The plant hormones gibberellin and abscisic acid regulate gene expression, secretion and cell death in aleurone. The emerging picture is of gibberellin perception at the plasma membrane whereas abscisic acid acts at both the plasma membrane and in the cytoplasm – although gibberellin and abscisic acid receptors have yet to be identified. A range of downstream-signalling components and events has been implicated in gibberellin and abscisic acid signalling in aleurone. These include the G_{alpha} subunit of a heterotrimeric G protein, a transient elevation in cGMP, Ca^{2+}-dependent and Ca^{2+}-independent events in the cytoplasm, reversible protein phosphorylation, and several promoter cis-elements and transcription factors, including GAMYB. In parallel, molecular genetic studies on mutants of Arabidopsis that show defects in responses to these hormones have identified components of gibberellin and abscisic acid signalling. These two approaches are yielding results that raise the possibility that specific gibberellin and abscisic acid signalling components perform similar functions in aleurone and other tissues.

GA and ABA influence a range of other events during growth and development^{1-3}, and studies of Arabidopsis mutants defective in GA and ABA signalling have led to the identification of some components of these signalling pathways. These recent developments in GA and ABA signalling in aleurone are reviewed here, together with insight gained from molecular genetic studies with Arabidopsis (see also Refs 5–8).

How do aleurone cells sense GA and ABA? Evidence from two different experimental approaches suggests that GAs are perceived at the aleurone plasma membrane. First, GA, covalently coupled to Sepharose beads is membrane-impermeant, but nevertheless stimulates high-level α-amylase gene expression and protein secretion in aleurone protoplasts. Second, microinjection of GA into aleurone protoplasts does not stimulate α-amylase gene expression, and it is only when GA is present in the protoplast incubation medium that they respond. Other studies also support a plasma membrane site of GA action (Fig. 1).

Microinjection of ABA into GA-treated barley aleurone protoplasts does not appreciably inhibit GA-induced expression of an AmyGUS reporter or α-amylase production, whereas ABA in the incubation medium does. Thus, ABA inhibition of GA-action is mediated by perception at the plasma membrane. However, induction of the ABA-responsive AmyGUS in barley aleurone protoplasts can be achieved either by microinjecting ABA or by adding it to the medium, suggesting that in this response ABA is perceived inside the protoplast (Fig. 1). Stomatal guard cells also appear to have internal and external sites of ABA perception.

The current challenge is to identify GA and ABA receptors unequivocally. There has been some progress in the identification of GA binding proteins in aleurone, although no ABA-binding proteins have been found in this tissue.
GA-binding proteins at the plasma membrane

The development of a method for isolating large quantities of highly purified aleurone plasma membrane, combined with the use of a high-specific-activity $[^3]S$-labelled GA-photoaffinity probe, led to the detection of two plasma membrane polyproteins of ~18 kDa and 68 kDa (Ref. 15). Photoaffinity labelling of these was competed by biologically active GA$_4$, GA$_1$, and unlabelled photoaffinity probe, whereas biologically inactive GA$_{48}$ did not compete. Polyproteins of 18 kDa and 68 kDa were also GA-photoaffinity labelled in plasma membrane from vegetative tissue of pea, sweet pea (Lathyrus odoratus) and Arabidopsis. The GA-binding proteins could not be detected on intracellular membranes and 2D-gel analysis demonstrated their low abundance15. Preliminary N-terminal amino acid sequence from the 18-kDa polyproteins purified from Arabidopsis has yet to identify a potential function. Nevertheless, the possibility that the polyproteins are involved in GA action is suggested by the observation that they are less intensely labelled in the sweet pea semi-dwarf GA-sensitivity mutant lb compared with wild-type Lb (Ref. 15). On non-reducing SDS gels, the 18 kDa and 68 kDa polyproteins are both resolved, indicating that they are not disulphide-linked as part of the same protein (A. Lovegrove, unpublished). The fact that both polyproteins are labelled by the GA-photoaffinity probe suggests that the GA receptor might be a complex of at least two proteins (Box 1).

