Genetics of Morphogenesis in Basidiomycetes

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In this chapter, our aim is to discuss the current knowledge of the genetics of morphogenesis in basidiomycetes. We begin by outlining some features that are shared among fungi in general and those that are unique to basidiomycetes. With this background of basic fungal morphology and physiology, we focus our discussion on the genetics of morphogenesis and the fascinating biology of three broad groups of basidiomycetes: the smuts, the rusts and the mushrooms. Where the smuts and rusts are considered, there is ample discussion on pathogenesis, as these two broad groupings of fungi are particularly destructive to plants worldwide. Of course, mushrooms represent a valuable commodity in their own right. Each of these broad sections encapsulates a current assessment of the environmental cues and the genes regulating morphological change during development. Each of these three taxon-focused sections also concludes with a section discussing current trends and/or future directions.

1. INTRODUCTION

The kingdom fungi lies on the opisthokont lineage of the Eukaryotes and thus is a closer relative to the animals than to the plants. The fossil record of the fungi is estimated to date back as far as 900 million years with all major classes represented about 300 million years ago (Taylor et al. 1994). The basidiomycetes are one of the two ultimate branches of the true fungi, the other being the ascomycetes. About 30,000 basidiomycete species have been described. Among the basidiomycetes are the well known mushrooms of the Hymenomycetes, the rusts of the Uredinomycetes known through recorded history as devastating pathogens of staple grain crops, and the smuts of the Ustilaginomycetes that cause persistent crop losses. In this chapter, we present an overview of some of the central processes particular to the basidiomycetes. We then
 delve more deeply into exemplars of each of the three above major groups and provide a current snap shot of what is known regarding the genetics of their developmental processes.

2. SPECIALIZED FEATURES

In the following sections, we present overviews of some of the developmental features common among the basidiomycetes. Some of these such as dimorphic growth and sporulation are found in other fungi while others like clamp connections, peculiarities of their mating systems and the surreal complexity of some of their host interactions, are specific to the basidiomycetes.

2.1 Budding vs. Filamentous Growth

Basidiomycetes display wide morphological diversity, having both multicellular filamentous forms as well as budding unicellular yeast forms. Dimorphic basidiomycetes exhibit a switch between yeast-like growth and filamentous growth (Alexopolous et al. 1996). Alternating phases of budding and filamentous growth are commonly observed in the dimorphic tremelloid and smut fungi. On the other hand, in most basidiomycetes, such as those that are found in the rusts and gasteromycetes, a yeast-like budding phase is not present. Because the dimorphic smut fungus Ustilago maydis will be discussed in great detail below in the exemplary model systems section 3.1, interesting examples of other fungi exhibiting dimorphic growth will be presented here.

One group of dimorphic fungi is found in the order Tremellales (Alexopolous et al. 1996). The well-studied fungus Cryptococcus neoformans, a microbe found on trees and a facultative pathogen of animals including humans, is a tremelloid fungus that infects the central nervous system, causing meningocencephalitis. C. neoformans grows as a budding yeast with a conspicuous polysaccharide capsule (Alexopolous et al. 1996; Hull and Heitman 2002). But in response to nutrient-limiting conditions or mating pheromone, yeast cells of opposite mating type produce conjugation tubes and fuse, forming a filamentous heterokaryon (Hull and Heitman 2002). Under the appropriate conditions, yeast cells of the alpha mating type can undergo haploid filamentation, producing monokaryotic filaments with unfused clamp connections (discussed below). Like the ascomycete fungal pathogens of animals, C. neoformans exhibits thermal dimorphism (Sia et al. 2000). In C. neoformans, yeast cell fusion and maintenance at 37°C leads to the formation of stable diploid yeasts. A switch to 25°C leads to the formation of monokaryotic diploid hyphae with unfused clamp connections. In contrast, yeast cell fusion and maintenance at 25°C leads to the formation of dikaryotic filaments with fused clamp connections (Sia et al. 2000).

Another group of dimorphic basidiomycete fungi are the Sporidiales. These fungi, represented here by Rhodosporidium sphaerocarpum, are taxonomically more closely related to the rust fungi than either the Ustilaginales or Tremellales based on septa
structure and rDNA analyses, and exhibit both a yeast phase and a filamentous phase in their lifecycles (Alexopolous et al. 1996). Compatible yeast cells of *R. sphaerocarpum* fuse, forming a filamentous dikaryon that eventually gives rise to intercalary and terminal teliospores. Teliospores germinate and meiosis occurs, giving rise to a four-celled septate promycelium from which basidiospores develop and bud in a manner strikingly similar to the developmental program characterized in the smuts. As a result, these have been mistakenly identified as such (Alexopolous et al. 1996).

Some ants in the genus *Cyphomyrmex* maintain basidiomycetes in the family lepiotaceae as unicellular masses of yeasts. These yeasts form a monophyletic clade within a larger filamentous clade (Mueller et al. 1998) suggesting that these yeasts were derived from mycelial ancestors and switched to yeast-like growth upon association with *Cyphomyrmex* yeast specialists (Mueller 2002). As Mueller (2002) pointed out, the ants may have evolved a feature to manipulate their cultivars, allowing the ants to keep their fungus in a yeast-like state. It would certainly be of interest to examine the growth of these fungi for filamentation under various culture conditions, away from the nests. In contrast, most other fungus growing ants propagate and maintain their cultivars in the filamentous form (Mueller et al. 1998; Mueller 2002). Although some filaments have been observed with clamp connections, these fungi traditionally have defied identification because they rarely produce a sexual form (Alexopolous et al. 1996). But DNA-based phylogenetic analysis has revealed a close relationship between many of these fungi and a group of poorly understood tropical mushrooms (Mueller et al. 1998).

### 2.2 Mating Types

Basidiomycetes generally have four functional mating specificities that combine to generate full sexual compatibility. Their cousins of the Ascomycota classically have 2 mating types (eg. a and α in *Saccharomyces* and A and a in *Neurospora*). Many excellent reviews are available on the topic of fungal mating types (Brown and Casselton 2001; Casselton and Olesnicky 1998; Casselton 2002; Fraser and Heitman 2004) and so the issue will not be belabored here, rather we will give a brief outline for the basidiomycetes as the mating type genes are crucial developmental factors for these fungi. The basidiomycetes have taken sexual promiscuity to new heights. For example it is estimated that *Coprinopsis cinerea* (*Coprinus cinereus*) and *Schizophyllum commune* have 12,000 and 20,000 different mating specificities all of which are inter-compatible but self-incompatible, respectively (Brown and Casselton 2001). This makes the probability of coming upon a compatible partner nearly 100%. Most mushrooms species have no inhibition to anastomosis in the homokaryotic stage and this makes biological sense since nearly all fusions will be sexually productive.

There are two functional classes of mating loci in the basidiomycetes. These are classically described as the a and b or A and B loci. Unfortunately in the smut model *Ustilago maydis* and related species the mating loci are named in reverse for function when compared to the Hymenomycetes including the mushrooms. In the mushrooms, the A loci encode homeodomain containing transcription factors while the B loci encode lipopeptide pheromones and transmembrane pheromone receptors. In the smuts the a
locus encodes the pheromones and receptors and the b locus encodes the homeodomain containing proteins. In the smuts the a and b loci may be genetically linked or unlinked. This situation leads to two major groups the bipolar smuts in which there are only two mating specificities that segregate at meiosis and the tetrapolar smuts in which meiosis produces four mating specificities in the progeny. It has been shown that at least for some bipolar smuts the a and b loci are both present but are not separable by recombination (Bakkeren et al. 1992; Bakkeren and Kronstad 1994; Lee et al. 1999). The tetrapolar mating system of the smuts is described later in this chapter. The arrangement of the A and B genes in the mushrooms is quite complex often with linked sub-loci. In some species the A and B genes may be distributed between distinct subloci known as Aα, Aβ, Bα and Bβ (Fowler et al. 2004; Pardo et al. 1996). The paradigm in C. cinerea is that each A locus encodes three pairs on homeodomain containing proteins while each B locus encodes three cassettes each with a pheromone receptor gene and usually two pheromones (Brown and Caselton, 2001). The pairs are made up of canonical members called HD1 and HD2, which must form at least one nonself heterodimer with those of another monokaryon for complementation of successful A mating function. None of the HD1 proteins are able to form a productive heterodimer with the HD2 proteins encoded by the same monokaryon. Similarly at the B locus the pheromones produced by a monokaryon will not interact with any of the receptors produced by that strain. Thus the basidiomycetes are exquisitely designed to find compatible partners in nature and yet recognize self as incompatible.

2.3 Nuclear Condition

In the basidiomycetes, nuclear condition alternates between haploid forms and dikaryotic forms generated by mating processes. Karyogamy takes place in the basidium, generating the diploid stage of the cell cycle. Also in these structures, meiosis occurs, resulting in the production of haploid basidiospores. Interesting questions can be asked when comparing for example rusts and smuts and trying to correlate nuclear state, ploidy level and developmental program and/or infection of hosts. A simple pattern is found in the smut fungi. The haploid, mononucleate smut basidiospores are mostly saprobic and although able to live off their host, are unable to cause disease (i.e., sporulate). Mating and the formation of a binucleate dikaryon are necessary for pathogenicity. There are interesting variations among smut fungi regarding the putative role of the haploid sporidia in nature. Thus, in Tilletia species mating of compatible basidiospores takes place while they are still attached to the promycelium and upon discharge and germination the secondary sporidia develop a dikaryotic mycelium, which is capable of infecting plants. Therefore, the duration of the haploid phase in these species is reduced in time. It has been shown that very early in the infection of corn by U. maydis, the hyphae can have multiple (more than 2) nuclei per cellular compartment but that soon after a proper dikaryotic mycelium is produced (Snetselaar and Mims 1993). In the macrocyclic rusts, such as in Puccinia spp., both dikaryotic aeciospores and urdindiospores are pathogenic on the primary host. But in addition, haploid basidiospores, which are often binucleate after a subsequent mitotic
division (Gold and Mendgen 1991; Anikster 1983; Heath et al. 1996), are also pathogenic on the alternate host or on the same host as for autoecious Uromyces spp. (see section 3.2.2 for a description of a rust life cycle and spore stages). Haploid basidiospores often produce binucleate infection hyphae early during infections but one nucleus disintegrates soon after during subsequent fungal growth in planta producing haploid, mono-nuclear cells, as has been convincingly shown for U. vigneae (Heath et al. 1996). The fertilization of haploid, mono-nuclear pycniospores with ones of a different (opposite or "non-self") mating type in specialized structures, the pycnia, recreates the dikaryotic (n+n) mycelium which reprograms the fungus to produce aeciospores in analogy with the smuts (although these produce teliospores at that point). Dikaryotic urediniospores produce infection structures containing variable numbers of nuclei per "cell" compartment, depending on the species and/or differentiation stage, e.g., 2 nuclei in the germ tube, 4 in the appressorium, and various numbers in the substomatal vesicle (Heath et al. 1996). This can occur through various rounds of mitosis without septum formation, asynchronous nuclear divisions and/or septation and nuclear degradation. However, this is mostly seen during early stages of fungal development and established (n+n) mycelium appears to be uniformly binucleate/dikaryotic. Unusual situations exist in haustoria of P. coronata which have only a single nucleus whereas the multinucleate condition (up to 24 nuclei per cell) apparently persists in one studied isolate of P. striiformis (Chong et al. 1992). It seems that the nuclear state is not synchronized with the rapidly changing developmental programming necessary during the early infection. Karyogamy takes place within the plant producing diploid teliospores. Teliospores arise from dikaryotic hyphal compartments by different mechanisms. In some cases karyogamy occurs early during teliospores development so that even immature teliospores are uninucleate. In other species however, nuclear fusion happens at late stages of teliospore development. In smut and rust fungi, teliospores are considered the probasidium. Upon germination, the teliospore gives rise to a promycelium, into which the nucleus moves and undergoes meiosis, giving rise to the formation of haploid basidiospores directly on the surface of the promycelium (O'Donell and McLaughlin 1984).

2.4 Clamp Connections

In many basidiomycetous fungi, a stable dikaryotic state is established after a mating between two compatible homokaryons. The dikaryon is a specialized form of heterokaryon in which each cell has two haploid nuclei, one from each parent of the cross. These nuclei do not fuse, but are maintained as a pair in each cell in an "n + n" arrangement. This genetically balanced condition is characteristic of many, but not all basidiomycete fungi (Raper 1966; Raper 1983). The dikaryon is capable of indefinite propagation, and is found in such well-studied mushroom-producing fungi such as Coprinopsis cinerea (Coprinus cinereus) and Schizophyllum commune, but not in the white button mushroom, Agaricus bisporus (Raper et al. 1972) or rusts and smuts (which form stable dikaryons following mating and in host tissues). The cellular processes involved in maintaining the dikaryon are described below, and in Figure 1.
During the course of a compatible mating, the dikaryon is established by means of the reciprocal migration of nuclei from each mating partner into the hyphae of the other. This is followed by a precise pairing of the two different nuclei in each cell. In order to maintain a strict 1:1 ratio of the two nuclear types in each new cell of the dikaryon, each subsequent cell division is accompanied by the coordinated processes of hook cell formation and conjugate nuclear division. The hook cell, or clamp connection, starts as a lateral bulge that quickly grows in a manner curving away from the apex of the hyphal tip, forming a characteristic hook shape (Buller 1933; Niederpruem et al. 1971). The two parental nuclei in the apical cell divide synchronously, but the mitotic spindles are of different length. The nuclear division arising from the more apical nucleus has a shorter spindle, and generates a daughter nucleus that migrates into the hook cell. The companion daughter nucleus of this pair remains in the tip cell, and migrates towards its apex. A longer spindle is generated by the nuclear division of the more distal of the two parental nuclei, and is oriented in the same plane as the long axis of the dividing cell. This event generates one daughter nucleus that migrates towards the hyphal tip, during which time it moves past the nucleus of the other parental type. The other daughter nucleus moves backwards in a distal direction, in the soon to be subapical cell (Runeberg et al. 1986; Salo et al. 1989; Tanabe and Kamada 1994). There is a consequence of these precise patterns of nuclear division and movement. With each new conjugate division, there is an alternation in the order of which nuclear type takes the leading position in the apical cell (Iwasa et al. 1998). The next cellular event is that a septum is formed at the base of the hook cell, trapping the single nucleus within. A second septum is laid down in the hypha just distal to the hook cell, the result of which is a new apical cell with two haploid nuclei of each parental type (Buller 1933; Niederpruem et al. 1971). A lateral bulge or peg then forms in the subapical cell in close proximity to the septum (Buller 1933; Badalyan et al. 2004; Kues et al. 2002). This event is quickly followed by the fusion of the free end of the hook cell to the subapical cell at the location of the peg. The previously entrapped nucleus in the hook then moves into the subapical cell, restoring the dikaryotic state to this cell (Buller 1933; Tanabe and Kamada 1994). The presence of fused clamp connections is diagnostic of the dikaryotic state.

The processes involved in establishing and maintaining the dikaryotic state are genetically controlled at a fundamental level by the activity of the A and B mating-type genes (Raper 1966). This concept has been confirmed at the molecular level by a host of studies (Casselton and Olesnicky 1998; Kamada 2002; Kothe 1996; Kues 2000). Nuclear pairing, conjugate nuclear division, hook cell formation, and septation are regulated by the products of the A genes. Nuclear migration and hook cell fusion are controlled by the action of the B gene products. The formation of subapical pegs is attributed to the activity of both the A and B genes, with the latter likely to exert a greater influence on the process (Kues et al. 2002; Badalyan et al. 2004). The genes clp1 and pcc1 are downstream elements in the A-regulated pathway leading to clamp connection development (Kamada 2002). These two genes will be discussed later in the section on the genetic control of mushroom development.
2.5 Sporulation

In order to reproduce and to protect precious genetic material and safeguard it to allow for future generations, many eukaryotic organisms have evolved elaborate mechanisms. Fungi produce several kinds of spores, which can be divided roughly into asexual and sexual spores. The production of asexual spores, mainly meant for dispersal to increase the population under favorable conditions, seems to be more prevalent among ascomycetes although these play a major role in the rusts and the human pathogen Cryptococcus neoformans and can cause epidemics. Under certain

(sudden, unfavorable) conditions, some fungi produce chlamydospores, thick-walled cells that develop from single (dikaryotic) hyphal compartments. In some higher mushroom species like Coprinopsis cinerea, monokaryotic (aerial) mycelium can produce abundant single-celled mitotic spores (oidia) on oidiophores (Polak et al. 1997) (See Figures 4 and 5). C. cinerea can also produce chlamydospores, which are submerged, thick-walled mitotic spores found in older cultures of dikaryons and certain monokaryons (Kues 2000). These structures arise in areas of compressed cytoplasm, and are not found in the other model mushroom-forming basidiomycete, S. commune. Sexual spores are mainly produced under unfavorable conditions as a “resting” stage,

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**Fig. 1**: Cellular processes involved in dikaryon formation (from Kues 2000). Involvement of A and B loci depicted for homobasidiomycete/mushroom growth.
e.g., to prepare for draught or overwintering. A hall mark of the basidiomycetes is the production of a basidium in which karyogamy in the otherwise dikaryotic mycelial cells takes place, followed by meiosis and generally the production of four haploid basidiospores in which different (often two opposite) mating types also segregate (Alexopolous et al. 1996). Ironically for this chapter, the phytopathogenic rust and smut fungi are different in that they produce an additional spore type, the teliospore which is essentially a probasidium. The teliospore is a true resting structure with a thick, often pigmented or melanized (Butler and Day 1998) and ornamented wall in which karyogamy has taken place. This cell type represents therefore a true diploid stage. Teliospores can survive for many years and are also meant for dispersal such as for the rusts and smuts. There is a tremendous variation in morphology and ornamentation of teliospores and other spore types such as the urediniospores in rusts (e.g., see Fig. 20-19 in Alexopolous et al. 1996). For example, Puccinia species often produce two-celled teliospores whereas in Uromyces spp. mostly one-celled teliospores are found. In general, spore features are often used for taxonomic purposes. Teliospore germination can take place under favorable conditions either directly after they have been produced or after a required, prolonged dormancy period. Germination produces a promycelium (metabasidium) in which meiosis takes place. Four resulting haploid nuclei then move into four cells, but one or four mature basidiospores might eventually form on the basidium (Alexopolous et al. 1996; Goates and Hoffmann 1987; Mendgen 1984). Haploid basidiospores from many smuts reproduce by budding and are saprobic allowing them to be cultured and making them amenable to molecular techniques. In the obligate rusts, the probasidium from the germinating teliospore and the resulting basidiospores are the only stages not requiring a host plant. However, cultivating rusts in vitro has been unsuccessful or is difficult at best (Bose and Shaw 1974; Williams 1984; Boasson and Shaw 1988; Fasters et al. 1993).

