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Mélanie Legrand,[1] Priya Jaitly,[2] Adeline Feri,[1,3,4] Christophe d'Enfert,[1,*] and Kaustuv Sanyal[2,*]

***Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have served as uncontested unicellular model organisms, as major discoveries made in the field of genome biology using yeast genetics have proved to be relevant from yeast to humans. The yeast *Candida albicans* has attracted much attention because of its ability to switch between a harmless commensal and a dreaded human pathogen. *C. albicans* bears unique features regarding its life cycle, genome structure, and dynamics, and their links to cell biology and adaptation to environmental challenges. Examples include a unique reproduction cycle with haploid, diploid, and tetraploid forms; a distinctive organisation of chromosome hallmarks; a highly dynamic genome, with extensive karyotypic variations, including aneuploidies, isochromosome formation, and loss-of-heterozygosity; and distinctive links between the response to DNA alterations and cell morphology. These features have made *C. albicans* emerge as a new and attractive unicellular model to study genome biology and dynamics in eukaryotes.**

Highlights

C. albicans is a major human pathogen, with features that distinguish it from other yeast species.

Rather than meiosis, ploidy reduction upon mating occurs by a parasexual process of concerted chromosome loss.

Centromeres have unique organisation and are epigenetically regulated. Neither centromeres nor the DNA replication origins share any common defining DNA sequence.

Loss-of-heterozygosity events are frequent and widespread in the genome of *C. albicans*.

The DNA damage response is coupled to morphogenetic shifts.

C. albicans emerges as a new unicellular model to study eukaryotic genome biology and dynamics.

Candida albicans: A Model for Studying Genome Biology

Maintenance of genome integrity and the accuracy of DNA replication are at the core of cell function, survival, and propagation. Thus, deciphering the molecular mechanisms that underlie genome biology is of crucial importance, especially as they have relevance in numerous human diseases such as cancer but also because they underlie species evolution and adaptation. For more than 50 years, ***Saccharomyces cerevisiae*** (see Glossary) and *Schizosaccharomyces pombe* have served as uncontested yeast models for molecular understanding of the processes underlying eukaryotic genome biology [1–6]. Major discoveries in this field have benefited from the 'awesome power of yeast genetics' and proven to be relevant across eukaryotes. In addition, the ascomycetous yeast ***Candida albicans*** – a distantly related cousin of *S. cerevisiae* (Box 1) – has attracted considerable interest because of its dominant importance as a human pathogen. While normally a commensal of humans, *C. albicans* is also responsible for superficial infections – thrush, oropharyngeal candidiasis, vaginal candidiasis – in healthy individuals as well as disseminated infections in hospitalised patients that receive broad-spectrum antibiotic treatment and have debilitated immunity [7]. The investigation of putative *C. albicans* virulence factors, in particular the ability to alternate between yeast and filamentous forms; the exploration of *C. albicans* interactions with the host; and the search for new antifungal targets have been accompanied by the development of a molecular toolkit that allows gene function to be accurately characterised in this species (Box 1) [8–16]. Notably, this toolkit has enabled exploring other aspects in depth, especially the genome biology of *C. albicans*. A number of features distinguish *C. albicans* from other yeast species – a unique reproduction cycle where haploid, diploid, and tetraploid forms are observed; a distinctive organisation of chromosome hallmarks; a highly dynamic genome, with extensive karyotypic

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variations, including chromosomal loss and gain, as well as rearrangements, **isochromosome** formation, segmental duplication, and loss-of-heterozygosity (LOH); and distinctive links between the response to DNA alterations and cell morphology (Figure 1, Key Figure). In this review, we highlight these unique features that have helped *C. albicans* emerge as a new unicellular model to study genome biology and its evolution.

