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Review

Sex in fungi: lessons of gene regulation

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ABSTRACT. Fungi have been very useful for gene regulation studies. Mating implicates in a series of events influenced by many types of environmental input that are interpreted into regulatory pathways, including signal transduction. Although various aspects of mating and signal transduction in the yeast *Saccharomyces cerevisiae* have long been characterized, recent findings in filamentous fungi indicate that pheromones and pheromone receptors may be essential for mating partner recognition and also for nucleus recognition in sorting before meiosis. A brief overview on mating-type genes of ascomycete fungi and recent contributions to the understanding of their role in the regulation of multicellularity and sexual dimorphism is presented in this review.

Key words: Mating-type gene, Fungi

INTRODUCTION

Sex is especially regarded as the mechanism through which genetic diversity of a species is guaranteed. Genetic barriers have evolved in different ways to avoid selfcrossing, culminating, mechanistically, with sexual dimorphism. The X-Y system is the most familiar sex-determining mechanism; genetic recombination has been almost totally suppressed between the sex chromosomes and this ensures exchange of genetic material between two different individuals, at least for the other chromosomes. In many lower eukaryotes, such as fungi, sexual exchange of genetic material relies on the existence of simple cell identity mechanisms that stimulate outcrossing. Individuals having distinct cell identities can then be considered of different mating types.

As with any other complex mechanism in living organisms, the choice for entering or not the sexual cycle depends on many environmental inputs and regulatory proteins. Although the fungi as a group have diverse forms of life, one can generalize that they have a haploid genetic complement, and a (normally short) diploid phase, followed by meiosis. An organism can be perpetuated in the vegetative haploid state by successive mitotic divisions and also by producing haploid spores mitotically. Alternatively, crossing can be advantageous at a certain moment of the life cycle (changes in nutrient or CO_2 concentrations), and two haploid cells (usually with a different genetic content) can fuse. When the two nuclei fuse, the diploid state can be maintained by mitotic divisions, or else this cell goes through meiosis, producing four haploid spores.

The term 'mating type' is used to differentiate individuals that are sexually compatible. Sexual dimorphism is almost non-existent in fungi and in most cases (as in *Neurospora crassa*, for example) individuals are hermaphrodites, i.e., they produce both male and female reproductive structures. The existence of mating types was first recognized at the beginning of the twentieth century in the fungus *Rhizopus* by Blakeslee (1904), who also introduced the terms homothalism and heterothalism for self-fertile and self-sterile individuals, respectively. However, it was not until the early 80s that the first molecular characterization of mating-type loci was achieved for the yeast *Saccharomyces cerevisiae* (Astell et al., 1981). In 1988, the mating-type genes of the filamentous ascomycete *N. crassa* were also cloned (Glass et al., 1988). The development of new techniques, such as PCR, now allows easy isolation and characterization of mating-type genes of several fungi. Early DNA sequence analysis indicated that the term allele was not appropriate to denote alternate mating types since they consist of mainly dissimilar sequences that do not appear to have common evolutionary origins; the term idiomorph was proposed instead (Metzenberg and Glass, 1990). Analysis of these genes revealed that they mostly encode transcription factors, and in some fungi they also encode pheromones and pheromone receptors. The transcription factors are involved in the expression of specific proteins, giving the cell its mating identity. A transduction machinery, involving G-proteins, mitogen-activated protein kinases, adenylate cyclase and cAMP-dependent protein kinases, allows the cell to respond to pheromone signals and to integrate this response with the cell physiology (see next section).

The Ascomycotina and the Basidiomycotina are two of the three major groups of the kingdom Fungi. The structures of mating type loci in these two groups differ greatly in the number of genes and alleles. Mating types are classified as bipolar or tetrapolar, depending on the existence of two or four mating determinants, respectively. In the ascomycetes, there is one mating-type locus, with two alternate alleles; thus, mating is bipolar (reviewed in Kronstad and

Staben, 1997; Pöggeler, 2001). Mating types in basidiomycetes can be extremely complex, with both bipolar and tetrapolar mating systems (reviewed in Casselton and Olesnicky, 1998; Kothe, 1999; Casselton, 2002). The number of mating types varies, depending upon whether they are determined by two loci with two or more different alleles or by several loci bearing hundreds of alleles that result in several thousands of mating types.