2.6 Pathogenicity and Virulence Factors in Biotrophic Interactions of Smuts and Rusts

By definition, pathogens have the ability to cause disease on a host. Successfully completing their life cycle (i.e., producing viable progeny/survival structures) is the pathogen's only goal, selected for during evolution. A large arsenal of proteins and metabolites contribute to its armor to allow it to recognize certain organisms as hosts, be induced to germinate and risk its precious genetic material, and to wage chemical warfare in order to breach barriers, gain access, suppress recognition and defense responses and coax the invaded organism in supporting its development. The pathogen will achieve this at various levels of success resulting in the attacked host being classified as susceptible, partially resistant, resistant, or as a nonhost and the pathogen correspondingly as more or less virulent, as avirulent, or as an inappropriate pathogen. Traditionally, these interactions are described from a plant geneticist's perspective and often inadequately describe the wide range of interactions that can be observed microscopically in plant pathogenesis. Indeed, "modern" agriculture, selection and breeding (starting more than 10,000 years ago) have revealed a sizeable repertoire of
such pathogenicity and virulence factors contributing to their respective classification (reviewed in Bakkeren and Kronstad 1994). In some (rare) instances, the host-pathogen interaction leads to complete incompatibility in an otherwise compatible pathogen species. Genetic analysis of both pathogen and host identified (often dominant) single genes superimposed on compatibility which have been termed “avirulence” and resistance genes, respectively. It is now generally believed that these “avirulence” genes are maintained in pathogen species and populations because they are among the general arsenal of virulence factors of the pathogen, but have been co-opted by the plant's innate surveillance system to be recognized as “non-self” and trigger a resistance (immune) response. Thus, even though harboring “avirulence” genes bestows an obvious disadvantage on its bearer with respect to host infection, deleting or mutating them also comes at a cost, sometimes subtly and only apparent on a larger population level (Tian et al. 2003; Brown 2003; Wichmann and Bergelson 2004). Surveying and monitoring of population dynamics of large numbers of races (harboring different combinations of avirulence genes in a pathogen species) uncovered through numerous cultivars (resulting from breeding programs and harboring an equal number of cognate resistance genes capable of triggering defense after recognition) form the basis of modern crop resistance breeding.

To distinguish between virulence factors in sensu stricto, that is, contributing to the disease process, and genes merely involved in fitness and/or general metabolism is extremely difficult in the biotrophs and might be no more than a matter of definition and semantics (reviewed in Bakkeren and Gold 2004). During the infection process, including the establishment of intercellular hyphae and haustoria, true biotrophs inflict minimal damage to host cells and tissue integrity is maintained. Moreover, detection of ‘pathogen associated molecular patterns’ (PAMPs) or ‘non-self’ molecules by the host is minimized by cloaking or biochemical changes when in planta, and by active suppression of host defenses. An excellent recent review discusses the attributes of ‘true biotrophs’ vs. hemibirotrophs and necrotrophs (Oliver and Ipcho 2004).

3. EXEMPLARY MODEL SYSTEMS
3.1 Smuts
3.1.1 Introduction

The basidiomycete smut fungi belong to the order Ustilaginales, an order that includes plant pathogenic fungi. Smuts are facultative obligate biotrophs that affect approximately 4000 species of angiosperms belonging to over 75 families (Alexopolous et al. 1996). While these fungi have a saprobic, budding yeast phase that can be easily maintained in culture, these fungi employ an obligate dikaryotic phase to grow filamentously within the plant host, sometimes producing spectacular symptoms. One of the most striking symptoms (and particularly pronounced in the U. maydis-maize interaction) is the induction of galls or tumors in the host (Figure 2).

At maturity, these galls are associated with black dusty masses of teliospores that resemble soot or smut, hence the name. In this chapter section, the smut fungus *Ustilago maydis* is discussed in detail as an exemplary model system of development and
morphogenesis, a discussion propelled forward at the genetic level in the context with the fascinating biology of this fungus. U. maydis is among the most important models for fungal plant pathogenesis, morphogenesis, mating and signaling. It is currently the only well developed genetic model of plant pathogens among the basidiomycetes. *U. maydis* shares many features with the mushroom fungi and is an important comparative model for the important human disease caused by the basidiomycete *Cryptococcus neoformans*. In the following treatment we present the central aspects of *U. maydis* under current and continuing study.

### 3.1.2 Dimorphism

Fungal dimorphism is an interconversion of the yeast and mycelial morphologies, commonly termed the dimorphic switch. Although various genetic determinants and environmental stimuli have been characterized that govern this dramatic morphological alteration, one central theme in the control of dimorphism in fungi is the interplay between a pheromone-responsive mitogen-activated protein (MAP) kinase and a cyclic AMP (cAMP) pathways. Through signaling via the pheromone-responsive MAP kinase pathway, the yeast form of the smuts mate and form a filamentous dikaryon. In *U. maydis*, mate recognition is controlled by the master regulatory *a* locus, encoding both pheromone and pheromone receptors (Bolker et al. 1992). Cells of opposite mating types, differing at their *a* loci, secrete pheromone which induces the production of mating hyphae that grow toward the opposing mating partner and eventually fuse (Snetsalaar et al. 1996). Once cell fusion has occurred, production and maintenance of the filamentous dikaryon is dependent on master regulatory *b* genes from opposite mating types, e.g. *b1* and *b2* (Kamper et al. 1995) dependent on master regulatory *b* genes from opposite mating types, e.g. *b1* and *b2* (Kamper et al. 1995). Filamentous growth may facilitate penetration of plant cells, thereby aiding in the collection of nutrients from the host and providing an ideal environment for sexual maturation. Environmental signals also trigger filamentous growth or are involved in the maintenance of a yeast phase. As opposed to the filamentous dikaryon, the precise role of the haploid yeast phase remains enigmatic in *U. maydis*. A yeast phase, in which cell division occurs rapidly by budding, leading to geometric cell population growth, may potentially aid in the dispersal of these plant pathogens by rain and wind. The production of large numbers of budding cells increases the overall number of propagules available in the environment, ensuring that at least some compatible members of the population meet at the right place and time under conditions suitable for mating. Therefore, yeast-like growth, as opposed to filamentous growth, may be advantageous in the saprophytic phase of smuts.

As mentioned above, interplay between the pheromone-responsive MAP kinase pathway and the cAMP pathway controls dimorphism in *U. maydis*. In this section, we discuss dimorphic switching as it relates to environmental signals as well as alternative pathways regulating dimorphism in the exemplary model system of *U. maydis*. These signals impinge on various pathways or proposed regulatory components that will be discussed in great depth in upcoming sections of this chapter (see sections 3.1.3 through
3.1.5). The genetic determinants of dimorphism in the smuts induced by compatible mating partners will be described in the Mating section. Ultimately, the signaling pathways that control dimorphism must converge on the regulation of the cytoskeleton, the genetic control of which will be discussed in detail under Cell Cycle and Cytoskeletal Regulation.

![Diagram of Disease cycle of corn smut, caused by Ustilago maydis](from Agrios 1997)

Control of the dimorphic switch has been an area of intensive study in *U. maydis* and other pathogenic fungi (Sanchez-Martinez and Perez-Martin 2001). In *U. maydis*, cAMP is required for the maintenance of the budding form (Gold et al. 1994). Disruption of the *U. maydis* adenylate cyclase (*uac1*) gene led to the formation of constitutive filamentous strains from budding haploids (Barrett et al. 1993). A number of mutations, termed *ubc* for *Ustilago* bypass of cyclase, suppress the filamentous phenotype of the *uac1* mutant (Gold et al. 1994; Mayorga and Gold 1998). Complementation of one of these *ubc* suppressor mutations led to the restoration of the filamentous *uac1* phenotype and enabled the isolation of the *ubc1* gene encoding the regulatory subunit of cAMP-dependent protein kinase A (PKA) (Gold et al. 1994). In addition to *ubc1*, four additional *ubc* genes (*ubc2, ubc3, ubc4* and *ubc5*) were cloned by mutant complementation (Mayorga and Gold 1998; Andrews et al. 2000). The *ubc5/fuz7* gene was identified in an independent screen (Banuett and Herskowitz, 1994). The *ubc3* and *ubc4* genes were also identified in screens independently as *kpp2* and *kpp4*, respectively (Muller et al. 1999; Muller et al. 2003 b). The *ubc3/kpp2, ubc4/kpp4, and fuz7/ubc5* genes encode a MAP
kinase, MAPKK kinase and a MAPK kinase respectively and all are members of the pheromone-responsive MAP kinase cascade involved in mating, morphogenesis, and pathogenic development (Mayorga and Gold 1998; Mayorga and Gold 1999; Banuett and Herskowitz 1994; Andrews et al. 2000; Muller et al. 2003). The cAMP and MAP kinase pathways impinge on the transcription factor Prf1, which is differentially phosphorylated by PKA and the MAP kinase Kpp2/Ubc3 to activate pheromone and receptor gene expression as well as genes regulating filamentous growth (Kaffarnik et al. 2003).

The number and types of stimuli that can flip the dimorphic switch in fungi appears to defy a unifying theme. In *U. maydis*, in addition to pheromone response-induced and b gene-regulated filamentous growth, pH changes (Garrido and Perez-Martin, 2003; Martinez-Espinoza et al. 2004) exposure to air (Gold et al. 1994) and nutrient deprivation (Smith et al. 2003) and the presence of lipids (Klose et al. 2004) can trigger morphogenetic changes. In *U. maydis*, the cAMP and MAPK pathways play a key role in pH-regulated dimorphism (Martinez-Espinoza et al. 2004). On solid acid medium (pH3), haploid wild-type strains of *U. maydis* exhibit a mycelial phenotype. However, *ube2* and *ube5* MAPK pathway mutants grow as yeasts at pH 3. In contrast, *prf1* mutants remained in the mycelial phase at pH 3. These results strongly implicate the pheromone-responsive MAPK cascade in pH-regulated dimorphism and that this signaling is independent of the transcription factor Prf1, suggesting a branching of this signaling pathway upstream of Prf1. Further analyses revealed that *ube1* mutants, which display a mycelial morphology at neutral pH characteristic of cAMP pathway mutants, grew as yeasts at pH 3. Also, upon addition of cAMP to the medium, the *ube2* and *ube5* mutant strains grew as yeasts. Taken together, these results provide a strong indication of the role of the cAMP pathway in signaling pH-regulated dimorphism (Martinez-Espinoza et al. 2004). Interestingly, in the smut fungus *Ustilago hordei*, wild-type strains behave oppositely in response to pH changes. In contrast with *U. maydis*, *U. hordei* primarily maintains a yeast-like morphology at acid pH but more filamentous growth is observed at neutral and basic pH (Lichter and Mills 1998).

Like pH changes, nutrient sensing plays a critical role in modulating morphogenic changes in fungi. In *U. maydis*, a gene encoding a protein with high similarity to the high-affinity ammonium permease, Mep2p, from *S. cerevisiae* was identified as highly expressed in budding yeast cells as opposed to filamentous cells (Smith et al. 2003). *mep2* is involved in cAMP signaling and is required for pseudohyphal growth in response to low ammonium in *S. cerevisiae*. Interestingly, *ump2* was able to complement the pseudohyphal defect characteristic of the yeast *mep2* mutant. Furthermore, deletion of this gene in *U. maydis* eliminated the filamentous phenotype of haploid cells on low ammonium. Additionally, the importance of a PKA phosphorylation site in Ump2p was revealed by site-directed mutagenesis and complementation studies (Smith et al. 2003). Overall this work indicates a potential role of the cAMP-PKA pathway in signaling morphogenic changes through nutrient stress and specifically the important role of ammonium permeases in response to low nitrogen conditions. An additional example of nutrient sensing affecting morphogenesis in *U. maydis* is the presence of lipids, which
promote filamentous growth dependent on the cAMP and MAPK signaling pathways (Klose et al. 2004).

In addition to the cAMP and MAPK pathways and their relatively defined roles in regulating dimorphism in U. maydis, other pathways may operate in conjunction with or independently in signaling the dimorphic switch. Guevara-Olvera et al. (1997) revealed a role of polyamine biosynthesis as a determinant of the dimorphic switch. Mutants of the ornithine decarboxylase (odc) gene, which encodes a product that catalyzes the first step in polyamine biosynthesis, behave as polyamine auxotrophs. The dimorphic switch was inhibited in medium containing the minimum concentration of polyamines to support growth. Supplementation of the medium with additional polyamines led to the dimorphic transition (Guevara-Olvera et al. 1997). In contrast with the central role of polyamine biosynthesis, analyses of single and double chitin synthase (chs1-6) mutants revealed only slight alterations in morphology (reviewed in Martinez-Espinoza et al. 2002). Finally, and likely an additional contributor of the dimorphic switch in U. maydis, what is known of the role of calcium homeostasis in the morphogenesis will be discussed in detail in the Cell Cycle and Cytoskeletal Regulation section.

3.1.3 Mating

Genetic analysis of the sexual system has been a major focus of work on Ustilago maydis and this species has become a paradigm for the higher basidiomycetes (Casselton and Olesnicky, 1998; Casselton 2002). The heterothallism of U. maydis was recognized as early as 1927 (Stakman and Christensen 1927). Employing tetrad analysis, Hana (1929) showed conclusively that there were at least two pairs of segregating sex factors because in some cases four distinct mating types were derived in a single tetrad. He designated the diploid nucleus as possessing an AaBb genotype and the progeny as AB, Ab, aB and ab. Similar notations for the mating-type genes are still used. No distinction was made for the function of A or B in Hana’s work (Hana 1929). Rowell and DeVay (1954), designated these two mating factors as “a” and “b” and determined that 2 specificities of a and multiple specificities of b existed. Different specificities were necessary at both the a and b loci to generate productive maize infection in which teliospores (chlamydospores) were generated, and the currently used designations for these mating-type loci was established. In this same study it was determined that amphimixis progeny were occasionally generated that had both a specificities such that they could productively be paired with any strain possessing a different b allele. However, these amphimixis strains were not solopathogenic while diploids heterozygous at both a and b were solopathogens. This indicated to the authors that the a compatibility factor was clearly not a primary pathogenicity characteristic, a fact further corroborated by Banuett and Herskowitz (1989). Rowell clearly demonstrated that the roles of heterozygosity at a and b were in fusion and dikaryon vigor/stability, respectively (Rowell 1955). He also noted that alleles of both factors had to be different in the mating partners to generate the virulent pathogen.
Trueheart and Herskowitz (1992) generated a cytoduct assay in which cell fusion was strictly controlled by possession of unequal alleles at the $a$ locus while the $b$ locus played no role. Later, Laity et al. (1995) complemented this work showing that heterozygosity at the $b$ locus within a strain inhibits further mating (Laity et al. 1995). In summary then, $a1$ strains will fuse with $a2$ strains regardless of the condition at the $b$ locus except that once $b$ becomes heterozygous the cell will fuse no further with any other strain. Additionally, $a1a2b_u$ stains fuse promiscuously with any normal haploid mating type. By addition of charcoal to solid medium, the development of the functional dikaryon can easily be monitored in culture (Day and Anagnostakis 1971; Holliday 1974). White filamentous growth is observed on this medium only when both $a$ and $b$ differ in cospotted compatible strain pairs.

The $b$ locus is multiallelic (Rowell and DeVay, 1954). Puhalla (1970) found only 2 $a$ but 18 different $b$ mating-type alleles in 62 lines amongst 33 different isolations from the U.S. and Canada. He predicted that there should be no more than 25 distinct alleles of $b$ in the population (Puhalla 1970).

The master control genes of mating and pathogenicity, the $a$ and $b$ mating-type genes, have now been cloned and characterized (Kronstad and Leong 1989; Kronstad and Leong 1990; Schulz et al. 1990; Froeliger and Leong 1991; Bolker 1992). Holliday had noted that the $pan1$ gene was tightly linked at about 2.5 map units from the $a$ mating-type locus (Holliday 1961; Holliday 1974). Using this information, Froeliger and Leong (1991), cloned the $a2$ mating-type determinant by complementation of a $pan1$ mutant $a1$ strain with a cosmid from a prototrophic $a2$ strain. By mating $a1b1/a2$ transformant strains with an $a1b2$ strain to generate a filamentous and pathogenic dikaryon, the presence of the $a2$ allele was confirmed. The initial cloning of the $a$ locus indicated that the $a1$ and $a2$ allelic sequences were idiomorphs, (i.e. they lacked sequence homology). Homologous flanks were then employed to isolate the $a2$ mating-type idiomorph and similar methods used to confirm function (Froeliger and Leong 1991). The $a$ mating-type genes were then sequenced and Bolker et al. (1992), demonstrated that the mating-type specificity in each idiomorph was determined by two genes. One gene encodes a lipopeptide mating factor, and the other a pheromone receptor. Thus the $a$ locus encompasses $mfa$ and $pra$, two tightly linked genes that encode secreted pheromone and membrane spanning pheromone receptors, respectively (Bolker 1992). The pheromone encoded by the $mfa$ gene is thought to interact directly with the pheromone receptor product encoded by the $pra$ gene of the opposite $a$ mating specificity (Spellig et al. 1994). Synthetic pheromone causes cell cycle arrest in the G2 phase (Garcia-Muse et al. 2003). This is in contrast with the situation described in ascomycete yeasts such as S. cerevisiae and Schizosaccharomyces pombe where pheromone induces cell cycle arrest at G1. The function of the genes at the $a$ locus helps explain the fact noted above that $a^f$ (possession of two different allelic specificities of $a$) is required for a diploid heterozygous at $b$ to become filamentous on charcoal mating media (Banuett and Herskowitz 1989). In addition to its function as a mating attraction system, dikaryon heterozygosity at the $a$ locus (in addition to heterozygosity at $b$) also contributes to the in vitro production of the post mating dikaryotic filamentous form.
through an autocrine response in which the pheromones and receptors of opposite allelic specificity are present within the same cell and therefore may continually interact (Banuett and Herskowitz 1989; Spellig et al. 1994). Smuts having a bipolar mating system, such as the barley covered smut U. hordei, possess both a and b gene complexes homologous in sequence and function. When the gene complexes were tested across the species U. maydis and U. hordei, the a gene complexes were shown to determine interspecies compatibility as well and the b gene complexes to function properly in triggering filamentous growth thereby proving the existence of conserved pathways (Bakkeren and Kronstad 1993; Bakkeren and Kronstad 1994; Bakkeren and Kronstad 1996). The a and b gene complexes are separated by approximately 500 kb in MAT-1 and 430 kb in MAT-2 strains and the region in between these complexes displays some inversions and deletions compared to the other mating type possibly giving an explanation for the lack of recombination over this part of the chromosome and alleviating the need for multiple mating types; apart from the two opposite mating specificities for a similar to U. maydis, only two specificities seem to exist for b as well in Nature (Bakkeren and Kronstad 1994; Lee et al. 1999). The U. hordei MAT-1 region has been sequenced and analyzed and harbors apart from several genes, a large number of (partial) transposable element and repeat sequences which could have been involved in the evolution of this part of the chromosome (Jiang, Bakkeren and Kronstad, manuscript in preparation). The a and b gene complexes separated in the tetrapolar U. maydis, could have become fixed in bipolar smuts during the genesis of primitive sex chromosomes as has been suggested happened in several Cryptococcus species (Fraser and Heitman 2004).