Candida albicans: A Yeast Species with a Cryptic Reproduction Cycle

Sequencing of the *C. albicans* genome has revealed that this diploid species, long thought to be devoid of sex [17–20] is actually equipped with the majority of the genetic circuit required for sexual reproduction. Similar to mating type (***MAT***) loci in *S. cerevisiae*, mating type-like (***MTL***; Figure 2A) regions encoding the transcriptional regulators that control expression of mating specific genes [21] and elements of a functional pheromone response pathway exist in the *C. albicans* genome [22]. While many of the meiotic regulators from *S. cerevisiae* have counterparts in *C. albicans*, the latter lacks key meiotic components including ***IME1***, the master switch for entry into the meiotic pathway in *S. cerevisiae,* and *SPO13*, which in *S. cerevisiae* is essential for proper execution of **meiosis** I. Existence of functional analogues of these genes in *C. albicans* is difficult to rule out as extensive sequence divergence may cause difficulty in their *in silico* identification. While *IME1* is essential for meiosis in a number of yeast species, strikingly, this gene is absent from many sexual species in the CTG clade, suggesting a possible clade-specific rewiring of the meiotic cycle (Box 1) [23]. In addition, the highly conserved pheromone response pathways were detected in nonmating species [23]. The apparent plasticity of mating and meiosis pathways in *Candida* species reinforces the fact that knowledge of gene products involved in sexual reproduction is insufficient to accurately predict the reproductive behaviour of an organism.

Hints from *in silico* data have fuelled the quest for evidence of mating and sexual reproduction in *C. albicans*. While engineered *C. albicans* strains homozygous for ***MTL* loci** are able to mate and form tetraploids both in laboratory conditions and in a mammalian host [24,25], meiosis remains to be demonstrated in *C. albicans*. Tetraploid cells revert to diploidy by undergoing a parasexual process of concerted chromosome loss [26–29]. The combination of mating and subsequent concerted chromosome loss that allows *C. albicans* to alternate between diploid and tetraploid (but also haploid and diploid) has been referred to as a **parasexual cycle** (Figure 2B). The apparent lack of conventional meiosis in *C. albicans* suggests that the function of meiotic genes may have diverged [22,23,30,31]. A classic example is Ndt80, the meiosis-specific transcription factor and a key modulator of progression of meiosis and sporulation in *S. cerevisiae*, that functionally diverged to participate in the biofilm pathway in *C. albicans* [32,33]. Similarly, Ume6, a key transcriptional regulator of early meiosis-specific genes in *S. cerevisiae*, has been rewired towards autophagy and hyphal growth regulation in *C. albicans* [30,34]. The latest example of such rewiring is Rme1; the function of which diverged from preventing meiosis by repressing *IME1* in *S. cerevisiae* to regulating chlamydospore formation in *C. albicans* (Hernandez-Cervantes *et al.*, personal communication).

Although we cannot rule out the possibility of *C. albicans* undergoing meiosis in conditions that have not been explored thus far, the hypothetical absence of meiosis does not prevent *C. albicans* from generating genetic and phenotypic diversity necessary for this opportunistic human pathogen to adapt to new environments. In addition to extensive shuffling of parental chromosomes resulting in new combinations of homologues, completion of the parasexual cycle often gives rise to aneuploid strains and is accompanied by recombination events between homologous chromosomes [35,36]. Unexpectedly, recombination events during the nonmeiotic parasexual cycle are dependent on the DNA double-strand break (DSB)

Glossary

***Candida albicans*:** this ascomycetous yeast, normally a commensal of humans, is responsible for superficial infections in healthy individuals as well as disseminated infections in immunocompromised patients.
Centromere (CEN): an essential chromosomal element that facilitates sister chromatid separation via kinetochore formation during mitosis.
CRISPR-Cas9: Cas9 is an RNA-guided DNA endonuclease enzyme that associate with the CRISPR (clustered regularly interspaced short palindromic repeats) to target and cut specific sites in genomes. CRISPR-Cas9 can be exploited for genome editing in *C. albicans.*
DNA replication origins (ORIs): genetic elements bound by the origin recognition complex and where DNA replication initiates. In *C. albicans*, ORIs are categorized into arm ORIs and centromere ORIs.
Experimental evolution: use of laboratory experiments to study evolutionary dynamics in controlled conditions imposed by the experimenter.
Gene flow: transfer of genetic variation from one population to another.
Heterozygosity: presence of different alleles at one or more loci on homologous chromosomes in a diploid organism.
Homologous recombination (HR): pathway that repairs DSBs in DNA using a type of genetic recombination in which nucleotide sequences are exchanged between two identical DNA molecules.
Homozygosis bias: when one haplotype or even a part of it is never found in the homozygous state.
***IME1*:** master regulator of meiosis. The *IME1* gene is required for expression of meiosis specific genes and sporulation and in *S. cerevisiae*.
Isochromosome: abnormal chromosome whereby two identical chromosome arms flank a centromere.
Kinetochore: large multiprotein complex that assembles on centromere DNA and serves as the chromosomal attachment site of the spindle microtubules.

inducing **Spo11**; the meiosis-specific endonuclease that initiates meiotic recombination in *S. cerevisiae* [35].