The identification of components of pheromone and signal transduction systems in Basidiomycetes indicates similarities between some mating strategies in this group and the Ascomycotina. In contrast to the Basidiomycotina, the Ascomycotina exhibit specialized mating structures (male and female gametes) and the dikaryotic phase is very short compared to the prolonged vegetative dikaryotic stage of basidiomycetous fungi. Although some aspects of mating are essentially different (cell fusion, cell differentiation and multicellularity), it seems that the basic processes through which mating and meiosis occur are conserved in fungi.

MATING IN *SACCHAROMYCES CEREVISIAE*

The ascomycete *S. cerevisiae* has the best studied mating-type locus. Many eukaryotic gene regulatory pathways have been elucidated through the studies of yeast mating type. Various functions in the *S. cerevisiae* life cycle are interconnected due to mating-type locus regulation. Mating-type genes determine the cell type as haploid or diploid and also determine the choice between sexual or vegetative growth. The two haploid cell types are denominated a and α and their identity is determined by sequences present at the *MAT* locus. Each a and α cell contains sequence determinants for both cell types but, by a process of internal recombination and transcription silencing, a haploid cell can switch to the opposite mating type (Herskowitz et al., 1992). The diploid cell (a/α) results from pheromone-mediated fusion of two haploid cells. Under nutrient starvation the fate of the diploid cell is meiosis, with the formation of four haploid cells, two of which are a, and two are α.

Each *MAT*a and *MAT*α idiomorph contains two divergently transcribed genes: a1, a2 and α1, α2, respectively; these genes encode transcription factors that are involved in the determination of cell identity, except for a2 whose function is unknown (Herskowitz et al., 1992). In haploid cells, α1 regulates the expression of haploid-specific and cell type-specific genes, including α-specific pheromones and pheromone receptors, but a1 is not required for expression of any a-specific genes (Fields, 1990). Mating is triggered, following binding of pheromones to receptors. One of the earliest responses in the conjugation process is the induction of cell surface agglutinins that facilitate mating (reviewed in Lipke and Kurjan, 1992). The cells then synchronize their cycles by arresting at the G1 phase, i.e., before DNA replication. A diploid cell cannot express genes needed for mating but instead drives the cellular process to meiosis and sporulation, depending on environmental conditions (reviewed in Herskowitz et al., 1992). A novel regulatory factor is formed by the interaction of a1 and α2 proteins that represses both α1 and general haploid cell functions (Goutte and Johnson, 1988; Dranginis, 1990).

Cloning and characterization of the a and α mating-type locus showed that these regions are composed of non-homologous sequences (Strathern et al., 1980; Astell et al., 1981). The a1 and α1 proteins are encoded by totally dissimilar sequences of 642 and 747 bp, respectively, while a2 and α2 sequences have partial homology (Nasmyth and Tatchell, 1980; Strathern et al., 1980; Astell et al., 1981). The a1 and α2 proteins are transcriptional repressors (when both are present) and both contain a homeodomain DNA-binding motif (Shepherd et al.,

1984). The α1 protein has been shown to be a transcription activator (Sprague et al., 1983; Sengupta and Cochran, 1991) but its DNA-binding domain (the α-box) has yet to be characterized in detail.

