to exogenous GA than the wild type. The germination capacity of the gal-1 mutant can be restored, without the help of exogenous GA, by removing the surrounding testa and endosperm, or by incorporating this mutation into a testa-mutant background. Debeaujon and Koornneef (2000) concluded that dormancy and germination are the net result of a balance between many promoting and inhibiting factors, including GA and ABA, which have the embryo and the testa as targets. Their results support the view that the GA requirement for dormancy release and germination is determined by: (1) ABA produced in the developing seeds and/or the state of dormancy set by ABA; and (2) the amount of ABA produced upon imbibition, especially in dormant seeds. Furthermore, when the restraint to radicle protrusion imposed by the seed envelopes is weakened by testa mutations, the embryo growth potential threshold required for germination is lowered. Thus, the GA requirement for Arabidopsis seed germination is determined by testa characteristics, embryonic growth potential and by embryonic ABA.

GA appears to regulate dormancy release and germination positively, by a complex interaction with ABA and environmental conditions. Regarding temperature as an environmental condition, Gonai et al. (2004) concluded that ABA is an important factor in the regulation of thermoinhibition of lettuce seed germination, and that GA affects the temperature responsiveness of the seeds through ABA metabolism. In members of the Asteraceae (e.g. lettuce), Rubiaceae (e.g. coffee) and Solanaceae (e.g. tomato, pepper and tobacco), the micropylar endosperm and testa tissues impose a constraint to radicle protrusion (Ni and Bradford, 1993; Hilhorst, 1995; Bewley, 1997a; Leubner-Metzger, 2003b; da Silva et al., 2005). The assumption that the micropylar tissues impose a physical constraint to radicle protrusion is supported by puncture force measurements and surgical experiments. Removal of the micropylar testa and the endosperm tissues permits radicle growth under conditions that inhibit germination of intact seeds, e.g. of tobacco (Kincaid, 1935; Leubner-Metzger, 2003b), tomato (Liptay and Schopfer, 1983; Hilhorst, 1995) and lettuce (Bewley, 1997a; Dutta et al., 1997). Since GA-deficient tomato gib-1 mutant seeds germinate only upon treatment with GA, and isolated gib-1 embryos elongate without a GA requirement, it can be concluded that the GA is needed to overcome the constraint of the seed coverings (Bassel et al., 2004). In tomato and Arabidopsis, physiological, biochemical and genetic evidence suggests a role for GA in weakening the structures covering the embryo during germination (Bewley, 1997a; Yamaguchi and Kamiya, 2002; Yamauchi et al., 2004). Weakening of the micropylar endosperm appears to be a prerequisite for germination and is likely to be achieved by cellwall hydrolysis through the collaborative or successive action of several GA-induced cell-wall hydrolases (e.g. Bewley, 1997a; Nonogaki et al., 2000; Leubner-Metzger, 2003b; Mo and Bewley, 2003; da Silva et al., 2005). Endosperm rupture is inhibited by ABA, and endosperm weakening, at least in part and depending on the species, is also inhibited by ABA (Toorop et al., 2000; Wu et al., 2000; Leubner-Metzger, 2003b; da Silva et al., 2004; Müller et al., 2005). This direct or indirect GA–ABA antagonism is supported by physiological and biochemical experiments and by screens for suppressor mutants (Finkelstein et al., 2002; Finkelstein, 2004). Several of the non-dormant Arabidopsis mutants that originated from a screen for seedling emergence in the presence of the GA biosynthesis inhibitor uniconazol are new alleles of abi3 and aba2 (Table 1, Fig. 2; Nambara et al., 1992, 1998). Suppressor screens of the ABA-insensitive abi1-1 mutation not only identified *sleepy1* (*sly1*), but also *ga1* mutant alleles (Table 1, Fig. 2; Steber et al., 1998).

Among the GA-response mutants (Table 1) of Arabidopsis, the GA-insensitive gai mutant is characterized by a dwarf phenotype, increased GA content and complex seed effects that are consistent with a severely decreased GA sensitivity of dormancy release and germination (Derkx and Karssen, 1993; Koornneef and Karssen, 1994; Richards et al., 2001). No appreciable seed germination of gai occurs in the dark, and only a combination of light with either chilling or dry after-ripening causes dormancy release and germination. The Arabidopsis GAI gene and its orthologues in other species encode nucleus-localized proteins that act as transcription factors, and appear to be negative regulators of the GA-signal transduction pathway. The GAI protein belongs to the DELLA subfamily of GRAS regulatory proteins, which includes several other negative regulators of GA responses, e.g. in Arabidopsis RGA (repressor-of-ga1-3), RGL1 (RGA-like1), RGL2 and RGL3 (Silverstone et al., 1997; Richards et al., 2001; Peng and Harberd, 2002). The DELLA domain region is involved in modulating the GA response, and its deletion in the gai mutant causes a gain-of-function mutation characterized by dominant GA-insensitive repression of GA responses.