The majority of *C. albicans* isolates are found in the diploid state and diploidy is considered the preferred ploidy state of *C. albicans*. Nevertheless, nondiploid isolates have also been reported [37]. Changes in ploidy including haploidy, tetraploidy, or aneuploidy (primarily monosomy or trisomy) are thought to provide *C. albicans* with a rapid response to changing environments within the host [38]. Deviations from diploidy seem to harbour a fitness cost in the long term, as **experimental evolution** experiments using clinical and laboratory haploid, diploid, and polyploid *C. albicans* strains in complete medium and under nutrient-limited conditions have revealed that the stabilised genome nearly always reaches diploidy, a phenomenon termed as **ploidy drive** [39].

Large genomic changes, similar to the ones observed in products of *in vitro* parasexual genome reduction or long-term evolution experiments, are well tolerated by *C. albicans* and have been associated with acquisition of new phenotypic traits, such as drug resistance. Although population genetics approaches have recently confirmed the predominance of clonal reproduction in the *C. albicans* population [17,40,41], the work by Ropars and colleagues on genomes of 182 *C. albicans* isolates from diverse origins revealed the occurrence of **gene flow** in this population [42] (Figure 2C). These lines of evidence highlight the fact that parasexuality (or possibly sexuality) also occurs in nature and significantly contributes to *C. albicans* genetic and phenotypic diversities.

The *Candida albicans* Genome: An Organisational View

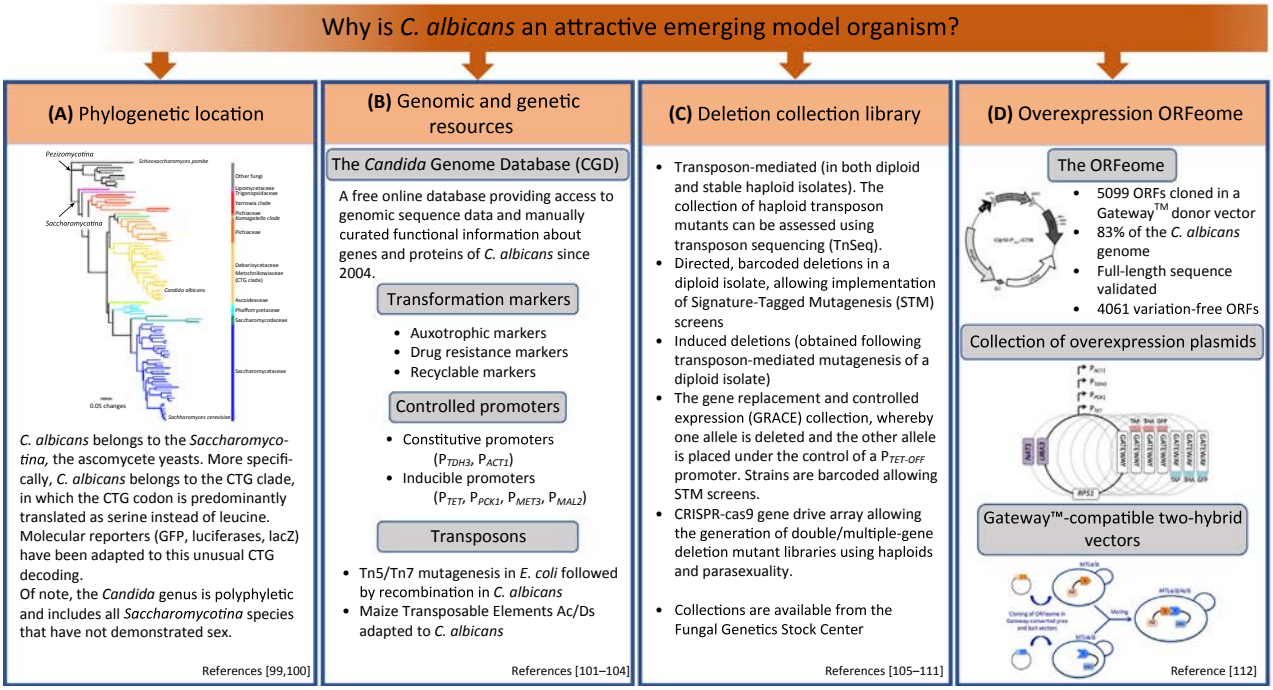
The essential elements of a eukaryotic chromosome – namely **centromeres**, **DNA replication origins**, and **telomeres** – have been identified in *C. albicans* (Figure 3). The centromere (CEN) is an essential chromosomal element that facilitates sister chromatid separation via **kinetochore** formation during mitosis. The CEN DNA sequences of *C. albicans* are 3–5 kb long and are all unique; devoid of any common sequence motif or repeat [43] (Figure 3A). The absence of a CEN-specific DNA sequence and the inability of the exogenously introduced CEN DNA to function as a native CEN suggest epigenetic regulation of CEN identity in *C. albicans* [44]. However, upon CEN deletion, **neocentromeres** are formed efficiently in *C. albicans*, mostly proximal and rarely distal to the native CEN [45–47]. Similar to the native CENs, neocentromeres also cluster in 3D with other functional CENs [47]. Gene conversion (GC) at the CENs can interchange the deleted CEN with the native CEN [45] and possibly explains the low frequency of SNPs across *C. albicans* CENs. Besides CEN clustering, structural integrity of the kinetochore is required for CEN function in *C. albicans.* Depletion of an essential kinetochore protein disrupts the integrity of the kinetochore architecture [48,49] and results in delocalisation and degradation of CENP-A [48] that forms centromeric chromatin.

