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# Control of filamentous growth by mating and cyclic-AMP in *Ustilago*

**James Kronstad, Guus Bakkeren, Scott Gold, Arthur Yee, Carole Laity, Gillian Duncan, Katherine Barrett, Luc Giasson, Roderick Campbell, and George Athwal**

**Abstract**: Mating between compatible haploid cells of the corn smut fungus, *Ustilago maydis*, results in a switch from budding to filamentous growth. We are analyzing the multiallelic *b* incompatibility locus that governs maintenance of the infectious, filamentous dikaryon. Specificity regions have been identified in the N-terminal portions of the *bE* and *bW* genes at the *b* locus. In addition, we have found that heterozygosity at the *b* locus attenuates fusion. This result suggests that *b* gene products may exert a negative influence on some processes, in addition to their generally recognized role in maintaining filamentous growth. The *b* genes have also been characterized in *Ustilago hordei*. This species has a bipolar mating system in which the *b* genes are linked to genes required for pheromone production and response to form one large mating-type region (*MAT* locus) with two allelic specificities. In a separate study, we have discovered that defects in adenylate cyclase result in constitutive filamentous growth and greatly reduce the virulence of *U. maydis* on corn seedlings. Mutations have been identified that suppress the adenylate cyclase defect and restore budding growth. Sequence analysis revealed that one of the suppressor mutations is in the gene encoding the regulatory subunit of cAMP-dependent protein kinase (PKA). Mutants altered in PKA activity have the interesting phenotype of multiple budding and frequent mislocalization of the bud site.

*Key words*: smut, dimorphism, *b* locus, sex.

**Résumé** : Chez le champignon du charbon du maïs, l'*Ustilago maydis*, l'accouplement entre deux cellules compatibles déclenche le passage de la croissance levuriforme à la croissance filamenteuse. Les auteurs analysent le lieu multigénique d'incompatibilité *b* qui contrôle le maintien à l'état mycélien dicaryote infectieux. Au lieu *b*, ils ont identifié des régions de spécificité dans les portions terminales N des gènes *bE* et *bW*. De plus, ils ont constaté que l'hétérozygocité au lieu *b* atténue la fusion. Ce résultat suggère que les produits du gène *b* peuvent exercer une influence négative sur certains processus, en plus de leur rôle généralement reconnu dans le maintien de l'état filamenteux. Les gènes *b* ont également été caractérisés chez l'*Ustilago hordei*. Cette espèce a un système d'accouplement bipolaire dans lequel les gènes *b* sont liés à des gènes nécessaires pour la production de phéromones et pour la réaction conduisant à la formation d'une grande région de type de compatibilité (lieu *MAT*) avec deux spécificités alléliques. Dans une étude distincte, les auteurs ont découvert que des défauts dans la cyclase de l'adénylate conduit a une croissance filamenteuse constitutive et réduit fortement la virulence de l'*U. maydis* sur plantules de maïs. Les auteurs ont identifié des mutations qui suppriment le défaut de la cyclase de l'adénylate avec retour à la croissance par bourgeonnement. L'analyse séquentielle montre qu'une des mutations suprimmante se retrouve dans le gène codant pour la sous-unité régulatrice de la protéine kinase (PKA) dépendant du cAMP. Les mutants altérés au niveau de l'activité PKA possèdent un phénotype intéressant, avec bourgeonnement multiple et de fréquentes localisation aberrantes du site de bourgeonnement.

*Mots clés* : charbon, dimorphisme, lieu *b*, sexe.
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## Introduction

Fungi of the genus *Ustilago* present an excellent opportunity for the molecular genetic analysis of fungal pathogenesis and morphogenesis. In particular, the species *Ustilago maydis* and *Ustilago hordei* have recently emerged as useful experimental organisms because of the ease with which these fungi can be manipulated in the laboratory. Specifically, these fungi grow with a yeastlike morphology, they are readily transformed with integrative and autonomously replicating vectors and it is possible to perform targeted gene disruption (Tsukuda et al. 1988; Wang et al. 1988; Kronstad et al. 1989; Gold et al. 1994*a*). One difficulty is that classical genetic analysis can be time consuming because the host plant must be inoculated to obtain teliospores. In *U. hordei*, this process can take 2−3 months.