In agreement with this, the loss-of-function allele gait6 confers increased resistance of stem growth to the GA biosynthesis inhibitor paclobutrazol and wildtype-like germination in the light; gai-t6 cannot rescue the non-germination phenotype of ga1-3 (Dill and Sun, 2001; Richards et al., 2001). The different negative DELLA-type regulators appear to possess separate, as well as overlapping, roles in GA responses (Fig. 2). It has been proposed that RGL1 plays a greater role in seed germination than do GAI and RGA (Wen and Chang, 2002), but RGL2 has been proposed to be the most important regulator of Arabidopsis seed germination in response to GA (Lee et al., 2002; Tyler et al., 2004; Cao et al., 2005). Loss-of-function rgl2 alleles suppress the GA-deficient seed germination phenotype conferred either by treatment with paclobutrazol or by ga1-3. Loss of function of the four DELLA genes (RGL2, RGL1, RGA and GAI) leads to light- and gibberellin-independent seed germination (Cao et al., 2005). Arabidopsis ga1-3 seeds lacking RGA + RGL1 + RGL2 or GAI + RGL1 + RGL2, confer GA-independent germination in the light but not in the darkness, while ga1-3 seeds lacking GAI + RGA + RGL2 germinate both in the light and darkness (Cao et al., 2005). This suggests that the destabilization or inactivation of RGA and GAI is not only triggered by GA, but also possibly by light. Therefore, DELLA proteins may act as integrators of environmental and endogenous cues to regulate seed germination.

RGL2 and the other DELLA-type proteins are transcription factors that inhibit GA responses and, thus, are considered to be negative regulators of GA signal transduction (Dill and Sun, 2001; Peng and Harberd, 2002). Therefore, GA should induce their degradation, as has been demonstrated for some DELLA-type regulators. Lee et al. (2002) found that RGL2 transcripts are undetectable in dry Arabidopsis seeds and increase rapidly following seed imbibition; this expression is restricted to elongating regions of radicles. In contrast, Tyler et al. (2004) and Bassel et al. (2004) found that RGL2 transcripts are abundant in dry Arabidopsis seeds, remain high during imbibition and germination, and decrease in emerged seedlings. An interpretative oversight of numerous Arabidopsis research papers is that early post-germination seedling growth is measured, and conclusions are extrapolated back to visible germination (radicle emergence), which occurs much earlier (Cohn, 1996). A careful time-course analysis of Arabidopsis seed germination showed that the RGL2 mRNA decline occurred after radicle emergence, i.e. after germination had been completed (Bassel et al., 2004). In tomato, there is only one DELLA gene (LeGAI); its mRNA is expressed during seed germination, during which there is an increase, but no decline, in its transcripts in either the embryo or in the endosperm (Bassel et al., 2004). LeGAI mRNA contents also increase in

GA-treated gib-1 mutant seeds (endosperm and embryo) of tomato. There is also no decline of two DELLA gene transcripts in the radicle of soybean seeds before and after germination. Bassel et al. (2004) conclude that the embryo of soybean has the potential to complete germination without a requirement for GA biosynthesis or response. However, in seeds such as those of tomato and Arabidopsis, where germination is constrained by the surrounding structures, i.e. coat dormancy, an appropriate amount of GA is required to overcome this. But even when germination does require the intervention of GA, this is not achieved by the suppression of DELLA gene transcription. An assumption that is often made is that there is a direct correspondence between transcript and protein amounts. While this occurs in many cases, it is not always so, especially in seeds. Tyler et al. (2004) also investigated DELLA protein contents, while Lee et al. (2002) and Bassel et al. (2004, unfortunately their antibody did not work) base their conclusions solely on transcript amounts. Tyler et al. (2004) conclude from their experiments: 'We showed that RGL2 protein in imbibed seeds is rapidly degraded by GA treatment'. Although this might be the case, it is not supported by the data provided in the publication. No germination time-course is presented, and what is shown in their immunoblot analyses is that GA causes protein degradation of RGL2 and RGA at >50 h, i.e. in seedlings and not in seeds.

The *Arabidopsis sleepy1* (*sly1*) and the *comatose* (*cts*) mutants exhibit marked seed germination reduction that cannot be rescued by GA (Steber et al., 1998; Russell et al., 2000; Steber and McCourt, 2001; Strader et al., 2004). The SLY1 and CTS proteins are proposed to be key factors involved in GA signalling of seeds. The cts mutant is impaired in seed dormancy release in the dry or imbibed state by after-ripening or cold treatment, respectively. CTS encodes a peroxisomal protein of the ATP-binding cassette (ABC) transporter class, and regulation of CTS function seems to be a major control point for the switch between dormancy and germination (Footitt et al., 2002). CTS may be involved as an importer of precursors in the biosynthesis of jasmonic acid (Theodoulou et al., 2005). Jasmonic acid inhibits germination of nondormant seeds and affects dormancy (e.g. Bogatek et al., 2002; Preston et al., 2002). The sly1 mutant was selected in a screen for suppressors of the ABAinsensitive *abi1-1* mutant. The SLY1 gene is a positive regulator of GA signalling and an F-box protein (McGinnis et al., 2003). RGA and RGL2 are substrates of SLY1, and this suggests that their degradation through the 26S proteasome pathway is involved in GA signalling. Tyler et al. (2004) show that the F-box protein SLY1 is required for RGL2 protein degradation in Arabidopsis seedlings. SLY1 has been postulated to be a 'key factor in GA reception' (Steber et al., 1998; McGinnis *et al.*, 2003). The F-box protein TIR1 is an auxin receptor that mediates auxin responses by promoting the degradation of the Aux/IAA transcriptional repressors (Dharmasiri *et al.*, 2005). We, therefore, postulate that the F-box protein SLY1 is a GA receptor that mediates GA responses by promoting the degradation of DELLA-type transcription repressors, e.g. RGL2, in seeds and seedlings.

