MINIREVIEW

Genome structure and dynamics of the yeast pathogen *Candida glabrata*

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Abstract

The yeast pathogen *Candida glabrata* is the second most frequent cause of *Candida* infections. However, from the phylogenetic point of view, *C. glabrata* is much closer to *Saccharomyces cerevisiae* than to *Candida albicans*. Apparently, this yeast has relatively recently changed its life style and become a successful opportunistic pathogen. Recently, several *C. glabrata* sister species, among them clinical and environmental isolates, have had their genomes characterized. Also, hundreds of *C. glabrata* clinical isolates have been characterized for their genomes. These isolates display enormous genomic plasticity. The number and size of chromosomes vary drastically, as well as intra- and interchromosomal segmental duplications occur frequently. The observed genome alterations could affect phenotypic properties and thus help to adapt to the highly variable and harsh habitats this yeast finds in different human patients and their tissues. Further genome sequencing of pathogenic isolates will provide a valuable tool to understand the mechanisms behind genome dynamics and help to elucidate the genes contributing to the virulence potential.

Introduction

In humans, during the recent years, several factors have increased the risk of fungal infections, like broad spectrum antibiotics, immune-compromised patients, cytotoxic chemotherapy, and transplantations (Malani *et al.*, 2005; Nucci & Marr, 2005). Among all fungal pathogens, *Candida* species remain the most common cause of invasive fungal infections (Pfaller & Diekema, 2007). Although *Candida glabrata* has been considered as a human commensal and can be often found in the oral cavity (Fidel *et al.*, 1999), it has recently emerged as the second most prevalent cause of *Candida* infections, just after *Candida albicans* (Pfaller & Diekema, 2004). Roughly, *C. glabrata* is responsible for 15–25% of disseminated candidiasis (Perlroth *et al.*, 2007).

Phylogenetically, *C. glabrata* is much closer to *Saccharomyces cerevisiae* than to *C. albicans* and its sister species (Dujon *et al.*, 2004). Indeed, *C. glabrata* belongs to post-WGD (whole genome duplication) yeasts (Fig. 1). *Candida glabrata* with three sister species, *Kluyveromyces*

delphensis, *Candida castellii* and *Kluyveromyces bacillisporus*, have been classified to belong to the *Nakaseomyces* genus (Kurtzman, 2003). Two additional clinical isolates, *Candida nivariensis* and *Candida bracarensis*, have later been added to the same genus (Alcoba-Florez *et al.*, 2005; Correia *et al.*, 2006; Sharma *et al.*, 2013). The *Nakaseomyces* has been divided into two subgroups, the first, 'glabrata group' includes the three pathogenic species and *N. delphensis*, and the second group includes *C. castellii* and *N. bacillisporus*.

Unlike *S. cerevisiae*, which is diploid and sexual, *C. glabrata* is believed to be obligate haploid and mating has never been observed (Kaur *et al.*, 2005; Muller *et al.*, 2008). *Candida albicans* can live in two different morphological forms (Sudbery, 2011), but the switching between the yeast form and hyphae has not been observed in *C. glabrata* (Kaur *et al.*, 2005; Fig. 2). Recently, there has been more focus on *C. glabrata* genome dynamics and several new sister species genomes have been reported, both providing additional insight into metabolism and virulence potential of the yeast species.
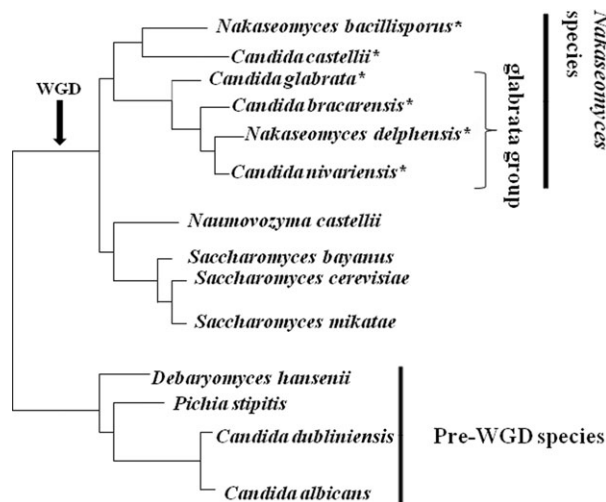
Fig. 1. A schematics phylogenetic tree (adopted from Gabaldón *et al.*, 2013) shows the *Nakaseomyces* species (marked with an asterisk) and some other yeasts. *Candida glabrata, C. nivariensis, C. bracarensis* are pathogenic fungi within the 'glabrata group'. The whole-genome duplication event (WGD), which took place app. 100 million years ago, is arrowed.
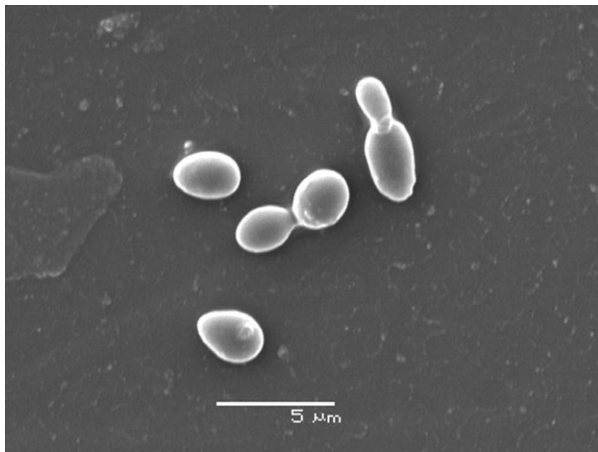

