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The Role of GAMYB Transcription Factors in GA-Regulated Gene Expression

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Abstract

A gibberellin- and abscisic acid-regulated MYB, GAMYB, was first identified as an activator of GA-regulated genes in cereal aleurone. Here we review recent advances made in delineating the signaling events related to GAMYB expression and function in aleurone. In addition, there is a growing body of evidence that GAMYB plays an important role in other aspects of plant growth and development, including anther development, stem elongation, floral initiation and seed development.

Key words: Gibberellin; GAMYB; Germination; Aleurone; Abscisic acid; α-amylase; Signal transduction

Introduction

Gibberellins (GA) play an important role in mobilization of endosperm reserves following germination of cereal grains. Soon after germination, gibberellins are released from the embryo into the aleurone layer where they induce the expression of a number of genes encoding hydrolytic enzymes (Jacobsen and others 1995). The hydrolytic enzymes are secreted into the starchy endosperm, progressively hydrolyzing the starch, protein and cell wall reserves, which are then utilized by the rapidly growing seedling.

Over the last 10 years, rapid progress has been made in our understanding of the molecular mechanisms of GA-regulated gene expression in the cereal aleurone. Molecular, genetic, biochemical and proteomic techniques have all been used successfully to identify transcription factors and signal transduction components that regulate the expression of hydrolytic enzymes such as α-amylase, a key starch-degrading enzyme (Lovegrove and Hooley 2000; Olszewski and others 2002).

The demonstration that GA action in aleurone includes the activation of α-amylase gene transcription led to a number of studies designed to identify the relevant *cis*- and *trans*-acting factors (Lovegrove and Hooley 2000; Olszewski and others 2002). The functional analysis of α-amylase gene promoters has led to the identification of a 21 bp gibberellin response element (GARE) containing a conserved sequence, TAACAA/GA, which is found in the promoters of a number of GA-responsive genes (Skriver and others 1991).

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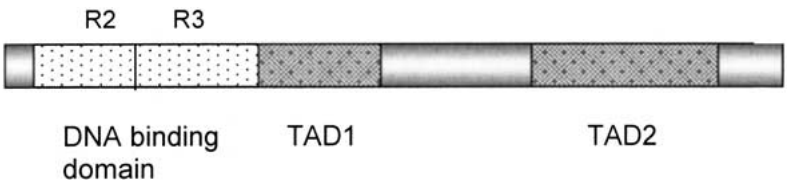

Figure 1. HvGAMYB has a conserved MYB DNA binding domain and two transcriptional activation domains (TAD).

The GARE has been shown to play a key role in mediating both the GA- and abscisic acid (ABA) regulation of these genes. A number of other *cis*-acting elements in α-amylase gene promoters have also been shown to act as enhancers within a GA-response complex. These include Box 2, pyrimidine and TATCCAT boxes (Gubler and Jacobsen 1992; Lanahan and others 1992; Rogers and Rogers 1994; Tregear and others 1995). The combination and relative positions of these *cis*-acting elements varies between GA-responsive promoters but all known GA-responsive promoters contain a GARE.

Trans-acting factors which bind to the *cis*-acting elements in α-amylase gene promoters have been identified but in some cases their roles in GA-regulated gene expression are not fully understood. Functional screening of Box 2 element-binding proteins identified two members of the WRKY family, ABF1 and 2, which do not appear to be transcriptionally regulated by GA in oat aleurone cells (Rushton and others 1995). BPBF (*barley prolamin-box binding factor*), a GA-upregulated DOF transcription factor, binds to pyrimidine boxes from α-amylase and protease promoters (Mena and others 2002). Transient expression experiments indicate that BPBF acts as a repressor of α-amylase transcription. The GARE appears to be the binding site of two transcription factors with opposing roles (Gubler and others 1995; Raventós and others 1998). HRT (for *Hordeum* repressor of transcription), a zinc finger protein that binds to a 21 bp sequence containing the GARE, represses α-amylase transcription in transient expression analyses (Raventós and others 1998). The role of a second GARE-binding factor, GAMYB, is the subject of this review and will be examined below.