The constitutive GA-response mutant *spindly* (*spy*) of Arabidopsis has been isolated in a screen for seeds able to germinate in the presence of paclobutrazol (Izhaki et al., 2001; Richards et al., 2001; Swain et al., 2001). Thus, the GA requirement for seed dormancy release and germination is decreased in the spy mutant. The elongated growth phenotype of spy mutant plants resembles wild-type plants treated with GA. Furthermore, *spy* mutations suppress the effects of GA deficiency on germination, and overexpression of (wild-type) SPY inhibits seed germination of Arabidopsis and petunia. The Arabidopsis SPY gene and its orthologues in other species encode tetratricopeptide repeat proteins that might function as Olinked *N*-acetylglucosamine (*O*-GlcNAc) transferases (Hartweck et al., 2002). SPY appears to act as a negative regulator of GA responses upstream of GAI. A possible function of SPY is to regulate GAI function by O-GlcNAc modification and thereby influence the nuclear localization of GAI (Richards et al., 2001).

The release from photodormancy and promotion of germination of light-requiring seeds of many species are regulated by phytochrome (Kamiya and Garcia-Martinez, 1999; Yamaguchi and Kamiya, 2002). Red light up-regulates the biosynthesis of bioactive GA₁ and GA₄ by inducing GA biosynthetic genes in germinating seeds of lettuce and Arabidopsis (Toyomasu et al., 1993, 1998; Yamaguchi et al., 1998, 2001; Ogawa et al., 2003). Gene induction of GA 3-oxidases (i.e. GA 3β-hydroxylases) during imbibition is controlled by light via phytochrome. In lettuce, synthesis of a GA-degrading enzyme (GA 2-oxidase, LsGA2ox2) is suppressed by red light, which might lead to an additional increase in bioactive GA in these seeds (Nakaminami et al., 2003). Light-induced GA biosynthesis during Arabidopsis seed germination appears to occur in two separate embryo tissues, the provascular tissue (early steps, CPS) and in the cortex and endodermis of the radicle (late steps, GA 3oxidase transcripts GA3ox1 and GA3ox2); the intercellular transport of a GA precursor has been proposed (Yamaguchi et al., 2001; Ogawa et al., 2003). Stratification (cold treatment at 4 °C), commonly used to promote and synchronize seed germination, caused GA3ox1 mRNA expression in the entire radicle and in the aleurone layer, and an increase in bioactive GA content (Yamauchi et al., 2004). Thus, light and low temperature can modulate the spatial expression pattern of GA biosynthetic genes. N. tabacum seed germination is also regulated by phytochrome, and GA substitutes for the red-light trigger needed to release photodormancy, inducing dark germination (Kretsch et al., 1995; Leubner-Metzger, 2003b). Similar results have been obtained for N. attenuata seeds, where GA treatment causes germination in the dark (Schwachtje and Baldwin, 2004). Normally, those seeds require smoke for breaking their dormancy, but smoke treatment in the dark without addition of GA is not sufficient to cause germination. Therefore, smoke exposure may increase the sensitivity of N. attenuata seeds to GA. Far less is known about the role of GA sensitivity during the after-ripening-mediated release of N. tabacum photodormancy. Fresh N. tabacum seeds are photodormant, i.e. they do not germinate during incubation in darkness, and even prolonged incubation in the dark does not induce testa rupture, βGlu I accumulation or endosperm rupture (Leubner-Metzger, 2002, 2003b). After-ripening contributes to the release of photodormancy, but its effect varies greatly between different seed batches, as reported for several tobacco cultivars. The GA requirements for photodormancy release of fresh and completely after-ripened photodormant seed batches are equal.

Non-photodormant tobacco seeds have lost the GA requirement for dark germination, which could be due to increased GA sensitivity and/or increased endogenous GA (Leubner-Metzger, 2001, 2002, 2005). GA treatment of dark-imbibed non-photodormant tobacco seeds increases the rate of germination in the dark, demonstrating that GA is also a positive regulator of germination speed. Thus, GA not only releases dormancy, it promotes germination rate and onset, as also supported by other studies with GA biosynthesis inhibitors and with non-dormant seeds (Steinbach et al., 1997; Tadeo et al., 1997; Kamiya and Garcia-Martinez, 1999; Yamaguchi and Kamiya, 2002). Endogenous GAs are required for embryo cell elongation and endosperm cap weakening (e.g. Bewley, 1997a; da Silva et al., 2005). Taken together, GA releases coat and embryo dormancy, promotes germination and counteracts inhibitory ABA effects, directly or indirectly.

