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Primer

Fungal mating-type loci

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Fungi form one of the most ubiquitous and successful kingdoms of life on Earth. There are more than 100,000 known species of fungi, divided into four phyla — ascomycetes, basidiomycetes, zygomycetes and chytrids — and these have populated a vast array of biological niches, including those that are pathogens of animals (including humans and insects), plants and even other microorganisms. The morphological diversity among species is staggering, from the budding yeasts and filamentous fungi to the mushrooms and wood rotting organisms. Fungi also serve as powerful tools for our understanding of cell biology, and several have emerged as model systems, including most notably the budding yeast *Saccharomyces cerevisiae*. We shall review here our current understanding of the role of a specialized region of the fungal genome known as the mating-type locus, which plays a central role in the sexual cycle.

The mating-type, or *MAT*, locus is a unique region of the fungal genome that governs the establishment of cell-type identity and orchestrates the sexual cycle. It is this region that differs in DNA sequence between cells of opposite mating-type. The *MAT* locus encodes global transcription factors, which establish cell-type identity by controlling the expression of developmental cascades, and this commonly involves homeodomain or other classes of transcriptional regulatory elements. Some fungi exist in only two mating-types (bipolar), whereas others occur in hundreds or thousands of mating-types (tetrapolar). Some fungi are also endowed with the capacity to switch mating-type (homothallic), whereas others are not (heterothallic). The mechanisms that give rise to only two versus many mating-types, and those that enable mating-type switching are now understood in considerable molecular detail.

The mating-type locus of fungi has evolved into two different organizational paradigms: the bipolar and tetrapolar systems. In organisms with a bipolar mating-type system, cells are of two opposite mating-types — commonly **a** and α or plus and minus — and cell identity is dictated by a single *MAT* locus that has two alternative alleles. For sexual reproduction to occur, mating partner cells must have different *MAT* alleles. Bipolar systems promote inbreeding, and can lead to the rapid homozygosis of recessive mutations in organisms that are predominantly diploid, such as *S. cerevisiae*.

Fungi with a tetrapolar system, by contrast, arrange their mating-type information in two distinct unlinked regions of the genome, commonly known as the *a* and *b* loci. Both of these regions must differ for mating to occur. In many tetrapolar fungi, the mating-type loci are multiallelic, giving rise to thousands of mating-types in the most extreme examples. Tetrapolar systems promote outbreeding, as any given meiotic segregant can only interbreed with 25% of its siblings from a cross: *a1 b1* can mate with *a2 b2*, but not with *a1 b1*, *a1 b2* or *a2 b1*. The evolution of these paradigms in part reflects the evolution of different phyla of fungi, in that ascomycetous fungi have bipolar mating-type systems, whereas basidiomycetous fungi may be either bipolar or tetrapolar.

Saccharomyces cerevisiae — the yeast *MAT* paradigm

The first paradigm for understanding fungal cell identity came from studies of the mating-type locus of *S. cerevisiae*. This organism has two haploid mating-types, **a** and α, which signal via secreted peptide pheromones that trigger cell and then nuclear fusion to produce stable **a**/α diploid cells. In response to adverse nutritional conditions, **a**/α diploid yeast cells undergo meiosis and sporulate to produce two **a** and two α haploid progeny.

A notable feature of budding yeast is the capacity to switch mating-type, which makes it a homothallic organism. The *S. cerevisiae MAT* locus (Figure 1) resides on chromosome III, and the unique region of the active *MAT* locus spans only 642 bp in **a** cells and 747 bp in α cells, a small fraction of the 316,613 bp chromosome on which they reside. Chromosome III also contains two other copies of the mating-type cassette at the telomeric *HML* and *HMR* loci, and these *MAT* alleles are transcriptionally repressed by a process known as silencing. Mating-type switching is restricted to cells that have budded at least once (mother cells), and is effected by an endonuclease, HO, which cleaves *MAT* and triggers recombination between the active and silent cassettes. This process is thought to promote mating of siblings following meiosis to rapidly re-establish the diploid state and possibly also homozygose recessive mutations.