Fig. 2. *Candida glabrata* yeast under electron microscope. The figure shows two single cells and two mother–daughter pairs during the budding process.

Candida glabrata genome potential

Dujon *et al.* (2004) have reported that *C. glabrata* CBS 138 has 13 chromosomes with the genome size of 12.3 Mb. In comparison with *S. cerevisiae*, which has 5807 protein-coding genes (Dujon *et al.*, 2004), *C. glabrata* has 5283 coding sequences, with an average size of 493 codons. Thus, this yeast lineage has reduced its coding potential upon separation from other post-WGD yeasts.

Candida glabrata has two intrachromosomal rDNA repeats loci in subtelomeric regions compared to *S. cerevisiae*, which has a single intrachromosomal rDNA repeat locus. As *S. cerevisiae*, *C. glabrata* has 42 tRNA-encoding genes, which are scattered throughout the genome (Dujon *et al.*, 2004).

In *C. albicans*, the CUG codon is translated as serine instead of leucine, but this genetic codon alteration is not found in *C. glabrata*. In general, most genes transcribed by RNA polymerase III and even other noncoding RNA genes do not have well-conserved size among yeasts (Gabaldón *et al.*, 2013). For example, in *C. glabrata*, RNase P RNA (RPR1) gene is 1149 nucleotides long, whereas in *S. cerevisiae* it is 568 nucleotides long (Kachouri *et al.*, 2005). This large ncRNA seems to be present in *C. glabrata* and sister species, and *K. delphensis* has a 1368 nucleotide long copy (Gabaldón *et al.*, 2013). *Candida glabrata* and other related species have a lower number of intron containing protein-coding genes than *S. cerevisiae* (Neuvéglise *et al.*, 2011).

It has been found by Conant & Wolfe (2007) that 551 duplicated gene pairs are conserved between *C. glabrata* and *S. cerevisiae*, among them six gene pairs encoding glycolytic enzymes *ENO1/ENO2, PYC1/PYC2, GLK1/ EMI2, HXK1/HXK2, TDH2/TDH3*, and *CDC19/PYK2*. Therefore, this yeast has a genetic capacity for a high glycolytic flow. Indeed, *C. glabrata* has recently been reported as an efficient alcohol producing yeast even under aerobic conditions (Hagman *et al.*, 2013). However, it is so far unclear whether this trait plays an important role during yeast pathogenicity. For example, *C. albicans* and its sister species are Crabtree-negative yeasts and do not produce ethanol under aerobic conditions (Hagman *et al.*, 2013).

Comparative genomics of *C. glabrata* and phylogenetically related species

Recently, several *C. glabrata* sister species have been sequenced. All the species in *Nakaseomyces* have transposon-free genomes that are smaller than *S. cerevisiae* and contain fewer genes (Gabaldón *et al.*, 2013). Moreover, all species in this group have a haploid genome, the size of roughly 10–12.5 Mb, with the exception of *N. bacillisporus* which is a diploid. The chromosomal number ranges from 8 in *C. castellii* to 15 in *N. bacillisporus*, while the 'glabrata group' yeasts have from 10 to 13 chromosomes. Note that chromosome number in *C. glabrata* isolates varies (Ahmad *et al.*, 2013). The gene-order conservation is very high between *N. delphensis*, *C. bracarensis* and *C. nivariensis*, but in general, it is low compared with *S. cerevisiae* (Gabaldón *et al.*, 2013). The divergence between orthologous proteins varies from 53% between