Pheromone and pheromone-receptor genes are specific for each cell type but haploid-specific genes are equally expressed in a and α cells. In a cells, a-specific genes are constitutively expressed after co-operative binding of Ste12 and a general transcription activator Mcm1 to a-specific genes. Because *STE12* and *MCM1* are also expressed in α cells, an obvious explanation of the absence of expression of a-specific genes in α and a/α cells is the presence of transcription repressors of these a-specific genes. This repression depends on a complex formed by α2, Ssn6, Tup1, and Mcm1 that binds to a-specific promoters (reviewed in Johnson, 1995). Ste12 and Mcm1, when in a complex with α1, positively regulate α-specific genes by binding to their promoter at sequences known as the QP' element. In diploid cells, the a1/α2 complex directs the Tup1/Ssn6 repressor to the promoter of haploid-specific genes. The completion of the genome sequencing has permitted *in silica* analyses of target genes of mating-type regulators, such as those regulated by Mcm1/α2 complex genes (Zhong et al.,1999).

While pheromone-mediated intercellular communication permits the recognition of a and α cells prior to fusion, later processes depend on a G-protein signaling pathway (reviewed in Leberer et al., 1997; Bölker, 1998). The yeast G-protein involved in mating consists of three subunits (α, β, γ) and many genes that code for components of the signal transduction pathway have been identified, such as *STE5*, *STE7*, *STE11*, *STE12*, *FUS3* and *KSS1* (reviewed in Marsh et al., 1991). Mutations in these genes lead to an inability to respond to pheromone. Binding of pheromone to receptors causes the trimeric G-protein to dissociate, which activates a cascade of events (through kinases and transcription factors) that ultimately arrest the cell cycle at G1. The decision to enter mitotic or meiotic division depends on nutritional factors. A cAMP-dependent signal transduction pathway is involved in sensing nitrogen and glucose levels and this cascade exerts its effects on a gene responsible for meiosis induction, *IME1* (reviewed in Klein et al., 1994). *IME1* is under the repressive control of *RME1* (repressor of meiosis). Transcription of *RME1* is repressed in diploid *MAT*a/*MAT*α cells by the action of the heterodimer a1/α2, so that meiosis and sporulation can proceed (Mitchell and Herskowitz, 1986).

MATING IN FILAMENTOUS ASCOMYCETES

The two best studied mating types of filamentous ascomycetes are those of *N. crassa* and *Podospora anserina*, probably because they were cloned only a few years after *S. cerevisiae* mating sequences were first isolated. More recently, several new mating-type genes have been isolated and they show similarities with these initially isolated mating-type genes (see following section). *Neurospora crassa* is self-sterile and has two alternate mating types, termed *A* and *a.* Both the *A* and *a* idiomorphs have been cloned and characterized (Glass et al., 1988, 1990; Staben and Yanofsky, 1990). *Podospora anserina* is closely related to *Neurospora* and it is pseudohomothallic. It also has two mating types, *mat*+ and *mat*-, and crosses produce ascospores with two opposite mating-type nuclei or with only a single nucleus. Sequencing analyses of *mat*- have shown structural and sequence similarities between genes encoded by the *N. crassa* (Debuchy and Coppin, 1992). The *N. crassa mat a* idiomorph is a 3.2-kb DNA segment and contains a 747-bp open-reading frame (ORF) corresponding to the *mat a-1* gene (Staben and Yanofsky, 1990). The *N. crassa* MAT a-1 and the *P. anserina* FPR1 polypeptides contain a