Heterotrimeric G proteins transduce GA signals

Evidence is emerging that functional counterparts of several components of the G protein-signalling pathway exist in plants16. Recent results from two independent lines of enquiry have revealed a possible role of heterotrimeric G proteins in GA signal transduction in wild oat and rice aleurone. The mastoparan analogue Mas7 stimulates GDP-GTP exchange by heterotrimeric G proteins, and is thought to mimic activated G protein-coupled receptors. When wild oat aleurone protoplasts are incubated with Mas7, they synthesize and secrete amylase in a dose-dependent manner and with an identical time course to GA (Ref. 17). As with GA, the response is largely overcome by ABA. Mas7 induces a-amy lase mRNA and expression of a-Amy2/54:GUS. This raises the possibility that Mas7 is activating a heterotrimeric G protein in the GA-signalling pathway. Further evidence supporting this theory has come from studying the effects of hydrolysis-resistant guanine nucleotides on GA-induction of a-Amy2/54:GUS expression17. The hydrolysis-resistant guanine nucleotide analogues GTP-$\gamma$-S and GDP-$\beta$-S bind to G subunits and hold them in either the activated (GTP-$\gamma$-S-bound) or inactivated (GDP-$\beta$-S-bound) form. GTP-$\gamma$-S introduced into aleurone protoplasts during transfection with reporter gene constructs prevents GA induction of a-Amy2/54:GUS expression, whereas GTP-$\gamma$-S stimulates expression slightly. Taken together the Mas7 and guanine nucleotide data strongly suggest that a heterotrimeric G protein(s) is involved at an early stage of the GA-signalling pathway in wild oat aleurone. It predicts that aleurone cells should contain G protein subunits, which was confirmed by PCR and northern analysis for a partial G$_{\alpha}$ subunit cDNA and two related G$_{\alpha}$ cDNAs (Ref. 17). ABA inhibition of GA-induction of $\alpha$-amy lase can partly be overcome by treating wild oat aleurone protoplasts with choloro or pertussis toxin (H.D. Jones and R. Hooley, unpublished), perhaps indicating G protein involvement in ABA signalling in aleurone.

Embryo-less half seed of the Dwarf 1 mutant of rice produce little $\alpha$-amylase when treated with GA, and Dwarf 1 seedlings elongate only slightly in response to GA (Ref. 16). These observations are consistent with Dwarf 1 being a GA-sensitivity mutant18, although we await measurement of the endogenous GAs in Dwarf 1 to see if they are elevated, as would be expected for such a mutant. Mutations giving rise to Dwarf 1 are now known to be in the G subunit GPA1 (Refs 19,20). Because these clearly affect GA sensitivity of rice aleurone, these observations add further support to the theory that a G protein is involved in GA signalling in aleurone.

Precedents from well characterized G protein-signalling pathways make it tempting to speculate that GA, perceived by a receptor at the plasma membrane, signals through a heterotrimeric G protein. Although this hypothesis requires testing experimentally, it is clear that the theory that plant hormone signals might be transduced by G protein-signalling pathways is further supported by the discovery of GCR1 – the first plant G protein-coupled receptor homologue – and the evidence that it might be involved in cytokinin signal transduction21 (Box 2).

Ca$^{2+}$/CaM-dependent and -independent events

The earliest event following GA treatment of aleurone is an increase in cytoplasmic calcium concentration ([Ca$^{2+}$]). In barley aleurone protoplasts, it occurs after 4–6 h (Ref. 23). In intact wheat aleurone cells, a faster response (2–5 min) has been observed22, which does not occur in response to the inactive GA$_{48}$. Elevation of [Ca$^{2+}$] by GA is quicker in some cells than others23,24, perhaps because of heterogeneity in the sensitivity of individual aleurone cells and protoplasts25,26. Provided GA is present continuously, it causes sustained elevations in [Ca$^{2+}$] that, when averaged over the whole cell, amount to increases of 100–500 nm above a resting level of 100–250 nm. Localized [Ca$^{2+}$] can be much higher. Combined temporal and spatial analysis of [Ca$^{2+}$] (S. Gilroy, pers. commun.; www.bio.psu.edu/faculty/gilroy/algal.html) coupled with a known dependency of these changes on extracellular Ca$^{2+}$, has led to the suggestion that GA alters Ca$^{2+}$ flux at the plasma membrane27,28,29. The mechanism is unknown, although one hypothesis is that the GA receptor might, directly or indirectly, regulate a plasma membrane Ca$^{2+}$-channel, possibly through a heterotrimeric G protein. Release of Ca$^{2+}$ from intracellular stores is another possibility30. However, it is unlikely to be mediated by IP$_3$ (Ref. 28), even though there is some evidence that GA causes a transient elevation in IP$_3$ in rice aleurone27. In barley, ABA prevents the GA-stimulated increases in [Ca$^{2+}$] and, in the absence of GA, high concentrations of ABA have been proposed to lower [Ca$^{2+}$] by activating a plasma membrane Ca$^{2+}$-ATPase (Ref. 29).