C. castellii and *N. bacillisporus* to higher identity level among 'glabrata group' species (77–88%).

After the WGD event, many genes have been lost in *C. glabrata*, for example, galactose metabolism (five genes), phosphate metabolism (four genes), cell defense and virulence (three genes), and nitrogen and sulfur metabolism (three genes; Dujon *et al.*, 2004). *Candida glabrata* genome has also lost genes involved in de novo biosynthesis of nicotinic acid (*BNA*), and this has been initially interpreted as a way to adapt to the host environment (Domergue *et al.*, 2005). However, the species among this clade have different habitats, and the loss of *BNA* has been observed in all of them and likely occurred in the common progenitor when it was still a free-living yeast organism. Another example of gene loss in *C. glabrata* and 'glabrata group' is loss of the six genes cluster involved in allantoin catabolism, *DAL*, which is conserved in *S. cerevisiae* (Wong & Wolfe, 2005). On the other hand, the *DAL* cluster is present in both *C. castellii* and *N. bacillisporus* (Gabaldón *et al.*, 2013).

Apparently, similar to *C. glabrata*, the duplicated genes encoding glycolytic enzymes, for example *ENO1/ENO2* and *PYC1/PYC2* are conserved in *Nakaseomyces* (Merico *et al.*, 2007). In contrast to 'glabrata group' where the glycolytic enzymes encoding genes are in pairs, pyruvate kinase, enolase, and glyceraldehyde-3-phosphate dehydrogenase encoding genes are present as a single copy in *C. castellii* and *N. bacillisporus*. Compared to all *Nakaseomyces* species that encode four hexokinase (*HXK*) genes, *C. castellii* genome encodes two *HXK* genes. Moreover, all *Nakaseomyces* genomes encode two copies of *ADH* genes, which have orthologs in *S. cerevisiae*, *ADH1* and *ADH3*, and are involved in conversion of acetaldehyde into ethanol. *Saccharomyces cerevisiae* genome also encodes *ADH2*, which involves in the conversion of the ethanol into acetaldehyde, and this gene has no ortholog in *Nakaseomyces*.

The genome of *C. glabrata* type strain (CBS 138) contains a family of 18 *EPA* genes (homologues of the *FLO* genes in *S. cerevisiae*), coding for glycosylphosphatidylinositol (GPI)-anchored cell-wall proteins (CWP), which are known to be adhered to human epithelium (Cormack *et al.*, 1999; Gabaldón *et al.*, 2013). Similarly, *C. bracarensis* has 12 members of the *EPA* family, while *C. nivariensis* genome harbors nine genes. On the other hand, the non-pathogenic yeasts in *Nakaseomyces*, *C. castellii*, has three homologs of the *EPA* genes, whereas *N. bacillisporus* has only one homolog, which is distant and clusters together with the *PWP* (PA-14 containing wall protein adhesion gene) and adhesion-like protein genes in *C. glabrata*. Although the 'glabrata group' has a common ancestor, the genome of *N. delphensis* has a single copy of the *EPA* gene (Gabaldón *et al.*, 2013).

Mini and megasatellites

Minisatellites are DNA tandem repeats that show size variation among different yeast species (Richard *et al.*, 2008). In *S. cerevisiae*, it has been found that several genes which are involved in cell wall formation contain minisatellites with variable sizes. These genes belong to the *FLO* family of mannoprotein-encoding genes. The mannoprotein-encoding genes show a variable number of repeats among different yeast isolates and the length of the repeat is directly related to flocculation and cell adhesion. For example, strains with *FLO1* carrying a longer repeat show better adhesion and increased flocculation (Verstrepen *et al.*, 2005).

The minisatellites within the hemiascomycetous yeasts are not conserved among their host genes. Moreover, the diversity of minisatellite sequences within the orthologous genes may indicate that the acquisition and loss of minisatellites have been fast during evolution (Richard & Dujon, 2006). Despite similar genome size, the number of minisatellites in *C. glabrata* is significantly higher than in *S. cerevisiae*. In addition, *C. glabrata* has two more unusual minisatellites, namely, compound minisatellite, with different alternative repeats and megasatellites, with unusually long motifs (Thierry *et al.*, 2008).

Haber & Louis (1998) suggested that minisatellites originated by strand slippage during replication of two short (5–10 bp) sequences or by unequal crossing over. This replication slippage has been reported in *S. cerevisiae* (Richard & Dujon, 2006) but not observed in *C. glabrata* where other mechanisms might be involved (Thierry *et al.*, 2008). Some authors proposed that the megasatellite motifs propagate by motif jumps, which may explain the presence of motifs between nonorthologous gene families (Rolland *et al.*, 2010).