Ethylene promotes seed germination and counteracts ABA effects

Ethylene is implicated in the promotion of germination of non-dormant seeds of many species (Corbineau *et al.*, 1990; Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000). In some species (e.g. peanut, sunflower), ethylene releases dormancy. In many species, ethylene alone is not sufficient to release seed dormancy, even when it promotes germination of non-dormant seeds of a given species. For example, inhibition of ethylene biosynthesis or action inhibits germination in the light, but treatment with ethylene does not release photodormancy and permit subsequent germination in darkness (e.g. Saini et al., 1989; Leubner-Metzger et al., 1998). Increased ethylene evolution is associated with the germination of eudicot seeds, e.g. Cicer arietinum (Matilla, 2000; Gómez-Jiménez et al., 2001), Pisum sativum (Gorecki et al., 1991; Petruzzelli et al., 1995), L. esculentum (Lashbrook et al., 1998), N. tabacum (Leubner-Metzger et al., 1998), and Lactuca sativa (Saini et al., 1989; Matilla, 2000). The need for endogenous ethylene for optimal germination of non-dormant seeds has been demonstrated in several of these studies by utilizing the ethylene action inhibitors 2,5-norbornadiene (NBD) or 1-methylcyclopropene (1-MCP) (Sisler and Serek, 2003). Ethylene production is higher in nondormant compared to dormant seeds (Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000), and is positively correlated with the germination rate of non-dormant pea seeds (Gorecki et al., 1991). A major peak of ethylene evolution coincides with the completion of germination, but ethylene production is already detectable very early during imbibition and before radicle protrusion through the covering layers.

Several hypotheses have been proposed to explain the mechanism(s) of ethylene action in germinating seeds (Esashi, 1991; Kepczynski and Kepczynska, 1997; Matilla, 2000). The primary action of ethylene could be the promotion of radial cell expansion in the embryonic hypocotyl, increased seed respiration or increased water potential. High-level induction of ABA-sensitive class I β -1,3-glucanase (β Glu I) gene expression in the micropylar endosperm of tobacco requires endogenous ethylene and promotes endosperm rupture (Leubner-Metzger et al., 1998). The molecular mechanisms of gene regulation by ethylene have been thoroughly studied in vegetative tissues, during flower senescence and fruit ripening (Hall et al., 2001; Klee, 2004; Stepanova and Alonso, 2005). However, there are also a few reports for ethylenealtered gene expression during seed germination: three members of the ETR gene family encoding putative ethylene receptors in tomato (Lashbrook et al., 1998), cysteine proteinase in chick-pea (Cervantes et al., 1994), ßGlu I in tobacco and pea (Leubner-Metzger et al., 1998; Petruzzelli et al., 1999), and 1-aminocyclopropane-1-carboxylic acid (ACC, the ethylene precursor) oxidase (ACO, the ethylene-forming enzyme) of pea (Petruzzelli et al., 2000, 2003b) and of Brassica rapa (Puga-Hermida et al., 2003).

Ethylene is perceived by a family of receptors related to ETR1 of *Arabidopsis*, and ethylene binding inhibits the signalling activities of these receptors (Hall *et al.*, 2001; Klee, 2004; Stepanova and Alonso, 2005). An increase in responsiveness to ethylene is associated with the germination of non-endospermic

and endospermic seeds, e.g. pea (Petruzzelli et al., 2000) and tomato (Lashbrook et al., 1998). The transcript amounts of three tomato ethylene receptor genes increase during seed germination. Ethylene biosynthesis and sensitivity are both important for the germination of tobacco (Leubner-Metzger et al., 1998) and Arabidopsis (Beaudoin et al., 2000; Ghassemian et al., 2000; Gallardo et al., 2002a, b; Siriwitayawan et al., 2003). ABA inhibits the seed germination of Arabidopsis, an effect that can be partially reversed by treatment with ACC (Ghassemian et al., 2000). The etr1 mutant seed of Arabidopsis exhibits poor germination (Bleecker et al., 1988; Chiwocha et al., 2005), and the seeds are also hypersensitive to ABA (Beaudoin et al., 2000). In the absence of ethylene, the ETR1 activates CTR1, which is a negative regulator of downstream signalling components. CTR1 is inactive in the presence of ethylene. Etiolated seedlings of the constitutive triple response1 (ctr1) mutant of Arabidopsis (Table 1, Fig. 2) are characterized by a constitutive ethylene triple response, i.e. inhibition of shoot elongation, shoot swelling and intensified curvature of the apical hook. Seed germination of ctr1 is less sensitive to ABA, and freshly harvested *ctr1* seeds germinate slightly faster than wild-type seeds (Beaudoin et al., 2000). CTR1 functions as a RAS-like mitogen-activated protein kinase kinase kinase (MAPKKK), and is the start of an ethylene-activated MAPK pathway (Chang, 2003; Ouaked et al., 2003). Additional ethylene signalling pathways exist, and an ethylene receptor complex is proposed to consist of ETR1, CTR1 and phosphorelay intermediates (Hall et al., 2001; Lohrmann and Harter, 2002). This type of phosphorelay signalling, which is likely to function via histidine protein kinases, involves signal receptors for ethylene, cytokinins, light and osmolarity. The phosphorelay permits the conversion of hormonal and environmental signals into biochemical reactions, allows the linking of the different signals to form a complex signalling network and provides signal integration.