GAMYB is a Transcriptional Activator of GA-Regulated Genes in Aleurone Cells

The importance of the TAACAA/GA sequence in the 21 bp GARE contained in GA-responsive promoters has been confirmed by functional analysis. Mutations introduced into the TAACAA/GA sequence virtually abolish the GA responsiveness of the α-amylase promoter in transient expression analyses (Gubler and Jacobsen 1992; Rogers and Rogers 1992; Tregear and others 1995). The similarity of the TAACAA/GA sequence to plant and animal MYB binding sites led to the isolation of a barley aleurone cDNA encoding a GA-regulated *Myb* called *HvGAMYB* (Gubler and others 1995). Members of the MYB family of transcription factors are highly conserved in the N-terminal regions but show great variation in the C-terminal regions (Stracke and others 2001). The N terminal region of HvGAMYB contains a typical R2/R3- MYB DNA-binding domain, consisting of 2 helix-loop-helix repeats, and *in vitro* binding studies have shown that HvGAMYB binds specifically to the TAACAA/GA sequence of the GARE (Figure 1) (Gubler and others 1995). Furthermore, nucleotides that are critical for HvGAMYB binding are also important for GARE function. HvGAMYB binding specificity has been determined using a PCR-based random site selection technique (Gubler and others 1999). The deduced consensus binding sequence YAACSRMM conforms closely with the TAACAA/GA sequence in the GARE.

The finding that transiently expressed HvGAMYB strongly activates the α-amylase gene promoter indicates that HvGAMYB functions as a transcriptional activator of α-amylase (Gubler and others 1995; Gubler and others 1999; Gómez-Cadenas and others 2001). Mutations within the TAACAA/GA sequence reduce the ability of HvGAMYB to transactivate the promoter, confirming that the TAACAA/GA sequence is the site of HvGAMYB binding. The constitutive expression of HvGAMYB in aleurone cells in the absence of GA is sufficient to trigger α-amylase promoter activity and indicates that HvGAMYB can replace the requirement for GA. Transient silencing of HvGAMYB through RNA-interference causes a large reduction in α-amylase promoter activity in GA-treated aleurone cells (Zentella and others 2002). This provides strong evidence that HvGAMYB expression is necessary as well as sufficient for the GA induction of α-amylase gene expression.

A functional map of the HvGAMYB protein (Figure 1) has been generated by analyzing the ac-

tivity of a series of C-terminal HvGAMYB deletions co-expressed in barley aleurone with α-amylase promoter-GUS fusions (Gubler and others 1999). Two transcriptional activation domains spanning the regions in HvGAMYB from amino acid positions 150 to 230, and 356 to 490 have been defined. This domain organization is supported by the observed activity of a series of COOH-terminal HvGAMYB deletions in a yeast one-hybrid system (Gubler and others 1999).

The presence of TAACAA/GA-like sequences in promoters of other GA-regulated hydrolase genes expressed in aleurone cells indicates that HvGAMYB may also transactivate expression of these genes. Transient expression experiments with protease and *EII*(*1-3*, *1-4*)-β-*glucanase* promoters have provided direct support for such a role (Cercós and others 1999; Gubler and others 1999). The *HvGAMYB* gene promoter may also be a target for HvGAMYB transactivation (F. Woodger unpublished data). The identification of a TAACAA/GA-like sequence within the *HvGAMYB* promoter and the finding that HvGAMYB strongly transactivates the *HvGAMYB* promoter provides evidence that HvGAMYB may be involved in a positive feedback loop which results in enhanced *HvGAMYB* gene expression. There are known examples of MYBs autoactivating their own gene expression (Schaffer and others 1998; Sala and others 1999).