high mobility group (HMG) DNA-binding motif. Different from *FPR1*, mutations in *mat a-1* abolish *a* mating identity and heterokaryon incompatibility (Griffiths and DeLange, 1978; Staben and Yanofsky, 1990). A MAT a-1 polypeptide synthesized in *Escherichia coli* has been shown to specifically bind *in vitro* the sequence 5'-CTTTG-3' through its HMG domain (Philley and Staben, 1994). The *mat a-1* gene was shown to be sufficient to confer all *a* functions by a gene replacement of the *A* idiomorph with the whole *a* idiomorph or the *mat a-1* ORF only (Chang and Staben, 1994). Recently, a second ORF (*mat a-2*) has been characterized in the *a* idiomorph, which is co-transcribed with *mat a-1* (Pöggeler and Kück, 2000). The *A* idiomorph is a 5.3-kb region that has very little DNA sequence similarity to the *a* idiomorph (Glass et al., 1990). The *mat A* idiomorph contains three genes, *mat A-1*, *mat A-2* and *mat A-3* (Ferreira et al., 1996). The *mat A-1* gene is required for *mat A* mating identity, postfertilization functions, and vegetative incompatibility with *mat a* strains (Glass et al., 1990; Saupe et al., 1996). *FMR1*, *SMR1* and *SMR2* are encoded by the *mat-* mating-type locus of *P. anserina* (Debuchy et al., 1993). MAT A-1 and FMR1 have similarity to the *S. cerevisiae* mating-type transcriptional activator MATα1 (Sprague et al., 1983). MAT A-2 shows about 20% identity with the mating-type polypeptide SMR1 (Ferreira et al., 1996) but does not share similarities with other known protein domains. MAT A-3 has an HMG domain and shows approximately 20% identity with both MAT a-1 and SMR2 from *P. anserina* (Debuchy et al., 1993; Ferreira et al., 1996). In the *N. crassa* idiomorph flanking regions there are several ORFs (*eat*) that have varied expression and may also be involved in mating and sexual development (Randall and Metzenberg, 1998).

A study involving the reciprocal introduction of mating-type genes in *P. anserina* and *N. crassa* was undertaken to test whether structural similarities in mating types also reflect functional correspondence (Arnaise et al., 1993). The results indicated that the *N. crassa* and *P. anserina* mating-type idiomorphs are interchangeable in relation to mating reactions but not for heterokaryon incompatibility or postfertilization functions. Postfertilization functions can be restored in the *P. anserina mat+* deletion strain, even with ectopic copies of *mat+* or *mat-* (Coppin et al., 1993). It is possible that ectopically positioned *N. crassa A* and *a* idiomorphs cannot rescue postfertilization functions in *P. anserina*, as do *mat-* and *mat+* (Arnaise et al., 1993).

Mutation analyses of the *mat* A idiomorph suggest that *mat A-1* and *mat a-1* are the critical factors for both mating and sexual development in *N. crassa*; *mat A-2* and *mat A-3* increase the efficiency of the process but are not essential for the production of ascospore progeny (Glass and Lee, 1992; Ferreira et al., 1998). Further studies with *N. crassa* mating-type genes have been hampered by the fact that ectopic copies of mating type do not fully restore fertilization functions in transformation experiments. However, *P. anserina* has been used intensely in the investigation of the involvement of mating-type genes in cell recognition (Zickler et al., 1995; Coppin and Debuchy, 2000; Arnaise et al., 1997, 2001). It was initially understood that *FPR1*, *FMR1* and *SMR2* were involved in internuclear recognition and led to the production of uniparental progeny and aberrant meiosis; *SMR1* seemed to be involved only in postfertilization functions (Zickler et al., 1995; Arnaise et al., 1997). Deregulated or overexpressed genes can lead to mortality of the ascospores, suggesting a fine regulation (repression and/or activation) of these genes, being essential for the proper functioning of the sexual machinery (Coppin and Debuchy, 2000). Recently it has been shown by genetic analysis that mutations in *FPR1*, *FMR1* and *SMR2* can result in self-fertile strains (Arnaise et al., 2001). These mating-type mutants have been isolated, using suppression of sterility due to SMR1 mutations as the selectable phenotype. It has been suggested from this study that FMR1 and

SMR2 act as repressors of *mat*+ functions and as activators of *mat*- functions. Conversely, FPR1 would be a repressor for *mat*- and an activator for *mat*+ functions. These data can explain the previously observed uniparental asci if one assumes that in *MAT* mutants expression of the opposite nuclear identity triggers self recognition and that there is competition for pairing of identical versus different nuclei during pre-meiosis migration of two nuclei to the tip cell. Though interaction of mating-type proteins of *N. crassa* and of *S. macrospora* has been demonstrated *in vitro* (Badgett and Staben, 1999; Jacobsen et al., 2002), it was not possible to determine whether mating-type proteins act directly as repressors or, as in yeast, repression is achieved by the activation of a repressor protein by the mating-type protein.