Box 1. Gibberrellin and abscisic acid perception: key points

- Gibberrellin (GA) is perceived at the aleurone plasma membrane.
- Plasma membrane GA-binding protein (18 kDa and 68 kDa) occurs in aleurone and other tissues.
- Abscisic acid (ABA) inhibition of GA action is mediated by perception at the aleurone plasma membrane.
- ABA induction of Em:GUS expression is mediated by perception inside the cell.
- No ABA-binding proteins have been identified in aleurone.

Box 2. Heterotrimeric G proteins: key points

- G protein agonists and antagonists can mimic and block gibberrellin (GA) action.
- Mutations in the rice GA receptor $\alpha$-subunit reduce GA-insensitivity in aleurone and other tissues.

Reviews
These alterations in $[\text{Ca}^{2+}]_{i}$ indicate a potential signalling role, and the fact that calmodulin (CaM) protein levels are stimulated two- to fourfold by GA, and reduced by ABA (Ref. 30), suggest that a $\text{Ca}^{2+}$-CaM system might operate. This, and earlier data 1, strongly implicate CA-induced changes in $[\text{Ca}^{2+}]_{i}$ and CaM in the secretion of hydrolyases that are induced by GA (Fig. 2). Likely targets of $\text{Ca}^{2+}$-CaM are:

- **An ER Ca$^{2+}$-ATPase** that can be activated by CaM, and probably supports the demand for endomembrane $\text{Ca}^{2+}$-transport 
- **A tonoplast $\text{Ca}^{2+}$ transporter**
- **A storage vacuole membrane slow-vacuolar (SV)-type cation channel**

Cyclic nucleotide monophosphates such as cGMP are thought to affect phosphorylation at two sites on the SV-type channel, regulating its activity in a complex way. CaM-affinity screening of an aleurone cDNA library has been used in an attempt to identify other targets of Ca$^{2+}$/CaM (Ref. 34). This led to the isolation of a plasma membrane-located CaM-binding transporter that might potentially be regulated by cyclic nucleotide monophosphates such as cGMP.