Infection of the host by smut pathogens is initiated by a filamentous, dikaryotic cell type that is formed by mating between haploid cells (Mills and Kotze 1981; Agrios 1988). The budding, haploid cell type is generated by germination of diploid teliospores, meiosis, and subsequent mitotic divisions. The dikaryon is obligately dependent on infection of the host plant to proliferate and to form the teliospores. Teliospore formation can occur in localized galls on stems and leaves or on floral parts of the host plant. For example, *U. maydis* causes local infections and galls on many different parts of corn plants and sporulates within the gall tissue. In contrast, *U. hordei* infects seedlings, grows systemically within the plant and sporulates predominantly within floral tissue (Fischer and Holton 1957).

The smut fungi have attracted considerable attention recently because of the role that mating interactions play in the disease process (Froeliger and Kronstad 1990; Banuett 1992). Specifically, the infectious dikaryon is usually formed by mating between compatible haploid strains; these haploid mating partners are nonpathogenic by themselves (Rowell and DeVay 1954; Rowell 1955*a*). In the best characterized species, *U. maydis*, a tetrapolar mating system exists in which two different genetic loci, *a* and *b*, control mating or compatibility. The *a* locus appears to control initial fusion events and to influence filamentous growth after mating has occurred (Banuett and Herskowitz 1989; Trueheart and Herskowitz 1992; Spellig et al. 1994). The *a* locus has two alternative idiomorphic sequences called *a1* and *a2*. These sequences encode pheromones and pheromone receptors; recently, the pheromones encoded at *a* have been isolated and characterized (Bolker et al. 1992; Spellig et al. 1994). The *b* locus encodes two polypeptides bE and bW; these contain homeodomain motifs and are, therefore, believed to be regulatory factors (Kronstad and Leong 1990; Schulz et al. 1990; Gillissen et al. 1992). In *U. maydis*, there are at least 25 different specificities at the *b* locus (Puhalla 1970; Silva 1972). In general, haploid mating partners must have different specificities at both the *a* and the *b* loci to fuse and form a stable dikaryon. For the *b* locus, specificity (recognition of different specificities) appears to be mediated by the variable N-terminal regions of the bE and bW polypeptides (Yee and Kronstad 1993; unpublished data). Interestingly, many smut fungi, such as *U. hordei*, have a bipolar mating system in which a single *MAT* locus with alternate specificities has been identified genetically (Fischer and Holton 1957).

In addition to the intriguing connection between mating and pathogenesis in the smut fungi, mating interactions and environmental conditions also control dimorphic growth. In the case of mating, compatible haploid budding cells fuse to form a filamentous cell type, the infectious dikaryon. Nutritional factors have also been reported to influence whether cells bud or grow as filaments (Kernkamp 1939). Dimorphic growth is a feature of many saprophytic and pathogenic fungi. For example, budding cells of *Cryptococcus neoformans* also form a filamentous dikaryon on mating (Kwon-Chung and Bennett 1992). Furthermore, many important animal pathogens switch morphology in response to a variety of environmental factors such as pH, carbon source, and temperature (see Maresca and Kobayashi 1989). The dimorphic growth displayed by *Ustilago* species provides an opportunity to characterize genes, in addition to the mating-type genes, involved in the regulation of budding and filamentous growth.

