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The mating type-like loci of *Candida glabrata*



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ABSTRACT

Candida glabrata, a haploid and opportunistic fungal pathogen that has not known sexual cycle, has conserved the majority of the genes required for mating and cell type identity. The *C. glabrata* genome contains three mating-type-like loci called *MTL1*, *MTL2* and *MTL3*. The three loci encode putative transcription factors, **a1**, $\alpha 1$ and $\alpha 2$ that regulate cell type identity and sexual reproduction in other fungi like the closely related *Saccharomyces cerevisiae*. *MTL1* can contain either **a** or α information. *MTL2*, which contains **a** information and *MTL3* with α information, are relatively close to two telomeres. *MTL1* and *MTL2* are transcriptionally active, while *MTL3* is subject to an incomplete silencing nucleated at the telomere that depends on the silencing proteins Sir2, Sir3, Sir4, yKu70/80, Rif1, Rap1 and Sum1. *C. glabrata* does not seem to maintain cell type identity, as cell type-specific genes are expressed regardless of the type (or even absence) of mating information. These data highlight important differences in the control of mating and cell type identity between the non-pathogenic yeast *S. cerevisiae* and *C. glabrata*, which might explain the absence of a sexual cycle in *C. glabrata*. The fact that *C. glabrata* has conserved the vast majority of the genes involved in mating might suggest that some of these genes perhaps have been rewired to control other processes important for the survival inside the host as a commensal or as a human pathogen.

This manuscript is part of the series of works presented at the “V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi” (Oaxaca, Mexico, 2012).

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Los loci de apareamiento de *Candida glabrata*

RESUMEN

Candida glabrata, una levadura patógena haploide y oportunista, que carece de ciclo sexual conocido (asexual), conserva la mayoría de genes ortólogos requeridos en los procesos de apareamiento, esporulación y la identidad del tipo celular. El genoma de *C. glabrata* contiene 3 loci de apareamiento llamados *MTL1*, *MTL2* y *MTL3* que codifican los presuntos factores de transcripción **a1**, $\alpha 1$ y $\alpha 2$ que controlan la reproducción sexual e identidad celular en otros hongos, como *Saccharomyces cerevisiae* con el cual tiene una estrecha relación filogenética. *MTL1* puede contener información **a** o α ; *MTL2* contiene información **a**, y *MTL3* que contiene información $\alpha 1$ y $\alpha 2$ son loci próximos a 2 telómeros. *MTL1* y *MTL2* son activos transcripcionalmente mientras que *MTL3* está sujeto a un silenciamiento que no es completo, que proviene del telómero y depende de las proteínas Sir2, Sir3, Sir4, yKu70/80, Rif1, Rap1 y Sum1. *C. glabrata* parece no mantener identidad de tipo celular ya que varios genes específicos de un tipo celular se expresan en todas las células con independencia del tipo de información de apareamiento en los loci *MTL*, o incluso, en su ausencia. Estos datos ilustran varias diferencias importantes entre la levadura no patógena *S. cerevisiae* y *C. glabrata* que podrían explicar la característica asexual en esta última. El hecho de que en *C. glabrata* se hayan conservado los genes necesarios para el apareamiento podría indicar que es posible que algunos de estos genes se hayan «reorganizado» para controlar otros procesos importantes en la supervivencia de *C. glabrata* en su huésped, como comensal o como patógeno.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

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Sexual reproduction is thought to be advantageous in spite of the high cost in energy associated with it. Sex is thought to provide a means to eliminate deleterious mutations and promote genetic recombination, which in turn might produce progeny with a combination of beneficial traits that may be better adapted to changing environmental conditions.²⁶ Indeed, recent experiments in the model yeast *Saccharomyces cerevisiae* support this idea. The data indicate that under stressful conditions, sexual cells have an advantage over the obligate asexual congenic strain that only differed in their respective ability to mate. Instead, when these strains were grown under mild non-stressful conditions, there was no advantage for the obligate sexual strain.¹³ Sexual reproduction is widely spread among eukaryotic organisms, even in many microbes that can reproduce asexually. However, there are some eukaryotes, including some fungi that appear to lack a sexual cycle. Only a few of the known species of fungi are associated with human disease, and many of the human pathogens have long since been thought to be asexual since no sexual reproduction has been observed. It is thought that sex in these pathogens might lead to loss of particular combinations of genes required to survive within the host.