Much of what we know about the fungal bipolar system is based on a detailed understanding of the *S. cerevisiae MAT* locus, which encodes either one or two key cell fate determinants: **a1** or α2, homodomain proteins which can homodimerize (α2–α2) or heterodimerize (**a1**–α2) to repress **a**-specific or haploid-specific genes, respectively (Figure 2). A third product is α1, another DNA-binding protein that activates α-cell specific genes in α cells. The central feature is that cell identity is controlled by only one or two principal determinants, homeodomain or α-domain proteins which bind DNA to control expression of other genes. This theme has been revisited in many other fungi in which cell identity determinants are homeodomain or HMG box transcriptional regulators (sometimes both).

Candida albicans — the *MAT* locus of a pathogenic yeast

The recent discovery that the genome of *Candida albicans*

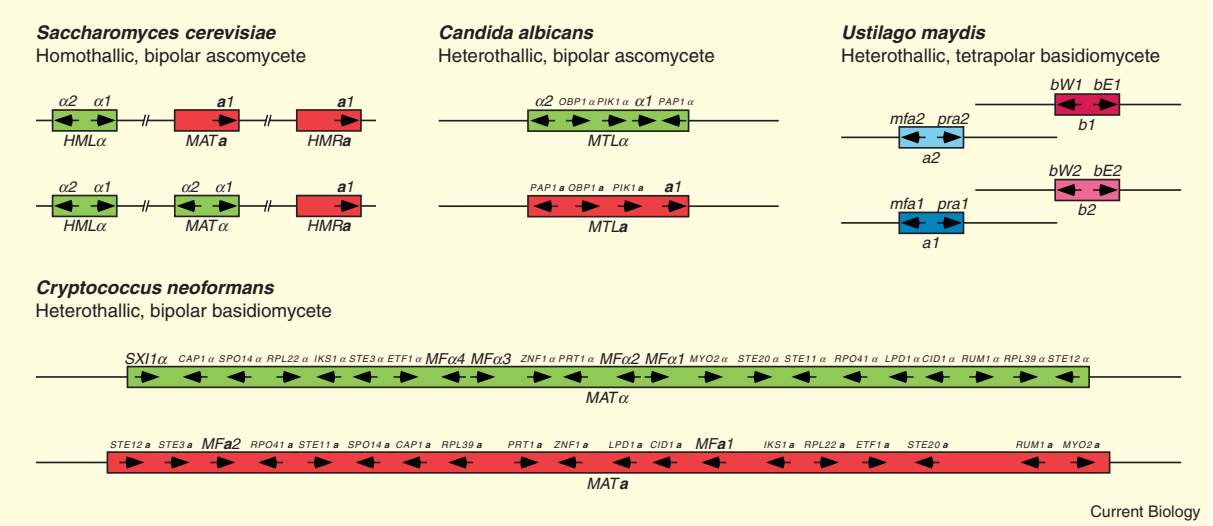

Figure 1. The structure of fungal *MAT* loci.
Fungi have either bipolar or tetrapolar mating-type systems which direct the interaction of partners during the sexual cycle. *S. cerevisiae* is a homothallic ascomycete with a bipolar mating system. Cells have the ability to undergo mating-type switching in response to cleavage by the HO endonuclease, allowing the active *MAT* cassette to be replaced with the silent cassette of opposite mating-type. The closely related pathogenic yeast *C. albicans* also has a bipolar system, but lacks silent cassettes or the HO endonuclease, and does not undergo mating-type switching. The phytopathogen *U. maydis* is a tetrapolar, heterothallic fungus with two mating-type loci: one, *b*, encodes homeodomain transcription factors, while the other, *a*, encodes pheromones and pheromone receptors; these are multi-allelic loci, giving rise to many different mating-types. In contrast, the pathogenic basidiomycete *C. neoformans* has a bipolar system with only two mating types, though it is also heterothallic. The *C. neoformans* mating-type locus encodes a homeodomain transcription factor, pheromones and pheromone receptors, and other elements of the pheromone activated MAP kinase cascade in addition to many genes whose role in mating, if any, is unknown.