To identify Ca$^{2+}$- and CaM-dependent and -independent signalling events in aleurone, the $[\text{Ca}^{2+}]_{i}$, and CaM levels were modified by microinjecting CaCl$_2$, caged Ca$^{2+}$, Ca$^{2+}$-chelators, CaM and CaM antagonists into barley aleurone protoplasts 11. Blocking the GA-induced increase in $[\text{Ca}^{2+}]_{i}$, using the Ca$^{2+}$ buffer diazo-2 inhibits Ga-induced $\alpha$-amylase secretion but does not appreciably affect Amy:GUS expression. CaM antagonists also inhibit $\alpha$-amylase secretion. Attempts to mimic GA-induced $[\text{Ca}^{2+}]_{i}$ and CaM changes by means of microinjection do not stimulate Amy:GUS expression. In addition, GA induction of Em:GUS is independent of changes in $[\text{Ca}^{2+}]_{i}$, and CaM (Ref. 13). These data therefore exclude changes in $[\text{Ca}^{2+}]_{i}$, CaM from GA signalling, which leads to the induction of $\alpha$-amylase gene expression and secretion, and which lead to the induction of $[\text{Ca}^{2+}]_{i}$ in GA-treated barley aleurone 11. However, a study with rice aleurone 16 concludes that $[\text{Ca}^{2+}]_{i}$ and CaM are important intermediaries in the GA induction of gene expression. Furthermore, the fact that $[\text{Ca}^{2+}]_{i}$ is elevated within minutes after GA treatment of wheat aleurone cells, whereas hydrolase synthesis and secretion does not take place until several hours later, argues that changes in $[\text{Ca}^{2+}]_{i}$ might be involved in events that precede hydrolase secretion. Further complexity has been uncovered by the discovery that if the decrease in $[\text{Ca}^{2+}]_{i}$ caused by ABA is blocked in GA-treated barley aleurone protoplasts, ABA is prevented from inhibiting GA-induced Amy:GUS expression and $\alpha$-amylase secretion 11. This presents a paradox: artificially reducing $[\text{Ca}^{2+}]_{i}$ in GA-treated protoplasts does not affect Amy:GUS expression, although the ABA-driven reduction in $[\text{Ca}^{2+}]_{i}$ is essential for ABA-inhibition of GA-induced Amy:GUS expression (Fig. 3). The inhibitory effect of ABA on GA-induced Amy:GUS expression and $\alpha$-amylase secretion can also be overridden by microinjecting CaM, although it is not clear what effect the CaM has on $[\text{Ca}^{2+}]_{i}$. These experiments highlight an additional level of potential complexity that might be explained by temporal or spatial dynamics of the $[\text{Ca}^{2+}]_{i}$ signature. A further glimpse of such complexity might be the observation that overexpression of a putative ER Ca$^{2+}$-ATPase in rice aleurone leads to GA-independent Osamy-c:Luciferase expression, possibly by disrupting spatial or temporal aspects of $[\text{Ca}^{2+}]_{i}$ signals 17.

Taken together these data suggest that for both GA and ABA there are specific $\text{Ca}^{2+}$-CaM-dependent and -independent signalling events. In the case of ABA, these correlate with responses

![Diagram](image-url)  

**Fig. 2.** Principal components in $[\text{Ca}^{2+}]_{i}$ and CaM signalling in aleurone. Gibberellin (GA), perceived by a receptor (red) at the plasma membrane (green line), elevates $[\text{Ca}^{2+}]_{i}$, and CaM elevates ER $[\text{Ca}^{2+}]_{i}$ maintenance. Ca$^{2+}$ stimulates secretory vesicle fusion, probably via a monomeric G protein. ABA stimulation of Em gene expression (via an intracellular ABA receptor (pale blue)), and GA stimulation of $\alpha$-amylase and CaM gene expression, are Ca$^{2+}$ and CaM-independent.
secretion. LY also prevents DNA degradation and cell death35,36. Nevertheless, they certainly precede the increase in transcription and, not unexpectedly, there is both molecular and pharmacological evidence for the involvement of protein kinase cascades in GA and ABA action. ABA treatment of wheat and barley aleurone induces the expression of a serine/threonine protein kinase, PKABA1 (Ref. 37). A possible role for this kinase in ABA inhibition of GA-regulated gene expression is suggested from experiments in which constitutive overexpression of PKABA1, strongly inhibits GA induction of α-Amy1, α-Amy2 and cysteine proteinase promoter:GUS constructs (Fig. 4). Overexpression of a null mutant of PKABA1, or of a different CDPK, has no effect on GA induction of the α-Amy2 promoter:GUS construct. Overexpressing the kinase also has a minor effect on ABA induction of HvVIA1 gene expression39. This could indicate a broader signalling role for PKABA1, or might be an artifact of overexpression.