Examples of asexual fungal pathogens are *Sporothrix schenckii*,²⁹ *Coccidioides immitis*, *Coccidioides posadasii*,¹¹ *Candida parapsilosis*,⁴⁰ *Candida glabrata* and others. However, in recent years there has been a surge in genomic studies that show that several of the human fungal pathogens contain the genes required for sexual reproduction, suggesting that they may have a cryptic sexual cycle, one that is controlled by very specific conditions, reviewed in some bibliographic references in this article.^{21,24,32,33} This is the case of *C. immitis*, *C. posadasii*,¹¹ *Candida tropicalis*,⁴⁰ *Aspergillus fumigatus*,³⁷ *Candida albicans*¹⁷ and *C. glabrata*.^{9,44} For some of these fungal pathogens, a cryptic sexual or parasexual cycle was later discovered, like the case of *C. albicans*,¹⁸ *C. tropicalis*,³⁸ *A. fumigatus*³⁴ and *Paracoccidioides brasiliensis*.⁴² These pathogens reproduce sexually under very particular conditions. In the case of *C. albicans* and *C. tropicalis*, in order to mate cells first have to undergo a morphological switch, which is the mating-competent stage.³⁰ For *A. fumigatus*, the sexual cycle can be observed in the laboratory after prolonged incubation (over 6 months) in the dark.³⁴ In *Cryptococcus neoformans* even though a sexual cycle has been documented in the laboratory for a long time,²² mating is limited by the almost unisexual geographic distribution of only the α mating type.²⁵ In this way, these pathogens have retained the ability to generate genetic variation through controlled sexual or parasexual cycles in response to specific or changing conditions.^{2,33}

For sexual reproduction to occur, two cells of opposite mating type must recognize each other through mating-specific pheromone and receptor signals. This is followed by cell–cell fusion, nuclear fusion and in many fungi by meiosis, although in some fungi like *C. albicans*, no meiosis has been observed, instead mating products undergo gradual chromosome loss until haploid chromosome content is achieved.¹

In this review we will focus on the common opportunistic pathogen *C. glabrata*, an asexual haploid yeast, that shares a closer phylogenetic relationship with *S. cerevisiae* than to other *Candida* species.^{5,14} *C. glabrata* is a commensal in healthy individuals but can become a successful pathogen associated with high mortality rates in immunocompromised patients.³⁶

Control of sexual reproduction and cell type identity

Sexual reproduction and cell-type identity in most fungi are controlled by the genes encoded in the mating type locus called *MAT* (or *MTL* in some fungi). This locus encodes transcription factors

that regulate the expression of genes that determine cell type identity and the signaling cascades that enable the cell to respond to the pheromone secreted by cells of the opposite mating type. These transcription factors are usually proteins containing a homeodomain or other types of regulatory domains like a α -domain or HMG (high mobility group) domain. Early studies in the non-pathogenic yeast *S. cerevisiae* have led to a detailed molecular mechanism for cell type identity control and mating. This organism, which can reproduce both sexually and asexually, contains three mating type loci (*MAT*, *HML* and *HMR*) of which only *MAT* is transcriptionally active and the other two loci are maintained repressed by a mechanism known as silencing.¹⁵ Information present at the *MAT* locus can be either **a-type** or α -type while *HML* and *HMR* contain α and **a** information respectively, in over 97% of the strains studied. Control of cell type and mating involves a regulatory circuit determined by the **a1** protein and the $\alpha1$ and $\alpha2$ proteins encoded in the *MATa* and *MAT α* loci, respectively. The **a1** and $\alpha2$ genes encode homeodomain-containing transcription factors while the $\alpha1$ gene encodes a protein containing a α domain. In *MATa* haploids only **a**-specific genes (**asg**) are expressed whereas in *MAT α* haploids only α -specific genes (α sg) are expressed. In both types of haploids a set of genes specific for haploid cells (*hsg*) is also expressed. After mating, the resultant diploid (**a**/ α) forms a heterodimer with the proteins **a1** and $\alpha2$ that represses $\alpha1$, the *hsg* and some other genes involved with certain types of stress.^{12,15,19} This regulatory circuit with some modifications also controls cell type identity in *C. albicans*, a diploid opportunistic human pathogen. Notably, a heterodimer composed of **a1**/ $\alpha2$ proteins is also formed in this organism and negatively regulates the phenotypic switch required for mating, therefore, indirectly controlling mating.³⁰