EIN2 is a downstream signalling component that, in the absence of ethylene, is down-regulated by CTR1. As with *etr1*, the *ethylene insensitive2* (*ein2*) mutants of Arabidopsis are characterized by higher seed dormancy and by hypersensitivity to ABA (Table 1; Fig. 2). Interestingly, ctr1 and ein2 mutants have been recovered as enhancer and suppressor mutants, respectively, of the ABA-insensitive seed germination phenotype of the *abi1-1* mutant (Beaudoin *et al.*, 2000). Arabidopsis enhanced response to ABA3 (era3) mutants are characterized by increased sensitivity of the seed to ABA and by overaccumulation of ABA (Ghassemian et al., 2000). The era3 alleles turned out to be new alleles of the EIN2 locus (Fig. 2). The ein2-45 allele increases seed dormancy, but this effect is completely counteracted by severely ABA-insensitive mutations, such as abi3-4 (Beaudoin et al., 2000). As a consequence, the ein2-45 abi3-4 double mutant is as nondormant as the abi3-4 single mutant. The non-dormant phenotype of the ein2 abi3-4 double mutant suggests that ethylene suppresses seed dormancy by inhibiting ABA action. Thus, EIN2 is a possible negative regulator of ABA response. However, as the abi3-4 allele causes vivipary, pleiotropic effects may mask real interactions. The enhanced dormancy of etr1 and ein2 seeds suggests that endogenous ethylene is a negative regulator of Arabidopsis seed dormancy. The finding that ein2 mutations can suppress, and ctr1 mutations can enhance, the ABA-insensitive germination phenotype of abi1 suggests that ethylene also influences the sensitivity of the seed to ABA. These results indicate a strong interaction between the ethylene and ABA signal transduction pathways, and a key conclusion is that ethylene can promote germination by directly interfering with ABA signalling (Beaudoin et al., 2000; Ghassemian et al., 2000). ABA-ethylene interactions are also evident in seeds and seedlings of other species (e.g. Leubner-Metzger et al., 1998; Spollen et al., 2000). In summary, ethylene alone probably cannot act as a positive regulator of germination, but can only act by interfering with ABA signalling.

Genetically downstream of EIN2 are transcription factors that regulate gene expression in response to ethylene (Fig. 2). Ethylene-ABA interactions are also manifested at this level of ethylene signalling (Ghassemian *et al.*, 2000). EIN3 and EIN3-LIKE proteins bind to the promoter of the Arabidopsis ethylene responsive factor 1 (ERF1) gene, which encodes an ethylene-responsive element binding protein (EREBP)-type transcription factor, and thereby confers a hierarchy of transcription factors involved in ethylene signalling (Solano et al., 1998; Lee and Kim, 2003). The EREBP-type transcription factors mediate ethylene regulation of gene expression (Figs 1, 2). They bind to the GCC box within the positively acting ethylene-responsive element (ERE) of target promoters, and this *cis*-regulatory element is necessary and sufficient for the regulation of transcription by ethylene. Ethylene is involved in endosperm rupture and high β Glu I expression during tobacco seed germination, but it does not affect the spatial and temporal pattern of BGlu I expression (Leubner-Metzger et al., 1998). A promoter deletion analysis in germinating tobacco seeds suggests that the distal region, which contains the ERE, is required for high, ethylene-sensitive expression; the proximal region is necessary and sufficient for low-level micropylarendosperm specific expression; and both regions contribute to down-regulation by ABA. Transcription factors, like the tobacco EREBP-3 (Leubner-Metzger et al., 1998), the Arabidopsis bromodomain protein IMB1 (Duque and Chua, 2003) and the tobacco EIN3-LIKE homologues (Lee and Kim, 2003), are involved in mediating ABA–ethylene regulation of β -1,3-glucanase expression. Transcripts of EREBP-3 and IMB1 are stored in air-dry seeds, induced during early seed imbibition, down-regulated during late germination and regulated by ABA (Leubner-Metzger *et al.*, 1998; Duque and Chua, 2003). Interestingly, sequences that are homologous to the GCC box are also present in the promoters of some ACO genes (see below, and references in Petruzzelli *et al.*, 2000).

Ethylene promotes ethylene biosynthesis during pea seed germination by positive feedback regulation of ACC oxidase (ACO), which catalyses the final step of ethylene biosynthesis (Petruzzelli et al., 2000, 2003b). An early onset and sequential induction of ACC biosynthesis, Ps-ACO1 mRNA, a 36-kDa Ps-ACO1 protein, ACO activity and ethylene production are localized almost exclusively in the embryonic axis. Within the axis, ethylene biosynthesis and responsiveness are localized in the cell elongation and differentiation zones of the radicle. Maximal contents of ACC, Ps-ACO1 and ethylene evolution occur when radicle emergence is just completed. Accumulation of *Ps-ACO1* mRNA, protein and ACO enzyme activity in the embryonic axis during late germination requires ethylene, whereas Ps-ACS1 mRNA and overall ACC contents are not induced by ethylene. Ethylene does not induce ACO in the embryonic axis during early germination. Similar observations have been made for the highly homologous ACO in chickpea seeds (Nicolas et al., 1998; Matilla, 2000; Gómez-Jiménez et al., 2001). ABA inhibits chickpea ACO expression, ACC accumulation and ethylene production during germination, but not after radicle emergence. This suggests that interference of ethylene and ABA signalling confers an opposing feedback regulation of ethylene production in the embryonic axis. Calcium is required for ethylene and ABA responses during pea and chickpea seed germination (Nicolas et al., 1998; Petruzzelli et al., 2003b). Although ABA and ethylene act antagonistically on seed germination, they both inhibit root elongation of Arabidopsis seedlings (Beaudoin et al., 2000; Ghassemian et al., 2000; Petruzzelli et al., 2003b). Thus, seeds and vegetative tissues differ with regard to the ethylene-ABA interactions and integrated responses.