Genome-wide approaches such as microarrays offer the possibility of identifying other genes transactivated by GAMYB in aleurone cells (Fath and others 2001). In rice aleurone cells, microarray analyses have identified 75 genes with increased expression in response to GA. Searching the promoters of GA-upregulated genes for OsGAMYB binding sites may identify new genes that are transactivated by OsGAMYB. The recent isolation of an *OsGAMYB* knockout mutant in rice will also provide a useful tool to identify OsGAMYB target genes in aleurone cells (M. Matsuoka personal communication).

GAMYB Expression is Regulated by GA and ABA

Timecourse studies have shown that GA induces a rapid increase in *HvGAMYB* gene expression prior to α-amylase gene expression in barley aleurone layers. Within 1–2 h of GA treatment, the level of *HvGAMYB* transcript and protein starts to increase and remains high over the next 12 h (Gubler and others 1995, 2002). There is evidence that the increase in *HvGAMYB* transcript is at least due in part to an increase in the rate of transcription. In nuclear run on experiments, a two-fold increase in the rate of *HvGAMYB* transcription occurs in response to GA (Gubler and others 2002). Furthermore, the activity of a HvGAMYB promoter:GUS fusion responds to GA in transient expression experiments (Gómez-Cadenas and others 2001). Attempts to identify the cis-acting elements within the *HvGAMYB* promoter which confer GA-responsiveness have failed due to the relatively low responsiveness of this promoter to GA (F. Gubler unpublished data). There is indirect evidence that GA may also regulate HvGAMYB expression at the posttranscriptional level (Gubler and others 2002). The large increase in amounts of GAMYB protein (10-fold) compared to transcript (3-4-fold) indicates that GA may promote HvGAMYB translation in addition to transcription although it is possible that HvGAMYB protein may be more stable than *HvGAMYB* transcript.

Analysis of GA response mutants in rice and barley has led to the identification of upstream GA signal transduction components that regulate GAMYB expression in aleurone cells (Olszewski and others 2002). The aleurone of the rice dwarf *d1* mutant, which is defective in the α-subunit of a heterotrimeric G-protein, has reduced sensitivity to GA compared to the wild type, and higher levels of GA are required to induce increases in α-amylase compared to the wild type (Ueguchi-Tanaka and others 2000). Similarly, in *d1* aleurone cells, amounts of *OsGAMYB* transcript increase only slightly compared to wild-type cells when incubated with 10^{-7}M GA_3. The GAI/RGA-like proteins, such as barley SLN1, rice SLR1 and wheat RHT are putative transcription factors which function as negative regulators of the GA response in cereal aleurone (Peng and others 1999; Ikeda and others 2001; Chandler and others 2002). Aleurone layers from the barley *Sln1d* mutant, which contains a dominant mutation in the conserved DELLA region of SLN1, require 100-fold more GA than the wild type to induce expression of both *HvGAMYB* and α-amylase (Chandler and others 2002).

Recently, progress has been made in understanding how GA modulates SLN1 repression of *HvGAMYB* and α-amylase genes. SLN1 and related proteins RGA1 and SLR1 are rapidly degraded in response to GA, effectively 'derepressing' GA signaling (Dill and others 2001; Fu and others 2002; Gubler and others 2002; Itoh and others 2002). In aleurone cells the response is very fast, complete degradation of SLN1 protein being observed within 10 min of GA application (Gubler and others 2002). A lag time of 1 h between SLN1 degradation and the

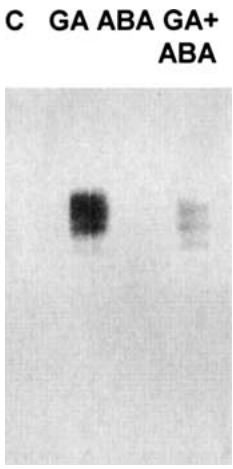

Figure 2. Effect of GA and ABA on HvGAMYB protein in barley aleurone layers. Aleurone layers incubated without hormone (C), and with 10^{-6}M GA_3 (GA), 5×10^{-5}M ABA (ABA), and 10^{-6}M GA_3 and 5×10^{-5}M ABA (GA + ABA) for 12 h. HvGAMYB protein was detected using affinity-purified antibodies as previously described (Gubler and others 2002).