In an ingenious assay, protein kinase substrate peptides have been microinjected into barley aleurone protoplasts in the expectation that they would compete with the endogenous protein phosphorylation targets and block kinase cascades38. One peptide, syntide-2, selectively inhibits GA induction of α-amylase/GUS, α-amylase secretion and protoplast vacuolation. The peptide does not prevent GA-stimulation of [Ca\(^{2+}\)]\(_i\), indicating that it is acting downstream of this early event in GA-signalling. Syntide-2, is a specific substrate for mammalian CaM-kinase II. To date, there is no direct evidence for a CaM-kinase II in plants therefore the identity of the aleurone kinase, or kinases, blocked by syntide-2 is not clear. Nevertheless, on the basis of a series of in vitro phosphorylation and protein-labelling assays, a putative Ca\(^{2+}\)-/CaM-kinase II has been identified in barley aleurone cytosol38. At this stage it is not known if syntide-2 blocks one or more events in the GA-signalling

### Box 3. Calcium and calmodulin: key points

- Gibberellin (GA) stimulates [Ca\(^{2+}\)]\(_i\) and CaM, abscisic acid (ABA) prevents this.
- Ca\(^{2+}\)/CaM maintain secretion in GA-treated protoplasts.
- Up-regulation of gene expression by GA and ABA is Ca\(^{2+}\)/CaM-independent.
- Down-regulation of α-amylase gene expression by ABA is Ca\(^{2+}\)/CaM-dependent.

### Protein kinase cascades

Reversible protein phosphorylation is a universal signal-transducing mechanism and, not unexpectedly, there is both molecular and pharmacological evidence for the involvement of protein kinase cascades in GA and ABA action. ABA treatment of wheat and barley aleurone induces the expression of a serine/threonine protein kinase, PKABA1 (Ref. 37). A possible role for this kinase in ABA inhibition of GA-regulated gene expression is suggested from experiments in which constitutive overexpression of PKABA1, strongly inhibits GA induction of α-Amy1, α-Amy2 and cysteine proteinase promoter:GUS constructs (Fig. 4). Overexpression of a null mutant of PKABA1, or of a different CDPK, has no effect on GA induction of the α-Amy2 promoter:GUS construct. Overexpressing the kinase also has a minor effect on ABA induction of HvVIA1 gene expression39. This could indicate a broader signalling role for PKABA1, or might be an artifact of overexpression.

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- Up-regulation of gene expression by GA and ABA is Ca\(^{2+}\)/CaM-independent.
- Down-regulation of α-amylase gene expression by ABA is Ca\(^{2+}\)/CaM-dependent.
Pharmacological studies have provided further evidence for kinases and phosphatases in GA and ABA signalling. Okadaic acid (OA) inhibits GA-induced increases in \([\text{Ca}^{2+}]_i\), α-amylase mRNA, α-amylase secretion and aleurone cell death at concentrations that inhibit the activity of serine/threonine protein phosphatases (PP) PP1 and PP2B (Ref. 39). The effect of OA on the elevation of \([\text{Ca}^{2+}]_i\) caused by GA suggests that a reversible phosphorylation event is an essential part of this particular \([\text{Ca}^{2+}]_i\) signature, although not of the \([\text{Ca}^{2+}]_i\) elevation caused by hypoxia\(^{17}\). Although the target of this is not currently known it might prove useful to search for protein phosphorylation events that occur within minutes of GA treatment. Because OA also inhibits GA-induction of α-amylase gene expression and cell death – events independent of the elevation of \([\text{Ca}^{2+}]_i\) – OA could be acting in several ways (Fig. 5).

In barley aleurone protoplasts, OA partially inhibits ABA-induced PHVA1 gene expression, and tyrosine and serine/threonine phosphatase inhibitors prevent ABA-induced RAB gene expression, leading to tyrosine hyperphosphorylation of two 40 kDa polypeptides\(^{40}\). A current hypothesis is that ABA regulation of sub16 gene expression in barley aleurone involves rapid stimulation of a putative mitogen-activated protein kinase (MAP kinase) via a tyrosine phosphatase\(^{41}\).

In summary, a range of molecular and pharmacological data supports the hypothesis that protein kinases and phosphatases are involved in GA and ABA signalling in aleurone. The identity of the enzymes, their targets and their precise relationships to GA-stimulated events are poorly defined. For ABA signalling, the apparent role of reversible protein phosphorylation in aleurone is supported by molecular genetic evidence that two OA-insensitive serine/threonine phosphatase inhibitors are involved in ABA signalling in Arabidopsis\(^{42}\). In the case of GA, investigations have highlighted that the role of \([\text{Ca}^{2+}]_i\) in GA-regulation of gene expression is not fully understood (Box 4).