Downstream events generating the final response to pheromone appear to involve components similar to those encountered in S. cerevisiae. In this budding yeast, signal transduction from the pheromone-receptor interaction to the final cellular responses involves a trimeric G protein and a MAP kinase cascade with the final phosphorylation and activation of two critical proteins. These proteins are the Ste12p transcription factor, which when activated regulates transcription of target genes, and Far1p which causes cell cycle arrest by inhibition of the kinase activity of the G1 cyclin complex Cdc28-Cln (Banuett 1998; Valdivieso et al. 1993). In U. maydis none of the four cloned Ga subunits of the trimeric G proteins appear to be directly involved in transmission of the pheromone signal (Regenfelder et al. 1997; Kruger et al. 1998). As is the case in Schizosaccharomyces pombe (Sipiczki 1988), a ras gene (ras2) functions to stimulate filamentous growth through the pheromone responsive MAP kinase cascade (Lee and Kronstad 2002). Additional work suggested that the cdc25 homolog Slq1 may function as an activator of Ras2 (Muller et al. 2003 a). An additional finding in this work was that activated Ras1, the product of a second ras gene, increased pheromone gene expression. The three members of the pheromone responsive MAP kinase cascade have been identified. These are ubc4/kpp4 encoding the ste11p MAPKK kinase homolog (Mayorga and Gold 1998; Andrews et al. 2000; Muller et al. 2003 b) fuz7/ubc5 encoding the ste7p MAPK kinase homolog (Banuett and Herskowitz 1994; Mayorga and Gold 1998; Andrews et al. 2000), and the fus3p and kss1p MAP kinase homolog ubc3/kpp2
A putative adaptor protein Ubc2 may link the MAP kinase cascade with the upstream components of signaling through Ras proteins (Mayorga and Gold 2001). Preliminary two-hybrid screens with Ubc2 as bait identified a strong interaction of Ubc2 and Ubc4 MAPKKK SAM domains (Klosterman et al. unpublished) confirming a previously observed genetic interaction (Mayorga and Gold 1998). This is analogous to the interaction of the SAM domains of Stc11p and Ste50p in S. cerevisiae (Jansen et al. 2001). A gene designated prf1 encodes an HMG family transcription factor that links the pheromone response pathway to the expression of the b locus and thus to pathogenicity (Hartmann et al. 1996). The prf1 protein has potential phosphorylation sites for both a MAP kinase (presumably ubc3/kpp2, see below) and for the cyclic AMP dependent protein kinase (Kahmann et al. 1999; Muller et al. 1999; Kaffarnik et al. 2003). The putative MAP kinase phosphorylation sites appear important for the biological function of the protein (Muller et al. 1999). The prf1 gene is required for pathogenicity due to its essential function in the regulation of the b mating-type genes. Constitutive expression of the b genes restores pathogenicity in prf1 mutants (Hartmann et al. 1996). Additional transcription factor(s) are likely involved in transmitting the pheromone responsive MAP kinase and/or cAMP pathway signals besides Prf1. As noted by Lee and Kronstad (2002) epistasis experiments indicated that Ras2 may regulate filamentation via the pheromone responsive MAP kinase cascade including Ubc3, but not through the activation of Prf1. Recently it has also been shown that although the pheromone response pathway is required for conjugation tube formation in the mating reaction, Prf1 is not required for this process to occur (Muller et al. 2003 b). Additionally, work from our laboratory indicates that the MAP kinase cascade is required for acid-induced filamentation while prf1 is not (Martinez-Espinoza et al. 2004).

The b locus controls events after cell fusion necessary for establishment of the infectious filamentous dikaryon. lga2, a gene of unknown function (Urban 1996 b) located within the a2 idiomorph, is directly and positively regulated by the b-heterodimer (Romeis et al. 2000). Employing inducible promoters to replace those native to b, both positively and negatively b transcriptionally regulated genes have been identified (Brachmann et al. 2001). However, deletion of a number of these genes did not produce any discernible effect on morphology or pathogenicity, indicating that the ones characterized so far do not individually play a major role in pathogenesis and development. Additionally, genes, that when mutated, induced expression of the b genes in haploid cells as well as other dikaryon specific genes, have been identified using another reporter system (Quadbeck-Seeger et al. 2000; Reichmann et al. 2002). Mutants deleted for these genes (run1 and lida1) are able to colonize plants apparently normally but are fully defective in teliosporogenesis a process discussed in more detail below.

3.1.4 Signaling

Signaling pathways in plant pathogenic fungi clearly play central roles in environmental sensing, mating processes, morphogenesis and communication with the
host. The pathways most well studied and most significant in the fungi, as judged by the frequency with which they are encountered in mutants affected in these processes, are the cAMP activated protein kinase A (PKA) and MAP kinase pathways. The importance of signaling involving calcium as a messenger molecule for these processes is clearly a common phenomenon. These pathways tend not to stand alone but rather frequently crosstalk and can be viewed as a web of interconnected pathways, often several of which impact a single phenotypic outcome. A common problem with interpreting these studies is that perturbing one pathway is like cutting a single strand of a spider’s web, consequently deforming many unintended interconnected strands. Several excellent reviews have recently been published that deal with these topics in great detail. cAMP signaling and its interaction with MAP kinase pathways in phytopathogenic fungi was very recently well reviewed (Lee et al. 2003). Thus here we provide a few well-characterized illustrative examples.

In general, mutations inactivating PKA lead to debilitation of a fungus. For example, in *U. maydis*, mutation in adenylate cyclase, Uac1 (Barrett et al. 1993; Gold et al. 1994), the catalytic subunit of PKA, Adr1 (Durrenberger et al. 1998) or in a G-protein alpha subunit Gpa3, required for activation of adenylate cyclase (Regenfelder et al. 1997) cause similar consequences, freezing this dimorphic fungus in the filamentous phase and eliminating the ability to colonize maize. Nonetheless, upon close comparison of these mutants, they do differ in several subtle phenotypes. For example, the adenylate cyclase mutant is much more invasive on agar than is the PKA catalytic subunit mutant (Gold, unpublished). This indicates that cAMP likely plays a role beyond the function of *adr1*.

Mutations that activate PKA such as mutations in the regulatory subunit of PKA (a PKA inhibitor protein) or activated alleles of specific G-alpha proteins tend to cause phenotypes of opposite character to those of inactivating mutations. For example, in *U. maydis*, ubc1 encoding the regulatory subunit of PKA, is an epistatic suppressor to the uac1 mutation (Gold et al. 1994). Likewise an activated allele of *gpa3* generates several phenotypes similar to a ubc1 mutant but is hypostatic to uac1 (Regenfelder et al. 1997; Kruger et al. 1998). However, mutations activating the PKA pathway still tend to be detrimental to virulence. Null ubc1 mutants are able to colonize maize leaves but are unable to induce gall formation (Gold et al. 1997). Mutants with intermediate activation of PKA caused further progression toward the wild type infection (Kruger et al. 2000). These results indicate that a delicate balance of PKA activation must be maintained for progression through the various phases of the infection cycle. Perturbation in one or another direction, activation or inactivation, leads to detrimental effects on the fungus.

Mitogen activated protein kinases have often been encountered in phytopathogenic fungi as required for morphogenesis and for full virulence. This topic has been reviewed in some detail (Xu 2000). *S. cerevisiae* has five functional (and partially overlapping) MAP kinase cascades. These are involved in mating, filamentation, cell integrity, high osmotic growth stress response, and ascospore formation. The environmental triggers activating these pathways are commensurate with their function, eg, pheromone for mating. In the genomes of plant pathogenic fungi (e.g., in
M. grisea), and filamentous fungi in general for that matter (e.g., in N. crassa), often only three MAP kinase enzymes appear to be present. These fall into three families relative to yeast: pheromone responsive (Fus3/Kss1), osmoregulation (Hog1) and cell integrity (Slt2). Mutations in these MAP kinases tend to cause differential defects in pathogenicity. For example, in M. grisea PMK1 (pheromone response) and MPS1 (cell integrity) are both required for disease on unwounded leaves but mps1 mutants can colonize wounded leaves while pmk1 mutants cannot (Xu and Hamer, 1996; Xu et al. 1998). In this fungus the third MAP kinase, OSM1, is not required for virulence under laboratory conditions. Pheromone responsive MAP kinase genes have been repeatedly found to be critical for full virulence in the fungi (Xu 2000). MAP kinase genes from other families have also been shown to be important for virulence in plant pathogenic fungi but tend to be more variable in their effect than the Fus3 family. The pheromone responsive MAP kinase in U. maydis ubc3/kpp2 (Mayorga and Gold 1999; Muller et al. 1999) was initially defined as of relatively minor importance in virulence. However, it now appears that this was primarily due to partial functional redundancy because when only the kinase activity is mutated but the protein still synthesized a much more dramatic effect on virulence is observed (Muller et al. 2003b). This situation is reminiscent of the complementation of fus3 deletion by kss1, which is unable to complement the fus3 kinase mutant (Madhani et al. 1997). In U. maydis, formation of swollen appressorium-like structures and their production of invading hyphae which penetrate epidermal cells, appear to be distinct steps in the infection process. A mutant strain defective in Kpp6 activity, a b mating-type gene-regulated MAP kinase, has recently been constructed, which is able to produce appressoria but is unable to penetrate plant cells (Brachmann et al. 2003). Microscopic observations of plant surfaces after inoculation with compatible strains both carrying an inactivated mutant allele, kpp6\textsuperscript{1355A,1357F}, showed appressorium formation. However, from the majority of those appressoria only short filaments that failed to penetrate plant cells emerged.

Cross talk between the cAMP and MAP kinase cascade signaling pathways has been well documented (D'Souza and Heitman 2001; Lee et al. 2003). In U. maydis the interaction of the cAMP and MAP kinase pathways was made evident by the finding that a number of suppressors of the filamentous phenotype of an adenylate cyclase (uac1) mutant were members of the pheromone responsive MAP kinase cascade (Mayorga and Gold 1998; Mayorga and Gold 1999; Andrews et al. 2000; Mayorga and Gold 2001). Also in U. maydis, it has recently been demonstrated that the differential phosphorylation of Prf1 by the pheromone responsive MAPK and the cAMP dependent protein kinase regulates the activity of this transcription factor toward its various promoter targets including the a and b mating-type genes (Kaffarnik et al. 2003). Induction of the a mating-type genes requires the PKA phosphorylation sites while induction of the b genes requires both the PKA and MAPK phosphorylation sites. Another integrator of cAMP and MAP kinase signaling is apparently the crkl encoded kinase (Garrido and Perez-Martin 2003). The transcription of this kinase is oppositely affected by the function of the MAPK and cAMP pathways.
3.1.5 Cell cycle and cytoskeletal regulation

Detailed analyses of the cell cycle in *U. maydis* have been conducted in relation to budding cell morphology. Cells of *U. maydis* produce one polar bud per cell cycle (Jacobs et al. 1994). When the bud nears maturity, the nucleus divides in the bud of the daughter cell and following retrograde movement of one nucleus to the mother cell, cytokinesis occurs (Banett and Hirskowitz 2002; O’Donnell and McLaughlin 1984). Analyses of nuclear density in asynchronously growing populations revealed that *U. maydis* cells exhibit both prereplicative DNA content (1C) typical of G1 phase of the cell cycle and postreplicative DNA content (2C) of G2 phase (Snetselaar and McCann 1997). When haploid *U. maydis* cells are exposed to mating pheromone, they undergo G2 arrest and produce conjugation tubes (Garcia-Muse et al. 2003) in contrast with the budding yeast *S. cerevisiae*, which generally lacks a G2 phase (O’Farrell 2001).

The genetic determinants of cell cycle control are beginning to be uncovered in *U. maydis*. In eukaryotes, cyclin dependent kinases (CDKs) and their cognate regulatory subunits known as cyclins control cell cycle events such as the onset of mitosis and S phase. In *S. pombe* and *S. cerevisiae*, one CDK in each of these fungi and different cyclins control these events of the cell cycle (O’Farrell 2001). In *U. maydis*, using a PCR-based approach, the CDK, Cdk1, was identified along with two B-type cyclins, Clb1 and Clb2 (Garcia-Muse et al. 2004). The *cdk1* gene product was isolated from cell lysates together with the Clb1 and Clb2 proteins, which together form the mitotic CDK. Levels of Clb1 and Clb2 were determined to fluctuate during the cell cycle, falling in G1 and rising at entry to S/G2/M. Conditional mutants revealed Clb1 depletion resulted in cell cycle arrest at two points, one at which cells exhibited a prereplicative 1C DNA content and one in which the cells exhibited a postreplicative 2C DNA content. Clb2 depletion on the other hand arrested the cells when they exhibited a 2C DNA content. This suggested that Clb1 is required for the G1 to S and G2 to M transitions while Clb2 is required for entry into mitosis, a finding supported by the observation that overexpression of *clb2* caused early entry into mitosis. Additionally, cells overexpressing *clb2* or those with a single dosage of *clb2*, divided by septation and grew filamentously but did not form buds, nor could they produce tumors in the plant. However, both of these mutants were capable of filamentous growth within the plant (Garcia-Muse et al. 2004). Interestingly, filamentous growth within the plant but lack of tumor formation is also observed in *ubc1* and *ubk1* mutants that exhibit defects in bud site selection (Gold 1997; Abramovitch 2002).

Further searches for Cdk genes in *U. maydis* revealed the presence of a novel gene, *crk1* (cdk related kinase 1), that bears limited identity with mitotic cyclin-dependent kinases and the putative encoded protein most closely matches Imc2, a kinase involved in morphogenic control in *S. cerevisiae* (Garrido and Perez-Martin 2003). Disruption of the *crk1* gene in *U. maydis* revealed morphological defects such as the production of shorter and rounder cells. At low pH, *crk1* defective cells were unable to form the multicellular chains like wild type cells. However, complementation with wild-type *crk1* was capable of inducing filamentation. The activity of CRK1 was also correlated with both the cAMP and MAP kinase pathways since inactivation of *crk1* suppresses
filamentous growth of cAMP pathway defective mutants. Strains deficient in gpa3 produce highly elevated levels of the crkl message, suggesting that the cAMP pathway negatively regulates crkl expression. Further experiments revealed that the MAPK pathway counteracts the negative regulation of crkl by the cAMP pathway (Garrido and Perez-Martin 2003).

Coupled with the findings of the Cdk and Cdk-related genes in U. maydis, two genes involved in cell separation also have been identified. Weinzierl et al. (2002) identified don1 and don3 (donuts) by complementation of a mutant colony morphology that resembled a donut. Sequence analysis revealed that don1 encodes a protein containing a nucleotide exchange domain (GEF), a PH domain and a FYVE zinc finger domain while don3 encodes a highly conserved Ser/Thr-protein kinase domain characteristic of Ste20-like kinases, targets of small GTP-binding proteins of the Rho/Rac family, including Cdc42. Two-hybrid assays revealed Don1 and Don3 both interact with U. maydis Cdc42. Because don1/don3 double mutants and individual mutants exhibited the same phenotypes, it was suggested that Don1 and Don3 both interact with Cdc42 in the same signaling pathway. Further analyses revealed that both wild-type and don1/don3 mutant budding cells form a primary septum at the bud neck between mother and daughter cells following bud formation and mitosis. don1 and don3 mutants were unable to form a secondary septum and consequently did not form a fragmentation zone. In conclusion, don1 and don3 appear necessary for secondary septum formation and cell separation. The targets of Don3 phosphorylation are presently unknown (Weinzierl 2002).

In U. maydis, the cell cycle has been studied in relation to changes in cytoskeletal organization (Steinberg et al. 2001; Banuett and Herskowitz 2002). These studies have revealed that transitions in the cell cycle require rearrangement of the microtubule (MT)- and F-actin-based cytoskeleton. These changes in MTs are highly dynamic and determine cell polarity (Steinberg et al. 2001; Banuett and Hirskowitz 2002). The appearance of lateral budding and buds at opposing poles of the cell following benomyl (known to affect MTs and frequently used as a systemic fungicide) treatment or in a U. maydis α tubulin conditional mutant strain indicates MTs play a key role in determining cell polarity (Steinberg et al. 2001). The assembly of the asymmetric dimers of α and β tubulin subunits confer MT polarization, having a plus end and a minus end (reviewed in Steinberg and Fuchs 2004). Motors such as dynein and kinesin use ATP for movement along microtubules (MTs) (Straube et al. 2001; Wedlich-Soldner et al. 2002 a; Wedlich-Soldner 2002 b) in a directional manner.

Morphological changes in U. maydis and other fungi are ultimately linked to the molecular motors that drive cytoskeletal rearrangement and move cargo to the growing tip to support polar growth of the cell. Searches of the U. maydis genome sequence revealed the presence of at least 14 such motors (Basse and Steinberg 2004). A PCR-based approach was used to identify the dyn1 and dyn2 genes (Straube et al. 2001) encoding the two components of the dynein heavy chain, a minus-end-directed MT motor (reviewed in Xiang and Plamann 2003). The dyn1 gene encodes the predicted site of ATP hydrolysis while dyn2 encodes the putative MT binding site. Interaction
between Dyn1 and Dyn2 was confirmed by coprecipitation from growing budding cells and colocalization was confirmed by fluorescence microscopic analyses of Dyn1 and Dyn2 proteins. Following a shift of conditional mutants to restrictive conditions whereby dyn1 and dyn2 expression was repressed, cells with two or more nuclei began to appear. Additionally, under these conditions, the mutants exhibited more and longer MTs, suggesting a function of dynein in MT dynamics. These studies indicated Dyn1 and Dyn2 control nuclear migration to the neck region of budding cells where the minus ends of MTs are localized in polar MT organizing centers (Straube et al. 2001).