expression of *HvGAMYB* indicates that the SLN1 is not a direct repressor of *HvGAMYB*. Instead, it may act through an intermediary that has been proposed to be cGMP (Penson and others 1996; Gómez-Cadenas and others 2001; Gubler and others 2002). Like many of the GA responses in cereal aleurone, *HvGAMYB* gene expression is also inhibited by ABA (Figure 2) (Gubler and others 2002). Experiments with *sln1* loss of function mutants indicate that ABA acts downstream of SLN1 to block GAMYB transcription (Gómez-Cadenas and others 2001). The failure of ABA to prevent GA-stimulated SLN1 degradation is consistent with this model (Gubler and others 2002).

Regulation of GAMYB Function by GAMYB-Binding Proteins

Although it is well established that the ratio of GA to ABA regulates *HvGAMYB* gene expression in aleurone, posttranslational regulation is also likely to be an important mode of control of HvGAMYB activity. There are multiple isoforms of HvGAMYB in aleurone, and HvGAMYB is detectable in non-GA-treated barley aleurone without the expected accumulation of α-amylase activity (Gubler and others 2002). Also, reversible phosphorylation and acetylation are already known to modulate the function of other MYB transcription factors and MYBs are increasingly understood to operate as part of large transcriptional complexes (Vorbrueggen and others 1996; Colgin and Nyborg 1998; Wong and others 1998; Tomita and others 2000). HvGAMYB-binding proteins that are expressed in barley aleurone have been identified. Potentially, these factors could be involved in the posttranslational regulation of HvGAMYB and, in a number of cases, functional evidence for such a role has been obtained.

It has recently been reported that the DOF transcription factor, BPBF, can interact with HvGAMYB in yeast and that these two transcription factors coordinately activate the expression of genes encoding seed storage proteins during seed development (Diaz and others 2002; see below). This work has also been extended into barley aleurone with the finding that BPBF is expressed in this tissue following seed germination (Mena and others 2002). In this study the authors showed that the expression of *Pbf* (the gene encoding BPBF) is GA-responsive but ABA-repressible in isolated aleurone. It was noticed also that the pyrimidine box, 5′-CTTTT-3′, contained in the GARC of many GA-responsive grain hydrolase promoters (see above) is complementary in sequence to the core-binding sequence found in the promoters of seed storage genes regulated by BPBF (Mena and others 1998). BPBP binds to this motif *in vitro* in the promoters of the *AL21* protease and *Amy2/32b* genes, both of which are GA-inducible in aleurone (Mena and others 2002). In co-bombardment assays, expression of BPBF inhibits both GA-induction of the *AL21* protease promoter and the transactivation of this promoter by constitutively expressed HvGAMYB (Mena and others 2002). Unlike their apparently co-operative role in the transactivation of seed storage gene expression in developing seeds, the authors propose that HvGAMYB function in aleurone can be negatively regulated by BPBF, possibly as part of programmed cell death. This is consistent with the observation that BPBF is expressed later than HvGAMYB in GA-stimulated aleurone (Mena and others 2002).

To isolate HvGAMYB-binding partners from barley aleurone, we have conducted a yeast two-hybrid screen using the RAS-recruitment system (RRS; Woodger and others 2003). The RRS is a yeast two-hybrid system where protein-protein interactions are monitored at the inner face of the plasma membrane rather than in the nucleus (Broder and others 1998). This system is suitable for the study of transcription factors such as HvGAMYB, which autoactivate the transcriptional readout used to monitor interactions in the conventional yeast-two-hybrid system (Gubler and others 1999). Four