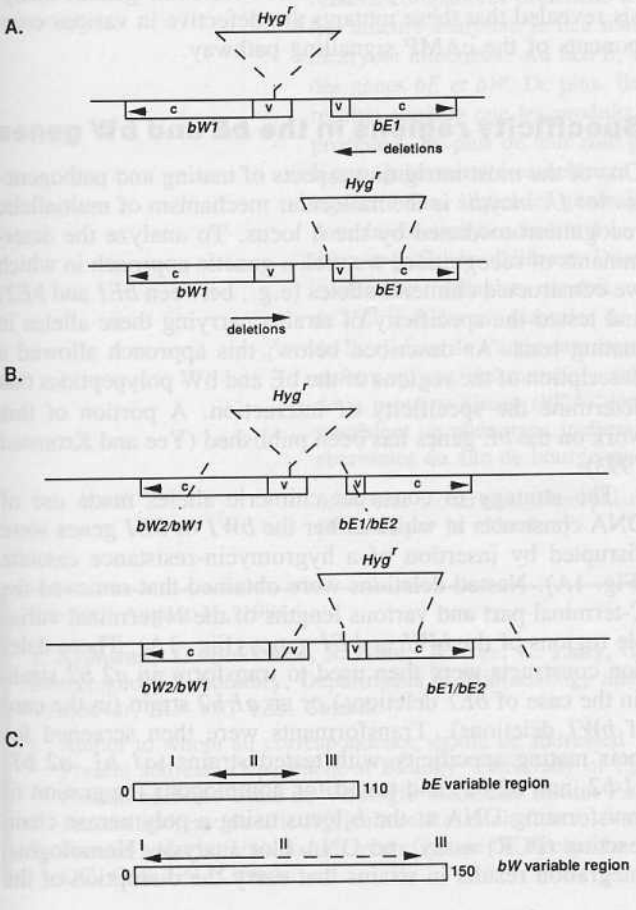
In this article, we review recent results on the genetic analysis of dimorphic growth in *U. maydis* and *U. hordei*. We have focused on both mating type and environmental control of morphogenesis. In particular, the *b* mating-type genes in both *U. maydis* and *U. hordei* have been characterized and the determinants of specificity have been identified in the variable N-terminal regions of the *bE* and *bW* genes of *U. maydis*. The role of the *b* locus in the regulation of fusion has also been explored, as well as the organization of the *b* locus relative to the *MAT* locus in *U. hordei*. This work has general implications for the control of mating in basidiomycetes. In an attempt to explore additional factors controlling filamentous growth in *U. maydis*, we have isolated mutants altered in morphogenesis. Molecular genetic analysis revealed that these mutants are defective in various components of the cAMP signalling pathway.

## Specificity regions in the *bE* and *bW* genes

One of the most intriguing aspects of mating and pathogenesis for *U. maydis* is the molecular mechanism of multiallelic recognition mediated by the *b* locus. To analyze the determinants of recognition, we took a genetic approach in which we constructed chimeric alleles (e.g., between *bE1* and *bE2*) and tested the specificity of strains carrying these alleles in mating tests. As described below, this approach allowed a description of the regions of the bE and bW polypeptides that determine the specificity of interaction. A portion of this work on the *bE* genes has been published (Yee and Kronstad 1993).

The strategy to construct chimeric alleles made use of DNA constructs in which either the *bW1* or *bE1* genes were disrupted by insertion of a hygromycin-resistance cassette (Fig. 1A). Nested deletions were obtained that removed the C-terminal part and various lengths of the N-terminal variable regions of the *bW1* or *bE1* genes (Fig. 1A). These deletion constructs were then used to transform an *a2 b2* strain (in the case of *bE1* deletions) or an *a1 b2* strain (in the case of *bW1* deletions). Transformants were then screened for their mating specificity with tested strains (*a1 b1*, *a2 b1*, *a1 b2*, and *a2 b2*) and tested for homologous integration of transforming DNA at the *b* locus using a polymerase chain reaction (PCR) assay and DNA blot analysis. Homologous integration results in strains that carry the disruption of the

**Fig. 1.** Construction and classes of chimeric *bE* and *bW* genes. (A) Plasmid constructs carrying disruptions and deletions of the *bE1* and *bW1* genes. The inserts of the plasmids are shown along with the position of a hygromycin resistance marker, which was inserted in the *bW1* gene (top) or *bE1* gene (bottom) to create nonfunctional genes. Arrows indicate the directions of deletions constructed by exonuclease digestion. A set of nested deletions containing varying amounts of the N-terminal variable (*v*) regions was obtained for both *bE1* and *bW1*. For transformation, each construct was digested with restriction enzymes that cut within or just outside of the disrupted gene and within the variable region (at the site of the deletion). This digestion yielded linear fragments with *bE* and *bW* sequences flanking the hygromycin resistance marker to promote gene replacement at the *b* locus. (B) The genomic organization is shown for chimeric alleles resulting from homologous integration of the constructs from (A). Note that the integration events result in disruption of one gene (*bE* or *bW*) with the hygromycin-resistance cassette. The *bE* chimeric alleles were constructed in a strain that was originally *a2 b2* and the *bW* alleles were constructed in an *a1 b2* strain. (C) Maps of the variable regions of *bE* (codons 0–110) and *bW* (codons 0–150) showing the approximate positions of the specificity regions defined by the analysis of the chimeric alleles. Class II transformants have a specificity different from the *bE1/bE2*, or *bW1/bW2*, specificities of the starting alleles and, thus, define the specificity regions. Class I transformants do not have altered specificity and Class III transformants have a specificity that is switched from *b2* to *b1*. Note that among the Class II chimeric alleles of *bW* there were two (recombination at codons 48 and 116) that appear to interact constitutively with all of the *bE* specificities tested (*bE1*, *bE2*, and all chimeric *bE* genes). Therefore, a broken line is used to indicate potential novel properties of the chimeric alleles that define this portion of the specificity region.