Despite the similarity in the organization and sequences on the mating-type loci of *N. crassa* and *P. anserina*, some strategies seem to be quite different in these two fungi. A promising strategy has risen that may facilitate studies of the function of *N. crassa* mating-type genes in internuclear recognition. Recently, it has been shown that due to suppression of a phenomenon reported so far only in *N. crassa*, termed meiotic silencing of unpaired DNA (MSUD), a mating-type deletion strain containing an ectopically integrated copy of *MAT* gene may be complemented for postfertilization functions (Shiu et al., 2001; Shiu and Metzenberg, 2002). When a mutant (*sad-1*), deficient in such silencing, is crossed to a strain containing sequences of opposite mating type in an ectopic location, the cross is at least 100 times more fertile than when the cross is done with the *sad-1*$^+$ counterpart (Shiu et al., 2001). This strain will enable the study of the functions of the mating-type proteins and will help in understanding whether the four genes are *bona fide* mating types and their exact role in nuclear recognition. This may clarify whether, despite the similarities in the structure and sequences of the mating-type genes in *N. crassa* and *P. anserina*, the mechanisms for cellular and nuclear recognition have evolved differently in these two closely related strains.

Signal transduction is involved in the mating-type response in yeast and seems to be an important part of the mating response in *N. crassa*. Three genes coding for G-proteins have been identified in *N. crassa*: *gna-1*, *gna-2* and *gna-3* (Turner and Borkovich, 1993; Kays et al., 2000). As in *S. cerevisiae*, putative pheromone precursor genes (Pöggeler and Kück, 2000; Bobrowicz et al., 2002) and pheromone receptors (Pöggeler and Kück, 2001) have been identified in *N. crassa*. The gene *ccg-4* encodes a polypeptide containing multiple repeats of a putative pheromone sequence bordered by Kex2-processing sites and is expressed exclusively in *mat* A strains. In *mat* a strains another gene, termed *mfa-1*, is expressed that codes for a pheromone precursor whose mature pheromone is predicted to have a C-terminal carboxy-methyl isoprenylated cysteine. Both pheromone sequences seem to be very similar to those encoded by other filamentous ascomycetes. The expression of the pheromone precursor genes is mating-type specific and is under the control of the mating-type locus (Bobrowicz et al., 2002). The putative receptors, PRE1 and PRE2, show significant similarity to the yeast pheromones Ste2 (α-factor) and Ste3 (a-factor). Interestingly, these two products seem to be expressed in both mating types, as shown in Northern and RT-PCR analyses with RNA isolated from several phases of the sexual cycle. Similar results have been obtained with the homothallic species *S. macrospora* pheromone genes (Pöggeler and Kück, 2001). As suggested previously by Debuchy (1999), it may be that pheromones and their receptors in filamentous ascomycetes are required, not only for recognition of the cells before mating, but also for premeiotic recognition of nuclear identity; thus, its expression in homothallic species is explained. The *N. crassa* homologue of *TUP1*, *RCO-1* (Yamashiro et al., 1996), does not seem to affect pheromone expression in the

same fashion as in *S. cerevisiae* (Bobrowicz et al., 2002). Isolation of strains containing mutations in the pheromone precursor and pheromone receptor genes will clarify eventual peculiarities of the pheromone response in *N. crassa* and other ascomycetes.