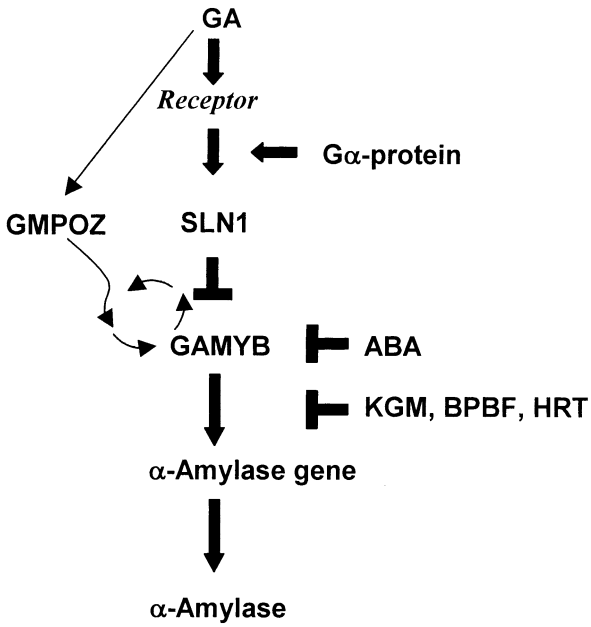

Figure 3. Model of GA and ABA regulation of GAMYB and α-amylase gene expression in cereal aleurone cells.

partial cDNAs with potential functions in signaling or transcriptional regulation were identified as interacting with HvGAMYB in yeast: (i) a novel WD40-domain protein, (ii) a homolog of the human nuclear coactivator SKIP, (iii) a leucine-acidic domain-rich protein, and (iv) a ser-thr kinase (Woodger and others 2003; GenBank accession numbers AY167560, AY167563, AY167562, and AY167561, respectively).

The full length protein encompassing the ser-thr kinase binds HvGAMYB *in vitro* and was termed KGM (*kinase associated with GAMYB*). KGM is a member of the emerging Mak-subgroup of protein kinases, which are related to the cdc2- and MAP kinases (reviewed in Miyata and Nishida 1999). Co-bombardment assays revealed that KGM can specifically inhibit GA-inducible α-amylase promoter activity by repressing the transactivation of this promoter by HvGAMYB (Woodger and others 2003). This activity is partly dependent on the integrity of a conserved tyrosine residue in the activation loop of KGM, a site that must be phosphorylated in classical MAP kinases to facilitate substrate recognition and catalytic activation (Cobb and Goldsmith 1995). Thus, it is possible that phosphorylation and dephosphorylation are important in the regulation of KGM activity. Despite the functional and physical interaction observed between KGM and HvGAMYB, recombinant GST-KGM fusion protein does not phosphorylate HvGAMYB in *in vitro* kinase assays. Further investigation of this possibility *in vivo* is required. It is also possible that KGM modulates HvGAMYB function by a kinase-independent mechanism as other kinases are known to regulate transcription factor activity through sequestration rather than phosphorylation in a manner which nonetheless depends on phosphorylation of the kinase activation loop (Bardwell and others 1998).

We have also characterized a second HvGAMYB-binding protein from the group of barley aleurone proteins isolated in the yeast two-hybrid screen described above. The original protein (GenBank accession number AY167562) features leucine-acidic domains similar to those found in animal nuclear co-activators (reviewed in Leo and Chen 2000), but we have also found that the full-length protein contains a BTB/POZ domain and so we have termed this protein GMPOZ (*GAM*YB-associated *POZ* protein) (F. Woodger unpublished data). The BTB/POZ domain is an NH_3-terminal domain of about 120 amino acids found in a variety of transcriptional regulators and cytoskeletal modifiers and is thought to provide a scaffold for the organization of higher-order structures such as the cytoskeleton and chromatin (reviewed in Collins and others 2001). Our preliminary data suggest that *GMPOZ* is a GA-inducible activator of α-amylase promoter activity but not at the level of HvGAMYB transactivation (F. Woodger unpublished data). As described above, HvGAMYB may also function to autoactivate *HvGAMYB* gene expression and thus we speculate that GMPOZ and HvGAMYB may function to co-regulate *HvGAMYB* gene expression (unpublished data).