*bW1* or *bE1* gene and chimeric alleles (i.e., *bE1/2* or *bW1/2*) of the intact mating-type gene as diagrammed in Fig. 1B.

Three classes of transformants were identified from the mating tests used to assess specificity. First, the majority of the transformants had no change in mating specificity, i.e., they retained the specificity of the resident *bE2* or *bW2* alleles present in the recipient strain. A second class displayed altered allelic specificity. These strains behaved as if they carried *b* alleles with specificity different from either *b1* or *b2* such that they were able to give a positive mating reaction with strains carrying either parental allele. The third class showed a complete switch from the *b2* specificity of the recipient strain to the *b1* specificity of the transforming DNA.

To characterize the organization of the chimeric *b* alleles in the transformants representing each class, the variable regions of the chimeric *bE* and *bW* genes were recovered by PCR and the nucleotide sequences of these regions were determined. This analysis allowed maps to be constructed that defined specificity domains in the variable regions of the *bE* and *bW* genes (Fig. 1C). As expected, class I transformants contained very little of the *b1* sequences. That is, recombination between the incoming *b1* DNA and the resident *b2* DNA occurred near the 5′ end of the coding region such that insufficient *b1* sequences were integrated to change specificity. The class II transformants contained chimeric alleles with recombination points within the central part of the variable region; these alleles defined the specificity region for *bE* and *bW*. The class III transformants contained alleles in which most of the variable region of the *b2* genes had been replaced by the variable region from the incoming *b1* allele.

Overall, the analysis of the chimeric alleles of *bE* and *bW* has allowed a description of the regions that define specificity. Presumably, these regions mediate the interaction between bE and bW polypeptides and determine whether an active *b* regulatory factor will be formed. We speculate that the determinants of specificity may actually lie at the borders of the specificity domains identified by the chimeric alleles. That is, the actual size of the specificity region may be less important than the sequences at the borders. We have previously described comparisons of borders between chimeric alleles of *bE* that suggest that relatively small changes in amino acid sequence (i.e., in one or two residues) can alter specificity (Yee and Kronstad 1993). It is also important to note, when considering the size of the specificity regions, that we have analyzed only one combination of *b* genes (*b1* and *b2*). The positions and sizes of the specificity domains for *bE* and *bW* probably differ between different allele pairs. Thus, the sizes of the specificity domains must be large enough to accommodate the interactions that occur between the 25 or more naturally occurring *b* specificities. It is possible that the entire variable regions of *bE* and *bW* fulfill this role, although only

