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Translational Triggering and Feedback Fixation in the Control of Fungal Development

William E. Timberlake

Myco Pharmaceuticals Inc., Building 300, One Kendall Square, Cambridge, Massachusetts 02139

INTRODUCTION

The asexual reproductive pathway of the filamentous fungus *Aspergillus nidulans* serves as a model for the study of the molecular genetic controls of morphogenesis. Figure 1 shows the major developmental steps leading to formation of the multicellular sporophore, the conidiophore, and the uninucleate, mitotically derived spores, the conidia. Conidiation begins when vegetative cells (hyphae) that have acquired developmental competence (Axelrod et al., 1973) are subjected to poorly understood inductive signals that include reduced nutrient availability (Saxena and Sinha, 1973), red light (Mooney and Yager, 1990), and exposure to an air–surface interface (Axelrod, 1972). The first manifestation of development is the production of an aerial hypha by the anchoring cell, called a foot cell. This hypha is similar to vegetative hyphae in that it undergoes polarized extension by the apical addition of new cell wall material. However, instead of growing indeterminately, this conidiophore precursor ceases apical extension after reaching a height of ~100 μm and undergoes a dimorphic switch: its tip begins to expand by the depolarized addition of cell wall material (Figures 1A, 1B, 1F, and 1G; Mims et al., 1988). All subsequent developmental steps entail nonpolar, or budding, growth.

The swollen, multinucleate tip of the developing conidiophore, the vesicle (Figure 1B), produces a tier of buds referred to as metulae. The nuclei in the vesicle undergo division, and one daughter nucleus enters each metula (Figures 1C and 1H). The metulae undergo one to several budding divisions to produce the final cell type of the conidiophore, the sporogenous phialides (Figures 1D and 1I). Phialides are a type of stem cell because they repeatedly produce a new differentiated cell type, the conidium, by mitotic division while retaining their own identity (Mims et al., 1988; Sewall et al., 1990a; Figures 1E and 1J). Conidia are nearly spherical spores with thick, impermeable, hydrophobic cell walls that promote their dispersal and survival over years or decades.

Conidiation in *Aspergillus* is under convenient experimental control (Law and Timberlake, 1980), facilitating molecular and biochemical studies of development. It is also readily subjected to mutational and molecular genetic analyses (Clutterbuck, 1969; Timberlake, 1990), leading to identification of numerous genes that regulate the conidiation process or encode essential enzymes or structural proteins that contribute directly to morphogenesis. This review focuses on two regulatory genes, *bristle* (*brlA*) and *abacus* (*abaA*), whose activities are essential for conidiophore development. These genes encode transcriptional regulators that activate expression of developmentally important structural genes and are themselves developmentally regulated. Recent data support the hypothesis that *brlA* expression is initially activated by a translational control mechanism and in turn activates *abaA*. *abaA* next activates additional structural genes, *brlA*, and downstream regulatory genes. Moreover, *abaA* is subject to positive autoregulation and may be capable of retaining itself in the active state. Thus, it is likely that an initial developmental trigger working at the level of translation activates a set of feedback loops that fix the core developmental pathway in the active state. This is likely to represent the molecular basis for developmental determination in this system.

A MODEL FOR GENETIC CONTROL OF CONIDIATION

Figure 2 presents a model for the genetic control of conidiophore development and spore maturation that is based on results from numerous genetic studies (reviewed by Timberlake, 1990; Timberlake and Clutterbuck, 1993). Mutational analysis of the conidiation pathway has led to the identification of only a limited number of loci that are candidate developmental regulators (Clutterbuck, 1977; Clutterbuck and Timberlake, 1992). Of these, mutations in only three loci, *brlA*, *abaA*, and *wetA*, result in an essentially asporogenous phenotype, demonstrating that the genes are indispensable for the process. Loss-of-function mutations in two other genes, *stuA* and *medA*, result in the formation of deformed conidiophores that nevertheless are able to produce significant numbers of viable conidia. Thus, these genes are not absolutely required for conidiation but are needed for correct spatial organization of the conidiophore. Several other genes whose products contribute directly to the form or function of conidiophore cell types have been identified, including *wA*, encoding a polyketide synthase (Mayorga and Timberlake, 1992), and *yA*, encoding a *p*-diphenol oxidase (Clutterbuck, 1972; Aramayo and Timberlake, 1990), both of