A model summarizing current understanding of the hormonal regulation of the α-amylase gene, featuring the role of HvGAMYB, is presented in Figure 3. In this model, HvGAMYB expression occurs in a positive feedback loop, possibly featuring GMPOZ, which is stimulated by GA but repressed by SLN1 and by ABA downstream. The HvGAMYB-binding partners BPBF and KGM repress HvGAMYB function at the level of α-amylase gene transactivation, and HRT is a GARE-binding factor that is also a potential repressor of α-amylase transcription.

The GAMYB Family of Transcription Factors

To extend studies of GAMYB beyond barley, homologs have been isolated from a number of other species. In cereals, partial or complete GA-

MYB homolog cDNAs have been isolated from *Oryza sativa* (Gubler and others 1997), *Triticum aestivum* (Chen and others 2001), *Avena sativa* (unpublished genebank # CAB40189) and *Lolium temulentum* (Gocal and others 1999). The proteins encoded by these homologs all have greater than 95% amino acid identity to that of HvGAMYB. GAMYB homologs, or *GAMYB*-like genes, have also been identified in dicotyledonous plants, primarily *Arabidopsis.* However, unlike the cereals, which each appear to only have a single copy of GAMYB, *Arabidopsis* has a small family of *GAMYB*-like genes, namely, *MYB33*, *MYB65*, *MYB97*, *MYB101* and *MYB120* (Stracke and others 2001). In transient expression assays *MYB33*, *MYB65* and *MYB101* have been shown to functionally substitute for HvGAMYB in transactivation of the α-amylase promoter in barley aleurone (Gocal and others 2001). Thus it appears that the GAMYB gene family may have undergone expansion in dicotyledonous plants. These *Arabidopsis* genes all have very strong amino acid identity with HvGAMYB in the R2R3 domain, and in three sequence signature motifs, defined as Box1, Box2 and Box3 (Gocal and others 2001). However, identity drops off dramatically outside these regions, where proteins share no greater than 37% identity with HvGAMYB. Nonetheless, all these genes contain an intron located at the 3′ end of the open reading frame that is unique to this class of MYB genes. This further supports the claim that the cereal and *Arabidopsis GAMYB*-like genes were all derived from a common ancestral gene. Lastly, the proteins encoded by all the GAMYB family members are significantly larger (ranging from 389 to 581 amino acids) than the majority of the other members of the *Arabidopsis* R2R3 MYB transcription factor family (mainly 200–300). These three characteristics, sequence similarity, exon/intron structure and protein size are an argument for the fact that the GAMYB gene family belongs to a distinct subclass in the MYB superfamily of genes.

Functions of GAMYB Outside the Aleurone

Over the last several years there has been evidence suggesting that GAMYB functions in GA-mediated processes outside the aleurone including floral initiation, stem elongation, anther development and seed development. Primarily this evidence lies in the observation that GAMYB is selectively expressed in plant cells where these GA-mediated processes occur and that GAMYB gene expression increases in these tissues when exogenous GA is applied. Thus, GAMYB may be part of a common GA response pathway controlling diverse developmental processes in the plant.

Floral Initiation

Floral initiation has been studied extensively in the grass *Lolium temulentum* and occurs when the plant is exposed to long-day conditions. GAs increase with the long-day exposure and are thought to be transported to the apex where they induce the vegetative to floral transition (King and Evans 2003). The finding that exogenous GA application substitutes for long-day induction provides further evidence that floral induction involves GAs (King and Evans 2003). To assess whether GAMYB has a role in this process, the expression of the *Lolium* homolog *LtGAMYB* has been examined (Gocal and others 1999). Like *HvGAMYB*, *LtGAMYB* expression is upregulated in response to GA in the seed (Gocal and others 1999). *LtGAMYB* expression is also induced at the shoot apex during floral initiation, increasing significantly 100 h after long-day exposure. Furthermore, this expression is associated with increases in endogenous GA levels (5–20-fold) in the plant. Thus, the authors suggest that LtGAMYB plays an important signalling role in *Lolium* flowering (Gocal and others 1999).