Cross-talk between ethylene and GA during the transition from seed dormancy to germination has been postulated from the hormonal regulation of a GA 20-oxidase in *Fagus sylvatica* (Calvo *et al.*, 2004). GA treatment induces *ACO* expression in imbibed *ga1-3* seeds (Ogawa *et al.*, 2003). An excess of ethylene can bypass the GA requirement and induce germination of *Arabidopsis ga1* mutant seeds imbibed in the light, the effect being much weaker in darkness (Karssen *et al.*, 1989; Koornneef and Karssen, 1994). The GA–ethylene interaction seems reciprocal, because high

GA concentrations restore the germination of etr1 mutant seeds to wild-type levels (Bleecker et al., 1988). By contrast, ethylene does not break dormancy of the tomato gib-1 mutant, although it promotes the germination of tomato wild-type seeds (Nelson and Sharples, 1980; Groot and Karssen, 1987). Treatments with GA, ethylene or cytokinins alone are not able to overcome lettuce thermoinhibition in the dark (Saini et al., 1989; Matilla, 2000). In addition to exogenous ethylene, at least one other hormone, or light, is required. Ethylene biosynthesis is essential for the release of thermoinhibition in the dark following application of GA or cytokinin, as well as for the lightinduced release of thermoinhibition. The cytokinin or ethylene requirements for overcoming thermoinhibition of lettuce germination can also be eliminated or diminished by removing or weakening the endosperm. Thus, ethylene biosynthesis is involved as a negative regulator of coat dormancy. Furthermore, thermoinhibition of lettuce is correlated with the accumulation of ABA (Yoshioka et al., 1998).

Taken together, promotion of seed germination by ethylene appears to be a general phenomenon, while in some species ethylene also appears to be required for dormancy release. Ethylene seems to counteract the inhibitory effects of ABA on seed germination by interfering with ABA signalling.

Brassinosteroids promote seed germination

Brassinosteroids (BR) and GA interact with light in regulating elongation of shoots and photomorphogenesis of seedlings by apparently independent pathways (Altmann, 1999; Szekeres, 2003). Endogenous BR have been identified in seeds of several species (e.g. Adam and Marquardt, 1986; Schmidt et al., 1997). They are perceived by the plasma membrane-localized leucinerich repeat receptor kinase, BRI1 (BR insensitive 1) in Arabidopsis, and several homologues have been identified utilizing BR-insensitive mutants of other species (Szekeres, 2003; Kinoshita et al., 2005). Whereas BR application enhances germination of certain parasitic angiosperms (Takeuchi et al., 1991, 1995), cereals (Yamaguchi et al., 1987), Arabidopsis mutants (Steber and McCourt, 2001) and tobacco (Leubner-Metzger, 2001), it does not affect the germination of non-photodormant, non-endospermic cress seeds imbibed in the dark (Jones-Held et al., 1996). Germination of the endospermic seeds of parasitic Orobanche and Striga species is, in contrast to Arabidopsis and tobacco, inhibited by light (Takeuchi et al., 1991, 1995; Rugutt et al., 2003). BR, ethylene and GA cannot substitute for the conditioning treatment with strigol, which is needed for inducing germination of unconditioned (i.e. dormant) seeds of those species. Conditioning removes the restriction on the

ethylene biosynthetic pathway and increases the capacity to produce ethylene (Babiker et al., 2000). In Striga hermothica the transcripts of ACC synthase and ACO increase after treatment with the strigolactone GR24 and after conditioning, supporting the idea that endogenous ethylene is involved in the germination of Striga seeds (Sugimoto et al., 2003). ACO enzyme activity of Striga asiatica seeds can only be stimulated by conditioning, but not by strigol treatment (Mohamed et al., 2001). Germination of conditioned (i.e. non-dormant) Orobanche and Striga seeds imbibed in the light and in the dark is promoted by treatment with BR. ABA inhibits germination of Orobanche seeds. Consistent with this, inhibition of carotenoid biosynthesis by norflurazon or fluridone promotes strigolinduced germination of Orobanche seeds. Unexpectedly, this effect is not due to a decreased ABA content of the seeds and therefore must be attributed to perturbations other than inhibition of ABA biosynthesis (Chae et al., 2004).

BR promotes the germination of pre-chilled (i.e. non-dormant) seeds of the BR-deficient biosynthesis mutant *det2-1* and the BR-insensitive response mutant bri1-1 of Arabidopsis (Table 1) imbibed in the light (Steber and McCourt, 2001). Seed germination of det2-1 and *bri1-1* is more strongly inhibited by ABA than is germination of the wild type, and therefore BR is able to partially overcome the inhibition of germination by ABA. BR treatment rescues the germination phenotype of the severe GA-deficient biosynthesis mutant ga1-3, which normally requires GA treatment for dormancy release and germination. BR treatment also partially rescues the germination phenotype of the severe GA-insensitive response mutant sly1, which also cannot be rescued by treatment with GA. Interestingly, a new allele for *sly1* has been identified in a screen for BR-dependent germination that suggests interactions between BR and GA signalling in seeds (Steber et al., 1998; Steber and McCourt, 2001). These results point to a role for BR in stimulating germination of Arabidopsis seeds. This is further supported by the germination phenotype of the gpa1 mutant of Arabidopsis (Ullah et al., 2002). The GPA1 gene encodes the α -subunit of a heterotrimeric G protein. Seeds with the gpa1 null mutation are 100-fold less responsive to GA, and GPA1 overexpressing seeds are hypersensitive to GA. The gpa1 mutant seeds are also completely insensitive to BR rescue of germination when the seed GA content is reduced. These findings support the view that there is a complex interaction between GA and BR in regulating seed germination of Arabidopsis.