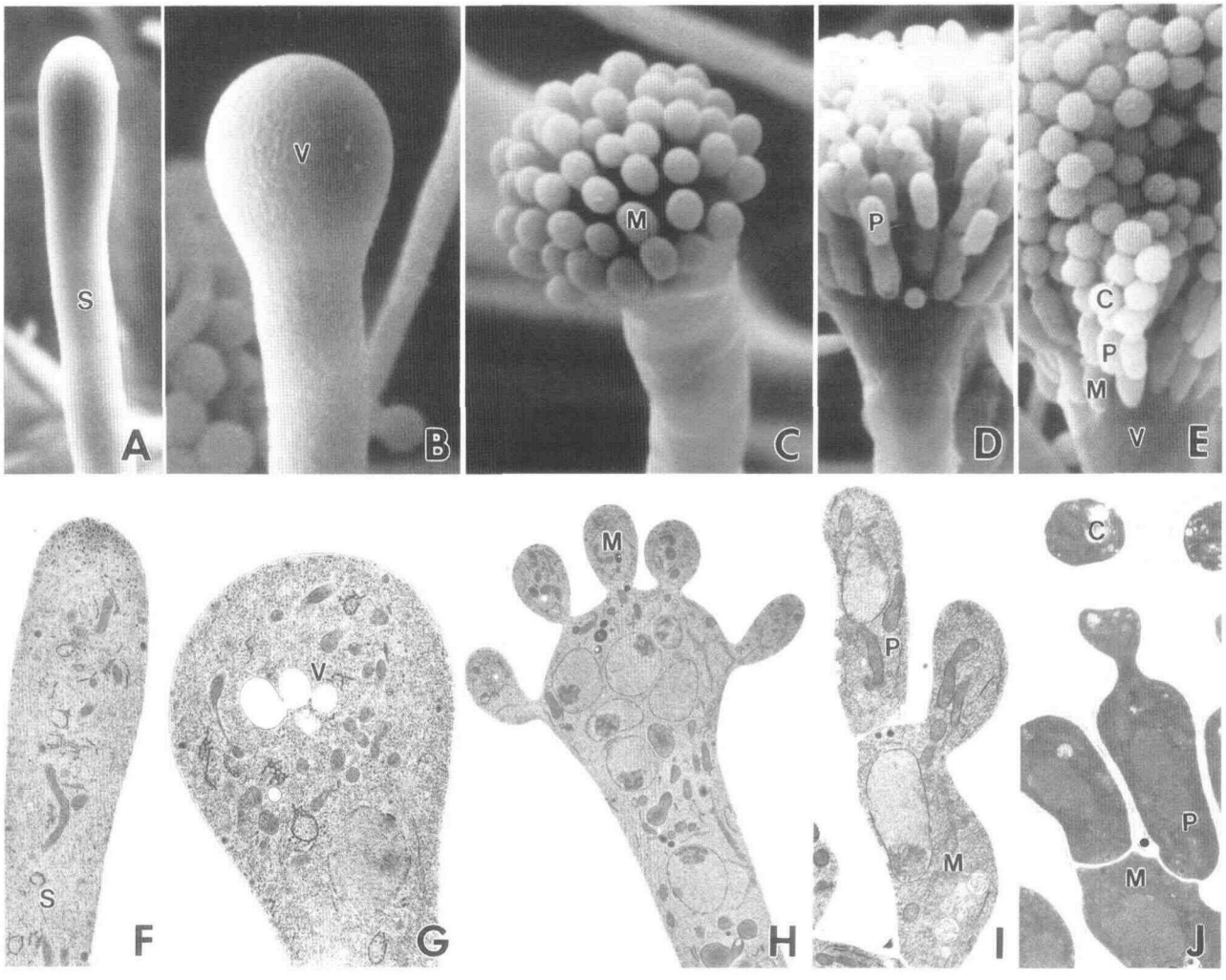

Figure 1. Electron Microscopic Analysis of Conidiophore Development in *Aspergillus*.