Structure of the *C. glabrata* mating type-like loci *MTL1*, *MTL2*, and *MTL3*

Early studies reported that *C. glabrata* contains three mating type-like loci (*MTL*), in a similar configuration to that of the *MAT*, *HML* and *HMR* loci in *S. cerevisiae*.⁴¹ *MTL1* corresponds to *MAT* while *MTL2* and *MTL3* to *HMR* and *HML*, respectively. In *C. glabrata*, *MTL1* and *MTL3* are in chromosome B, and *MTL2* is in chromosome E, whereas in *S. cerevisiae* the three loci are in chromosome III. It was initially proposed that *MTL1* is the expression locus and that *MTL2* and *MTL3* are transcriptionally silent, analogous to the situation in *S. cerevisiae*.⁴¹ In the vast majority of *C. glabrata* isolates (approximately 97%), the information at *MTL2* is **a**, and α in *MTL3*. In contrast, the information in *MTL1* can be of either type, for example in the sequenced strain CBS138 (<http://www.genolevures.org/cagl.html#>), *MTL1* contains α whereas the BG14 strain⁷ contains **a** information in this locus.³⁹ It has been reported that there is a bias toward **a**-type of information at *MTL1* in a collection of 190 *C. glabrata* clinical isolates from Africa, Europe, North America and South America where it was found that approximately 80% of the isolates contain **a** information and only 20% are α at this locus.⁴ However, in our collection of 79 clinical isolates from three hospitals in Mexico, the α containing isolates are more frequent, since 64% of isolates contain α information at *MTL1* and 36% contain **a** information²³ (and Robledo-Márquez and Castaño, unpublished data). Therefore, it seems that the distribution of mating types varies depending on the geographical sites where the *C. glabrata* isolates are collected. It is not known whether there is a correlation between the information at *MTL1* and the pathogenicity in *C. glabrata* as there seems to be in *C. neoformans* where the α strains are more virulent than the very uncommon **a** strains.^{10,25}

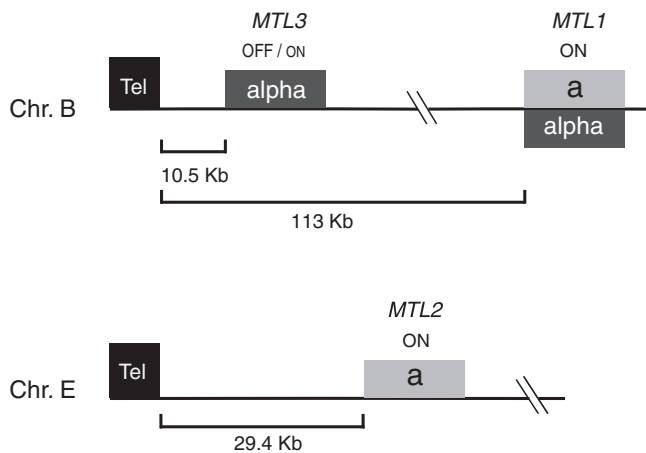


Fig. 1. Structure of the MTL loci of *Candida glabrata*. *C. glabrata* contains three mating type like loci (MTL). *MTL1* is located in chromosome B at an internal position and can contain mating information type **a** or type α as indicated. *MTL2* localized at 29.4 kb from the left telomere of chromosome E generally contains **a** information and *MTL3* which is localized at 10.5 kb from the left telomere of chromosome B usually contains α information. *MTL1* and *MTL2* are transcriptionally active (indicated by ON) and *MTL3* is subject to incomplete silencing spreading from the telomere (indicated by OFF/on).