BR promotes seedling elongation and germination of non-photodormant tobacco seeds, but does not appreciably affect testa rupture and the subsequent induction of β Glu I in the micropylar endosperm (Leubner-Metzger, 2001). Treatment with BR, but not

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GA, accelerates endosperm rupture of tobacco seeds imbibed in the light. In contrast, in dark-imbibed seeds, both BR and GA promote endosperm rupture, but only GA enhances βGlu I induction. Promotion of endosperm rupture by BR is dose-dependent, and 0.01 µM brassinolide is most effective. BR and GA promote ABA-inhibited dark-germination of nonphotodormant seeds, but only GA replaces light in inducing BGlu I. These results indicate that BR and GA promote tobacco seed germination by distinct signal transduction pathways and distinct mechanisms. GA and light act in a common pathway to release photodormancy, whereas BR does not release photodormancy. BGlu I induction in the micropylar endosperm and release of coat dormancy appear to be associated with the GA/light pathway, but not with BR signalling. Xyloglucan endo-transglycosylase (XET) enzyme activity accumulates in the embryo and the endosperm of germinating tobacco seeds, and this appears to be partially controlled by BR (Leubner-Metzger, 2003a). A GA-regulated XET mRNA also is expressed exclusively in the micropylar endosperm of tomato seeds (Chen et al., 2002). Therefore, it is possible that the XET induction in the tobacco embryo is controlled by BR. These findings suggest as a model for the endosperm-limited germination of tobacco: (1) Photodormancy is released exclusively by the GA/ light pathway. (2) Promotion of subsequent endosperm rupture by the BR and the GA/light signal transduction pathways is achieved by independent and distinct mechanisms. (3) ABA inhibits endosperm rupture by interfering with both pathways. (4) The GA/light pathway regulates β Glu I induction in the micropylar endosperm and controls endosperm weakening. (5) The BR pathway promotes endosperm rupture of non-dormant seeds, at least in part, by enhancing the growth potential of the embryo (Leubner-Metzger, 2001, 2003b).

Taken together, these findings suggest that GA and BR act in parallel to promote cell elongation and germination and to counteract the inhibitory action of ABA. Since BR stimulates germination of the GAinsensitive mutant *sly1*, it is unlikely that BR acts by increasing GA sensitivity. It is possible that BR acts by stimulating GA biosynthesis in Arabidopsis seeds imbibed in the light (Steber and McCourt, 2001). However, this stimulation is unlikely to occur in tobacco, because BR does not promote the expression of β Glu I, which is induced by GA in the dark (Leubner-Metzger, 2001). It is known that BR can stimulate ethylene production, and ethylene treatment can rescue the germination phenotype of the GAdeficient Arabidopsis ga1-1 mutant (Karssen et al., 1989; Koornneef and Karssen, 1994; Steber and McCourt, 2001). However, there are several arguments against the hypothesis that BR acts via ethylene: (1) Ethylene does not increase in cress seedlings following BR treatment of seeds (Jones-Held *et al.*, 1996). (2) Endogenous ethylene promotes β Glu I accumulation in the micropylar endosperm of tobacco, but BR treatment promotes endosperm rupture without enhancing β Glu I accumulation (Leubner-Metzger *et al.*, 1998; Leubner-Metzger, 2001). (3) Ethylene rescue of *ga1-1* seed germination results in seedlings exhibiting a triple response, but BR rescue of *ga1-3* seed germination results in seedlings that do not (Steber and McCourt, 2001). Another possibility is that BR action occurs via auxin, because auxin response factors are subject to BR regulation (Müessig *et al.*, 2003). If BR promotes germination via embryo expansion, this effect is probably specific to embryos and is not found in seedlings.

Cytokinins and auxins

Cytokinins are present in developing seeds and accumulate predominantly in the liquid endosperm (Emery et al., 2000; Fischer-Iglesias and Neuhaus, 2001; Mok and Mok, 2001). It has been proposed that the endosperm is a source of cytokinins needed for the promotion of cell division in the embryo. They may have roles in embryogenesis, in embryonic pattern formation, in the early period of grain filling of cereals, and in enhancing sink strength. In sorghum grains the cytokinin content is high in the embryo, low in endosperm, and it declines during imbibition (Dewar et al., 1998). After radicle protrusion a cytokinin peak is associated with α -amylase accumulation. This cytokinin might play a role in cell division and elongation of the emerged root. It may also be redistributed within the embryo to regions where it effectively concentrates and directs root growth. There is a high ABA content in the embryo during germination, very low amounts of bioactive GA, and it has been proposed that the cytokinin/ABA interaction plays a role in controlling Sorghum germination (Dewar et al., 1998). Confirmation of these trends in other species may revive interest in the concept of a permissive role of cytokinins in the regulation of germination, as developed by Khan (1975). For example, the ability of cytokinins alone (GA is without effect) to overcome ABA-inhibited germination of isolated Grand Rapids lettuce embryos (Bewley and Fountain, 1972) merits reinvestigation at the molecular level. It would not be surprising to learn that there is extensive cross-talk between cytokinins and other plant hormone signalling systems in the regulation of dormancy and germination.