The genome of *C. glabrata* has 40 megasatellites mapped to 33 genes, which are distributed on 11 chromosomes with strong preference toward the subtelomeric region. The megasatellites have long motifs ranging from 135 up to 417 nucleotides and belong to two large families, 'SHITT' and 'SFFIT' (the names come from conservation of five amino acid in each motif in the megasatellite; Thierry *et al.*, 2008). In a recent study, which covered 20 completely sequenced fungal genomes, 216 peptide motifs encoded by megasatellites and located in protein-coding genes have been extracted (Tekaia *et al.*, 2013). The detected megasatellites have been characterized according to the motif sequence similarity and their amino acid composition. Based on this analysis, there is a weak match between SHITT and FLO motifs despite their similar size (Tekaia *et al.*, 2013).

The originally sequenced *C. glabrata* has three *EPA* genes containing megasatellites (*EPA2*, *EPA11*, and *EPA13*), another three genes (*EPA1*, *EPA3*, and *EPA15*) contain minisatellites, but there are no tandem repeats in *EPA6*, *EPA7*, and *EPA8* (Thierry *et al.*, 2008). A recent study has shown that the chromosomal translocations that occurred among different strains of *C. glabrata* are associated with deletions ranging from 130 bp up to 12 kb and two of them were located within megasatellites (Muller *et al.*, 2009). Even translocations and segmental duplications may operate through mini- and megasatellites (Poláková *et al.*, 2009).

Genome rearrangements

Candida glabrata isolates have been reported to show variable karyotypes (Shin *et al.*, 2007). Apparently, the genome of *C. glabrata* is frequently rearranged and some authors consider these rearrangements as an adaptive mechanism. Muller *et al.* (2009) have reported that the most frequent changes in karyotype belong to chromosomal length polymorphism (CLP), mainly due to chromosomal translocations and copy number variations within tandem gene repeats. These gene tandem repeats encode putative proteins like yapsins, cell wall aspartyl proteases (Kaur *et al.*, 2007). Muller *et al.* (2009) have also shown that there is size variation in the subtelomeric *EPA* genes, which is most likely due to the differences in the number of minisatellites within the genes, as proposed by Thierry *et al.* (2008). In general, Muller *et al.* (2009), based on their French strain collection, claim that the *C. glabrata* genome is comparatively stable. This is in contrast to many other works, which propose a very dynamic nature of the *C. glabrata* genome.

Poláková *et al.* (2009) have found that among Danish isolates, chromosomal size variation is associated with either gross intrachromosomal translocations or interchromosomal segmental duplications. Some novel chromosomes have been observed and shown to originate by segmental duplications. These novel chromosomes carry duplicated genes, which are potentially involved in yeast–host interaction, and they include the ABC transporter family genes, which plays a role in multidrug resistance. The formation of new chromosomes has also been reported in a new study analyzing over 200 clinical isolates; Ahmad *et al.* (2013) have found several extra chromosomes originating through at least two different mechanisms (Fig. 3). Remarkably, even phylogenetically related isolates have shown rearranged chromosomes, implying that *C. glabrata* frequently remodels its genome. It has been speculated that in this way, the yeast can 'improve' its fitness when exposed to new environmental conditions (Ahmad *et al.*, 2013). A striking observation

of genome dynamics has been made on recent derivatives from a single laboratory strain. Apparently, among these very closely related strains, different phenotypic groups correlated with specific karyotypic changes (Bader *et al.*, 2012). Thus, genome aberrations and functional adaptations may occur not only during infection, but also under laboratory conditions during a very short time span and without extreme selective pressures.

Three large gene families are associated with the cell wall, the *YPS* gene cluster, consisting of six genes encoding the yapsins (extracellular GPI-linked aspartyl proteinases), eight alpha-1,3-mannosyltransferase, and five beta-mannosyltransferase gene clusters (Dujon *et al.*, 2004; Dujon, 2010; Jawhara *et al.*, 2012). Apart from the chromosome remodeling, also gene duplications and cluster and gene family formation apparently play an important role in the specialization to specific environmental condition, virulence, and interaction with the host (Butler *et al.*, 2009; Moran *et al.*, 2011).

The genome of *C. glabrata* encodes Sir complex including Sir2p, Sir3p, and Sir4p. These proteins are responsible for subtelomeric silencing in *C. glabrata* (Castaño *et al.*, 2005; Maestre-Reyna *et al.*, 2012). Recently, it has been reported that *C. glabrata* isolates were more adherent to the epithelial cells with overexpression of the *EPA1* gene and, moreover, these strains contain several polymorphisms in Sir3p, which might cause reduced silencing (Martinez-Jimenez *et al.*, 2013). On the other hand, it has been shown that the deletion of *HST1* (homologue of *SIR2*) altered the *C. glabrata* response to stressful conditions with an increase in the fluconazole resistance and decrease in the susceptibility to hydrogen peroxide (Orta-Zavalza *et al.*, 2013).