A role for GAMYB in flowering has also been investigated in *Arabidopsis*, a plant in which flowering is also induced by exposure to long-days or GAs. Like *LtGAMYB*, the timing and location of *MYB* gene expression in *Arabidopsis* has been found to be consistent with roles in GA-mediated processes. For example, an increase in expression of *MYB33* at the shoot apex coincides with the onset of flowering, either when endogenous GA levels had increased in the plant or when GA was applied to the plants (Gocal and others 2001). Furthermore, mRNA *in situ* analysis has shown that *MYB33* has the same temporal and spatial expression pattern as the floral meristem-identity gene *LEAFY* (Gocal and others 2001). In addition to overlapping expression patterns, the MYB33 protein binds specifically to a MYB-binding site (5′-CAACTG-3′) within the *LEAFY* promoter (−375 to −88 bp). This element is known to be essential for the GA activation of the *LEAFY* promoter (Blázquez and Weigel 2000). Thus, in the apex it is possible that GA up regulates *MYB33*, which then binds to the *LEAFY* promoter inducing LEAFY expression, which induces the transition from a vegetative to floral state.

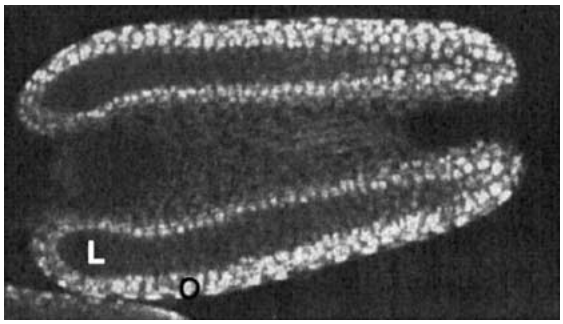

Figure 4. GFP expression in an anther from barley transformed with a HvGAMYB:GFP fusion gene. GFP was detected in nuclei of outer cell layers (O) including the tapetum but not in the locule (L). Anthers from barley plants expressing HvGAMYB:GFP fusion protein were analyzed with a confocal microscope (Murray and others 2002).

Stem Elongation

A partial wheat cDNA homologous to *HvGAMYB*, which is expressed in the first (sub-crown) internode of several varieties of wheat, has been isolated (Chen and others 2001). The subcrown internode of one variety, Hong Mang Mai, elongates strongly in response to applied GAs compared to other varieties of wheat tested. *TaGAMYB* expression correlates with internode length and is higher in Hong Mang Mai than in the other varieties. Furthermore, *TaGAMYB* expression in the internodes of Hong Mang Mai and one other variety, Hope, is induced by the application of GA. Although endogenous GA levels are not greater in Hong Mang Mai than in the other varieties of wheat, internode elongation in Hong Mang Mai appears to be much more sensitive to GA application, and this is reflected in the enhanced level of TaGAMYB expression. These preliminary data suggest that *TaGAMYB* may have a role in subcrown internode elongation but further work is required to develop this hypothesis. A number of genes are known to be induced by GA during elongation (Lee and Kende 2002) and it seems likely that the involvement of GAMYB in these processes in both mono- and dicotyledenous plants will soon be described.

Anther Development

To further investigate the expression of HvGAMYB in barley, northern blot analysis of RNA from different barley tissues has been conducted (Murray and others 2003). Outside the aleurone, expression is strongest in anthers and in transgenic barley plants expressing a HvGAMYB: Green Fluorescent Protein (GFP) fusion. HvGAMYB is expressed in all four anther cell layers – the epidermis, endothecium, middle layer and tapetum (Figure 4). Expression occurs early in development and, in the epidermis and endothecium, persists until anther dehiscence. In the middle layer and tapetum, expression persists until those cell layers degrade during the normal course of development. GA is known to be required for anther development, and HvGAMYB expression is also induced in anthers that have been incubated in GA_3. Furthermore, transgenic barley plants over-expressing the *HvGAMYB* gene have reduced male fertility, with the severity of the phenotype depending on the level of HvGAMYB expression (Murray and others 2003). Anthers that contained more than a four-fold increase in HvGAMYB protein relative to nontransgenic controls fail to dehisce, whereas transgenic plants expressing lower amounts of protein are able to shed some pollen. There are no other obvious morphological alterations in these transgenic plants.