MATING TYPE IN OTHER ASCOMYCETES

Cochliobolus heterostrophus is a pathogenic ascomycete that infects corn leaves. It is heterothallic, and mating is controlled by the *MAT-1* and *MAT-2* idiomorphs, later named *MAT1-1* and *MAT1-2*, respectively (Turgeon and Yoder, 2000). Unlike *P. anserina* mating-type sequences, *C. heterostrophus* mating-type genes do not hybridize to *N. crassa* idiomorphs, even under low stringency conditions (Turgeon et al., 1993). Isolation of *MAT1-1* genes has been made based on the change from the uni-mating phenotype to dual mating upon transformation of a *MAT1-2* strain with a cosmid library from a *MAT1-1* strain. The opposite mating type was isolated after screening of a cosmid library with the *MAT1-1* clone. The *C. heterostrophus* idiomorphs are considerably smaller than the *P. anserina* and *N. crassa* counterparts. A single ORF has been characterized in each idiomorph, *MAT1-1* and *MAT1-2* (Turgeon et al., 1993). The predicted MAT1-1 and MAT1-2 proteins have regions of similarity with MAT A-1/FMR1 and MAT a-1/FPR1, corresponding to the α-box and HMG domains, respectively. Furthermore, *mat*-specific homologs are functionally interchangeable in relation to mating identity among species of *Cochliobolus*, *Neurospora* and *Podospora* (Turgeon et al., 1993; Arnaise et al., 1993). By hybridization with the *C. heterostrophus* mating-type sequences, it was shown that several other heterothallic *Cochiobolus* sp. have either *MAT1-1* or *MAT1-2* homologs and two homothallic species hybridized to both idiomorphs (Turgeon et al., 1995). *Bipolaris sacchari*, which is related to *C. heterostrophus*, was the first asexual species to have mating-type genes cloned (Sharon et al., 1996). It contains a *MAT1-2* homologue that confers dual mating when transformed into a *MAT1-1 C. heterostrophus* strain. It seems that *B. sacchari* lacks genes other than *MAT* genes that are important for the sexual cycle because it fails to cross, even when carrying transgenic *MAT1-1* or *MAT1-2* from *C. heterostrophus.* Idiomorphic regions that correspond to the sporulation control region of *P. anserina* (and *N. crassa*) are absent in *C. heterostrophus*; thus, if genes controlling postfertilization functions work the same way in this ascomycete, they must be present in locations other than at the mating-type locus.

The mating-type genes of the homothallic *Sordaria macrospora* have also been cloned (Pöggeler et al., 1997). It has one mating-type locus containing four genes homologous to the *N. crassa* mating-type *genes (Smt a-1*, *Smt A-1*, *Smt A-2* and *Smt A-3*). Similarly to *N. crassa* (Badgett and Staben, 1999), *Smt A-1 and Smt a-1* have been shown to act as activators and they can interact with each other in yeast reporter systems (Jacobsen et al., 2002).

CLONING OF NEW *MAT* GENES

Although mating-type genes of different species may have diverged relatively quickly, similarities of some functional regions (such as HMG and α-box) can reach as much as 90%. Arie et al. (1997) have developed a method for efficiently cloning mating-type genes based on such regions and PCR techniques. Primers were based on similarities of the HMG regions of *C. heterostrophus*, *N. crassa* and *P. anserina*. During the last couple of years, mating types from

other ascomycetes have been cloned using this same technique or based on homology of the flanking or idiomorphic regions (Arie et al., 2000; Yun et al., 2000; McGuire et al., 2001).

A sexual phase has not been described for the phytopathogenic fungi *Fusarium oxysporum* and *Alternaria alternata*. The *MAT1-2* idiomorphs of both fungi were cloned using the PCR approach (Arie et al., 2000). The *MAT1-1* gene was cloned based on the hypothesis that the flanking sequences of both idiomorphs were similar. *MAT1-1* has similarities with the *N. crassa mat* A-1 (α-domain) and *MAT1-2* codes for an HMG domain protein similar to *N. crassa mat a-1*. The *F. oxysporum MAT1-1* has 5,220 bp and *MAT1-2*, 5122. *Alternaria alternata MAT1-1* and *MAT1-2*, are 2,667 and 2,978 bp, respectively, including the flanking regions.