encodes a mating-type locus (*MTL*) overturned the long-held dogma that this pathogenic yeast is asexual. *MTL* of *C. albicans* shares striking features with *MAT* from *S. cerevisiae*, also existing in two opposite alleles: *MTL***a**, which encodes a homolog of the budding yeast **a1**protein; and *MTL*α, which encodes homologs of the yeast α1 and α2 proteins. The *C. albicans MTL* alleles are larger than those of budding yeast (8742 and 8861 bp), and encode three additional genes with divergent mating-type specific alleles. These additional *MAT*-specific genes encode homologs of poly(A) polymerase, an oxysterol binding protein, and the phosphatidylinositol kinase Pik1, whose roles in mating, if any, are currently unknown.

Most isolates of *C. albicans* are **a**/α diploids which, like *S. cerevisiae* diploids, are not capable of mating. But *C. albicans* strains engineered to be either homozygous (**a/a** or α/α) or hemizygous (**a**/Δ or α/Δ) at *MTL* mate at low efficiency both *in vitro* and during infection in an animal model. More recent studies have shown that mating can occur with high efficiency, but this requires a developmental switch in which the normal 'white' cells of the organism convert to mating-specialized 'opaque' cells. How this occurs *in vivo* was unknown, as the opaque cell form is unstable at 37°C, but *C. albicans* has recently been found to mate with high efficiency on skin, where the temperature is several degrees cooler. Furthermore, the identification of naturally occurring **a/a** and α/α homozygous diploid strains amongst clinical isolates further supports the idea that mating may indeed occur during commensal or pathogenic growth of this ubiquitous human fungal pathogen.

Although there are striking parallels in the arrangement of the mating-type locus in *S. cerevisiae* and *C. albicans* despite the ~200 million years of evolution separating them from a common ancestor, there are two marked differences. First, *S. cerevisiae* is a homothallic yeast that readily switches mating-type, whereas *C. albicans* is a heterothallic yeast that does not switch mating-type — there is no sign in the genome sequence of *C. albicans* of any silent versions of the *MTL* alleles, nor of a homolog of the HO endonuclease that drives switching. A second striking difference between the two yeasts is that the mating-type locus of *S. cerevisiae* controls not only mating but also meiosis, whereas *C. albicans* has never been observed to undergo meiosis or sporulate. The **a/a**/α/α tetraploid cells produced by mating in *C. albicans* have recently been found to undergo chromosome loss to produce **a/a**, **a**/α and α/α diploid progeny, and thus might have evolved an alternative version of the sexual cycle that involves cell fusion and ploidy reduction but not meiosis and sporulation, possibly as a means to benefit from mating yet survive continuous assault by the host immune system.

Cryptococcus neoformans — a step in the evolution of sex chromosomes

Cryptococcus neoformans is a basidiomycete, and so quite divergent in evolution from *S. cerevisiae* and *C. albicans*, which are both ascomycetes. And yet