Two genes from a tobacco pollen cDNA library, *NtmybAS1* and *NtmybAS2*, have been isolated that have high sequence similarity to GAMYB. Like *HvGAMYB*, the expression of these genes appears predominantly in the anthers of plants (Yang and others 2001). As revealed by *in situ* hybridization, these genes are most strongly expressed in the tapetum, where they are thought to regulate phenylalanine ammonia-lyase (PAL). This is because the expression of the *MYB* genes overlaps with *PAL* gene expression and *NtmybAS1* actives PAL promoters in cotransfected tobacco leaf protoplast assays. However, whether NtmybAS1 and NtmybAS2 are GA-regulated has not been investigated. It seems likely from these studies in both tobacco and barley that a function in anther development is one of the major roles of GAMYB. Interestingly, two GAMYB-binding proteins, KGM and GMPOZ (see above), are strongly expressed in anthers as well as aleurone, suggesting they may regulate GAMYB function in this tissue as well (Woodger and others 2003; F. Woodger unpublished data).

Seed Development

Several lines of evidence implicate HvGAMYB in the transactivation of the endosperm-specific genes, B-Hordein (*Hor2*) and trypsin-inhibitor BTI-Cme (*Itr1*) (Diaz and others 2002). Firstly, *HvGAMYB*, *Hor2* and *Itr1* have overlapping temporal expression patterns in the seed during development including aleurone, starchy endosperm, nucellar projection,

vascular tissue and the immature embryo. In gel shift assays recombinant HvGAMYB protein binds specifically to the motifs 5′-TAACAAC-3′ and 5′-CAACTAAC-3′ from the promoters of the *Hor2* and *Itr1* genes, respectively. These promoter motifs resemble the HvGAMYB binding sites in the α-amylase promoter (see above). HvGAMYB transactivates the *Hor2* and *Itr1* promoters *in vivo* but not when these binding motifs are disrupted. These results indicate a function for HvGAMYB in endosperm development. It was previously known that the DOF transcription factor BPBF binds to the *Hor2* promoter and activates Hor2 expression (Mena and others 2002). The disruption of the BFPF binding site prevents HvGAMYB transactivation of the promoter, indicating a functional interaction between these two proteins (Diaz and others 2002). Using a two-hybrid assay, the two proteins were found to physically interact in yeast.

As described above, in contrast to the positive regulatory interaction observed between BPBF and HvGAMYB in the control of endosperm gene expression during seed development, HvGAMYB function in aleurone is proposed to be negatively regulated by BPBF, possibly as part of programmed cell death (Mena and others 2002). Perhaps BPBF recruits other factors in a tissue-specific fashion, which in turn mediate the alternative activities of these transcriptional complexes within the correct developmental window. Certainly, the maize DOF1 protein is known to participate in a variety of protein-protein interactions (Yanagisawa 1997).

Concluding Remarks

Although GAMYB is clearly a wide-ranging activator of hydrolase gene expression in cereal aleurone, until recently very little was known about either the regulation of GAMYB function or the role of GAMYB in other tissues. However, with the identification of multiple GAMYB binding proteins, some with proven regulatory effects on GAMYB function, many new avenues are open for understanding how the activity of this transcription factor is controlled. Also, research in this field is rapidly moving beyond the study of post-germination events in aleurone cells to other GA-mediated processes including floral initiation, internode elongation and anther and seed development. Further study of GAMYB in these various tissues and processes seems likely to show that it functions widely in growth and development and is involved in many, if not all, GA-regulated processes.

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