The upper panels show scanning electron micrographs of the major stages of development, whereas the lower panels show transmission electron micrographs of freeze-substituted samples at equivalent stages of development.
(A) and **(F)** Aerial hypha nearing the end of the polarized growth stage.
(B) and **(G)** Swelling of the hyphal tip to form the globose conidiophore vesicle (V).
(C) and **(H)** Vesicular budding to form uninucleate metulae (M).
(D) and **(I)** Metular budding to form the sporogenous phialide cells (P).
(E) and **(J)** Repeated production of conidia (C) by phialides.
See Oliver (1972), Mims et al. (1988), Sewall et al. (1990a, 1990b) for details.
Reproduced, with permission, from Timberlake (1991).

which direct synthesis of conidial pigment, and *rodA*, encoding a hydrophobic component of the conidial wall (Stringer et al., 1991). Numerous other developmentally regulated genes have been identified by molecular methods (Timberlake, 1980; Zimmermann et al., 1980) but have not yet been assigned functions.

The model in Figure 2 was proposed based on the following observations. Null *brlA* mutants make only conidiophore stalks that elongate indeterminately, failing to produce any of the other conidiophore cell types (Clutterbuck, 1969; Boylan et al., 1987). Null *abaA* mutants produce metulae that proliferate abnormally, never forming any phialides or spores (Sewall et al., 1990b). Null *wetA* mutants produce normal conidiophores but the spores fail to undergo normal maturation and autolyse (Clutterbuck, 1969; Sewall et al., 1990a). Thus, the mutant phenotypes suggested the order of gene action *brlA*→*abaA*→*wetA*. This order was confirmed by epistasis tests that examined conidiophore morphology in double mutants (Martinelli, 1979), studies

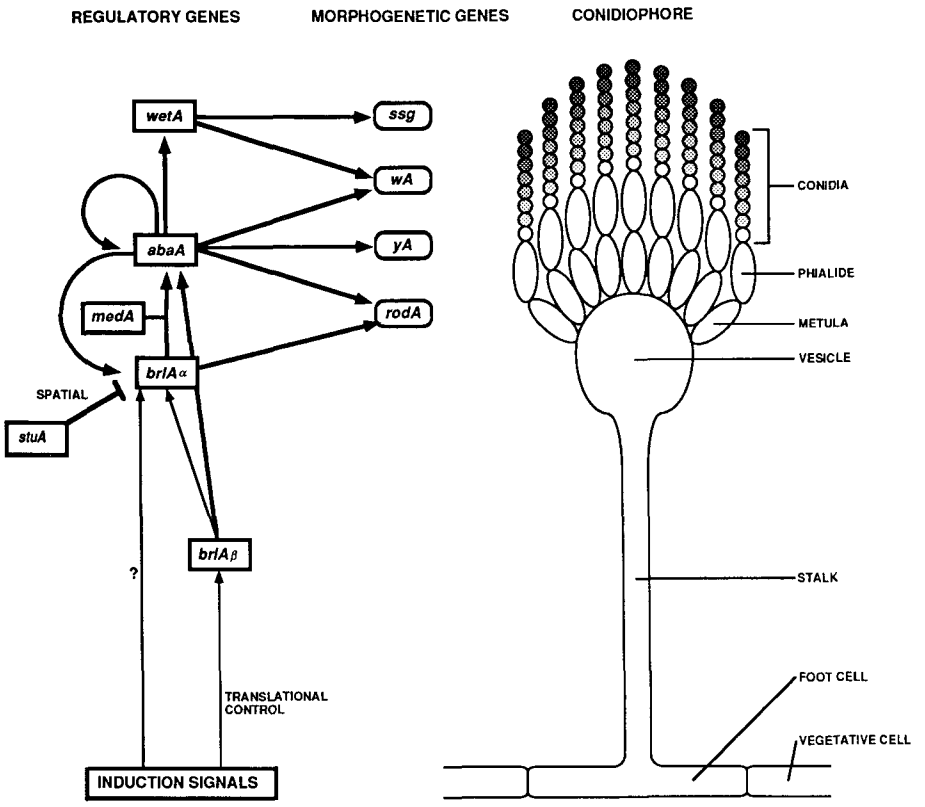

Figure 2. Proposed Regulatory Pathway for Conidiophore Development.