Other motors essential for appropriate control of morphogenesis have been identified in *U. maydis*. Lehmler et al. (1997) identified the kin2 gene encoding the heavy chain of kinesin, a plus-end-directed MT motor important for pathogenicity (Lehmler et al. 1997; Steinberg et al. 1998) and discussed later. kin3 was identified by a PCR-based approach and encodes a member of the UNC-104/KIF1 family, consisting of an N-terminal kinesin motor domain, a forkhead-associated domain, and a C-terminal pleckstrin homology (PH) domain (Wedlich-Soldner et al. 2002 b). The kin3 mutant cells form tree-like aggregates due to a cell separation defect and a defect in the bipolar budding pattern. These morphological defects most likely arise from the disruption of bidirectional transport of early endosomes, evident from the finding that unlike wild-type cells, kin3 mutants do not exhibit endosomal clustering at the septa and distal cell pole. Kin3-GFP was localized to endosomes, organelles likely involved in the delivery of components for cell growth, and its movement was associated with MTs in a plus-end-directed manner. Further analysis of a dyn2 temperature-sensitive mutant in a kin3 mutant background revealed a role of dynein in minus-end-directed movement of endosomes. Thus, a balance of between Kin3 and dynein activity organizes polar endosomes in opposing directions during budding (Wedlich-Soldner et al. 2002 b). This is in agreement with previous studies of the *U. maydis* yup1 gene, encoding a target soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (t-SNARE) (Wedlich-Soldner et al. 2000). In this study, a Yup1-GFP fusion protein was localized at vesicles, revealing rapid bidirectional motion. At nonpermissive temperature, yup1ts mutants do not accumulate Yup1 carrying vesicles at cell poles like that observed in wild type, resulting in an abnormal distribution of wall components and striking morphological defects characterized by elongated multicellular structures with multiple growth sites. Taken together, these studies indicate a recycling of membranes via Yup1-mediated endo- and exocytosis and that this action, driven by dynein and Kin3, contributes to polar growth (Wedlich-Soldner et al. 2000; Wedlich-Soldner et al. 2002 b).

To examine the overall pattern of MT organization during the cell cycle of *U. maydis*, Straube et al. (2003) used fluorescent protein variants fused to MT plus-end-binding (Peb1) and α-tubulin (Tub1) proteins and minus-end-SPB-specific γ-tubulin (Tub2) from *U. maydis*. Fluorescence microscopic analysis of these fusion proteins at various stages of the cell cycle revealed that MT bundles containing antipolar-oriented MTs span the length of the unbudding cells of G1 and S phase and those with small buds in G2. When the daughter cell reaches about 15 percent of the length of the mother cell, MTs are
nucleated and anchored at the bud neck. This nucleation occurs at a polar microtubule organizing center (MTOC) so that polarization occurs and most MT plus ends grow away from the bud neck in both mother and daughter cells in G2 (Straube et al. 2003). Given this arrangement of MTs in G2, Garcia-Muse et al. (2003) suggested that G2 phase may be especially suited to serve as a decision point for a polar morphogenic response because the polar MT arrangement of G2 could support conjugation tube formation. Disruption of MTs with benomyl and following their regrowth revealed there are multiple dispersed MT nucleating centers in unbudded cells of G1 and S, indicating the spindle pole body (SPB) is inactive during interphase. In contrast, multiple MT nucleation sites were localized near the neck region of budding cells. At the onset of mitosis, the cytoplasmic network of MTs disassembles and SPBs nucleate MTs, forming the spindle and asters (Steinberg et al. 2001; Straube et al. 2003; Bannuett and Hirskowitz 2002). Following mitosis and septum formation, the MTs are again elongated toward both poles of each cell. This work also clearly illustrated that MTOCs organize MTs during budding and thus MTs do not reach the growth region by stochastic chance, evident from the finding that lateral budding mutants contain MTs that bend out of the growing bud and emanate from paired tubulin structures. Overall, this work indicates that MT nucleation in \textit{U. maydis} occurs away from the nucleus at an MTOC near the bud neck (Straube et al. 2003). In many fungi, including \textit{S. cerevisiae}, MTs are nucleated at the SPB (reviewed in Xiang and Pliamann 2003; Steinberg and Fuchs 2004). In contrast, in the fission yeast \textit{S. pombe} and in \textit{U. maydis}, the SPB is inactive during interphase and MT nucleation begins at the onset of mitosis where MTs are important for chromosomal segregation. In support of this idea, overexpression of the \textit{U. maydis} B-cyclin Clb1 resulted in hypersensitivity to benomyl and FACS analysis revealed that these cells had unusual DNA contents less than 1C or greater than 2C, suggesting that interference with microtubule assembly may be responsible for alterations in chromosomal segregation (Garcia-Muse et al. 2004).

Calcium homeostasis plays a role in modulating morphogenesis in fungi and other eukaryotes. \textit{U. maydis} is no exception as indicated by the analysis of \textit{ucn1} and \textit{upa2} mutants, which encode the catalytic subunit (protein phosphatase 2B) of the Ca$^{2+}$-dependent holoenzyme encoding and the calcineurin catalytic subunit (protein phosphatase 2A), respectively (Egan and Gold, unpublished). The \textit{U. maydis} \textit{ucn1} gene encodes a calcineurin phosphatase. Mutation of \textit{ucn1} leads to a dramatic phenotype characterized by large clusters of cells that exhibit a multiple budding pattern (Egan and Gold unpublished) similar to the \textit{eca1} mutants (Adamikova et al. 2004), originally identified by the complementation of a temperature sensitive mutant. The gene \textit{eca1} encodes a protein sharing highest identity with endoplasmic reticulum (ER)-resident Ca$^{2+}$ ATPases (SERCA). Analysis of \textit{eca1} deletion mutants revealed a temperature-dependent morphological defect. At 30$^\circ$ C, \textit{eca1} mutant cells exhibited growth at both poles and multiple septa were formed while a switch to 22$^\circ$ C restored wild type growth. Low external concentrations of Ca$^{2+}$ suppressed the \textit{eca1} mutant phenotype at 30$^\circ$ C while high Ca$^{2+}$ levels increased morphological defects at 22$^\circ$ C. As an underlying basis for this morphological defect, \textit{eca1} mutants displayed longer and highly
disordered MTs at 30°C as opposed to 22°C. Further studies revealed that the calcineurin phosphatase activity was not inhibited in eca1 mutant cells. But the altered Ca²⁺ homeostasis in eca1 mutants caused increases in Ca²⁺/calmodulin dependent kinase (CamK) activity. This increased activity was proposed to deregulate MT dynamics, which underly defects in morphology in eca1 mutants. Consistent with this notion, suppression of CamK activity had a dramatic effect, restoring wild type budding morphology and MT organization. Similarity was observed in the phenotypes of eca1 and dynein mutants. Moreover, eca1/dynein double mutants do not exhibit an increased mutant phenotype, suggesting both Eca1 and dynein participate in the same pathway. Dynein was previously shown to modulate motility of the peripheral tubular ER network in *U. maydis* (Wedlich-Soldner 2002 a) and eca1 mutants are defective in this organellar motility (Adamikova et al. 2004).

In addition to MTs, studies have indicated a role of F-actin in polar growth of fungi (reviewed in Xiang and Plamann 2003; Steinberg and Fuchs 2004). In *U. maydis*, studies of Bannett and Hirskowitz (2002) revealed the presence of actin patches that are concentrated at sites of bud emergence, the small bud, and the bud tip in large buds. Overall, this resembles the localization pattern of actin patches observed in yeasts at sites of polarized growth and secretion (reviewed in Xiang and Plamann 2003). Weber et al. (2003) also noted actin patches at sites of apical growth in *U. maydis*. The *U. maydis* actin cytoskeleton also consists of fine and thick cables (Bannett and Hirskowitz 2002). Some of these cables may cooperate with a newly identified *U. maydis* class-V myosin (Myo5) motor involved in actin transport of vesicles and organelles to actively growing regions of the buds and filaments (Weber et al. 2003). Deletion of *myo5* results in a phenotype characterized by thicker cells that fail to separate, forming cellular aggregates that are divided by septa and retain growth polarity. Localization studies revealed that a GFP-Myo5 fusion protein accumulates in a polar manner near the bud tip. This localization was disrupted by addition of the F-actin inhibitor lantrunculin A, indicating a dependency on F-actin. Further studies indicated that temperature sensitive *myo5* mutants exhibit reduced dikaryon formation likely attributable to impaired pheromone perception and conjugation tube formation. As a result, *myo5* mutants were also reduced in mating and pathogenicity (Weber et al. 2003). While MT and actin-based transport must function cooperatively in morphological transitions in *U. maydis*, the level of this cooperativity is presently unknown (Basse and Steinberg 2004).

### 3.1.6 Differential gene expression

Due to its central role in regulating morphogenesis and pathogenicity and its postulated function as a transcriptional regulator of gene expression, considerable effort has been made to identify genes whose expression is regulated by the bW/bE heterodimer. This follows from the hypothesis that these downstream genes could play important roles in morphogenesis and pathogenicity. Several studies identified a number of genes either up- or downregulated upon dikaryon formation; *eg11* encoding an endoglucanase (Schauwecker et al. 1995) and *rep1* and *hum1*, coding for a repellent and a hydrophobin, respectively (Wosten et al. 1996) were all identified as upregulated
in dikaryotic cells. \textit{lga2}, a gene of unknown function present at the \textit{a2} locus, is also strongly upregulated in the presence of an active \textit{bE/bW} heterodimer (Urban et al. 1996a). However, deletion of these genes did not affect dikaryon formation/stability and/or pathogenicity. Among the genes known to be repressed in the presence of an active \textit{bW/bE} heterodimer are the pheromone and pheromone receptor genes (Urban et al. 1996a). Recently, higher throughput differential screening methods have been applied to identify genes whose expression is controlled by the \textit{b} heterodimer. A general limitation to identify genes differentially expressed in budding versus dikaryotic cells is the lack of synchronization of the mating process and the fact that only a small fraction of cells fuse when two compatible strain are crossed \textit{in vitro} (Urban et al. 1996a). To overcome this problem, engineered haploid strains were constructed in which a functional \textit{bW/bE} heterodimer can be induced in the appropriate carbon or nitrogen sources (Brachmann et al. 2001). Using these strains and an RNA fingerprint method, Brachmann et al. (2001) identified 10 new \textit{b} regulated genes; five \textit{b}-induced and five \textit{b}-repressed. In a follow up study using the same approach, Brachmann et al. (2003) identified the \textit{kpp6} gene, which encodes a MAP kinase required for efficient plant penetration. Among all the genes reported as \textit{b}-regulated so far, \textit{kpp6} is the only one shown to be crucial for pathogenicity.

To characterize binding sequences for the \textit{b} heterodimer, binding \textit{in vitro} of a synthetic \textit{b} heterodimer to the putative promoter region of \textit{lga2} was investigated and a \textit{b}-protein binding sequence, termed \textit{bbs1}, was identified (Romeis et al. 2000). This work furnished the first direct evidence that the \textit{bW/bE} heterodimer can function as a transcriptional activator. A similar sequence (identical for 16 out of 23 nucleotides), present in the putative promoter region of the \textit{b}-induced gene \textit{frb52}, is bound by an active \textit{bW/bE} heterodimer \textit{in vitro} (Brachmann et al. 2001). However, these or similar sequences are absent in some of the \textit{b}-regulated genes identified. It may be possible that sequences very divergent from the ones identified so far are also targets for the \textit{b} heterodimer. However, a more plausible explanation is that some of the genes whose expression is highly induced upon dikaryon formation are only indirectly regulated by the \textit{bW/bE} heterodimer through the action of other transcription factors in the \textit{b}-regulatory cascade. Some indirect regulation is likely to go through the transcription factor \textit{Prf1}. For instance, \textit{kpp6} contains no potential binding sites for \textit{bW/bE}; however, two putative binding sites for \textit{Prf1} (PREs) are found upstream of the start codon.

Several studies have identified downstream components of the \textit{b}-dependent regulatory cascade. As explained above, \textit{egi1} is specifically expressed in the dikaryon, identification of mutants that bypass the requirement of an active \textit{b} heterodimer for \textit{egi1} expression in haploid cells and complementation of such mutants led to the identification of two genes encoding putative repressors of \textit{b}-regulated genes, \textit{rum1} and \textit{hda1} (Quadbeck-Seeger et al. 2000; Reichmann et al. 2002). Deletion of either gene resulted in expression in haploid cells of several genes known to be \textit{b}-regulated as well as induction of the \textit{bE} and \textit{bW} genes themselves. These mutations also had an effect on disease development leading to arrest of teliospore formation after karyogamy and therefore producing galls lacking mature teliospores. It has been hypothesized that
Hda1 functions in a complex with Rum1. However, detailed microscopic observations show that the block in teliospore development appears to occur earlier in hda1 mutants than in rum1 mutants. This, together with the fact that repression of plant induced genes such as mig1 and ssp1 in haploid cells is relieved in a hda1 but not in a rum1 mutant background (Huber et al. 2002; Torreblanca et al. 2003) suggest a Rum1-independent function for Hda1. These studies show that temporal or spatial misexpression of a set of genes prevents the completion of the disease cycle, illustrating how tight regulation of gene expression is critical for disease development.

Another major regulator of morphogenesis and pathogenicity in U. maydis is the cAMP signaling pathway. Two recent papers from our laboratory have specifically focused on the identification of genes either up- or downregulated in filamentation induced by disruption of cAMP production (Andrews et al. 2004; Garcia-Pedrajas and Gold 2004). For these screenings, subtractive cDNA libraries were constructed in which the two growth conditions compared were budding haploid wild type cells and the constitutively filamentous uac1 mutant. These studies led to the identification of 26 genes upregulated in filamentous growth and 37 downregulated in filamentous growth, the vast majority of which have not previously been reported in U. maydis. Interestingly, rep1, encoding a repellent protein and identified as highly induced in b-dependent filamentous growth was repeatedly encountered in our screening for genes upregulated in the constitutively filamentous uac1 mutant (Andrews et al. 2004). Similarly, in screening for genes downregulated in the filamentous uac1 mutant strain frb124, previously identified as b-repressed gene (Brachmann et al. 2001) was found. This suggests that these genes are specific for particular growth forms, budding or filamentous, and that there is some overlap in gene expression in filamentation induced by either a low level of cAMP or an active b-heterodimer. Deletion of two genes upregulated in filamentous growth; uor1 encoding a putative member of the aldo-keto reductase family and ufu1 with no similarity to known genes, produced no detectable mutant phenotype for morphology, mating or pathogenicity (Andrews et al. 2004). Among the genes downregulated in the filamentous uac1 mutant an interesting case is ump2 with similarity to ammonium transporters. Although highly expressed in budding cells and repressed in filaments its deletion impairs filamentous growth in response to low nitrogen (Smith et al. 2003). Interestingly, several genes with putative roles in extracellular matrix formation and adhesion were identified as downregulated in the uac1 filamentous mutant (Garcia-Pedrajas and Gold, 2004). These genes could play a role on adhesion to plant surfaces prior mating. Deletion of one of these genes, which exhibit high similarity to UDP-glucose dehydrogenase, in a wild type haploid background, resulted in loss of adhesion properties in vitro (Garcia-Pedrajas and Gold, unpublished).

A topic that has received considerable attention due to its implication in development during parasitic growth is the study of U. maydis genes whose expression is induced during growth within plant tissue. The identification and characterization of these genes will be discussed in section 3.1.7. Although not directly aimed to screen for differentially expressed genes, production of EST libraries from various developmental
stages is also useful in the identification of genes differentially expressed in various developmental conditions. Two such libraries have been produced and analyzed recently; one containing ESTs from a diploid strain (Nugent et al. 2004) and another containing ESTs from genes expressed in germinating teliospores (Sacadura and Saville 2003). The identification and deletion of genes whose transcription appears to be regulated by pathways playing critical roles in morphogenesis and pathogenicity has made it obvious that not all differentially expressed genes play detectable roles in these processes. Indeed, to date, for most identified differentially expressed genes, deletions have not produced any discernable mutant phenotype. This could be the result of functional redundancy; alternatively, these genes may play roles dispensable for pathogenicity or morphological transitions.

3.1.7 Making a home for one’s self

*U. maydis* is fully dependent on the plant to complete its life cycle. After mating the dikaryon can only develop parasitically. An approximate time frame of plant colonization in laboratory inoculations has been established. The early stage of colonization after penetration is characterized by rapid growth of the fungal tip leaving behind compartments devoid of cytoplasm that are sealed off and collapse. It is interesting to note that disease symptoms such as chlorosis and anthocyanin production are not uncommon at this stage, sometimes observed well in advance of the colonizing hyphae (Callow and Ling 1973) suggesting release of toxins and/or degradative enzymes by the fungus. Three or 4 days post infection dikaryotic hyphae start branching and are filled with cytoplasm. This change in growth mode coincides with the beginning of tumor development, which is induced approximately 5 days after inoculation. Branch primordia that resemble the clamp connections of other basidiomycetes are observed. However these structures do not appear to play the role of true clamp connections in maintaining the dikaryotic stage; nuclear migration into these structures has not been observed and they do not fuse with adjacent cells. As the fungus proliferates within the plant tumor it branches profusely with the formation of increasingly shorter branches. These changes appear to signal the switch from vegetative to sporogeneous hyphae. About nine days after inoculation hyphae are embedded in a mucilaginous material, presumably derived from hyphal walls, and tend to stick together. The tip of the hyphae became lobed followed by hyphal fragmentation into segments of one to several cells. Karyogamy probably takes placed at this stage, followed by rounding of individual cells and deposition of ornamented secondary cell walls (Snetselaar and Mims 1992; Snetselaar and Mims 1993; Snetselaar and Mims 1994; Banuett and Herskowitz 1994). Little is known about how *U. maydis* acquires nutrients during this biotrophic development inside the host. Intracellular structures somewhat resembling haustoria described in rust fungi have been observed (Luttrell 1987; Snetselaar and Mims 1994). However, these structures do not show a clear demarcation of an interface analogous to the one present in true haustoria; they are not consistently observed and when present are very irregularly branched. Conceivably, these irregular structures may correspond to the multilobed sporogenous
hyphae that give rise to spores (Banuett and Herskowitz 1996) rather than to structures formed to obtain nutrients. Although the identification and characterization of the genetic components governing the switch from saprophytic to pathogenic development have been major research topics in *U. maydis*, characterization of the genetic programs acting during fungal growth within plant tissue is still at an early stage. Questions such as how the fungus perforates cell walls to travel within plant tissue, what signals triggers the switch between the various morphological stages observed during colonization, or how the fungus acquires nutrients during the growth *in planta*, remain unanswered.