**Putative serine/threonine-O-linked N-acetylgalactosamine transerase is a negative regulator of GA signalling**

Recessive mutations at the spindly (spy) locus of Arabidopsis confer a largely GA-independent phenotype characterized by a reduced GA requirement for germination, increased internode length and altered flowering time. All spy alleles tested partially suppress the phenotype of the GA-deficient mutant ga1-1. SPY is therefore thought to act as a negative regulator of GA signalling in Arabidopsis\(^{43}\). The barley homologue of SPY is expressed at a low level in aleurone cells. When it is over expressed behind the CaMV35S promoter in a transgenic assay, SPY inhibits GA-induced expression of an α-amylase promoter-reporter construct\(^{43}\). Intriguingly, overexpression of SPY in aleurone stimulates the expression of an ABA-regulated dehydrin promoter. These data suggest that SPY has a conserved function as a negative regulator of GA responses in vegetative tissues and aleurone cells, and that it might be a positive regulator of ABA signalling in aleurone. Nevertheless, overexpression of signalling proteins might not reveal their true function in vivo, or accommodate subtleties of post-transcriptional regulation that might be essential to their action. Further studies will no doubt address such issues.

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**Fig. 4.** The abscissic acid (ABA)-inducible protein kinase PKABA1 negatively regulates gibberellin (GA)-inducible gene expression. GA is perceived by a receptor (red) at the plasma membrane (green line). GA-induction of α-Amy1, α-Amy2 or cysteine proteinase/GUS constructs is strongly suppressed by constitutive overexpression of PKABA1. ABA induction of PKABA1 gene expression might be mediated by ABA receptors at either the plasma membrane (dark blue) or inside the cell (pale blue). 

**Fig. 5.** Scheme depicting possible protein phosphorylation events revealed by okadaic acid (OA) and syntide-2. Gibberelinin (GA) is perceived by a receptor (red) at the plasma membrane (green line). Because OA inhibits the GA stimulation of \([\text{Ca}^{2+}]_i\), α-amylase gene expression and programmed cell death (PCD) it might be inhibiting PP1 or PP2B at a point in GA signalling upstream of these events (1), or at multiple sites downstream of this (2 and 3). Syntide-2 identifies a protein phosphorylation event downstream of the effect of GA on \([\text{Ca}^{2+}]_i\) that could be in the pathway to gene expression or α-amylase secretion.
SPY has significant amino acid sequence identity to a family of serine and threonine-O-linked N-acetylgalactosamine (O-GlcNAc) transferases (OGTs) that extends over both the characteristic tetratricopeptide repeat (TPR) and catalytic domains. Although OGT activity has yet to be demonstrated for SPY, it is likely that this signalling protein interacts with other proteins via the TPR domain and that O-GlcNAc modification affects their signalling potential42. However, there are numerous potential targets of OGTs: two possible targets of SPY O-GlcNAc modification are GAI and RGA.

GAI and RGA
To date there is no direct evidence for a role for GAI or RGA in GA signalling in aleurone cells. GAI and RGA are members of a small gene family in Arabidopsis, encoding nuclear-localized putative transcription factors43. GAI and RGA are GA-derepressible repressors of GA-mediated growth responses. The N-terminal region of these proteins contains a so-called DELLA region, which is thought to play a critical role in GA-derepression. It is now known that the maize dwarf-8 (db) gene and two Reduced height loci of wheat, Rht-B1b (formerly Rht1) and Rht-D1b (formerly Rht2), are orthologs of GAI, which contain nucleotide substitutions resulting in the mutant genes encoding N-terminally truncated proteins44. The related Reduced height locus Rht-B1c of wheat is known to affect GA sensitivity in aleurone45, raising the possibility that this class of proteins might have a role in GA signalling in aleurone (Box 5).