The core regulatory genes are *brlA*, *abaA*, and *wetA*. The activities of these genes are refined or modified by auxiliary regulatory genes such as *stuA* and *medA* (Miller et al., 1992). Morphogenetic genes such as *rodA*, *yA*, and *wA* produce products that contribute directly to the specialized phenotypes of differentiated conidiophore cells. *ssg* indicates spore-specific genes with unknown functions. Arrows indicate positive regulation; bars indicate negative regulation. In two cases (*brlA*, *abaA*), regulation has been shown to be mediated by direct interactions between transcription factors encoded by the regulatory genes and upstream promoter elements in the morphogenetic or regulatory genes. Initial regulation of *brlA* expression may occur at the level of translation. Positive feedback regulation of *brlA* by *abaA* and autogenous regulation of *abaA* could provide a mechanism to maintain the activity of the regulatory pathway independent of the environmental signals that initially activated it.

of gene expression patterns in the wild type and mutants (Zimmermann et al., 1980; Boylan et al., 1987), and determination of the effects of forced expression of the genes on hyphal morphology and gene expression under conditions that normally prevent sporulation (Adams et al., 1988; Mirabito et al., 1989; Marshall and Timberlake, 1991). Results from work by Mirabito et al. (1989) further indicated that *abaA* is a feedback regulator of *brlA*. The same experimental approaches were used to infer the connections between regulatory and structural genes.

The phenotypes of *stuA* and *medA* mutants indicate that the activities of these genes impinge upon the central regulatory pathway and refine its activities. *stuA* mutants form reduced conidiophores that produce conidia by direct vesicular budding or through production of aberrant metulae and phialides (J. Aguirre and W.E. Timberlake, unpublished results; Miller et al., 1992). *medA* mutants produce supernumerary tiers of sterigmata, some of which ultimately differentiate into productive phialides, others of which redifferentiate to produce abnormal conidiophores (J. Aguirre and W.E. Timberlake, unpublished results). Spatial control of transcription of both *brlA* (J. Aguirre and W.E. Timberlake, unpublished results) and *abaA* (Miller et al., 1992) is lost in *stuA* mutants, and this may be the underlying cause for the morphological abnormalities. *medA* mutants resemble mutants containing certain hypomorphic alleles of *brlA*, suggesting that the genes may have related or overlapping functions. This notion was confirmed by the finding that a null *medA* mutation was suppressed by the addition of an extra copy of *brlA* (B.L. Miller, personal communication). This result suggests that *medA* augments the activity of *brlA* to yield full activation of *abaA*. This idea is supported by the effects of *medA* mutations on *brlA* and *abaA*

transcription (J. Aguirre and W.E. Timberlake, unpublished results) and the similarity of *medA* and hypomorphic *brlA* mutants.

In summary, as shown in Figure 2, *brlA* is activated early during development and in turn activates sporulation-specific structural genes, such as *rodA* and *yA*, and the next regulatory gene, *abaA*, with assistance from *medA*. Expression of *brlA* and *abaA* is spatially restricted by the activity of *stuA*. *abaA* enhances the expression of *brlA*-induced structural genes, activates additional structural genes, for example *wA*, and activates the final regulatory gene in the pathway, *wetA*. *wetA* then activates spore-specific genes and may be involved in inactivating *brlA* and *abaA*, which are not expressed in differentiating conidia. This is a genetic model, and the proposed connections between genes could be indirect. However, molecular studies of *brlA* and *abaA* have shown that these genes encode positively acting transcription factors that interact directly with upstream sequences of both regulatory and structural genes. The nature of these interactions predicts that the core regulatory pathway maintains itself in the active state once initiated by external signals, leading to determination of the developmental state.

brlA IS A TRANSCRIPTIONAL ACTIVATOR

Several lines of evidence support the idea that *brlA* encodes a positively acting transcriptional regulator. First, null *brlA* mutations interrupt development at an early stage and block expression of numerous genes that are activated specifically during conidiogenesis (Clutterbuck, 1977; Boylan et al., 1987). Because this block occurs at the level of mRNA accumulation, it is likely that *brlA* is required for transcriptional activation of developmentally regulated target genes. Moreover, hypomorphic *brlA* mutations permit more extensive, although abnormal, development and prevent expression of a subset of the genes whose expression is affected by null mutations. This observation suggests that the *brlA* product (BrlA) interacts with target genes with different affinities so that some genes are affected more than others in partial loss-of-function mutants. Second, forced expression of *brlA* in hyphae inhibits growth (Adams and Timberlake, 1990) and leads to cellular differentiation that mimics several aspects of normal development, including production of viable conidia (Adams et al., 1988). Associated with these morphological alterations is the activation of many conidiation-specific genes, including the downstream regulatory genes *abaA* and *wetA* (Mirabito et al., 1989), consistent with the proposed function of BrlA as a transcriptional activator. Finally, the inferred amino acid sequence of BrlA contains tandem Cys_2-His_2 zinc fingers near the carboxy terminus that (Adams et al., 1988) are required for *brlA* activity (Adams et al., 1990), implying that BrlA is a nucleic acid binding protein.