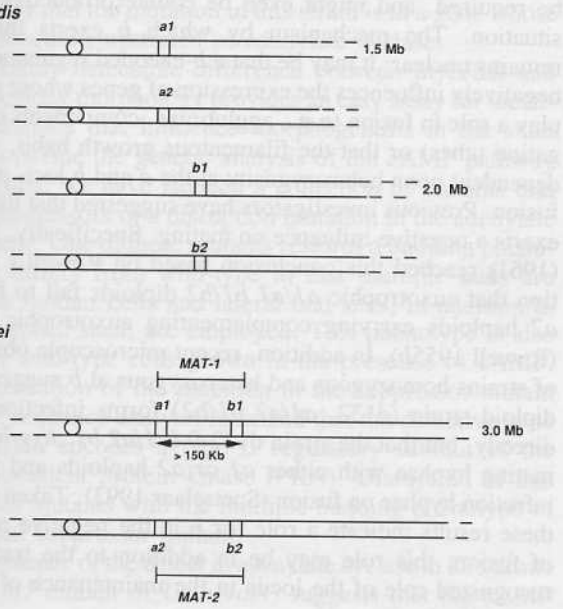



**Fig. 2.** Chromosomal organization of *a* and *b* gene complexes for *U. maydis* and *U. hordei*. (A) The genomic organization of the *a* and *b* loci in an *a1/a2 b1/b2* dikaryon of *U. maydis*. Note that the *a* and *b* sequences are located on different chromosomes and that the sizes of the chromosomes are given for strains 521 (*a1 b1*) and 518 (*a2 b2*). The sizes of the chromosomes carrying these sequences may be different in other strains. (B) The genomic organization of the *MAT-1* and *MAT-2* regions in a dikaryon of *U. hordei*. The *a* and *b* sequences are linked in this species. The distance from *a1* to *b1* in one *MAT-1* strain has been estimated to be at least 150 kb; these sequences are on the largest, 3.0 Mb, chromosome in this strain. Note that an arbitrary orientation and distance for the *MAT* regions to the centromere is shown.

smaller portions are identified when a single combination of alleles is analyzed.

## Tetrapolar versus bipolar mating

As mentioned earlier, two genetically different mating systems, termed bipolar and tetrapolar, govern compatibility in the smut fungi. Most of the molecular-genetic work on mating in *Ustilago* species has been carried out with the tetrapolar species *U. maydis*, although other species, such as the bipolar smut *U. hordei*, have recently been examined (Martinez-Espinoza et al. 1992, 1993; Bakkeren and Kronstad 1993, 1994). The availability of cloned *b* genes from *U. maydis* prompted us to search for and identify related functional *b* sequences in *U. hordei* (Bakkeren et al. 1992; Bakkeren and Kronstad 1993). It is interesting to note that a multiallelic function like *b* had not been identified genetically in bipolar smut fungi; mating in these fungi is controlled by a single *MAT* locus with two alternate specificities (*MAT-1* and *MAT-2*). Therefore, we sought to define the physical and genetic relationship between the *b* genes and the genetically identified *MAT* locus in *U. hordei*.

Initially, we discovered a restriction fragment length polymorphism (RFLP) marker, identified with a *bE* probe from *U. maydis*, that cosegregated with the *MAT* locus of *U. hordei* (Bakkeren et al. 1992; Bakkeren and Kronstad 1994). In fact, we were unable to detect recombination between the RFLP marker (*b* sequences) and the *MAT* locus. We also employed sequences from the *a* locus of *U. maydis* to isolate a region with *MAT-1* mating-type activity from *U. hordei*. Sequence analysis revealed that this region encoded a pheromone receptor with high sequence similarity to the *pra1* pheromone receptor gene from the *a1* locus of *U. maydis*. Thus, it appears that *U. hordei* has a genetic locus that is equivalent to the *a* locus of *U. maydis*. Using hybridization probes for the *a1* and *b* loci from *U. hordei* and DNA blots of chromosomes separated by pulse-field electrophoresis, it was possible to demonstrate that both loci are present on the largest (3 Mb) chromosome of *U. hordei*.

These data indicated that the difference between the bipolar mating system of *U. hordei* and the tetrapolar system of *U. maydis* is simply a matter of the genomic organization of the genes required for fusion (*a* locus) and dikaryon maintenance (*b* locus). In *U. maydis*, these loci are present on different chromosomes (*a* locus; 1.5 Mbp and *b* locus; 2.0 Mbp), and in *U. hordei*, the loci are linked together to form a large *MAT* region (Fig. 2). Preliminary physical mapping of the distance between the *a1* and *b1* sequences in *U. hordei* indicated that they are at least 150 kb apart (G. Bakkeren, unpublished results). Recombination appears to be suppressed in this region because it was not possible to identify progeny, among 2182 screened, that failed to mate with either parent. That is, one would expect that recombination occurring in the *MAT* region during a cross between parents of genotypes *a1 b1* and *a2 b2* would generate progeny of genotypes *a1 b2* and *a2 b1*. We assumed that these progeny would be unable to mate with either parent. Recombination is suppressed in other eukaryotic microbes. For example, Ferris and Goodenough (1994) have found that the mating-type region in *Chlamydomonas reinhardtii* is suppressed for recombination and contains translocations, inversions, and deletions across 190 kb, when the two mating types are compared.