Regulation of gene transcription
The expression of a variety of aleurone hydrolase genes is induced or stimulated by GA, and this is overcome by ABA. Investigations have concentrated on the transcriptional regulation by GA and ABA of barley, wheat, rice and wild oat gene promoters. Functional analysis of α-Amy1 and α-Amy2 gene promoters by transient expression and gel retardation assays in combination with DNase-1 footprinting, have led to the identification of several cis-elements and sites at which nuclear proteins bind. In some cases, the trans-acting factors that bind to specific sequences have been cloned and characterized. It appears that the regulation of α-Amy1 and α-Amy2 gene promoters involves the interplay of several classes of transcription factor (Fig. 6). As yet, relatively little is known about comparable elements in other GA-regulated genes.

GA response element GAMYB and HRT
The GA response element (GARE) has been identified as a 21-nucleotide region of the barley Amy 1/6-4 promoter that, when present as six copies in a chimeric promoter driving a reporter gene, confers GA-responsive expression that could be overcome by ABA (Ref. 47). This region of the promoter in both α-Amy1 and α-Amy2 genes contains the sequence element TAACA/GA, which has been shown in functional studies to be an important component of the GARE (Refs 48,49). The GARE appears to bind GA-inducible nuclear proteins50. A potential similarity between the TAACA/GA element and c-MYB and v-MYB recognition sequences led to the isolation of a barley aleurone cDNA, encoding a MYB transcription factor that binds specifically to the TAACA/GA element and is able to transactivate an α-Amy1 gene promoter in a transient assay51. A rice GAMYB appears to have a similar function52 (Fig. 7).

GAMYB expression is stimulated by GA in advance of α-amylase gene expression. GAMYB functions within the GA-response complex (GARC) to transactivate α-Amy1 and α-Amy2 promoters. The GA response element (GARE) of α-Amy1 promoters binds the repressor HRT. Box 2 is a binding site for the WRKY proteins ABF1 and ABF2 in α-Amy2 promoters, although the regulation of ABF1 and ABF2 is not understood.
induces the expression of α-Amy1/genes. Because GAMYB expression is super-induced by cycloheximide, one theory is that GA signalling might act on a rapidly turned-over repressor of GAMYB expression although the identity of this is unknown. Nevertheless, post-transcriptional regulation of GAMYB activity by phosphorylation, or interaction with other proteins, is also probably based on models of how this class of transcription factors is regulated in other signalling systems. Is GAMYB involved in stimulating the expression of other GA-regulated genes in aleurone? A recent study has demonstrated that GAMYB can trans-activate the expression of GUS fused to promoter fragments of three other GA-regulated genes: an α-Amy1 gene, an EII(1-3,1-4)-β-glucanase gene and a cathepsin B-like protease gene. Analysis of these promoters reveals that the α-Amy2 gene contains a TAACAA/GA element, which is probably the GAMYB-binding site. However, neither the EII(1-3,1-4)-β-glucanase nor the cathepsin B-like protease promoters contain a TAACAA/GA element and it is likely that GAMYB transactivates these promoters through closely related, although as yet not unambiguously defined, elements.

GAMYB is not the only transcription factor that binds to the 21 nucleotide GARE (Ref. 47). Southwestern screens of a barley aleurone cDNA library using fragments of the Amy1/6-4 promoter have identified a novel, nuclear-located, zinc-finger protein, HRT, which binds the 21 nucleotide GARE and can repress GA-induced expression from α-Amy1 and α-Amy2 promoters. This HRT is therefore another control element acting on the GARE, although its regulation and relationships to GAMYB are not understood. The maize transcriptional activator VP1 can repress GA-induction of an α-Amy1 gene promoter, although it is not clear whether VP1 interacts directly with the α-amylase promoter or with other transcription factors bound to it.

GA-response complex

Although the GARE clearly plays a central role in GA activation of α-amylase gene expression, additional cis-elements are associated with the GARE and act as enhancers within a GA-response complex (GARC) that mediates GA- and ABA-responsive expression in both high and low pI α-amylase promoters. In barley α-Amy1 genes, it is thought that the GARE and TATCCAT box comprise a GARC (Ref. 49). In α-Amy2 genes the GARC is thought to comprise the GARE and Box 2 (Ref. 59).