Despite the evidence that BrlA could act as a transcriptional activator, efforts to detect sequence-specific DNA binding have not yet succeeded, perhaps due to low levels of BrlA polypeptide in *Aspergillus* complicated by proteolysis during extraction and the insolubility or inactivity of *Escherichia coli*–produced BrlA (Y.C. Chang, J. Mooney, and W.E. Timberlake, unpublished results). To get around the difficulties associated with in vitro DNA binding assays, Chang and Timberlake (1993) used the yeast *Saccharomyces cerevisiae* to test for the ability of *brlA* to transactivate gene expression in a heterologous system in which unidentified, intervening genetic elements were less likely to be a problem. The approach they took was to fuse the *brlA* coding region to the galactose-inducible GAL1 promoter in a CEN plasmid. Inactive mutant alleles of *brlA* were used as control. They then inserted the promoter region of the *rodA* gene upstream of a minimal yeast promoter fused to *E. coli lacZ* on an episomal plasmid. *rodA* upstream sequences were chosen because several lines of evidence made this gene a good candidate for direct activation by *brlA*. First, *rodA* transcripts accumulated during development in *abaA* and *wetA* mutants but not in *brlA* mutants, and second, forced expression of *brlA* in hyphae led to accumulation of *rodA* transcripts even in $abaA^-$ or $wetA^-$ strains (Stringer et al., 1991).

Yeast strains carrying both plasmids expressed β-galactosidase in the presence of galactose as carbon source but not glucose, and this expression was dependent upon the presence of a wild-type copy of *brlA*. Thus, *brlA* was capable of transactivating gene expression in yeast by interacting with an upstream element from a target *Aspergillus* gene. The ability to be transactivated by *brlA* mapped to an ~100-bp fragment of the *rodA* promoter. Additional evidence of the importance of this sequence was that its insertion upstream of a minimal, developmentally nonregulated *Aspergillus* promoter imparted developmental regulation on it, and deletion of this region from the *Aspergillus rodA* gene dramatically reduced the extent to which it was induced during development. These data strongly support the hypothesis that *brlA* acts by transcriptional activation, with *rodA* upstream sequences acting as direct targets.

Chang and Timberlake (1993) identified additional targets for *brlA* transactivation by inserting random DNA fragments from the *Aspergillus* genome into a site upstream of a minimal yeast promoter fused to *lacZ*. They then screened for yeast strains that showed galactose- and *brlA*-dependent expression of β-galactosidase. Several clones were isolated and the responsive fragments were sequenced. They all contained multiple copies of the sequence 5′-MRAGGGR-3′ (M = C or A; R = G or A), as did the *rodA*-responsive element, but no other obviously similar sequence elements. Synthetic oligonucleotides containing three or more copies of this sequence element mediated *brlA*-dependent induction in yeast, and the element was therefore designated BRE for *bristle* response element.

Even though these data indicate that BrlA binds directly to the BRE and interacts with the *Aspergillus* or yeast transcriptional machinery to stimulate transcription, it remains possible that the response is indirect. Nevertheless, BREs are present upstream of several developmentally regulated genes in addition to *rodA*, including *yA* (Aramayo and Timberlake, 1990), *abaA* (Mirabito et al., 1989), which is the next regulatory gene

in the pathway, and *brlA* itself (Adams et al., 1988). Thus, it is likely that *brlA* directly activates both morphogenetic loci and downstream regulatory loci and could be involved in regulating its own activity. In support of the latter idea is the observation that the *brlA* (α; see final section) promoter requires $brlA^+$ for full activity (J. Aguirre and W.E. Timberlake, unpublished results; Han et al., 1993).

abaA IS A TRANSCRIPTIONAL REGULATOR

Results from both in vivo and in vitro studies strongly support the hypothesis that *abaA* encodes a positively acting transcription factor. First, as with *brlA*, *abaA* mutants fail to accumulate numerous developmentally regulated transcripts (Boylan et al., 1987). Forced expression of *abaA* in hyphae leads to the activation of many conidiation-specific genes, including the downstream regulatory gene *wetA*, the upstream regulatory gene *brlA*, and *yA* (Figure 2; Mirabito et al., 1989). In addition, the inferred amino acid sequence of AbaA showed that it is a member of the ATTS(TEA) class of DNA binding proteins (Andrianopoulos and Timberlake, 1991; Bürglin, 1991).