Many species are known where cytokinins alone break seed dormancy (summarized in Cohn and Butera, 1982). During the conditioning of parasitic *Orobanche* and *Striga* species and the release of lettuce thermoinhibition, cytokinins appear to contribute to the promotion of dormancy release and subsequent germination by enhancing ethylene biosynthesis (Saini *et al.*, 1989; Babiker *et al.*, 2000; Matilla, 2000). A cytokinin–ethylene connection is also supported by the discovery that the *Arabidopsis cytokinin-resistant1* (*ckr1*) mutant is insensitive to ethylene and allelic to the ethylene-insensitive mutant *ein2* (Fischer-Iglesias and Neuhaus, 2001). Cytokinin-resistant mutants of *N. plumbaginifolia* have been isolated that exhibit reduced seed dormancy and pleiotropic seed effects suggestive of cytokinin–ABA interactions (Rousselin *et al.*, 1992).

Auxins seem to play a major role in embryogenesis, providing positional information for the coordination of correct cellular patterning from the globular stage onwards (Fischer-Iglesias and Neuhaus, 2001; Teale et al., 2005). While recent molecular and genetic data support an essential role for auxins in apical-basal pattern formation during embryogenesis, very little is known at the molecular level about the role of auxin during seed germination. Free indoleacetic acid (IAA) decreases during the imbibition of Sorghum grains (Dewar et al., 1998), and auxin regulates catalase expression in the scutellum of germinating maize kernels (Guan and Scandalios, 2002). A relationship between IAA, dormancy and preharvest sprouting of wheat has been reported (Ramaih et al., 2003).

IAA is released from conjugates stored in seeds of Scots pine during germination (Ljung et al., 2001). A peak of free IAA occurs prior to the initiation of root elongation and coincides with initial seed swelling during imbibition. In bean seeds, increasing synthesis of IAA occurs during germination (Bialek et al., 1992). Free IAA contents decline during radicle emergence, and new IAA synthesis is established in the emerged seedling. An IAA-modified protein from bean seeds, IAP1, is associated with rapid growth during seed development (Walz et al., 2002). Moreover, this protein undergoes rapid degradation during germination. IAA-modified proteins represent a distinct class of conjugated phytohormones and appear to be the major form of auxin in bean seeds. The postgermination accumulation of ACO and ethylene production of chick-pea seedlings is promoted by IAA (Gómez-Jiménez et al., 2001). IAA has no appreciable effect on ACO expression prior to or during germination. Several auxin-resistant mutants of N. plumbaginifolia have been isolated that exhibit reduced seed dormancy, and pleiotropic effects suggest auxin-GA interactions (Rousselin et al., 1992). However, there appears to be no auxin effect on the seed germination of GA-deficient Arabidopsis and tomato mutants (Koornneef and Karssen, 1994).

Whether or not auxins play a role in *Arabidopsis* germination is unclear; however, exogenous GA upregulates the early expression of several auxin-related genes (Ogawa *et al.*, 2003) that putatively encode auxin

carrier proteins, as well as genes that may be associated with auxin biosynthesis. This suggests that GA causes substantial changes in auxin content and transport during *Arabidopsis* seed germination.

Summary and conclusions

Dormancy and germination are complex phenomena that are controlled by a large number of genes, which are affected by both developmental and environmental factors (Bewley, 1997b; Koornneef et al., 2002). We are only beginning to understand part of the intensive cross-talk between the hormones implicated in these processes (Brady and McCourt, 2003; Finkelstein, 2004). A crucial role for ABA has been identified in inducing seed dormancy. Factors determining spatial and temporal ABA content and sensitivity patterns in seeds positively regulate induction of dormancy and probably its maintenance, and negatively regulate dormancy release and germination. GA releases dormancy, promotes germination and counteracts inhibitory ABA effects, directly or indirectly. GA is required for embryo cell elongation, for overcoming coat restrictions to germination of non-dormant and dormant seeds, and for inducing endosperm weakening. BR and ethylene also counteract the inhibitory effects of ABA on seed germination, but in most species they appear to act after dormancy has been released by GA. Ethylene seems to counteract the inhibitory effects of ABA on seed germination by interfering with ABA signalling. This is completely different from the situation in seedling root growth, which is inhibited by both hormones. The ABAethylene antagonism in seeds shows that hormonal interactions and responses differ between germinating seeds and vegetative tissues.

Molecular approaches using the angiosperm model species, *Arabidopsis thaliana*, have provided valuable data sets from transcriptome and proteome approaches. An absolute requirement for this type of evaluation is that it must be based on solid seed physiology. Cohn (1996) has discussed many of the relevant issues; additionally, numerous, more recent studies indicate that mutant seeds must be compared to corresponding wild-type seeds harvested from plants grown simultaneously under the same conditions. A good example of a 'solid-physiology' proteome approach is the work of Rajjou *et al.* (2004, http://seed.proteome.free.fr); it is based on germination time courses, in which germination *per se*, i.e. radicle emergence, was counted.

Exploiting natural variation and using clearly dormant *Arabidopsis* ecotypes, like Cvi, are promising areas for seed dormancy research (Koornneef *et al.*, 2002). However, the time has also come to move beyond *Arabidopsis* and apply molecular approaches like transcriptomics and proteomics to other seed model species. Seeds are an important factor of the enormous angiosperm biodiversity (Baskin and Baskin, 2004). The comparative investigation of representatives from each phylogenetic angiosperm clade offers the opportunity for seed research that enhances our understanding of the evolution of biodiversity.

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