Characterization of mutant strains affected in cAMP signaling has made it obvious that this regulatory pathway plays a critical role during penetration, induction of galls and teliosporogenesis. An active cAMP pathway is required for formation of infection structures and penetration. Thus, mutants with low PKA activity as a result of inactivation of genes in this pathway do not produce any symptoms in inoculated plants. Mutant strains lacking adenylate cyclase activity (*uac1*), the catalytic subunit of PKA (*adr1*), or the α subunit of the G-protein Gpa3 (*gpa3*) are all non-pathogenic (Barret et al. 1993; Gold et al. 1994; Regenfelder et al. 1997; Durrenberger et al. 2001). On the other hand, mutations leading to situations mimicking cAMP level above normal do not affect the early stages of infection but have a profound effect on gall formation and teliosporogenesis. In this sense, mutant strains with constitutive PKA activity achieved by disruption of *ubc1*, encoding the regulatory subunit of PKA, are able to infect and colonize plant tissue but they do not induce gall formation (Gold et al. 1997). Even lower levels of activation of cAMP pathway obtained in mutants with a constitutively active *gpa3* allele result in alteration of gall morphology. Tumors induced by these mutant strains show very reduced fungal proliferation and lack teliospores (Kruger et al. 2000). Taken together these results suggest that the necessary level of cAMP and PKA activity for initial colonization of plant tissue is relatively high, while a decreased cAMP concentration and PKA activity is necessary for induction and normal gall morphology. Few targets for PKA phosphorylation have been identified in *U. maydis*. Interestingly the *hgl1* gene encoding one such putative target was found to be important for teliospore maturation (Durrenberger et al. 2001). Inoculation of plants with *hgl1* mutant strains induced galls but they lacked darkly pigmented teliospores.

Identification of a gene encoding the heavy chain of conventional kinesin, *kin2*, and analysis of *kin2* mutant strains provided valuable information about the putative mechanism behind the mode of growth of the dikaryon in the initial steps of colonization. In both, dikaryons at early stage of plant colonization and in those formed *in vitro* a rapid growth with all the cytoplasm migrating to the hyphal tip leaving behind empty collapsed cells is observed. In contrast with dykaryons formed by wild type compatible strains, Lehmler et al. (1997) observed that in dikaryons formed by mutant *kin2* strains, hyphal structures remained short and filled with cytoplasm. Further investigation of dikaryotic hyphae formed by *kin2* mutants showed that they lack the large basal vacuole present in wild-type dikaryons and that instead they contain more 200-400 nm vesicles scattered within the hyphae (Steinberg et al. 1998).
These results strongly suggest that Kin2 is involved in vacuole formation and that the accumulations of these vacuoles at the basal end of the tip play a critical role in supporting cytoplasmic migration. Kin2 mutant strains are severely reduced in pathogenicity indicating that vacuolization plays an important role during normal dikaryon development in planta.

Another line of research likely to generate useful data to understand parasitic growth in *U. maydis* is the identification of plant upregulated genes. Among the genes identified as highly induced during fungal growth within plant tissue are mig1 and the mig2 gene cluster, all coding for secreted proteins (Basse et al. 2000; Basse et al. 2002). The mig genes have a number of features reminiscent of avirulence genes such as secretion, plant-inducible expression and an even number of cysteines presumably indicating the likely importance of desulphide bonds. However, they do not appear to play a critical role during pathogenic development since their deletion does not produce a discernible mutant phenotype. This makes it difficult to discern the roles of mig genes during pathogenic development. Recently, an approach that combines REMI (restriction enzyme mediated integration) mutagenesis with enhancer trapping by using green fluorescent protein as a reporter for *in planta* detection identified a new set of *in planta*-induced genes, the pig genes (Aichinger et al. 2003). As previously found for the mig genes, deletion of pig genes did not have an effect on virulence. The same is true for ssp1, a gene with similarity to dioxygenases identified as highly induced in mature teliospores (Huber et al. 2002). Again it has been difficult to determine the role of these genes in the biotrophic growth of *U. maydis* because deletions of these did not have an effect on pathogenicity. The search for genes upregulated in the plant continues, preliminary microarray analysis data indicate that more than 500 genes are plant-regulated (Kahmann and Kamper, 2004).

3.1.8 Lessons from the genome

The *Ustilago maydis* genome sequence was made publicly accessible in June, 2003 (http://www.broad.mit.edu/annotation/fungi/ustilago_maydis/). The genomic data is of very high quality and although the genome is not "finished" it is an outstanding tool for researchers of this fungus as well as the mycological research community at large. The genome data is primarily from a 10X shotgun assembly produced at the Broad Institute (previously the Whitehead). There is also data contributed from two private sector sequencing efforts, one from Bayer CropScience and the other from Excelixis, Inc. 15,389 ESTs have been generated primarily through the Bayer sequencing project and the efforts of Dr. Barry Saville at the University of Toronto. These ESTs are critical for the validation of the gene annotation. The genome is currently predicted to contain 6,522 coding genes. Most genes have no introns and where present introns are predicted to be relatively short with the bulk being less that 200 bp. Codon bias is quite apparent with nearly 6-fold differences in wobble base usage for several amino acids. A manual annotation project is currently underway at the Munich Information Center for Protein Sequences (MIPS) available at (http://mips.gsf.de/genre/proj/ustilago/). This effort was funded primarily through the efforts of Drs. R. Kahmann and J. Kamper. As
of November, 2004, 3.8 of the 20 Mb genome has been manually annotated at mips. The MIPS site offers a number of analytical tools for navigating the genomic data.

Functional genomics efforts are proposed and in some cases underway. Affymetrix microarrays have been generated based on the earlier Bayer CropScience sequencing efforts and are being employed to observe gene transcription under a number of specific conditions (Kahmann and Kamper 2004). Unfortunately, arrays are not currently available to the general research community. Construction of a publicly deposited gene deletion set has been discussed by the U. maydis research community but the logistics of this process and necessary funding have not yet been solidified. The International Ustilago maydis Research Conferences organized by Drs. F. Banuett and R. Kahmann are greatly helping in the planning and coordination of utilization of the genomic information.

Overall the availability of the genome is a permanent resource available to the scientific community at large and is of outstanding value to those of us that primarily work on this extraordinary fungus.

3.2 RUSTS
3.2.1 Introduction

Rust fungi belong to the order Uredinales, estimated to include from 4,000 to 6,000 species, belonging to 140-150 genera (Alexopolous et al. 1996; Hahn, 2000). Several well-known genera are Puccinia, Uromyces, Gymnosporangium and Cronartium. The divergence of the rust lineage from related basidiomycetes is estimated to have occurred 310 M years ago (Berbee and Taylor 1993). Savile and Baum (Savile 1976; Baum and Savile 1985; Savile 1990) hypothesized that rusts were very early parasites on early vascular plants and therefore have had a long time to co-evolve with their hosts resulting in a very intimate life style. The various rusts attack a wide variety of unrelated mono- and dicot plants. Many are economically important world-wide, such as the cereal, bean, pine, coffee, carnation and peanut rusts (Agrios 1997; Staples 2000). In particular Puccinia spp., infecting mainly monocots (as do most smuts), have been known since Biblical times because of the devastation they cause due to expanding cultivation of grains. Several rusts have been studied extensively over the last 100 years, such as cereal rusts Puccinia tritici (formerly, P. recondita) causing leaf or brown rust on wheat and rye, P. hordei on barley and P. sorghi on corn; stem rusts P. graminis f. sp tritici, avenae, or secalis on their respective hosts; P. striiformis causing stripe or yellow rusts of wheat, barley and rye; and P. coronata f. sp. avenae causing oat crown rust (Johnson et al. 1967; Bushnell and Roelfs 1984; Roelfs and Bushnell 1985; Kolmer 1996) flax rust, Melampsora lini (Agrios 1997; Ellis et al. 1997) bean rusts, Uromyces phaseoli/appendiculatus (Stavely 1984; Stavely et al. 1989); cowpea rust, U. vignae (Heath and Heath 1971); soybean rust, Phakopsora pachyrhizi (Bonde et al. 1976; Kuchler et al. 1984); cedar-apple rusts, Gymnosporangium spp. (Mims and Richardson 1989) and pine rusts, (Endo)cronartium spp (Hirt 1964; Hiratsuka et al. 1991).

Rusts generally cannot be cultured or with difficulty (Bose 1974; Williams 1984; Boasson and Shaw 1988; Fasters et al. 1993) although transient transformation of some
rusts has been achieved by biolistic methods (Bhairi and Staples 1992; Li et al. 1993; Schillberg et al. 2000) or microinjection (Barja et al. 1998). Attempts using Agrobacterium, commonly used for the genetic transformation of many fungi, have not yet been reported. General lack of such tools has been a major bottleneck in advancing the field, making common molecular genetic techniques to create targeted gene deletions or mutations or to clone genes by complementation currently impractical. In addition, even though some natural, easy-to-score color mutations have been described, no characterized rust mutants are available at this time. Within populations, changes in virulence revealed through extensive testing against large collections of host differential cultivars, have been noted many times in many rust species and it is assumed that these occur via mutation of specific ("avirulence") genes. This is thought to occur more readily in sexual populations (Kolmer 1992) but somatic mutations are likely frequent in asexual populations such as from wheat leaf rust (Samborski 1985). Although tedious, genetic analysis by (back-) crossing has been achieved in certain rust species. In this manner, the presence of (dominant) avirulence genes and their interactions with cognate host resistance genes ("gene-for-gene" interaction (Flor 1971; Samborski 1985) and species-specific elicitors (Chen and Heath 1993) has been validated. Crosses have also been used to construct genetic maps (Zambino et al. 2000; Dodds et al. 2004). It should be possible to mutagenize rust populations by other means but characterizing (morphological or metabolic) mutants in these obligate biotrophs will be very difficult. Despite the challenges in research on the rusts, there is hope and recent developments exploiting more random genomics approaches, seem to herald a breakthrough and are reviewed here. There are several excellent recent overviews on rust research (Staples 2000; Heath 2002; Hahn 2000).

3.2.2 Life cycles and spore stages

The rusts have the most complicated life cycles in the fungal kingdom and can include up to five stages and five different spore types produced on two unrelated plant hosts for the heteroeocious, macrocyclic forms (Figure 3). According to Savile (1976) this was the result of different adaptations throughout evolution caused by environmental stresses; the aecial spore stage was the final spore state to develop and arose as a result of the adaptation of heteroeicism (host-alternation), which itself was stimulated by climatic stress. Increased or diminished selection pressures caused by changing environmental climatic conditions also gave rise to less complex cyclic forms from which various spore stages and/or one of the hosts have been lost. This led to so-called demi- and micro-cyclic and autoecious rusts. It is thought that the sexual cycle is ancestral because it is more adaptable (Savile 1976) but some species are very successful by mainly relying on their asexual urediniospores (epidemic cycle), such as some cereal rusts that seem to survive in Mexico and travel each year north to Canada on the so-called 'Puccinia path', following the cereal growing season (Nagarajan and Singh 1990). It is easy to see how some species can occupy a niche successfully without the need for a sexual cycle, ultimately losing that dependency altogether or because it plays such a minor role that it has not been discovered. The cereal yellow rust, P.
striiforms, has no (known) sexual cycle and asexual populations have been described for
M. lini (Burdon and Roberts 1995) and P. triticina (Liu and Kolmer 1998).

Very extensive cell biological, light- and ultrastructural microscopic work has been
done on the many morphogenic stages, in particular of several cereal rusts and the bean

![Diagram of the life cycle of Puccinia graminis, wheat stem rust.]

**Fig. 3.** Life cycle of *Puccinia graminis*, wheat stem rust. Example of a macrocyclic (full-cycled), heteroecious rust. See text for description of stages. (Adapted from Alexopoulos et al. 1996)

and cowpea rust systems (Littlefield and Heath 1979; Gold and Littlefield 1979; Gold
and Mendgen 1984; Mendgen 1984; Harder 1984; Harder and Chong 1991; Hu and
Rijkenberg 1998 a; Hu and Rijkenberg 1998 b). Their life cycles have been described in
great detail. However, when comparing these rusts to the other model organisms
described in this chapter, no obvious sudden cell morphological switch from budding
to filamentous growth, such as described for the smuts when changing from
monokaryon to dikaryon after mating, or other morphologic mutants are apparent.

### 3.2.2.1 Stage 0 & I: pycniospores, sex and aeciospores

Interactions on and with hosts that produce pycnio- and aeciospores (in many
systems the alternate host) have been studied to a lesser degree even though these often
include the sexual stage (Harder 1984). Several studies have attempted to shed light on
the mating-type system in the rusts. Conclusions and speculations vary from rusts
having a simple bipolar system in several *Puccinia* and *Uromyces* species (Anikster et al.
1999) to a more complicated tetra-polar system with multiple allelic specificities in *M.*
lini (Lawrence 1980) and P. coronata (Narisawa 1994) similar to that in S. commune (see section 2.3). The mating system in Cronartium species seems to promote outcrossing (Yamazaki and Katsuya 1988; Gitzendanner et al. 1996). In the smuts, both bi- and tetrapolar systems exist, depending on the genetic arrangement and linkage of the two a and b gene complexes (see section 3.1.3). Rust pycnia consist of haploid, monokaryotic cells and produce haploid pycniospores of only one mating type embedded in a sugar-containing solution, called nectar. When transferred (by insects) to a pycnium of a different (opposite) mating type, among the very early mating events is the induction after 10 minutes of a dark-staining, proteinaceous polar cap on these pycniospores by a factor of a (glyco)-protanoeus nature found in the nectar of several Puccinia and Uromyces species. Among the many pairings of pycniospores, spore-free nectar and pycnia performed, somewhat less than 50% produced caps and aecia suggesting this factor was mating type-specific and eluded to the existence of a simple bipolar mating system (Anikster et al. 1999). However, hyphal confrontation-fusion assays of pure basidiospore cultures and microscopic observation of the production of dikaryons, suggested the existence of a tetra-polar system with multiple alleles for a similar rust, P. coronata (Narisawa 1994). It is conceivable that different rusts have different systems. More research is necessary and it will be very interesting to unravel the molecular basis of these mating systems to see whether they also harbor similar gene complexes in various arrangements as in the smuts.

After fertilization, that is, the fusion of one pycniospore to a receptive hypha in the pycnium of a different mating type and nuclear transfer, the newly formed dikaryon undergoes developmental reprogramming. Mycelium traverses the leaf and often forms aecia on the underside (Harder 1984). Aeciospores are dispersal structures and for heteroecious rusts need to land on the primary host.

3.2.2.2 Stage II: urediniospores and the “increase or epidemic” cycle

Rust pustules, ruptured through host surfaces, produce enormous numbers of urediniospores and generate self-inhibitors preventing their premature germination. In M. lini, the production of such self-inhibitors seems to be under the control of a recessively inherited, single gene trait (Ayliffe et al. 1997). Volatile chemicals have been described that can stimulate the germination of uredinio- and teliospores (Macko 1981; French 1992; French et al. 1993). Hydrophobic interactions contribute to the attachment of spores and sporelings to host surfaces (Clement et al. 1994) and glycoproteins and β-1,3-glucans are apparently also involved in adhesion (Epstein et al. 1987; Chaubal et al. 1991). Upon hydration, spores of U. viciae-faba seem to exude from pre-made surface components and actively secrete an adhesive substance; it is unclear whether recognition of the correct host, that is, chemical sensing of host components (possibly produced as a reaction to these fungal products) is involved. Cellulolytic enzymes, e.g., (endo-)cellulose, have been found on dormant spores (Heiler et al. 1993). Spores produce serine esterases, one of which is a cutinase (Deising et al. 1992; Clement et al. 1997). In Uromyces germlings, recognition and the mediation of extracellular signals occurs via transmembrane glycoproteins known as integrins, found in extracellular
matrix components and often exhibiting specific affinities to the tripeptide sequence Arg-Gly-Asp (RGD); competing synthetic RGD peptides inhibit the thigmostimulated cell differentiation (Correa et al. 1996).

Urediniospores of most rust fungi germinate on the host, form infection hyphae, which grow over the surface until they encounter a stomatal lip, triggering the formation of an appressorium. Extensive research has described the requirement of leaf topographical features and involvement of K+ and Ca2+ signaling, pH and host compounds such as sucrose to initiate this process (reviewed in Staples 2000, and see below). Presumably, perceived changes in or active reorientation of the microtubules and actin filaments making up the cytoskeleton, induce differentiation and dictate changes in cell morphology. However, appressoria and subsequently differentiated morphological structures during the infection process, such as the substomatal vesicle, infection hyphae and even haustorial mother cells can also be induced in vitro on artificial substrates, and by heat shock and/or chemicals; this might not represent the exact same differentiation process as that in planta because different signal transduction pathways and genes seem involved (Macko et al. 1978; Wanner et al. 1985; Hoch et al. 1986; Heath and Perumalla 1988; Bhai et al. 1990; Wietholter et al. 2003).

Once an appressorium has formed over a stoma, an infection peg forms basaly, which gains entry into the underlying cavity where a subsequent substomatal vesicle is produced (reviewed in Mendgen et al. 1996). Several fungal-produced enzymes have been implicated in the infection process such as superoxide dismutase, a catalase, a peroxidase and other, cell wall degrading enzyme classes such as extracellular metalloprotease, cellulase, xylanase, pectin methylesterase and polygalacturonate lyase. Some of these are linked to the fungal developmental program, expressed after appressorium formation and their expression gradually increases upon penetration of host cells by the haustorial mother cell (hmc); for reviews on this, see (Deising et al. 1995; Heiler et al. 1993; Lamboy et al. 1995; Rauscher et al. 1995; Xu and Mendgen 1997).

Once the vesicle is produced, new infection hyphae are protruding which when encountering mesophyll cells, differentiate into the haustorial mother cells. Here the first real contact is made with the host upon actual penetration of a mesophyl cell to produce an intracellular (D-, for dikaryotic) haustorium. Importantly, this leaves the host cell membrane intact and produces a seal, the neckband and a highly specific extra-haustorial membrane (Heath 1972; Harder and Chong 1991; Mendgen et al. 1996; Mendgen et al. 2000). It is often at this stage when incompatible interactions are visible and the Hypersensitive Response (HR) is activated, either due to avirulence gene products or nonhost combinations. However, recent studies indicate that host and nonhost responses are distinct and can occur as soon as spores land on leaf surfaces. Overall, differences between compatible and incompatible host and nonhost interactions have been described extensively and they involve host nuclear behavior, protoplast/vacuolar streaming, cell wall modifications and appositions, fungal encasement etc. (Niks and Dekens 1991; Heath and Skalamera 1997; Heath 1997; Munch-Garthoff et al. 1997; Hu and Rijkenberg 1998 a; Hu and Rijkenberg 1998 b; Mould and Heath 1999; Heath 2002; Christopher Kozjan and Heath 2003; Neu et al.
2003). These studies typically involve cytological descriptions sometimes using highly specific antibodies and including some pharmacological data but molecular data are mostly limited to host response genes. The study of the role of fungal genes during these interactions is in its infancy (see molecular interactions section 3.2.3).

Not all rust urediniospores or aeciospores for that matter gain entry through stomatal openings; for example, those from the soybean pathogen *Phakopsora pachyrhizi* penetrate host epidermis cells directly (Bonde et al. 1976). In the latter case, mesophyll cells are invaded and haustoria formed, similarly to the process described above (Littlefield and Heath 1979).