Aramayo and Timberlake (1993) mapped sites for *abaA* interaction in the upstream region of the *yA* gene by deletion analysis of the *yA* promoter fused to *lacZ*. They identified two elements, designated I and II, involved with developmental activation of *yA*. Promoter-distal element I contained multiple copies of the BRE consensus sequence and was required for full activation of the *yA* promoter in *Aspergillus*. However, 5′ deletions removing element I still showed strong developmental regulation until the deletions removed sequences from element II. Element II contained two potential binding sites for TEF-1, similar to the GT-IIC sites of the SV40 enhancer (5′-GTGGAATGT-3′; Davidson et al., 1988; Fromental et al., 1988; Xiao et al., 1991) in inverted orientation around a CCAAT box. Synthetic element I oligonucleotides imparted *abaA* responsiveness on minimal *Aspergillus* and yeast promoters, further supporting the idea that AbaA interacts directly with this sequence. Interestingly, mutation in the CCAAT box had opposite effects when assayed in *Aspergillus* and yeast. In *Aspergillus*, elimination of CCAAT resulted in premature and elevated expression of genes placed under element II control during development. In yeast, elimination of CCAAT resulted in loss of activity. Aramayo and Timberlake (1993) proposed that the CCAAT binding factor in *Aspergillus* is a negative modulator of *yA* expression and that it is displaced by binding of AbaA to the flanking ATTS sites. This displacement may be assisted by interactions between AbaA and BrlA bound at the upstream element I BREs.

Confirmation that the *yA* element II sites are direct targets for AbaA has come from in vitro DNA binding studies (A. Andrianopoulos and W.E. Timberlake, unpublished observations). AbaA polypeptide produced in *E. coli* either in the native form or as a fusion protein with β-galactosidase bound with high affinity and specificity to the ATTS sites in *yA* element I. DNase I and hydroxyl radical footprinting analyses and methylation interference and missing contact studies showed that AbaA binds specifically to the sequence 5′-CATTCY-3′, making essential major and minor groove contacts. In vitro binding of the nonfusion protein required only a single site, but two or more sites were needed for in vivo activity. Any variation from the consensus sequence resulted in greatly reduced binding, showing that AbaA, unlike many other eukaryotic transcription factors, possesses stringent sequence specificity.

A. Andrianopoulos and W.E. Timberlake (unpublished observations) also mapped AbaA binding sites in the upstream regions of several other developmentally regulated genes, including the structural genes *rodA* and *wA* and the regulatory genes *wetA* and *brlA*. In all cases, DNA fragments containing multiple AbaA binding sites mediated *abaA* induction of transcription in yeast, confirming the activity of the sites. Thus, the regulatory connections shown in Figure 2 are direct, and *abaA* acts as a positive feedback regulator of *brlA*. An unexpected finding from these studies was that the *abaA* promoter itself contains multiple AbaA binding sites and is capable of mediating *abaA*-directed gene activation in yeast. Thus, *abaA* is probably autogenously regulated, as shown in Figure 2. These results imply that the regulatory pathway is self-reinforcing. That is, after initial activation of *brlA* by external signals and subsequent activation of *abaA*, *abaA* acts as a developmental switch by maintaining its own expression and that of *brlA* and *wetA*: a phenomenon we refer to as feedback fixation. This could be the molecular basis for developmental determination in this system, a proposal that is consistent with the observation that conidiophore cells appear to lose their ability to return to vegetative growth once *abaA* has been activated.

brlA CONSISTS OF OVERLAPPING TRANSCRIPTION UNITS AND IS SUBJECT TO TRANSLATIONAL CONTROL

brlA was initially thought to have a simple structure, consisting of a single transcription unit with one exon (Boylan et al., 1987; Adams et al., 1988). However, Prade and Timberlake (1993) showed that the locus instead consists of overlapping transcription units, as shown in Figure 3. The downstream transcription unit, designated *brlA*α, corresponds to the originally identified transcription unit. The upstream transcription unit, designated *brlA*β, consists of two exons, with the intron spanning the promoter region of *brlA*α. The major translational reading frame of *brlA*β is identical to that of *brlA*α except for the amino-terminal appendage of 23 amino acid residues. In addition, *brlA*β contains an ATG-initiated reading frame of 41 amino acid residues, designated μORF, near its 5′ terminus. Existing *brlA* mutations that have been mapped occur in the region of the locus where *brlA*α and *brlA*β overlap (J. Clutterbuck, personal communication) and are therefore of no value in determining whether the two transcription units