The *MAT* genes of *Cryphonectria parasitica*, the cause of chestnut blight, have also been cloned (McGuire et al., 2001). They are structurally similar to other idiomorphs of ascomycetes and they have four structural genes. Differently from what happens in most ascomycetes, there is a gradual transition from idiomorphic to flanking sequences in *C. parasitica* idiomorphs. The *MAT1-1* idiomorph (4,691 bp) encodes MAT1-1-1, MAT1-1-2 and MAT1-1-3; MAT1-2 (2,810 bp) encodes a single gene, MAT1-2-1.

The *MAT1-2* idiomorph of *Mycosphaerella graminicola* has been cloned by heterologous hybridization with an 850-bp PCR fragment from the HMG region of the fungus *Tapesia yallundae*. The idiomorph is 2,772 bp long and as expected, shows high homology with *MAT* genes that encode HMG proteins. The *MAT1-1* idiomorph was identified by PCR, using primers designed from the previously cloned *M. graminicola MAT1-2*. *Mycosphaerella graminicola MAT1-1* has 2,839 bp and encodes an α-domain protein (Waalwijk et al., 2002).

Another fungus of medical importance is the diploid yeast *Candida albicans*. Until 1999, it was not believed that *C. albicans* had mating-type sequences or could undergo sexual reproduction. Using probes based on sequences from the Stanford *C. albicans* Sequencing Project that were similar to *S. cerevisiae MAT* loci, genes homologous to regulators of the sexual cycle (a1, α1 and α2) were identified (Hull and Johnson, 1999). These genes were termed *MTLa* and *MTLα* mating-type-like, analogously to the yeast nomenclature. Using different approaches, experimental matings with some *C. albicans* strains were performed by Hull et al. (2000) and Magee and Magee (2000). In both studies, strains with homozygous MTL (a or α) and auxotrophic markers for mating were constructed. Tetraploid cells, which contained both parental genetic contents, were recovered. Based on these data, it is suggested that *C. albicans* has a complete sexual cycle. Recently, it has been shown that the ability to switch colony morphology between white and opaque is also controlled by the MTL genes (Miller and Johnson, 2002). The mating rate in opaque cells is 10^6 times higher than for white cells. A brief review of these findings has recently been published (Hull and Heitman, 2002).

POSSIBLE TARGETS OF MATING-TYPE PROTEINS

Although the role of mating-type genes and regulation mechanisms on other genes is known with considerable detail in *S. cerevisiae*, in other ascomycetes not much is known about genes under the control of mating-type proteins. Although there is evidence for the control of some genes by mating-type genes, such as the pheromone genes (Bobrowicz et al., 2002), direct target genes of the mating-type products have not yet been described in ascomycetes. In *Neurospora* there are a few examples of genes known to be dependent on the expression of MAT A-1 and MAT a-1. A few genes regulated by MAT A-1 have been described through

reverse genetics (Nelson and Metzenberg, 1992). The expression analysis of some of those genes, in several different mating-type mutants, suggested that regulation of at least two of them needs the complexation of mating-type proteins in a manner analogous to what is known for *S. cerevisiae* (Ferreira et al., 1998). Some ORFs present in the flanking regions of the mating-type idiomorphs also exhibit mating type-dependent expression (Randall and Metzenberg, 1998). The analysis of promoter regions of such genes may give a clue towards consensus sequences for mating type-regulated genes. The identification of binding sites of mating-type products may be useful in genomic studies, as has been the case for the *S. cerevisiae* α2-Mcm1 complex (Zhong et al., 1999); the genome database could be scanned for potential target genes of the mating-type proteins.

CONCLUDING REMARKS

Studies on mating type in fungi have been helpful for the understanding of several eukaryotic regulation pathways, including cell cycle regulation, cellular and nuclear identity, and signal transduction. Mating may serve as a model for the study of developmental genetics and could help in elucidating regulatory mechanisms of multicellularity and sexual dimorphism.

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