### 3.2.2.3 Stage III: telial stage

Teliospores are produced in the uredium, often but not always under adverse conditions (e.g. upon draught or host senescence). Upon production of teliospores the uredium is by definition converted into a telial sorus and can be open or covered by the host epidermis. Teliospore ontogeny is not very different from that of the urediniospores but they subsequently differentiate into more condensed cells with thicker walls and in which vacuoles are absent, lipid droplets and glycogen-like material is present and the two nuclei pair if not fuse. Rust teliospores may be one- to five-celled and the germination of teliospores to produce the basidium may require dormancy periods of various lengths, depending on the species (Harder 1984; Mendgen 1984).

### 3.2.2.4 Stage IV: basidiospores

When formed on the basidium, mature basidiospores typically contain two haploid, homokaryotic nuclei (Gold and Mendgen 1991) and can be forcefully ejected and dispersed by wind. They can survive for a moderate period of time (days) and will readily germinate under humid conditions to form a short delicate germ tube. For many rust species such as for those in the genera *Puccinia* and *Uromyces*, these germ tubes penetrate the host cuticle and epidermal cells directly after forming an appressorium-like structure whereas others such as *Cronartium* spp., enter through stomata (Flor 1971; Mendgen 1984; Gold and Mendgen 1984; Hoch et al. 1987 b; Hoch and Staples 1987). Following penetration, an (M-, for monokaryotic) haustorium is produced which is merely an intracellular extension of intercellular hyphae without the significant morphological specialization as seen for the D-haustorium (Gold and Mendgen 1991).

Upon establishing a feeding relationship and presumably suppression of (alternate) host defense responses, the specialized pycnia are produced which generate the pycniospores from stage 0. See section 2.3 for a discussion of this topic.

### 3.2.3 Infection process: molecular aspects of interactions

Most genes described to date have been revealed through protocols using differential screening of cDNA libraries focusing on stage II: urediniospore infection and resulting biotrophic phase in the primary host. Urediniospores probably draw most of their energy from stored lipids when germinating (Staples and Wynn 1965). Indeed,
ultrastructural observations suggest that large lipid bodies in the cytoplasm of several spore types seem to degrade during germination (reviewed in Mendgen 1984). In the bean rust, *U. appendiculatus*, several genes were identified by expression during appressorium formation *in vitro* such as Inf56 and Inf24 (Bhairi et al. 1989; Xuei et al. 1992). They have been characterized to some degree but their role in the differentiation process is unknown. Microinjection of an antisense fragment to the ORF of *Inf24* strongly inhibited appressorium formation but it did not inhibit continued development of subsequent infection structures (a penetration peg and a substomatal vesicle) into already formed appressoria (Barja et al. 1998). Incidentally, this study also demonstrated that a gene knock-down approach could be successful in this rust. Several rif- ("rust infection-specific") genes of unknown function have been revealed by differential hybridization in *U. fabae* (Deising et al. 1995). *P. graminis* harbors a small gene family, *usp*, coding for small hydrophobin-like proteins that most likely function extracellularly (Liu et al. 1993). Two members are highly expressed during urediniospore germination but one seems to be a sporulation-specific gene.

### 3.2.4 Signaling

Cyclic AMP signaling plays an important role during differentiation in many fungi including in *U. maydis* (as detailed in section 3.1 of this chapter). Cyclic AMP and cGMP can induce differentiation in *U. appendiculatus* such as mitosis and septum formation and generally regulate appressorium development in urediniospore germlings (Epstein et al. 1989; Hoch and Staples 1984; Hoch et al. 1987 a). Ca$^{2+}$ signaling is very important in fungi (Zelter, 2004) and it was shown early on that K$^+$ and Ca$^{2+}$ signaling was involved in the morphological changes during the infection process (Hoch et al. 1987 a). In an early attempt to study the importance of Ca$^{2+}$ signaling, calmodulin was isolated from *U. appendiculatus* and shown to stimulate Ca$^{2+}$-dependent cyclic nucleotide phosphodiesterase (Laccetti et al. 1987).

### 3.2.5 Establishment of the biotrophic phase

In an effort to reveal biotrophic phase-specific genes, essential for "acclimatization" in a hostile environment such as establishing a feeding relationship and host defense-suppression strategies, several protocols to isolate haustoria from host leaves infected with *Puccinia* and *Uromyces* species have been developed (Hahn and Mendgen 1992; Tiburzy et al. 1992; Cantrill and Deverall 1993). Differential screening of a constructed haustorium-specific *U. fabae* cDNA library yielded many Plant-Induced Genes (PIGs) the analysis of which has produced some insight into the way the bean rust establishes a biotrophic state (Hahn and Mendgen 1997). Some genes might play a role in adapting to the hostile host environment such as overcoming oxidative and osmotic stress and detoxification of host compounds. Several genes involved in nutrient uptake and metabolism appear to be differentially regulated (Wirsel et al. 2001). Amino acid transporters have been identified (Hahn et al. 1997; Struck et al. 2002; Struck et al. 2004 a), of which *AAT1* and *AAT3* encode general amino acid permeases. Each prefers uptake of a different subset of scarce amino acids and are expressed in all infection
structures but upregulated in haustoria. In contrast, the amino acid transporter, AAT2p (formerly PIG2p), is exclusively expressed in haustoria and localizes to their plasma membranes (Mendgen et al. 2000). A gene involved in sugar uptake (Hexose Transporter 1, HXT1) is exclusively expressed in haustoria and HXT1p also localizes to their plasma membranes (Voegele et al. 2001). Interestingly, a *Saccharomyces cerevisiae* glucose uptake mutant and *Xenopus laevis* oocytes were used to functionally test this rust protein which was characterized as a proton-motive force driven monosaccharide (glucose, fructose, mannose) transport system. A fungal invertase was also found residing in the same subcellular location and suggested to convert sucrose to the monosaccharide substrates for HXT1p (Voegele and Mendgen 2003). Another proton-dependent transporter, AAT1p for ammonium, was discovered and cloned (Struck et al. 2002). This transporter is expressed during all stages of infection and thought to scavenge limited N-metabolites in the host. The presence of a H(+)-ATPase in the haustorial membrane has been suspected but the cloning and analysis of its gene (Struck et al. 1996; Struck et al. 1998) suggests that the fungus exerts control over the nutrient flux. The *BGL1* gene was revealed in a search for genes involved in sugar mobilization. It is expressed in all stages of growth, including haustoria. BGL1p is a fungal α-glucosidase, probably secreted in the extracellular space where it is found in the periphery of intercellular hyphae and haustoria. It may be involved in cellulose/cellobiose degradation and/or possibly defense (Haerter and Voegele 2004). Two of the PIGs (*THI1* and *THI2*) are involved in thiamine (vitamin B1) biosynthesis as revealed by complementation of *Schizosaccharomyces pombe* thiamine auxotrophic mutants. It was suggested that these essential co-factors for central carbon metabolism indicate the need for a very active metabolism through de novo biosynthesis (Sohn et al. 2000). Although many relevant genes have and will be revealed, such purified haustoria unless fixed immediately at the beginning of the extraction procedure, have undoubtedly changed their expression patterns during the isolation procedure which will hamper the construction of representative cDNA libraries or skew mRNA populations for transcript profile analyses.

Using suppression subtractive hybridization, Thara et al. (2003) constructed a fungal, *in planta*-specific cDNA library from *P. triticina*-infected wheat leaves 4 days after infection of compatible wheat and subtracted with mock-inoculated host sequences (Thara et al. 2003). They obtained 104 unique random sequences of which 69 were likely fungal. Among them, 25 represented ribosomal proteins and the remaining 44 encoded non-ribosomal fungal proteins. Some of these were novel and some represented previously found PIGs or virulence genes from other fungi. A targeted cDNA-AFLP technique allowed the selection of genes with interesting expression patterns during the infection process of *P. triticina* on wheat leaves form urediniospore inoculation up to sporulation (Zhang et al. 2003). Both up- and down-regulated genes from both fungus and host were revealed, 50% of which showed no homology to known genes. Another technique, differential display, was used to reveal 9 genes expressed in galls during the infection of southern pine by *Cronartium quercuum* f. sp. *fusiforme*; genes involved in metabolism and stress were identified (Warren and Covert
2004). The above-mentioned molecular studies confirm that rusts undergo a major reprogramming of their metabolism and transcriptome once inside the host. Illustrative is the apparent induction of a large number of ribosomal protein genes upon establishment in the host (Thara et al. 2003; Hu and Bakkeren unpublished). Moreover, these recent molecular studies have substantiated an old hypothesis that the haustoria divert nutrients and play a major role in the source-sink relationship with the host (Hahn 2000; Staples 2001; Szabo and Bushnell 2001; Wirsel et al. 2001; Struck et al. 2002; Voegele and Mendgen 2003).

3.2.6 Virulence factors

Many of the genes described in this section can undoubtedly be classified as pathogenicity or virulence genes, although their function as such has not been directly verified in a (targeted) mutational analysis as is common in other, more tractable pathogens. Factors eliciting the host defense as measured by the induction of (cell wall) autofluorescence and/or host PR genes, up to a full-blown HR including necrosis and DNA laddering, have been described for some rusts but their primary role in virulence is unknown (reviewed in Hahn 2000). The unfortunate elicitor functionality can reside within a true virulence factor produced to facilitate the infection process or be caused by "by-products" of the infection process (reviewed in Bakkeren and Gold 2004). Such elicitors triggering defense responses can be general, produced by a whole genus or by an inappropriate pathogen during a nonhost interaction, or more specific to a species or a certain race or isolate within a species. The mentioned avirulence factors elicit defenses resulting in complete restriction of the fungal life cycle on the host. For example, general chitin oligosaccharides, as well as a more specific glycopeptide elicitor have been described for *P. graminis*, which induce hypersensitive-like responses in several but not all wheat genotypes and stimulate lipoxygenase (LOX) activity (Sutherland et al. 1989; Beissmann et al. 1992; Bohland et al. 1997; Tada et al. 2001). In the latter category, race-specific elicitors have been isolated from *U. vigneae* (Chen and Heath 1990; D'Silva and Heath 1997) and *P. triticina* (Saverimuttu and Deverall 1998).

In the field of plant-microbe interactions, avirulence genes have long attracted attention because they represent often single dominant genes and trigger defense reactions. ‘Race-f cultivar specialization’ has been described for many biotrophic fungi including cereal rusts (Kolmer 1996; Chong et al. 2000; Chen and Line 2003; Brown and Casselton 2001; Long and Kolmer 1989), flax rust (Dodds et al. 2004) cowpea rust (Chen and Heath 1993) and the white pine-blister rust (Kinloch and Dupper 2002). The large number of races revealed for some rusts suggest the presence of many fungal factors that the respective host cultivars can detect. Efforts to construct genetic maps are underway for several rusts, e.g. *P. graminis* (Zambino et al. 2000) *M. lini* (Dodds et al. 2004) and *P. triticina* (McCallum and Mulock unpublished) and this has recently resulted in the isolation of the first basidiomycete avirulence gene, *AvrL567*, from *M. lini* (Dodds et al. 2004). The *AvrL567* gene is expressed in rust haustoria and encodes a 127 amino acid preproteon of unknown function. The N-terminal 23-amino acid signal sequence presumably is cleaved off and the mature protein is secreted in the plant cell
where it induces a hypersensitive response-like necrosis that is dependent on co-expression of the L5, L6, or L7 resistance gene. Elucidating the intended (virulence) function of such “avirulence” gene products is a biological challenge and of utmost importance.

Double-stranded RNAs have been found in many rust species. The number and size of these dsRNA molecules seem to be highly species-specific, but contrary to the effect they have on many other phytopathogenic fungi such as causing hypovirulence (Dawe and Nuss 2001), they don’t seem to be involved in pathogenicity, virulence, fitness or toxin production in the rusts (Pryor et al. 1990; Zhang et al. 1994).

3.2.7 Countering host defense

The existence of suppression of host defense response during rust infections is descriptive (microscopy; Harder and Chong 1991) or indirect through a phenomenon called ‘induced susceptibility’ (Niks 1989; Arz and Grambow 1995; Skalamera et al. 1997) and reviewed in Voegele and Mendgen (2003). Molecular studies are only just beginning for the rusts, but fungal factors suppressing host defense and increasing host susceptibility will by definition be encoded by virulence genes. In analogy with bacterial “effector” molecules that are transferred to the host cytoplasm/nucleus, a possible candidate is the *U. fabae* Rust Transferred Protein, RTP1, which can be found in the nucleus of infected bean cells. Its function, however, is unknown and there are no known homologs (Struck et al. 2002). Cro rl from *C. ribicola* is a small secreted protein that is preferentially expressed by the fungus growing in susceptible host seedlings and could have a role in moderating the interaction (Yu et al. 2002). Another, probably major strategy of biotrophs is avoidance of recognition of pathogen-associated molecular patterns or PAMPs and/or elicitation of host defense responses. In *U. appendiculatus* it was shown that the walls of germ tubes and appressoria contain chitin, the amount of which is reduced or masked in infection hyphae and haustoria. This may occur through deacetylation of chitin converting it to chitosan and it was suggested that this would prevent recognition by the host (Heath 1989; Freytag and Mendgen 1991; Deising et al. 1995; El Gueddari et al. 2002). The *U. fabae* β-glucosidase, BGL1p, could be involved in countering general host defenses by detoxifying phytoalexins such as saponins (Haerter and Voegele 2004).

3.2.8 Comparative biology, genomics and future direction

Whether genes shown to be involved in certain developmental stages on the primary host such as, sporulation, infection, metabolite diversion and the establishment of the source-sink relationship during biotrophism, or suppression of host defense, play roles in equivalent stages on the alternate host and the production of the other spore types is currently unknown. “Genomics”, the large-scale analysis of the genome, its genes and their expression, is in its infancy for the rusts but is expected to provide breakthroughs for these hard to manipulate obligate parasites. Large scale EST projects are underway for *P. triticina* (Hu and Bakkeren unpublished; Zhang et al. 2003). A large *P. triticina* EST database generated from cDNA libraries representing five distinct morphological
phases in Stage II, has revealed homologs of some of the genes discussed here and others that will expand our insights. For example, we identified sugar transporters (from resting and germinating urediniospores), a high-affinity leucine-specific transport protein, a manganese ABC transporter and a β-glucosidase, a homolog of *U. fabae* BGL1 (from germinating urediniospores). With respect to signaling, we identified a putative calcium transporting ATPase in resting spores and a calcium binding signal transducing protein, a putative calcium transporting ATPase, calmodulin, a calmodulin-dependent protein kinase, a calcium/proton exchanger and a potassium channel subunit, all in resting and germinating urediniospores. The discovery of several enzymes involved in lipid metabolism, such as lipases, acyl CoA dehydrogenase and fatty acid hydroxylase, in cDNA libraries from germinating urediniospores, might substantiate hypotheses regarding the physiology of germinating spores. However, no functional or transcript analyses have been done (Hu and Bakkeren manuscript in preparation). The comparison of global gene expression profiles using microarrays harboring large sets of genes representing several life cycle stages of *P. triticina* (Bakkeren lab in progress) might shed light on such questions. In addition to EST projects, total genome sequencing is underway for *P. graminis f. sp. tritici* (Dr. L. Szabo et al., USDA and the Broad Institute) and two soybean rust species, *Phakopsora pachyrhizi* and *P. meibomiae* (Dr. R. D. Frederick et al. USDA).

Ultimately, apart from the academic interest, we need to understand delicate interactions in order to develop "smart", very specific (bio)-fungicides to be used as sprays, or expressed in antagonists or in host plants to be used as crop protectants. In addition, if we find critical factors perturbed in the host during establishment of the feeding relationship, we can counteract these in the plant and possibly attain durable resistance. Understanding the molecular basis of nonhost resistance, widely thought to underpin more durable resistance, should open new avenues for crop protection.

3.3 Mushrooms
3.3.1 Introduction

The differentiation of fruiting bodies (mushrooms) in Basidiomycetous fungi represents what is arguably the most important developmental event in the life cycle of the species capable of forming such structures. Despite its' importance to these fungi, the process of mushroom development, also called fruiting, is poorly understood at the genetic level. It is not the purpose of this brief overview to systematically list and detail all of the pertinent research in the field: a number of excellent and thorough reviews have been recently published to which the reader is referred (Fischer and Kues 2003; Kamada 2002; Kues 2000; Kues and Liu 2000; Kues et al. 2004; Walser et al. 2003). This section briefly outlines mushroom development as it occurs in the two best-studied model systems, *Coprinopsis cinerea* (*Coprinus cinereus*) and *Schizophyllum commune*, and then describes what is currently known about the genetic control of this process. In an effort to make connections between genetic controls and signaling networks, special reference will be made to two well-studied non-mushroom forming Basidiomycetes, *U. maydis*, and *C. neoformans*. 

3.3.2 A double threat for use as models in the molecular study of mushroom development: *Coprinopsis cinerea* and *Schizophyllum commune*

For the most part, commercially valuable mushroom-producing fungi have been difficult subjects for both genetic and molecular biological studies of fruiting body development. However, it can be argued that this potential cloud has resulted in a silver lining, because not one, but two model systems have been studied to fill the gap. The concurrent utilization of two distinct model organisms for the study of mushroom development allows for the distinguishing of elements common to the process from those that are species-specific. The ink cap mushroom *Coprinopsis cinerea* and the common split-gill mushroom *Schizophyllum commune* both have a rich history of genetic study dating back to the early twentieth century (Buller 1933; Essig 1922). *C. cinerea* forms what can be termed a typical mushroom with a long stipe and well-developed cap, while the fruiting body of *S. commune* appears as a fan-shaped cup close to the surface of the substrate. Both of these fungi have life cycles (Figures 4 and 5) that can be completed on artificial media in the laboratory within two weeks. Each has independently propagatable haploid and dikaryotic (n + n) phases, facilitating genetic analyses. The molecular study of gene function has been enabled by DNA-mediated transformation (Binninger et al. 1987; Munoz-Rivas et al. 1986) and by the recent genomic sequencing of *C. cinerea* (www.genome.wi.mit.edu/cgibin/annotation/fungi/Coprinopsis_cinerea). Targeted gene disruption by homologous integration has been achieved in both systems (Aime and Casselton 2002; Binninger et al. 1991; Horton et al. 1999; Lengeler and Kothe 1999) although the frequency of these events are considerably lower than in other Basidiomycetes such as the plant pathogen *U. maydis* (Kahmann and Kamper 2004), or the human pathogen *C. neoformans* (Hull and Heitman 2002). Overexpression of genes has been demonstrated in *S. commune* through the use of recombinant constructs using strong promoters endogenous to this fungus (Yamagishi et al. 2002; Yamagishi et al. 2004). Current technical limitations include the lack of episomal plasmids for use in non-integrative transformation, and a well-developed GFP-tagging system for use as a reporter in cytological studies.