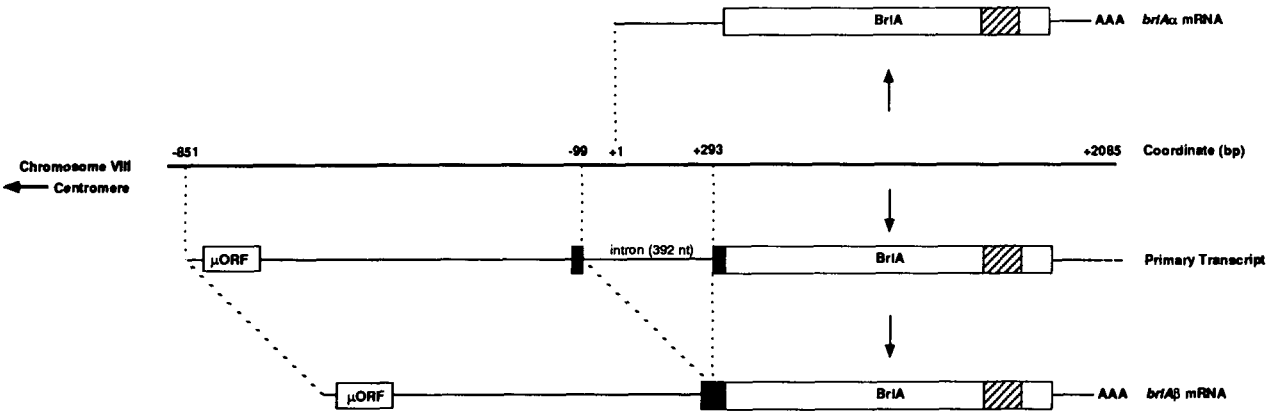

Figure 3. Structure of the *brlA* Locus.

The *brlAα* transcript is shown above the line representing chromosome VIII, and the *brlAβ* transcripts are shown below. *brlAa* consists of a single exon encoding a Cys_2-His_2 zinc finger polypeptide and is capable of driving conidiation when expressed from the strong, inducible *alcA* promoter (Adams et al., 1988). *brlAβ* consists of two exons encoding a polypeptide identical to BrlAα except for the addition of 23 amino-terminal amino acid residues. Deletion of the *brlAβ* intron eliminates *brlAα* transcription without altering *brlAβ* activity (Prade and Timberlake, 1993). *brlAβ* also contains a short, upstream reading frame designated μORF. Mutation of the μORF ATG leads to constitutive expression of the downstream reading frames, causing spontaneous development in cells maintained in submerged culture (Han et al., 1993). Boxes represent translational reading frames, with the shaded area in the BrlAα and β reading frames indicating the location of the zinc fingers.

have different functions. Putative *brlA* and *abaA* response elements occurred upstream of *brlAα* but not of *brlAβ* (Prade and Timberlake, 1993).

Prade and Timberlake (1993) constructed strains that were specifically defective in transcription of either *brlAα* or *brlAβ* to examine the roles of each transcription unit in development. *brlA*α^+,β^- mutants displayed an abnormal phenotype resembling the phenotype of *medA* mutants. The conidiophores produced supernumerary tiers of metulae-like cells, some of which produced complete, although abnormal, conidiophores by undergoing a heterochronic transformation. We refer to this phenotype as "*medusoid*." On the other hand, *brlA*α^-,β^+ mutants produced conidiophores that resembled weak *abaA* mutant conidiophores, producing extra metulae that sometimes produced phialide-like cells that formed a single spore. Spore chains were not observed. We refer to this phenotype as "*hypoabacus*." Strains that did not produce either transcript showed the null, *bristle* phenotype. As a control, a strain was constructed containing a *brlA*α^+,β^- allele at the native locus and a *brlA*α^-,β^+ allele at an alternative chromosomal site, and it produced essentially wild-type conidiophores. The conclusion was that the individual transcription units are needed for normal development but that their functions can be provided in *trans*. Surprisingly, however, multiple copies of a *brlA*α^-,β^+ allele in a *brlA*α^-,β^+ strain or multiple copies of a *brlA*α^+,β^- allele in a *brlA*α^+,β^- strain suppressed the mutant phenotype. Thus, even though the individual genes are essential for normal development in their wild-type configuration, they are individually dispensable in the abnormal situation in which multiple copies of mutant alleles are supplied. This result implies that the functions of the two BrlA polypeptides are largely redundant and that they can substitute for one another.