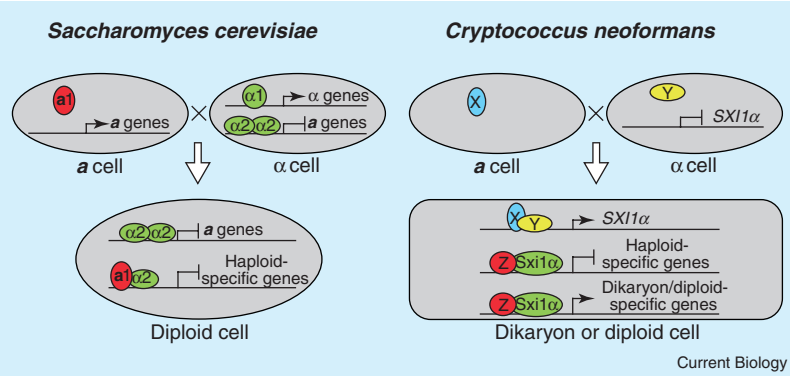

Figure 2. Two fungal mating paradigms: *S. cerevisiae* and *C. neoformans*.
In the budding yeast *S. cerevisiae*, cells express either of two homeodomain proteins, **a**1 or α2, depending on which cassette resides at the active *MAT* locus. In α cells, an α2–α2 homodimer represses **a**-specific genes, while another MATα-encoded product (α1) activates α-specific genes. Upon cell fusion, haploid-specific genes (including *α1*) are repressed by the **a**1–α2 heterodimer and **a**-specific genes continue to be repressed by the α2–α2 homodimer. In contrast, in the basidiomycete *C. neoformans*, the cell-identity factor Sxi1α is present at low levels in haploid cells. *SXI1*α expression is activated following cell fusion by factors that remain to be identified (X and Y), allowing it to participate with a partner from **a**-cells (depicted here as Z) in repression of haploid-specific genes and activation of unknown targets involved in dikaryon and diploid cell functions.

like *C. albicans*, *C. neoformans* is a ubiquitous human fungal pathogen to which we are all exposed early in life, as it occurs worldwide in association with pigeon guano and certain tree species. The organism can reside in a dormant latent state in granulomas and later emerge to cause life-threatening infections of the central nervous system.

The sexual cycle of *C. neoformans* involves a bipolar mating-type system with haploid **a** and α cells that do not switch mating-type. In contrast to *S. cerevisiae*, where cell fusion is immediately followed by nuclear fusion to establish the diploid state, nuclear fusion is delayed in *C. neoformans* and other basidiomycetes, and the fusion of yeast-like **a** and α cells that occurs in response to nutrient limitation produces a dikaryon, which grows filamentously. The filament tips ultimately produce a fruiting structure, the basidium, where nuclear fusion, meiosis and sporulation occur. Interest in the mating-type locus of this pathogenic fungus was fueled by early studies which revealed that mating-type is linked to physiology and virulence in *C. neoformans*. Strains of α mating-type are more common in nature and the clinical setting, can be more virulent than congenic **a** strains, and are endowed with the unusual ability to differentiate by a process known as haploid fruiting which involves filamentation and the production of spores of only the α mating-type.

The entire **a** and α alleles of the mating-type locus have recently been cloned and sequenced from two divergent varieties of *C. neoformans*. The *MAT* locus spans >100 kb and contains more than 20 genes, some of which function in differentiation and virulence, others having no clear link to either. The one gene unique to the α allele of the MAT locus encodes a homeodomain factor, Sxi1α, which functional studies have shown is a key cell fate determinant that establishes **a**/α cell identity (Figure 2). Sxi1α is related to other fungal homeodomain proteins, including **a**1 and α2 of *S. cerevisiae* and *C. albicans*. Remarkably, the **a** and α alleles of the *C. neoformans MAT* locus are otherwise composed of divergent sets of the same genes and so evolved from a common ancestral DNA region, which has been extensively remodeled. Comparison of the divergent *MAT* alleles has suggested molecular mechanisms by which the locus might expand or contract by transposition and inversions. These studies have established a novel paradigm for the arrangement of mating-type information in a fungal pathogen. The *MAT* locus of *C. neoformans* shares features both with the other known fungal *MAT* loci and the larger, more complex sex chromosomes of animals and plants.

The products encoded by the *MAT* locus of *C. neoformans* fall into three functional categories: the mating-type-specific cell identity determinant, elements of a specialized MAP kinase cascade that governs mating, and proteins of a variety of different functional groups which may or may not be involved in mating. In addition to encoding pheromones and pheromone receptors involved in the earliest steps in mate recognition — a feature shared with the *MAT* loci of several other basidiomycetes — the *C. neoformans MAT* locus also encodes several elements of the pheromone-activated MAP kinase cascade itself. These include homologs of the Ste20 PAK kinase, the Ste11 MEK kinase, and the transcription factor Ste12. Thus **a** and α cells have divergent alleles of these pathway elements, and so employ two somewhat different signaling cascades, which may contribute to the unique cell-type specific specializations involving mating, haploid fruiting and pathogenesis. The *MAT* locus also encodes a large number of other gene products, some essential. Whether these genes play mating- and virulence-specific roles, or have simply been entrapped in the *MAT* locus during its expansion, remains to be determined.