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**Fig. 4:** Life cycle of the mushroom-forming Basidiomycete *Schizophyllum commune* (Stankis et al. 1990)
3.3.3 Other mushroom-producing fungi of agricultural value

Mushroom species of agricultural importance include the button mushroom *Agaricus bisporus*, the oyster mushroom *Pleurotus ostreatus*, and the Shiitake mushroom *Lentinus edodes*. The worldwide market for these and other mushrooms for use as food, as dietary supplements and nutriceuticals, and in medicine was estimated to be over US $13 billion per year in 1996 (Chang 1996). The value of this market has spurred interest in the application of molecular genetic techniques for use in improving the characteristics of these fungi. Classical and molecular genetic studies have been hampered by the slow growth and the lack of fruiting of these fungi on synthetic media. An additional complication for genetic analyses is that in *A. bisporus*, basidiospores are binucleate and produce heterokaryotic mycelia upon germination. Recent advances in transformation technologies using *Agrobacterium tumefaciens* (de Groot et al. 1998; Chen et al. 2000) have opened up possibilities for molecular-based strain improvement, although these methods are still inefficient as compared to the model organisms. To date, there are no genomic sequencing projects for these fungi.

3.3.4 Overview of mushroom development

From a macroscopic point of view, the development of mushrooms represents the single most dramatic event in the life cycle of a fungus capable of producing such a structure. Under normal circumstances, only the dikaryotic phase is competent to form mushrooms; however, haploid fruiting strains do exist (Esser et al. 1979; Leslie and Leonard 1979; Murata et al. 1998; Uno and Ishikawa 1971; Verrinder-Gibbins and Lu 1984; Yli-Mattila et al. 1989). Mushrooms are also produced in haploids with

![Diagram of mushroom life cycle](image)

*Fig. 5:* Life cycle of the mushroom-forming Basidiomycete *Coprinopsis cinerea* (Kues 2000)
constitutive mutations in the A and B mating-type loci in both \textit{C. cinerea} and \textit{S. commune} (Raper et al. 1965; Swamy and Ishikawa 1984). One interesting difference between these two fungi is that the activation of the \textit{A} pathway alone (along with light) is sufficient to induce fruiting in \textit{C. cinerea} (Kues et al. 1994; Kues et al. 1998) but not in \textit{S. commune} (C. Raper and T. Fowler, personal communication).

The process of mushroom development can be divided into three main cellular events: aggregation, differentiation, and expansion. Since the cytological processes are best described in \textit{C. cinerea}, these are outlined here. For a more comprehensive description, see the excellent review by Kues (2000). Differences between what occurs in \textit{C. cinerea} and \textit{S. commune} will be noted. In both species, proper environmental conditions are required for normal fruiting to occur. These include nutrient limitation, periods of light and dark, and low CO$_2$ levels (reviewed in Kues 2000; Wessels 1992).

Mushroom development begins with the formation of what is called a primary hyphal knot. This undifferentiated structure is characterized by intense localized formation of short hyphal branches and restricted tip growth (Matthews and Niederprum 1972). The resulting cellular compartments are short, and in \textit{C. cinerea} the hyphal knot is also characterized by higher order side branches. A blue light signal results in hyphal aggregation and the formation of a compact ball called a secondary hyphal knot (Matthews and Niederprum 1973), which is the first fruiting-body specific structure (Lu 1974; Uno et al. 1974). In \textit{S. commune}, hyphal aggregation results in a stalk-like structure because of parallel growth of the adhering hyphae (Raudaskoski and Vauras 1982; Raudaskoski and Viitanen 1982; van der Valk and Marchant 1978).

The development of fruiting body primordia from secondary hyphal knots in \textit{C. cinerea} requires additional light signals, and it is within these structures that cellular differentiation occurs. There is an observable polarity of cells in the primordia, with roughly the upper third forming the cap, the middle third the stipe, and the lower third the basal plechtenchema. Cells in the latter region are randomly oriented, and do not get incorporated into the stipe, where cells have a varied spatial arrangement, depending upon their function (Hammad et al. 1993; Kues et al. 2004). From a genetic point of view, the key differentiation event is the development of the spore-bearing basidia within the gills of the cap, in a layer called the hymenium. It is within the basidia that karyogamy and meiosis occur, which in \textit{C. cinerea} is a synchronous event induced by a light signal (Lu 1974). At the same time, the mushroom grows by cell expansion and elongation (Kamada and Takemaru 1977). In \textit{C. cinerea}, the fruiting body is a short-lived structure because of autolysis of the cap tissue, which releases the basidiospores into the deliquescent milieu. The process of fruiting body formation in this fungus is quite rapid: from the first visible appearance of primordia to the autolysis of the mushroom cap takes about 4 to 5 days (Moore et al. 1979). In contrast, fruiting bodies of \textit{S. commune} are essentially perennial, and undergo karyogamy, meiosis, and sporulation in an asynchronous manner. The fan-shaped fruiting bodies enlarge primarily by cellular proliferation on the periphery, and not by cellular inflation. Spores are borne on the upper surface, and are dispersed directly into the air, and the mushrooms do not undergo autolysis.
C. cinerea exhibits much more plasticity than does S. commune in the program of fruiting body development. At various points in the program, other multicellular structures such as sclerotia and capless etiolated stipes can result in C. cinerea if light signals are not received at the appropriate times (Kamada et al. 1978; Kues et al. 1998; Lu 1974; Moore 1981). While light is necessary for the development of mushrooms in S. commune (Perkins 1969; Perkins and Gordon 1969) these other developmental "options" have not been observed.

3.3.5 Genetic control of mushroom development

The master regulatory genes responsible for the initiation of fruiting in the dikaryon reside at the mating-type loci A and B. In both C. cinerea and S. commune, the A mating-type genes encode homeodomain (HD) transcription factors, while the B genes encode lipopeptide pheromones and their receptors. The molecular genetic analysis of these genes in these fungi has been recently reviewed extensively elsewhere (Casselton and Olesnicky 1998; Kamada 2002; Kothe 1996; Kues 2000). The focus here will be on what is known about the genetic regulation of mushroom development downstream of the mating-type genes. Given the eclectic nature of the "fruiting" genes so far analyzed, it is informative to make reference to the genetics of sexual development in the better-studied non-mushroom forming Basidiomycetes. It is hoped that the concepts illustrated in these other species may inform us about the many gaps in our understanding with regards to the genetic control of mushroom development.

3.3.6 Experimental approaches for gene isolation

The genes characterized to date that are implicated in the genetic control of mushroom development are a heterogeneous lot; in many cases these genes do not have an obvious connection to one another. This may be a reflection of the fact that relatively few laboratories are currently studying this problem, and also the diverse manner of the approaches by which the relevant genes have been isolated. These approaches include: (1) complementation of recessive mutants defective or altered in the fruiting process, (2) differential mRNA expression under fruiting and non-fruiting conditions, (3) degenerate PCR, and (4) two-hybrid protein interaction screens. A high priority in the future will be to greatly expand our knowledge of the "players" found to be regulating mushroom development, and to integrate these genes into a set of genetic pathways that is much more definitive than is known at present.

The complementation of mutants defective in fruiting body development has been an effective genetic tool in efforts to isolate genes regulating this developmental process. Various mutagenesis procedures have been employed, including UV irradiation, mutagenic chemicals, and REMI or restriction enzyme mediated integration (reviewed in Kues 2000). Homokaryotic strains of C. cinerea and S. commune with constitutive mutations in the A and B mating-type loci reproduce the phenotype of the dikaryon, including the formation of clamp connections and fruiting bodies (Raper et al. 1965; Swamy and Ishikawa 1984). These self-compatible strains are called Anut Bnud and Acon Bcon; in C. cinerea and S. commune, respectively. There are considerable advantages
of using such strains over dikaryons in mutagenesis screens for fruiting-defective mutants. Both recessive and dominant mutations can be detected in the haploid genetic background, unlike in dikaryons. Without the complication of two different nuclei present in the same cytoplasm, the homokaryotic strain is by definition isogenic for all loci, including those genes that are necessary for fruiting. In *C. cinerea* (but not *S. commune*), uninucleate asexual spores called oidia can be used for mutagenesis (Pukkila 1993). Large scale UV or REMI mutagenesis of oidia from homokaryotic fruiting strains of *C. cinerea* results in a very high proportion (30-40%) of surviving clones with defects in fruiting (Granado et al. 1997; Muraguchi et al. 1999). These findings suggest that in addition to fruiting-specific genes, many other genes involved in cellular metabolism, hyphal growth, and general housekeeping play important roles in mushroom development (Kues 2000).

### 3.3.7 Early-acting genes in the control of fruiting

The master controlling elements of sexual development in mushroom-producing fungi are the products of the A and B mating-type genes. In the corn pathogen *Ustilago maydis*, the mating-type genes encode the same types of protein products (reviewed in Kahmann and Kamper 2004) as they do in *C. cinerea* and *S. commune*, but the nomenclature of a and b are “reversed” in *U. maydis*. DNA microarray analyses have set the number of b-regulated genes in *U. maydis* at 246 (Scherer and Kamper unpublished, cited in Kahmann and Kamper 2004). Only three genes to date have been shown to be direct targets of the bE/bW heterodimer, suggesting that the majority of the remaining genes are likely to be indirectly regulated. These genes have a b binding sequence (bbs) in their promoter regions, and have been designated class 1 b-regulated genes. It has been proposed that the bE/bW heterodimer triggers a regulatory cascade, with some of the class 1 genes encoding regulatory proteins, as well as being targets themselves (Kahmann and Kamper 2004). Unfortunately, none of the three class 1 genes characterized to date encode a likely regulatory protein, and none are essential for pathogenicity (Brachmann et al. 2001; Romeis et al. 2000; Weinzierl and Kamper, unpublished, cited in Kahmann and Kamper 2004).

In *C. cinerea*, one gene likely to be an immediate downstream target of the HD heterodimer encoded by the A mating-type loci is *clp1* (Inada et al. 2001). The *clp1*-l recessive mutant allele results in a truncated protein product, and has a “clampless” phenotype in a genetic background where the A mating-type pathway is activated (Inada et al. 2001). The promoter region of *clp1* contains a sequence (GATGX₉ACA) that is similar to the conserved sequence (GATGX₉ACA) found in the b-binding sequence or bbs of *U. maydis* (Romeis et al. 2000) and also to the hsg motif that is the target of the MATa2/MATa1 heterodimer in the budding yeast *S. cerevisiae* (Goutte and Johnson 1988). While it is a good candidate for a class 1 gene, *clp1* is unlikely to encode a transcription factor, as it has no obvious structural motifs, and does not show any extensive similarity to known DNA-binding proteins when used as a query sequence in BLAST (Altschul et al. 1997) searches. The most significant hit (E value = 3 X 10⁻⁹) is to a hypothetical protein of unknown function found in the *U. maydis* genome. As is the case
in *U. maydis*, other class 1 genes from *C. cinerea* need to be identified as potential regulatory genes in the HD-regulated pathway.

The gene *pcc1* was first identified in a homokaryotic strain of *C. cinerea* that was observed to produce fruiting bodies after prolonged culture (Murata et al. 1998). This spontaneous recessive mutation induced the formation of unfused clamp connections, also called pseudoclamps. This cell type is typical of *A*-activated heterokaryons, as is fruiting body development, although the occurrence of the latter is also dependent upon the genetic background of the individual (Kues et al. 1994; Kues et al. 1998; Kues et al. 2002). *pcc1* is predicted to encode a likely DNA-binding protein, and has an HMG domain with significant similarity to those found in ascomycete mating-type gene products (Murata et al. 1998). This region of the Pcc1 protein is also 43% and 29% identical to the HMG domain of the Rop1 and Prf1 proteins of *U. maydis*, respectively. Prf1 encodes a transcription factor that is a central regulator of both cell fusion and pathogenicity in this fungus (Hartmann et al. 1999). These effects are mediated by Prf1 binding to pheromone response elements, or PReS. PReS are present in the promoter of *prf1* itself, and also in the regulatory regions of the *a* and *b* mating-type genes (Hartmann et al. 1996; Urban et al. 1996 a). The former finding is highly suggestive of *prf1* autoregulation, while the latter result indicates that expression of the mating-type genes is regulated by Prf1. Interestingly, the promoter of *pcc1* also has a PRE-like sequence (Murata et al. 1998). Rop1 has been shown to regulate *prf1* gene transcription by directly binding the *prf1* promoter *in vitro* (Brefort et al. 2004). Rop1 is also essential for mating-type gene expression (Brefort et al. 2003). Given all of this information, it is possible that Pcc1 could represent the *C. cinerea* equivalent of either Rop1 or Prf1. If either of these is the case, it suggests that pheromone response as regulated by Pcc1 may be influenced by both cAMP and MAP kinase signaling, as is the case in *U. maydis* (Kahmann and Kamper 2004).

Pcc1 has been hypothesized to act as a repressor of *A*-regulated development in the homokaryon, and is also likely to be downstream of *clp1* (Kamada 2002). The latter idea is supported by the fact that *clp1* is not transcribed in the *pcc1* mutant, despite the observation that clamps are observed (Murata et al. 1998). It is important to note that forced expression of *clp1* with a β1-tubulin promoter induces clamp development in the absence of *A*-activation, thus releasing the *A* pathway from Pcc1 repression (Inada et al. 2001). This de-repression is likely to be post-transcriptional, as *pcc1* transcript levels are actually higher in *A*-on than in *A*-off mycelia (Murata et al. 1998). There exists the possibility that an as yet unidentified protein(s) mediates the regulation of Pcc1 by Clp1 (Kamada 2002). In addition to *pcc1*, the genes *hmg1* and *hmg2* from *C. cinerea* also encode proteins with HMG domains that have strong homology to that of Prf1 (Milner 1999). Gene disruption of *hmg1* indicates that the Hmg1 protein is necessary for nuclear migration, but not for clamp cell fusion (Aime and Casselton 2002). The *B* pathway of pheromone signaling induces both of these cellular events. Taken together, these findings suggest that HMG domain proteins might occupy central regulatory roles in both *A*-regulated (Pcc1) and *B*-regulated (Hmg1 and/or Hmg2) development in *C. cinerea*. 
A UV-induced mutant blocked after hyphal knot formation in the *A. pull B. mut* homokaryotic fruiting strain of *C. cinerea* was complemented by the gene *cfs1*. This gene is predicted to encode a eukaryotic counterpart to bacterial cyclopropane fatty acid synthases, which are a specific sub-family of S-adenosylmethionine-dependent methyltransferases (Liu et al. 2001; Liu 2001). This category of enzymes convert membrane-localized unsaturated phospholipids into cyclopropane fatty acids (Grogan and Cronan 1997), which are believed to be part of stress defense in bacteria. In mushroom-producing fungi, membrane alteration has been postulated to be a stress signal that helps tell the fungus to make the switch from vegetative to reproductive growth (Magae 1999; Oita and Yanagi 1993).

An unnamed gene has been identified that is essential for primary hyphal knot formation, the first step in fruiting body development (Clergeot et al. 2003). It appears to encode a protein related to *het-e* gene product of the Ascomycete Podospora anserine, and encodes a protein with a GTP-binding site and a WD-40 repeat domain similar to that seen in Gβ subunits (Saupe et al. 1995). In addition to being defective in hyphal knot formation, another phenotype of this mutant is a fast dark-brown staining of the agar around the growing cultures, which could indicate a de-regulation of laccase(s) (Clergeot and Ruprich-Robert unpublished results, cited in Kues et al. 2004). There are eight members of the laccase gene family in *C. cinerea*, the largest number identified so far in a single haploid fungal genome (Hoeffer et al. 2004). Although the literature is certainly not conclusive on the matter, it has been proposed that laccases could function in mushroom development by mediating hyphal aggregation, acting to form chemical crosslinks by oxidative polymerization of phenolic cell wall components (Broxholme et al. 1991; Zhao and Kwan 1999).

### 3.3.8 Signaling and fruiting body development

Signaling cascades involving the cAMP and MAP kinase pathways play definitive roles in mating, morphogenesis, and pathogenicity in the plant pathogen *U. maydis* (Kahmann and Kamper 2004), and in the human pathogen *C. neoformans* (Hull and Heitman 2002). It is likely that this will also be the case for the processes of mating and sexual development in the mushroom-forming Basidiomycetes as well. In *U. maydis*, the cAMP signaling pathway is controlled through the Gα subunit of a heterotrimeric G-protein, Gpa3 (Kruger et al. 1998; Kruger et al. 2000; Regenfelder et al. 1997). Neither the signal nor the receptor responsible for activating Gpa3 have been identified. The receptor that activates the Gα protein Gpa1 in a cAMP signaling pathway relevant to mating and virulence in *C. neoformans* has also eluded identification (Hull and Heitman 2002). There are likely to be four distinct Gα genes in *S. commune; SCGP-1* (GenBank accession AF157495, A. Pardo, M. Gorfer and M. Raudaskoski), *ScGP-A, ScGP-B, and ScGP-C* (Yamagishi et al. 2002). Dominant activating mutations were made in three of these Gα genes, and were introduced into homokaryotic strains under the control of a strong promoter. Over half of the recipients transformed with mutated *ScGP-A* or *ScGP-C* exhibited suppressed aerial hyphae formation (Yamagishi et al. 2002). Mutated *ScGP-B* transformants did not show any differences with wild-type strains. Contrary to
expectations, none of the constitutive mutant Gα constructs stimulated the pheromone response (β-regulated) pathway in A-on heterokaryotic matings, as evidenced by the absence of observable clamp connections in these mated strains. Both the mutated ScGP-A and ScGP-C genes also markedly suppressed fruit-body formation in A-on, B-on dikaryons (Yamagishi et al. 2002). The suppression of both fruiting and of aerial hyphae is reminiscent of the phenotype observed for strains carrying the transposon-mediated loss-of-function mutation in the \textit{thn1} gene of \textit{S. commune} (Fowler and Mitton 2000). The Thn1 protein is a likely member of the RGS domain family that regulate the activity of G-protein α-subunits, and it is possible that it may act upon the ScGP-A and/or the ScGP-C gene products. It is unclear exactly how the mutated G-proteins act to repress fruiting body formation, but it could be because of a disruption in cAMP signaling. Measured intracellular cAMP levels were elevated by 160-200% in monokaryons and dikaryons containing mutated ScGP-A and ScGP-C genes (Yamagishi et al. 2004). Taken together, these results suggest that both of these two Gα subunits may play a role in cAMP signaling, and that this pathway helps to regulate mushroom development in \textit{S. commune}. Two cDNAs encoding Gα subunits have been isolated from the mushroom-producing fungus \textit{Coprinellus congregatus}, but it has not been determined if either of these genes participate in cAMP signaling (Kozak et al. 1995).