These findings raised the question of why the locus evolved in this unusual way. The first clue was the observation that *brlAβ* transcript could be detected in hyphae by primer extension analysis (Prade and Timberlake, 1993). From the model presented in Figure 2, one would predict that if *brlAβ* were expressed in vegetative cells and if, as the genetics suggest, BrlAβ has the same activities as BrlAα, then the conidiation pathway should be constitutively and irreversibly activated and development should ensue. However, this is not the case, suggesting that BrlAβ protein is inactive in or absent from vegetative cells. Han et al. (1993) confirmed the presence of *brlAβ* mRNA in vegetative cells by making fusions of *lacZ* and the μORF. Strains containing these fusion constructs produced significant levels of β-galactosidase constitutively. By contrast, fusion of *lacZ* downstream of the *brlAα* reading frame resulted in tight developmental regulation of β-galactosidase accumulation. This suggests that even though the *brlAβ* mRNA is expressed in vegetative cells, the BrlA protein is not translated from this mRNA.

To investigate the role of the μORF in controlling translation of the downstream reading frames, Han et al. (1993) mutated away the μORF ATG in a construct containing a *brlAα–lacZ* fusion gene and then observed constitutive production of β-galactosidase. Internal mutations in the μORF failed to produce this effect. Thus, they concluded that translation initiation at the μORF represses translation from the downstream

reading frames. If this is the case, then a μORF ATG mutation in a construct containing intact *brlAα* and *brlAβ* reading frames should lead to BrlA production, activation of the core pathway (Figure 2), and spontaneous development in submerged culture. In an elegant experiment to test this hypothesis, Han et al. (1993) constructed a mutant allele lacking the μORF ATG and containing a *brlA42*^{ts} allele, reasoning that the lethal effects of uncontrolled *brlA* expression could be overcome by maintaining strains carrying the allele at the restrictive temperature. The dramatic result was that these strains grew normally at the restrictive temperature but when transferred to permissive temperature spontaneously produced fairly normal conidiophores in submerged culture.

Thus, translational repression of downstream translation of *brlA* reading frames is all that stands between vegetative growth and irreversible activation of the developmental pathway. Presumably, then, in the wild-type strain, an initial trigger for developmental activation involves subversion of the negative μORF translational control mechanism. Translational triggering could result from a reduction in aminoacyl-tRNA pools, thus making development sensitive to nitrogen deprivation, an environmental signal that often stimulates microbial sporulation. Alternatively, reduction in nitrogen availability could occur during growth of the aerial hypha to lead to *brlA* activation after an appropriate amount of aerial growth has occurred.

CONCLUSION

Conidiation in *Aspergillus* (Figure 1) is one of only a few developmental systems in which key regulatory genes have been isolated and characterized, their regulatory interactions worked out, and direct morphogenetic target genes identified (Figure 2). Two of the regulatory genes, *brlA* and *abaA*, encode transcription factors that activate the regulatory genes themselves as well as morphogenetic genes. The feedback systems of the core regulatory pathway reinforce expression of the pathway, making it independent of the external cues that initially activate it (feedback fixation) and resulting in developmental determination. One mechanism for initial activation of the pathway may involve subversion of a negative translational regulatory mechanism that controls *brlAβ* (translational triggering). Given that development precludes continued vegetative growth and represents a major commitment by the cells, it is perhaps surprising that turning off a single regulatory process is sufficient to irreversibly activate the developmental pathway. Thus, there may be additional fail-safe systems that have not yet been identified. The precise roles of auxiliary regulatory genes such as *medA* and *stuA* are not known. How environmental signals are sensed and transduced to activate the developmental pathway is poorly understood. The current understanding of the *Aspergillus* developmental regulatory pathway, coupled with the ability to carry out sophisticated molecular genetic manipulations, provides an exciting opportunity to learn much more about the mechanisms controlling development in this interesting system.

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