One implication of the finding that *a* and *b* sequences are linked in *U. hordei* is that only two types of *b* specificities would be required to establish the dikaryon. This is because fusion events between strains of opposite *a* specificity (*MAT-1/a1* and *MAT-2/a2*) would concomitantly bring together different *b* specificities. To test this idea, a collection of *U. hordei* strains was obtained and the DNA sequences of the variable regions of the *bE* and *bW* genes from each mating type (*MAT-1* and *MAT-2*) were determined. This analysis revealed very few differences between the *b* genes present in each mating type. That is, one class of *bE* and *bW* genes was always associated with the *MAT-1* mating type and the other class was always associated with *MAT-2* (Bakkeren and Kronstad 1994).

Taken together, the analysis of the organization of the *a* and *b* loci in *U. hordei* provides a simple explanation for the difference between bipolar and tetrapolar mating systems in the smut fungi. Although we don't know which mating system is ancestral, it is tempting to speculate that the bipolar system arose first because of its relative simplicity. In addition, it has been suggested that basidiomycetes, such as the smut fungi, evolved from ascomycetes, and tetrapolar mating systems have not been described in the latter group

Whitehouse 1949). Further exploration of the molecular organization of the *MAT-1* and *MAT-2* regions in *U. hordei* may shed some light on evolutionary questions. Specifically, we want to know the distance between the *a* and *b* sequences and the organization of the intervening region. This information may reveal the reason why recombination occurs rarely in this region. It will also be of interest to discover the orientations of the mating-type genes relative to flanking sequences and to compare the organization of the sequences outside of the *MAT* regions with sequences flanking the *a* and *b* genes in *U. maydis*. This analysis may contribute to an understanding of the molecular events that led to linkage or separation of the mating-type functions. The work from *C. reinhardtii* also raises the possibility that there are other genes with roles in mating, besides the *a* and *b* genes, in the *MAT* region of *U. hordei*. In *C. reinhardtii*, other genes that play roles in mating are found in the 190 kb region that shows differences between the two mating types; this organization may serve to keep functionally related genes together (Ferris and Goodenough 1994).

## Regulation of fusion by the *b* locus

In addition to the analysis of the specificity determinants for the *b* genes and a comparison of bipolar and tetrapolar mating, we have also been exploring the biological function of the *b*-encoded regulatory factor. Specifically, we have isolated a collection of UV and plasmid-insertion mutations that block filamentous growth in a pathogenic haploid strain (P6D, *a2 b2* [*a1 b1 ble*$^r$] of *U. maydis*. The initial goal of this work was to identify genes that were potentially regulated by the mating-type loci. The filamentous, pathogenic haploid strain was constructed by transformation of an *a2 b2* strain with a plasmid carrying the *a1* and *b1* mating-type sequences. In contrast to diploid or dikaryotic strains, this strain is useful for the isolation of recessive mutations that interfere with filamentous growth. The surprising finding from our initial analysis of the nonfilamentous mutants was that they were incapable of participating in fusion events because of heterozygosity at the *b* locus (Laity et al. 1995).

Initial attempts to perform plate mating assays with the nonfilamentous mutants revealed that these mutants are incapable of interacting with compatible haploid strains to give the aerial hyphae (filamentous growth) indicative of a positive mating reaction. This result suggested that the mutations in these strains, or the background of the parental strain, was interfering with fusion or with formation of infection hyphae. It was difficult to test directly whether the background of the parent strain (P6D; *a2 b2* [*a1 b1 ble*$^r$] was interfering with mating because this strain forms filamentous colonies by itself on mating medium. To circumvent this problem, a cytoduction assay developed by Trueheart and Herskowitz (1992) was employed to measure the ability of the parental strain to fuse with compatible partners. This assay revealed that the frequency of fusion was greatly reduced between strain P6D (*a2 b2* [*a1 b1 ble*$^r$] and an *a1 b1* tester strain. Similar results were obtained when haploid transformants carrying an extra *b* allele (*a2 b1/b2*) were employed in the cytoduction assay. In typical experiments, compatible *a1 b1* and *a2 b2* strains gave a frequency of cytoduction of approximately $1 \times 10^{-4}$. This value indicates the number of prototrophic cytoductants that received a mitochrondrial marker (*oli*$^r$; oligomycin resistance) from an *oli*$^r$ arginine auxotroph compared with the total number of prototrophic cells in the mixture. In contrast, mixtures of P6D and an *a1 b1* tester strain gave cytoduction frequencies of less than $1 \times 10^{-7}$. In support of these results, we also found that diploid strains (d132, *a1/a2 b1/b2*; d132-9, *a1/a2 b1::hyg/b2*) showed differences in their ability to fuse. Strain d132-9, which was derived from d132 by disruption of the *b1* locus, participated in fusion events with 100-fold greater frequency than d132, which has intact *b* genes.