MTL1 and MTL2 are both transcriptionally active

MTL1 is at an internal position on chromosome B (113 kb from the left telomere); *MTL2* and *MTL3* are both close to two telomeres, *MTL2* is 29.4 kb from the left telomere on chromosome E and *MTL3* is only 10.5 kb from the left telomere on chromosome B (Fig. 1). To determine whether *MTL1* is expressed and *MTL2* and *MTL3* loci are silenced as suggested initially,⁴¹ we introduced at least 5 independent *URA3* gene reporter insertions in each of the three loci and measured its expression using a plate growth assay on SC plates with 5-FOA, which is toxic to cells expressing *URA3*. Surprisingly, we found that *MTL2* is in a conformation active for transcription as all the reporter insertions in this locus were expressed giving rise to Ura⁺, 5-FOA^S strains. As expected, *MTL1* is also transcriptionally active and cells of all reporter strains in this locus are also Ura⁺, 5-FOA^S.³⁹ Muller et al.³¹ also reported expression from both *MTL1* and *MTL2* in *C. glabrata* as measured by quantitative RT-PCR. The **a1** gene contains two introns, and interestingly, the correct processing of the **a1** mRNA only occurs when the transcript originates from the *MTL1* locus, but not when transcription starts at *MTL2*.^{31,39} It is not known yet, what are the signals that direct processing of this mRNA since the sequence of the gene and the immediate flanking sequences are identical in the two loci (at least 370 bp on either the 5' or 3' end), although it is possible that relative transcription rates might regulate this process through a chromatin based mechanism.²⁸ The locus-specific processing of the **a1** transcript was thought to be a mechanism to maintain cell type identity. However, by using strains with different combinations of knockout mutations in the *MTL* loci, including a strain with no mating information [(*mtl1,2,3*) Δ], we found that several genes that are regulated in a cell type-specific manner in *S. cerevisiae*, are expressed in all strains in *C. glabrata* regardless of the information contained in the *MTL* loci and even in the absence of these loci. This suggests that *C. glabrata* might not maintain a cell type identity.³⁹

MTL3 is subject to subtelomeric silencing

The *MTL3* locus is the only *MTL* that is not transcriptionally active since in strains carrying reporter insertions throughout the locus, a large proportion of cells were able to grow on plates containing 5-FOA. This silencing is not complete because in every reporter

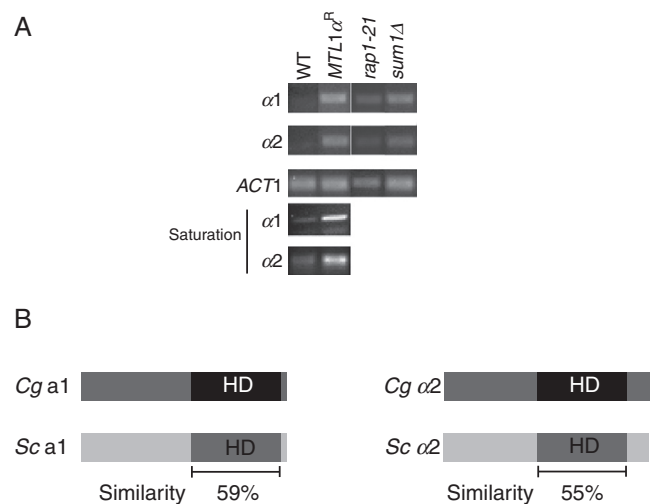


Fig. 2. Subtelomeric silencing of *MTL3* depends on Rap1 and Sum1 proteins. (A) RT-PCR of the $\alpha 1$ and $\alpha 2$ genes from *MTL3* from the wild-type (wt), the *rap1-21* or *sum1* Δ strains as indicated, or from *MTL1* in the *mtl(1, 2, 3)* Δ strain where $\alpha 1$ and or $\alpha 2$ were reconstituted at *MTL1* (*MTL1* α^R). The bottom part of the figure shows the RT-PCR performed at saturation conditions. RT-PCR for *ACT1* gene was used as loading control. (B) Schematic representation and comparison of the **a1** and $\alpha 2$ genes from *S. cerevisiae* and *C. glabrata*. HD represents the homeodomains of each protein and the similarity in this domain for each pair of proteins is indicated as percentage.

insertion tested, we also found a population of cells that expressed the *URA3* gene and were able to grow on plates without uracil.³⁹ Even though there is no detectable expression of the native $\alpha 1$ and $\alpha 2$ genes present at *MTL3* by RT-PCR,^{31,39} however, when the PCR is made under saturation conditions, a transcript is detected for both genes (Fig. 2A). These results are in contrast to the situation in *S. cerevisiae* where the two loci *HMR* and *HML* are very efficiently silenced through the activity of two cis-acting silencers flanking each locus where the silencing proteins Abf1, ORC and Rap1 bind.^{3,16,20}