In short, recent studies on *C. glabrata* genome polymorphism have pointed out that this property is clearly
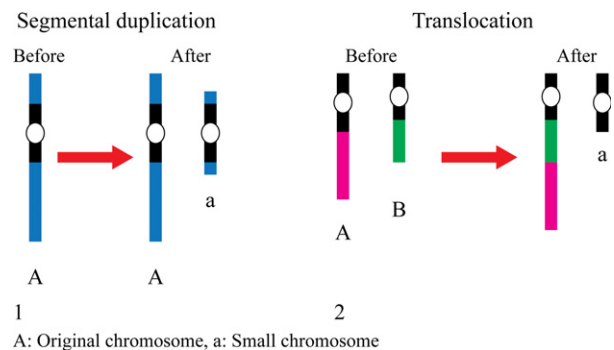

Fig. 3. Two possible mechanisms behind the birth of small chromosomes are shown (adapted from Ahmad *et al.*, 2013). (1) The small chromosome (a) originated by segmental duplication within chromosome A. (2) Interchromosomal translocation, where a large segment of chromosome A is translocated to join the chromosome B leaving the rest of chromosome A as a small chromosome.

connected to the virulence potential of this organism. Future genome sequencing of different isolates will likely provide even further insights into the interplay between genome dynamics and virulence.

OMICS aspects

The first genome structure has provided a background to develop different postgenomic tools and approaches. These could now in turn be used to analyze the connection between the genotype variability and different phenotypic traits. For example, the availability of commercial microarrays has provided a powerful tool to study global aspects of gene expression (Fukuda *et al.*, 2013) to identify potential virulence genes, as well as to determine the level of variation in gene expression among different clinical isolates (O.P. Ishchuk, J. Piškur, unpublished data).

Unlike *C. albicans*, the mechanism of virulence at the molecular level has so far not been well understood in *C. glabrata* (Silva *et al.*, 2011). The proteomic approach to study protein expression in *C. glabrata* has confirmed a previous observation that the virulence of *C. glabrata* increases when Ace2p, transcriptional factor, is inactivated (Stead *et al.*, 2010). In the same study, 32 of 123 proteins were overexpressed in the *ace2* mutant (Stead *et al.*, 2010). Moreover, it has been found that the expression of proteins involved in glucose metabolism, the TCA (Krebs) cycle, respiration and protein synthesis is low when *C. glabrata* grows at pH 7.4 or 8.0, whereas the expression of proteins involved in stress responses and protein catabolism was increased (Schmidt *et al.*, 2008). Proteomic approaches have also been used to study the *C. glabrata* biofilm. The stress-response protein Trx1p, a component of oxidative stress defenses of *C. glabrata*, was found to be upregulated (Seneviratne *et al.*, 2008, 2010). Hsp12p is another protein, which has been highly expressed in *C. glabrata* biofilm. In *S. cerevisiae*, the expression of this protein is induced by different kinds of stress including heat shock, oxidative stress and osmotic stress (Perez-Torrado *et al.*, 2005), and it is involved in azole antifungal resistance in *C. albicans* (Karababa *et al.*, 2004). The proteomic studies have also shown that several glycolytic enzymes including Fba1p, Tdh3p, Gpm1p, and Eno1p are downregulated in *C. glabrata* biofilm (Seneviratne *et al.*, 2010). It would also be interesting to apply the proteome approach on different clinical isolates to see whether the genome variation correlates with the proteome variation.

A new analysis based on high-resolution mass spectrometry has been conducted to identify new peptides, which were previously unknown in *C. glabrata* (Prasad *et al.*, 2012). By genome search for specific peptides (GSSP) and other comparative genomic strategies, the conservation and absence of protein-coding genes in *C. glabrata* and other related species has been elucidated. New postgenomic tools might also be helpful for early diagnosis and proper treatment, thereby reducing the mortality rate. To overcome the pathogen misdiagnosis, Perl *et al.* (2011) have suggested a new sensitive approach to study the volatile organic compounds, multicapillary column (MCC) equipped ion mobility spectrometer (MCC-IMS), to differentiate between different pathogenic fungi and their strains.

Current sequencing projects of tens of different pathogenic isolates, for example at the Cornell and Lund Universities, will provide further background for development of new postgenomic approaches and will help to reveal further genes behind *C. glabrata* virulence. The sequencing of their genomes will make these strains attractive models for postgenomic tool analysis.

Acknowledgements

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