There is a striking contrast between the compact mating-type locus of *S. cerevisiae*, which spans only ~700 bp and encodes only one or two key cell fate determinants, and the *C. neoformans* mating-type locus, which spans more than 100 kb and includes more than 20 genes. And while *S. cerevisiae* is a homothallic yeast that can switch mating-type, *C. neoformans* is heterothallic (like *C. albicans*) and does not switch mating-type. There are no silent mating-type cassettes or HO endonuclease in *C. neoformans*, and it would require a large amount of genetic coding capacity

to accommodate mating-type switching in this pathogenic basidiomycete. Moreover, because *C. neoformans* occurs predominantly as a haploid organism in which the diploid state is unstable and transient, it does not require a mating-type switching mechanism to rapidly reenter the diploid state. The spore chains produced by *C. neoformans* are also mixed, providing ready opportunity for siblings of opposite mating-type to again engage in mating. In summary, divergence into the homothallic and heterothallic lifestyles may have contributed to the evolution of two very different types of cell-identity-determining systems in these fungi. An interesting similarity between the *MAT* loci of *C. albicans* and *C. neoformans* is the inclusion of additional and essential genes, which likely affect both the evolution of this unique region of the genome and its biological functions.

Ustilago maydis — a tetrapolar paradigm

In contrast to the ascomycetes and the pathogen *C. neoformans*, many basidiomycetous fungi arrange their mating-type information in a distinctly different fashion. One example is the maize pathogen *U. maydis*, in which the filamentous dikaryon produced by mating is the infectious form of the organism. *U. maydis* arranges its mating-type information into two defined regions that lie on different chromosomes, giving rise to a tetrapolar mating system. The region of *MAT* known as the *b* locus contains a pair of divergently transcribed homeodomain encoding genes, *bE* and *bW*, with different alleles encoding alternative versions of the factors which can heterodimerize to govern the establishment of cell-type identity and completion of the sexual cycle. This region of the *MAT* locus shares features with the *MAT* loci of *S. cerevisiae* and *C. albicans*, and the *SXI1α* gene of *C. neoformans*.

In *U. maydis*, mating-type is also governed by a second region of the genome, the *a* locus, which encodes pheromones and pheromone receptors that direct the initial stages of mate recognition and cell fusion. Another unique feature of fungi with tetrapolar systems is that the *a* and *b* regions of the *MAT* locus are most commonly multi- rather than bi-allelic, giving rise to hundreds or even thousands of different mating-types. In the most extreme examples, such as the model mushroom fungi *Schizophyllum commune* and *Coprinus cinereus*, the control of cell–cell fusion by pheromones has been dispensed with: each encounter of two different strains results in promiscuous cell–cell fusion, and post-fusion steps in the sexual cycle require two nuclei of different mating-types. This unique association of pheromones and pheromone receptor genes with the *MAT* locus likely arose in the basidiomycete lineage, and in organisms with a bipolar mating system, such as *C. neoformans*, it is linked to the homeodomain region, whereas in those with a tetrapolar system, such as *U. maydis*, it is not.

Conclusions and outlook

We now understand at a molecular level how mating-type loci govern the establishment of bipolar and tetrapolar mating-type systems, and how unique adaptations have occurred in the budding yeast to enable it to switch mating-type. One conserved general feature of fungal *MAT* loci is the presence of genes encoding transcription factors which govern the establishment of cell type identity, and it is striking how the homeodomain proteins have been conserved in both ascomycetes and basidiomycetes. And yet in some examples, this role has been apparently supplanted by HMG box proteins. Thus far, the mating-type locus of *C. neoformans* is one of only a few examples in which the typically compact mating-type locus has been dramatically expanded, likely by chromosomal translocation and rearrangements. And while this *MAT* locus shares some features with those of other fungi that lie within the same phylum, it clearly establishes a novel paradigm. The diversity of these fungal mating-type loci, with both shared features and novel specializations, is a poignant example of the common theme of evolution and diversification that has occurred in biological systems as they evolve to exploit different environmental niches. The roles of the *MAT* locus in directing the establishment of cell-type identity and microbial virulence, and in the evolution of unique and specialized modes of sexual recombination, will likely continue to capture the imagination of biologists for many years to come.

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