Silencing at *MTL3* of *C. glabrata* was shown to be dependent on the Sir2, Sir3 and Sir4 proteins as well as Rif1 and yKu70 and yKu80 proteins; these data also differ markedly from the silencing of the *HM* loci of *S. cerevisiae* where yKu70 and yKu80 are not required for silencing, and only when *SIR1* is deleted, a modest effect of the yKu proteins is observed.^{35,43} The silenced chromatin structure is probably nucleated at the telomere (and not from flanking cis-acting silencers), and spreads over 11 kb to *MTL3*, because moving the entire *MTL3* locus to an internal position in chromosome L results in expression of both, the *URA3* reporter insertions and the $\alpha 1$ and $\alpha 2$ genes at that location.³⁹ We have also recently shown that silencing at *MTL3* also depends on Rap1, by using a strain containing allele *rap1-21* (a deletion of the 28 carboxy-terminal amino acids of the protein), which is defective for subtelomeric silencing at various telomeres in *C. glabrata*.^{6,8} In the *rap1-21* strain, but not in the wild-type strain, transcription of the α genes from *MTL3* can be detected (Fig. 2A), supporting the idea that silencing at *MTL3* comes from the telomere.

The transcriptional repressor Sum1 was discovered as an additional negative regulator of α -specific gene expression in *MATa* cells in *Saccharomyces bayanus* and *S. cerevisiae*. Repression by Sum1 in both organisms is important to prevent mating as α cells in *MATa* cells.⁴⁵ The *C. glabrata* and the *S. cerevisiae* Sum1 proteins are only 20% similar over their entire lengths. To investigate whether Sum1 in *C. glabrata* regulates $\alpha 1$ and $\alpha 2$ expression, we measured $\alpha 1$ and $\alpha 2$ expression in a *sum1* Δ strain by RT-PCR. Fig. 2A shows that a deletion of Sum1 in *C. glabrata* results in de-repression of $\alpha 1$ and $\alpha 2$ genes from *MTL3*, suggesting that Sum1 represses the α genes at *MTL3*.

The fact that in *C. glabrata* silencing at the *MTL3* locus is not complete might mean that in any given culture of strains of *C. glabrata* containing *MTL1a* and *MTL3a*, a small proportion of cells in the population could express both types of information and therefore a heterodimer between $\alpha 1$ and $\alpha 2$ could be formed in an analogous way to *S. cerevisiae*. This could result in the negative regulation of some genes if this putative heterodimer acts in a similar way to *S. cerevisiae*. In *S. cerevisiae*, the homeodomain of $\alpha 1$ interacts with 16 amino acids in the carboxy-terminal of the $\alpha 2$ protein.²⁷ The $\alpha 1$ proteins from *C. glabrata* and *S. cerevisiae* are 46% homologous across the entire protein, and in the homeodomain, where it interacts with $\alpha 2$, they are 59% homologous of which 32% are identities (Fig. 2B). In the case of $\alpha 2$, the *C. glabrata* and the *S. cerevisiae* proteins are 56% homologous and at the 16 amino acids in the C-terminal tail where the interaction with $\alpha 1$ takes place, they are 43% homologous (of which 25% are identical). Therefore, it is possible that when both types of mating information are expressed in *C. glabrata* a heterodimer could be formed; we are investigating this possibility.

From the data discussed, it is clear that there are important differences in the expression of the cell type-specific genes and the genes encoded in the *MTL* loci between *C. glabrata* and *S. cerevisiae* that might explain the absence of a sexual cycle in *C. glabrata*. However, the fact that the genes that are required for mating and cell type identity are conserved in *C. glabrata* could mean that some of these genes might have been rewired to control the expression of a different set of genes or perhaps participate in some process that is important to the survival within the host, either as a commensal or as a pathogen. Alternatively, *C. glabrata* might have a cryptic sexual cycle regulated by an as yet unknown mechanism. Further studies on the expression of cell type specific genes and pheromone response pathway genes will help clarify whether some of these genes control processes that are important to the survival in the host.

Conflict of interest

The authors have nothing to declare.

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