In \textit{C. cinerea}, it has been established that cAMP levels rise at the time of light-induced secondary knot and primordia formation, and then decline during fruiting body maturation until karyogamy and meiosis. Mature fruiting bodies (after maturation of basidiospores) are low in cAMP content (Uno et al. 1974; Uno and Ishikawa 1974; Kues et al. 2004). Indeed, addition of exogenous cAMP has been shown to induce fruiting in receptive strains of this fungus (Uno and Ishikawa 1971; Uno and Ishikawa 1973). Changes in cAMP levels have also been observed during the process of fruiting body formation in \textit{S. commune} (Kinoshita et al. 2002). Genes encoding adenylate cyclase, responsible for cAMP production, have been isolated from both \textit{C. cinerea} (Bottoli et al. 1999) and \textit{S. commune} (Horton et al. unpublished results). Transcripts for the \textit{C. cinerea} gene \textit{cac1} are constitutively expressed in vegetative mycelia, regardless of light conditions (Bottoli 2001). This finding suggests that enzyme activity is likely regulated at the post-translational level. The gene encoding a \textit{S. commune} adenylate cyclase was isolated by means of a two-hybrid screen as an expressed cDNA potentially interacting with a Frt1 "bait" (Horton et al. unpublished results). No experiments manipulating this adenylate cyclase have been reported to date. However, the gene \textit{frt1} has been implicated in the genetic pathway controlling fruiting, see below (Horton and Raper 1991).

Rising cellular cAMP levels that occur as a result of the activity of adenylate cyclase allows for the activation of cAMP-dependent protein kinases (PKAs). Binding of cAMP to the regulatory subunits of the PKA tetramer induces conformational changes that result in the release and subsequent activation of the catalytic subunits. In the \textit{U. maydis} pheromone response pathway, the PKA catalytic subunit Adr1 phosphorylates Prf1, which in turn induces the expression of both the \textit{a} and \textit{b} mating-type genes (Kaffarnik et al. 2003). A cDNA encoding a catalytic subunit of PKA has been isolated from \textit{S.}
commune, but to date there is no definitive evidence that links this gene with the fruiting pathway (Horton et al. unpublished results).

In U. maydis, a MAP kinase module is necessary for pheromone response, and is intimately associated with both mating and pathogenicity. Prf1 is a direct target of the MAPK enzyme Kpp2/Ubc3 (Muller 2003 b). No member of a MAPK signaling cascade has been isolated to date from a mushroom-producing Basidiomycete. The availability of the sequenced C. cinerea genome can clearly be of value in this effort. The use of two-hybrid technologies using Pcc1, Hmg1, or Hmg2 as bait could help to determine which MAPK is likely to interact with these putative transcription factors.

Ras proteins belong to a conserved family of small GTP-binding proteins, acting as molecular switches controlling a wide range of cellular processes (reviewed in Takai et al. 2001). Two Ras proteins are proposed to play roles in the MAPK and cAMP signaling pathways in U. maydis. Ras1 likely affects the activity of the adenylate cyclase protein Uac1, while Ras2 has been assigned to the MAPK cascade, upstream of the MAPK Fuz7/Ubc5 (Lee and Kronstad 2002; Muller 2003 b). In C. neoformans, the protein Ras1 is required for both pheromone production and pheromone response in C. neoformans, and has been implicated in the MAPK signaling pathway (Alspaugh et al. 2000; Waugh et al. 2002). A single ras gene has been isolated to date from C. cinerea (Ishibashi and Shishido 1993). A dominant active allele of ras has been constructed, and was observed to cause changes in both directional and invasive growth of homokaryotic hyphae (Bottoli et al. 2001). When this mutant allele of ras was expressed in the homokaryotic Amut Bmut mutant strain that mimics the dikaryon, the formation of primary hyphal knots was found to be repressed (Bottoli et al. 2001). This finding suggests that this Ras protein may participate in the genetic control of fruiting. Interestingly, expression of the dominant active ras allele also appears to cause defects in mating linked to the B mating-type pathway (Kues et al. 2004). A dominant activating allele of a S. commune ras gene had a similar repressing effect on fruiting in dikaryons, although cAMP levels were found not to be different from controls (Yamagishi et al. 2004). This suggests that this particular ras gene may not act through the cAMP signaling pathway, but perhaps through the MAPK cascade. A second ras gene is likely to exist in this fungus, as it appears that two different ras genes are in C. cinerea from BLAST searches of the translated genome.

As mentioned earlier, the gene frt1 has been implicated as impinging upon the fruiting pathway in S. commune. This is because the gene was originally isolated by its' ability to induce fruiting in certain homokaryotic transformation recipients (Horton and Raper 1991). The transgene also enhanced the mainstream developmental process of dikaryotic fruiting. It was hypothesized that homokaryotic fruiting was induced due to a difference in the frt1 allelic type between the transgene and the endogenous gene residing in the recipient. A rigorous test of this hypothesis would be to isolate the proposed alternate allele of frt1, and to demonstrate that it had the ability to induce fruiting in a homokaryotic strain containing the characterized allele (i.e. the reciprocal experiment to the original). Unfortunately, extensive efforts to isolate an alternate allele of frt1 have proven to be unsuccessful to date (Horton et al. unpublished). The frt1
translation product is predicted on the basis of motif analysis to be a small ATP-binding protein of 192 amino acids (Horton and Raper 1995). BLAST searches have shown extensive similarity to the C-terminal half of XPMC2, a Xenopus laevis protein that has been shown to rescue a mitotic catastrophe phenotype in the fission yeast S. pombe (Su and Maller 1995). A number of translated ORFs of unknown function derived from genomic sequencing projects (including C. neoformans, but interestingly not U. maydis or C. cinereus) also exhibit significant similarity to Frt1. All of these other polypeptides are considerably larger than Frt1: there are anywhere from 100 to over 200 additional amino acids upstream of the region of similarity. The overlap region between Frt1 and these other proteins contains both the putative nucleotide-binding region and a lengthy exonuclease domain found in proteins involved in a wide variety of cellular functions, such as transcription, DNA replication, repair and recombination, cell cycle progression, and RNA processing (Moser et al. 1997). However, the localization of Frt1 to the cell wall in hyphae of S. commune (Palmer and Horton in preparation), and the absence of several key conserved residues at active sites of the exonuclease domain make it unlikely that Frt1 has a functional domain of this type. In contrast, the other predicted proteins all have the conserved active site residues that Frt1 lacks, and the XPMC2 protein has been localized to the nucleus (Su and Maller 1995). To further define gene function, Δfrt1 null strains were constructed by homologous recombination and gene replacement. It was found that dikaryons homoallelic for Δfrt1 fruited normally, indicating that FRT1 is not essential to the production of fruiting bodies (Horton et al. 1999). Homokaryotic Δfrt1 null strains exhibited a profuse growth of aerial hyphae, yielding characteristic fluffy colony morphology. Steady-state transcript levels of normally dikaryotic-expressed genes such as Sc1, Sc4, and Sc7 were greatly elevated in these Δfrt1 null strains as compared to wild type (Horton et al. 1999). These findings suggested a rethinking of the role of frt1. It is likely that a major function of this gene in homokaryons is to act as a negative regulator of dikaryotic gene expression, an important event in the control of fruiting. Given this function for frt1, its absence in dikaryons would not be expected to impair mushroom development. The peripheral cellular location of Frt1 and its likely role as a negative regulator of fruiting suggest that there must be additional regulatory elements downstream of frt1 responsible for the genetic control of mushroom development in this fungus.

Although the mechanism by which frt1 affects the process of mushroom formation is unclear, the gene has proven useful in identifying some potential players in the genetic control of fruiting. frt1 cDNA was used as bait in a yeast two-hybrid screen of a S. commune cDNA library in order to select clones encoding proteins that potentially interact with Frt1 (Horton et al. 2001). Approximately 7 X 10^6 clones of the library were screened using the CytoTrap Ras-rescue two-hybrid approach (Aronheim et al. 1997; Aronheim 2004). Amongst the putative interactors isolated was a cDNA encoding a likely membrane-spanning sugar transporter with strong similarities to those isolated from yeasts and filamentous fungi. While some members of the sugar transporter superfamily act as receptors for monosaccharides such as glucose, others act as sensors for a variety of nutritional signals (Lalonde et al. 1999; Moriya and Johnston 2004;
Ozcan and Johnston 1999; Versele et al. 2001). For example, the product of the rco-3 gene of Neurospora crassa encodes a glucose sensor protein (Madi et al. 1997). mutants in which affect macroconidia formation, which can serve as either asexual spores or as male gametes. Transcript levels of a sugar transporter were found to markedly increase during mushroom growth in the commercially important species Agaricus bisporus (Molloy et al. 2001). What is clear from a variety of studies on yeast and filamentous fungi is that multiple signaling events (pheromones, nutritional signals) are coordinated to regulate developmental processes. The possible interaction between Frt1 and a sugar transporter/sensor protein may provide a molecular link with previous studies describing carbon starvation as a necessary trigger for mushroom development in Basidiomycetes (reviewed in Kues 2000; Kues and Liu 2000).

As stated earlier, light is a necessary environmental cue that regulates mushroom development in both C. cinerea and S. commune. Several “blind” mutants of C. cinerea have been identified (Yuki et al. 2003) which produce etiolated, non-fertile fruiting bodies in the presence of light. This phenotype is normally observed only if cultures are incubated in constant darkness after secondary hyphal knot formation (reviewed in Kues 2000). The dst1 gene from C. cinerea has been identified as a result of the complementation of one of these mutants (Yuki et al. 2003) and appears to encode a protein of high similarity to WC-1, a blue light receptor in the Ascomycete Neurospora crassa (Ballario et al. 1996; Froehlich et al. 2002; He et al. 2002). It is hoped that further characterization of this gene will result in the elucidation of important molecular connections between light signaling and mushroom development.

3.3.9 Genes affecting mushroom morphogenesis

In addition to those genes that are instrumental in the initiation of mushroom development, a number of genes have been isolated that play a role in the later stages of mushroom morphogenesis. The ich1 gene of C. cinerea was isolated from a spontaneous recessive mutant that when present in homozygous form in a dikaryon results in a capless fruiting body void of spore production. The gene encodes a 1353 amino acid protein predicted to have three nuclear targeting signals, and is expressed specifically in the mushroom cap in wild-type strains (Muraguchi and Kamada 1998). NCBI domain searches revealed a possible S-adenosylmethionine (SAM)-dependent methyltransferase domain in the Ich1 protein. It is unclear how the presence of both this domain and the putative nuclear localization signals in Ich1 can be reconciled. An enzyme from Aspergillus flavus sharing this methyltransferase domain is responsible for the conversion of sterigmatocystin to O-methylsterigmatocystin in the aflatoxin biosynthetic pathway (Keller et al. 1993). Aflatoxin is likely to play a role in Aspergillus development: when production is blocked, development of sclerotia and basidiospores are also inhibited (Guzman-de Pena and Ruiz-Herrera 1997; Trail et al. 1995). Kues (2000) speculates that Ich1 might help produce an analogous substance to aflatoxin, and that this substance may play a role in signaling within the fruiting body primordia. It is interesting to point out that the predicted protein product for the essential fruiting gene cfs1 from C. cinerea also has a SAM-dependent methyltransferase domain, although the
substrate for this enzyme is likely some sort of membrane phospholipid (Kues et al. 2004). BLAST searches revealed that the most significant similarity (E value = 5 × 10⁻³⁴) to Ich1 was to a hypothetical protein of unknown function translated from the U. maydis genome. Other significant hits were largely to plant cell surface proteins, due to the proline-rich C-terminal third of the Ich1 protein.

Two different mutants producing mushrooms with abnormally short stipes have been used to isolate the eln2 and eln3 genes in C. cinerea. eln2 encodes a novel type of microsomal cytochrome P450, and is constitutively expressed in both vegetative mycelia and developing mushrooms (Muraguchi and Kamada, 2000). The substrate(s) and the metabolite(s) of this particular enzymatic activity are unknown at present, as is the mechanism of how this protein participates in the process of mushroom morphogenesis. A cytochrome P450 gene has been reported to be specifically expressed in fruiting bodies of the commercial mushroom Agaricus bisporus (de Groot et al. 1997). eln3 encodes a predicted transmembrane protein with a general glycotransferase domain, and has high similarity to a number of hypothetical proteins from filamentous fungi (Arima et al. 2004). Kamada and co-workers have put forth the notion that the disorganization of stipe tissue in the loss of function mutant eln3 is the result of a defect in the mechanism responsible for cell-to-cell connections in that tissue. Eln3 might be involved in adding a carbohydrate moiety onto extracellular matrix proteins, which might be necessary for proper cellular organization within the stipe of the mushroom (Arima et al. 2004).

3.3.10 Structural genes

As mentioned earlier, the aggregation of hyphae is a prerequisite for fruiting body formation, and most certainly involves cell wall and extracellular matrix proteins (reviewed by Walser et al. 2003). Carbohydrate-binding lectins have been strongly implicated in both hyphal aggregation and mushroom development (reviewed in Wang et al. 1998). The genes cgl1 and cgl2 encode fruiting-specific galectins regulated by the A mating-type pathway in C. cinerea (Cooper et al. 1997; Boulianne et al. 2000). Galectins are a class of lectin that specifically bind β-galactoside sugars in a calcium-independent manner, and are conserved in sequence within their carbohydrate-recognition domain (Barondes et al. 1994 a; Barondes et al. 1994 b). The two galectin genes are differentially expressed during fruiting body development. Cgl1 is specifically expressed in primordia and mature fruiting bodies, while Cgl2 is initiated earlier, at the time of hyphal knot formation, and maintained until fruiting body maturation (Boulianne et al. 2000). This differential expression between the two galectins may in part be explained by the presence of two cAMP response elements (CREs, Conkright et al. 2003) in the promoter of cgl2, and their absence in cgl1 (Bertossa et al. submitted). cgl2 expression occurs concomitantly with a rise in cAMP levels at the time of hyphal knot formation, whereas cgl1 expression is only observed during the formation of fruiting body primordia, after cAMP levels peak (Bottoli 2001; Boulianne et al. 2000). Interestingly, each of the C. cinerea galectin genes have a promoter element (GATGX₁₁CAA) that is a potential binding site for the heterodimer of HD protein products of the A mating-type.
genes, and identical to that seen in the C. cinerea regulatory gene clp1. However, the replacement of this element by an unrelated linker sequence in the cgI2 promoter did not influence transcription of a heterologous reporter gene (Bertossa et al. submitted). This result suggests that transcriptional regulation of cgI2 (and perhaps by inference, cgI1) by A mating-type proteins is likely to be indirect. Both galectins have been localized primarily to the cell wall and extracellular matrix (ECM), but because they lack an obvious signal sequence, they are secreted by a non-classical pathway (Boullianne et al. 2000). Given the developmental regulation of their expression and their peripheral cellular localization, it would seem reasonable to propose that galectins may play a key role in the hyphal aggregation process necessary for fruiting body development. To date, neither cgI1 nor cgI2 have been disrupted, so no galectin-deficient strains have been generated to assess whether or not they are essential to mushroom development.

Another important class of structural proteins found in mushrooms, and indeed aerial structures of many fungi are the hydrophobins (reviewed in Wosten 2001; Wessels 1996). Hydrophobins were originally discovered in S. commune as products of genes that were highly expressed during the formation of fruiting bodies, and are small secreted proteins that contain eight cysteine residues in a conserved pattern (Mulder and Wessels 1986; Schuren and Wessels 1990). These proteins self-assemble at the outer surface of hyphae, covering them with an amphipathic membrane that lowers surface tension at the water/air interface, which in turn enables the hyphae to emerge into the air (Wosten et al. 1999). S. commune contains at least four hydrophobin genes, scI, sc6, sc3, and sc4, with the latter two being the best characterized. The SC3 protein is expressed in both homokaryons and dikaryons, while SC4 is dikaryon-specific (Wessels et al. 1995). SC3 is found in hyphae covering the outer surface of the fruiting bodies (Asgeirsdottir et al. 1995) whereas SC4 lines the air channels within (Lugones et al. 1999). Knockouts of sc3 and sc4 have shown that neither gene is absolutely necessary for the formation of aerial hyphae and fruiting bodies. In Δsc3 and Δsc3Δsc4 dikaryons fruiting was delayed, and the number of mushrooms were decreased by 50-80%, as compared to wild-type or Δsc4 strains (van Wetter et al. 2000). The greater effect of the Δsc3 knockout on fruiting is somewhat surprising, given that SC4 is localized exclusively in fruiting bodies, and SC3 is not.

3.3.11 Future prospects

While it is safe to say that the molecular genetic study of mushroom development is still in its early stages, the next decade promises to be a time where great strides will be made in our understanding of this process so fundamental to sexual reproduction in many Basidiomycete species. Connections are starting to be made between known elements of signaling pathways and the molecular mechanisms controlling fruiting. The long documented link between cAMP metabolism and mushroom development is finally getting some molecular genetic underpinning. Of high importance will be defining the roles of the individual elements of the cAMP and MAPK pathways, and integrating these into a coherent conceptual framework as they relate to each other, and
mushroom development. There are now some encouraging initial forays into exploring the molecular links between known environmental cues of fruiting, such as light and nutrition, and the initiation of the process itself. The comparison of the molecular genetic regulation of fruiting in two discrete model systems will be of particular use in applying these concepts to commercially important mushroom species.

Many of the tools used to date to explore the genetics of mushroom development will continue in their useful role. Mutants defective in the process will help elucidate still more of the key elements, especially in those cases concerning genes that might be unique to the mushroom-producing fungi, and therefore recalcitrant to comparative genomics approaches. The isolation of suppressor mutants has been under-utilized in the molecular study of fruiting. Existing experimental approaches will be accompanied by new resources such as the Coprinopsis cinerea genome, microarray analyses, the development of GFP-tagging for cytological studies, and RNAi for examining gene function, amongst others. There is every reason to be optimistic that our understanding of the genetic mechanisms controlling mushroom development will grow substantially in the near future.

4. CONCLUSIONS

In this chapter, we have presented an overview of the current knowledge of the genetic control of morphogenesis in several exemplary basidiomycete systems: smuts, rusts and mushrooms. The discussion presented herein reveals a staggering level of complexity and a multitude of questions that remain unanswered in this fascinating field. Therefore, it is highly encouraging and quite apparent that the future directions of research using the above-mentioned model systems will utilize the newly acquired and/or rapidly expanding genomic and EST database resources. Coupled with newly acquired tools such as microarray-based studies and general improved technology for transformation and gene specific mutagenesis, these resources will further enhance and rapidly accelerate these studies, providing a more unified model of basidiomycete development. As discussed in this chapter, such approaches have already begun to yield rapid advances of our knowledge in this area.

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