These results implicated heterozygosity at the *b* locus in the regulation of fusion during mating events. It is presumed that this regulation comes into play once an infectious dikaryotic cell type has been established; mating would no longer be required, and might even be counterproductive, in this situation. The mechanism by which *b* exerts this effect remains unclear; it may be that a *b*-encoded regulatory factor negatively influences the expression of genes whose products play a role in fusion (e.g., agglutinins, components of conjugation tubes) or that the filamentous growth habit, which is dependent upon heterozygosity at the *a* and *b* loci, precludes fusion. Previous investigators have suggested that the *b* locus exerts a negative influence on mating. Specifically, Holliday (1961) reached this conclusion based on Rowell's observation that auxotrophic *a1/a1 b1/b2* diploids fail to fuse with *a2* haploids carrying complementing auxotrophic markers (Rowell 1955*b*). In addition, recent microscopic observation of strains homozygous and heterozygous at *b* suggests that a diploid strain (d132, *a1/a2 b1/b2*) forms infection hyphae directly, but that the strain d132-9 (*a1/a2 b1::hyg/b2*) forms mating hyphae with either *a1* or *a2* haploids and produces infection hyphae on fusion (Snetselaar 1993). Taken together, these results indicate a role for *b* in the negative regulation of fusion; this role may be in addition to the traditionally recognized role of the locus in the maintenance of filamentous growth.

## cAMP and dimorphism

In *U. maydis* and *U. hordei*, it is possible to isolate mutants that forego budding and display a constitutively mycelial phenotype (Barrett et al. 1993; McCluskey et al. 1994). Although it is logical to assume that these mutants are altered in the *b*-regulated pathway that establishes filamentous growth, it is known that additional factors, such as nutritional status (Kernkamp 1939) and exposure to air (Gold et al. 1994*b*), control morphogenesis in the smut fungi. To begin an analysis of morphogenesis in *U. maydis*, Barrett et al. (1993) isolated 125 mutants that displayed a constitutively mycelial phenotype. Construction of diploids with nine of the mutants indicated that the mutation(s) were recessive. Complementation tests proved difficult because the filamentous phenotype of the strains interfered with diploid construction between different filamentous mutants. In addition, the mycelial phenotype of these strains proved to be unstable, with frequent reversion to budding growth. Overall, these problems made it difficult to define complementation groups.

The mutation in one of the relatively stable mutants was complemented with a cosmid library. The complementing region of one cosmid was identified by subcloning and dis-

ruption of this region in the genome yielded mutants with a constitutively mycelial phenotype. DNA sequence analysis of the complementing region indicated that the clone encoded a homolog of adenylate cyclase (Gold et al. 1994*b*). This result suggested that a low cAMP level, as a result of a defect in adenlyate cyclase, favors filamentous growth; in contrast, high cAMP levels must promote budding growth. In support of this idea, it was found that exogenous cAMP restored budding growth to the filamentous mutant. Exogenous cAMP also restores budding growth to a mycelial mutant isolated in *U. hordei* by D. Mills and coworkers (McCluskey et al. 1994). The mutation in this strain (*fil1*) does not seem to be in the gene for adenylate cyclase because compounds that stimulate adenylate cyclase (e.g., forskolin) restore budding growth to the mutant (D. Mills, personal communication). It seems likely that the mutation in this strain is in a gene whose product functions upstream of adenylate cyclase.