TATCCAT box

The TATCCAT box is present, and binds as yet uncloned nuclear protein, in both Amy1 and Amy2 genes. Functional studies suggest that this box, and sequence elements immediately downstream from it, play an important role in driving GA-regulated expression.

Box 2 and WRKY proteins

Functional analysis has demonstrated that for a single copy of the GARE to function in α-Amy2 promoters, Box 2 must be present as a coupling element. Box 2 binds two members, ABF1 and ABF2, of the WRKY family of DNA-binding proteins, which play an important role in driving GA-regulated gene expression.

Box 6. Gene transcription: key points

- The gibberellin response element (GARE) of α-amylase promoters plays a central role in gibberellin (GA)- and abscisic acid (ABA)-regulated gene expression.
- GAMYB is induced by GA and binds to the TAACAA/GA element within the GARE.
- The repressor HRT also binds to elements within the GARE.
- Other cis-elements and trans-acting factors are involved in GA-regulated gene expression.

Perspectives

Aleurone cells and protoplasts are an excellent system for studying GA regulation of gene expression, protein secretion, cell death and the antagonistic effect of ABA. The use of effector and reporter gene constructs, in combination with a range of pharmacological agents, has helped to define elements of signalling pathways that encompass events at the plasma membrane, in the cytoplasm and in the nucleus. Understanding the order and integration of signalling events in aleurone is a challenge that should become accessible through the generation and use of mutants.
Acknowledgements

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Formation and maintenance of the shoot apical meristem

John L. Bowman and Yuval Eshed

Development in higher plants is characterized by the reiterated formation of lateral organs from the flanks of shoot apical meristems. Because organs are produced continuously throughout the life cycle, the shoot apical meristem must maintain a pluripotent stem cell population. These two tasks are accomplished within separate functional domains of the apical meristem. These functional domains develop gradually during embryogenesis. Subsequently, communication among cells within the shoot apical meristem and between the shoot apical meristem and the incipient lateral organs is needed to maintain the functional domains within the shoot apical meristem.

P ot-embryonic development in higher plants is characterized by the reiterated formation of lateral organs from the flanks of apical meristems. A shoot apical meristem (SAM) is initially formed during embryogenesis, and derivatives of this meristem give rise to the above-ground portion of the plant. The SAM contains a population of pluripotent stem cells, which serve three primary functions:

1. Lateral organs, such as leaves, are produced from the peripheral regions of the SAM.
2. The stem cells of the SAM contribute to the formation of the stem.
3. The stem cells of the SAM must replenish those regions from which cells have been recruited and maintain the pool of stem cells required for further growth.

As a result of histological analyses the SAM has been subdivided into two different manners. First, three distinct zones of the SAM are defined by cytoplasmic densities and cell division rates:

- the peripheral zone
- the central zone
- the rib zone

These three zones might represent a functional subdivision of the SAM although direct evidence for this is lacking. Lateral organs are produced from cells recruited from the peripheral zone whereas stem tissue is derived from cells recruited from the rib zone. The central zone acts as a reservoir of stem cells, which replenish both the peripheral and rib zones, as well as maintaining the identity of the central zone. It should be noted that these cells do not act as permanent initials, but rather their behavior is governed by a position-dependent manner. Second, the SAM is also composed of clonally distinct layers of cells. The fact that the peripheral and central zones, as well as the lateral organs produced, contain cells from the three clonally distinct layers indicates that communication between cell layers is required to coordinate developmental processes. For example, leaves in most eudicots species are composed of derivatives from the epidermal layer (L1), the subepidermal layer (L2) and corpus (L3). One of the earliest markers of leaf initiation from the peripheral zone is the periclinal cell division in specific regions in the L2. Cells in the L1 and L3 adjust their growth accordingly, with the entire region acting coordinately to produce a leaf primordium.

In this review, we discuss some recent advances in our understanding of three aspects of meristem functioning: the origin of the SAM during embryogenesis, the maintenance of the stem cell population in the central zone, and the relationships between lateral organ primordia and the meristems from which they are produced. Several excellent reviews cover broader views of the biology of the SAM (Ref: 2-4).