The readily detectable difference between mycelial and yeastlike colony morphology provides an easy assay for identifying mutations that influence morphogenesis in the smut fungi. To pursue the genetic analysis of the cAMP pathway in *U. maydis*, we have isolated a collection of mutants that contain suppressors of a disruption mutation in the adenylate cyclase gene. One of these mutants restores a budding phenotype that differs from wild-type in that multiple buds are formed on mother cells and lateral bud sites, in addition to the usual apical sites, are employed. This phenotype is also seen with wild-type cells grown in the presence of cAMP. Complementation of the mutation in the suppressor mutant and DNA sequence analysis indicated that the defective gene in this strain encodes a type II regulatory subunit of the cAMP-dependent protein kinase (PKA). Disruption of this gene yields mutants with the multiple budding phenotype of the original suppressor mutant.

The influence of the defect in adenylate cyclase in *U. maydis* (and the *fil1* mutant of *U. hordei*) suggests that the cAMP pathway plays a key role in morphogenesis in the smut fungi. Defects in adenylate cyclase cause a variety of phenotypes in other fungi. For example, disruption of the gene encoding adenylate cyclase in *Schizosaccharomyces pombe* results in a slow growth and a tendency to enter the sexual reproduction pathway in rich medium (Maeda et al. 1990). *S. cerevisiae* cells carrying mutations in the *CYR1* gene arrest in the G1 phase of the cell cycle and exogenous cAMP restores growth (Casperson et al. 1985; Kataoka et al. 1985; Ishikawa et al. 1988). In *N. crassa*, mutants carrying the defective *cr-1* allele (encoding adenylate cyclase) form small compact colonies that lack extensive hyphal development and that display short aerial hyphae with tight clusters of dense conidia (Terenzi et al. 1976; Pall et al. 1981; Kore-eda et al. 1991).

A role for cAMP in the switch between budding and filamentous growth has been described for a number of different pathogenic and saprophytic dimorphic fungi (Maresca et al. 1977; Medoff et al. 1981, 1987; Paris and Garrison 1983; Brunton and Gadd 1989; Marques and Gomes 1991). For example, the intracellular level of cAMP increases during germ tube formation and subsequent hyphal growth in the pathogens *Histoplasma capsulatum* (Maresca et al. 1977; Medoff et al. 1981), *Candida albicans* (Niimi et al. 1980; Sabie and Gadd 1992), and *Blastomyces dermatitidis* (Paris and Garrison 1983). In contrast, cAMP levels drop during the emergence of hyphal germ tubes and the level of cAMP remains low during hyphal growth in *Mucor rouxii* (reviewed by Orlowski 1991). Our genetic evidence suggests that a similar relationship between cAMP levels and growth morphology exists for *U. maydis* and *M. rouxii*.

The finding that a defect in adenylate cyclase results in filamentous growth in *U. maydis* indicates that failure to activate PKA (due to a low level of cAMP) results in a defect in budding. Interestingly, budding growth is also eliminated by mating between haploid cells of *U. maydis*. In fact, there may be a connection between morphogenesis, cAMP and mating in *U. maydis* such that regulation by the mating pathway intersects with or impinges on the cAMP pathway. Presumably, the latter pathway is involved in sensing environmental conditions such as the nutritional status of the cells. In *S. cerevisiae*, regulatory pathways for mating and for budding pattern may overlap because the *BUD3* and *BUD4* genes, which establish an axial budding pattern, may be negatively regulated (directly or indirectly) by homeodomain-containing polypeptides encoded by the *MATa/MATα* loci (Chant and Herskowitz 1991). In *U. maydis*, the *b* mating-type locus also encodes polypeptides with homeodomain-like motifs, similar to those encoded by the *MAT* alleles of *S. cerevisiae* (Gillissen et al. 1992). We speculate that *U. maydis* may use the *b*-encoded regulatory factors to repress genes whose products are required for budding. Instead of altering budding pattern, as is the case in *S. cerevisiae*, the mating-type regulation would suppress budding and cause filamentous growth.

## Summary

Considerable progress has been made in the last 5 years towards a molecular understanding of mating in the smut fungi. For example, the *b* locus of *U. maydis* has been characterized in molecular detail, the organization of the *a* and *b* loci in the bipolar smut, *U. hordei*, has been described and a role for the *b* locus in controlling fusion has been uncovered. In addition, the cAMP pathway was found to control the decision between budding and filamentous growth. What is needed now is a concerted effort to identify and analyze other genes, besides those encoded at the *a* and *b* mating type loci, which may be targets of PKA phosphorylation or mating-type regulation, and which may encode other factors required for signal transduction, morphogenesis, and pathogenesis.

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