DNA replication initiates from multiple discrete genetic loci – the DNA replication origins (*ORI*). Based on the location, *ORI*s in *C. albicans* are of two types [50]: (i) arm *ORI*s, which are located on the chromosomal arms; and (ii) centromeric *ORI*s, which are present on [51] or close to the CENs [52] (Figure 3B). While a subset of arm *ORI*s are defined by a 15-bp AC-rich consensus motif and a nucleosome-depleted pattern, centromeric *ORI*s are defined by epigenetic mechanisms [50] and replicate earliest in the genome [51]. The centromeric *ORI*s together with the **homologous recombination** (HR) proteins, Rad51 and Rad52, play a key role in loading CENP-A onto the CENs [52]. While replicating CEN DNA, the moving replication forks from CEN-proximal *ORI*s stall at CEN due to the presence of the kinetochore acting as a physical barrier [52]. The fork stalling accumulates single-stranded DNA that attracts HR proteins Rad51

Loss-of-heterozygosity (LOH): genetic event resulting in the loss of one of the haplotype information in heterozygous diploid organisms.
***MAT* loci:** mating-type or *MAT* locus harbours genes that control sexual reproduction in fungi.
Major repeat sequences (MRS): special feature of the *C. albicans* genome, consisting of a long tract of repetitive DNA, which is present on all chromosomes, except chromosome 3.
Meiosis: cell division that reduces the number of chromosomes by half.
***MTL* loci:** mating type-like or *MTL* locus encodes transcription factors responsible for cellular identity in *C. albicans*.
Neocentromere: non-native centromere locus where a functional kinetochore assembles to allow chromosome segregation following disruption or inactivation of the native centromere.
Nondisjunction: failure of homologous chromosomes or sister chromatids to segregate equally in daughter cells during cell division.
Nonhomologous end joining (NHEJ): pathway that repairs DSBs in DNA by directly ligating the break ends without requirement for a homologous template.
Parasexual cycle: combination of mating and subsequent concerted chromosome loss that allows *C. albicans* to alternate between diploid and tetraploid (but also haploid and diploid).
Ploidy drive: process that brings an organism to its base ploidy level.
***Saccharomyces cerevisiae*:** commonly known as baker's yeast or brewer's yeast, *S. cerevisiae* is a single-celled eukaryote that operates in a manner similar to a human cell and therefore is used as an important model organism in genetics and molecular biology.
Spo11: endonuclease that initiates meiotic recombination by catalysing the formation of double-strand breaks in DNA.
Telomeres: regions of repetitive nucleotide sequences located at the termini of a eukaryotic chromosome to ensure chromosome end replication and protection from degradation or end-to-end chromosome fusion.

Box 1. *Candida albicans*, an Alternative Yeast Model with an Extended and Adapted Molecular Genetics Toolkit

Because *C. albicans* decodes the CUG codon as serine instead of leucine and is predominantly a diploid, numerous tools have been adapted for genetic engineering of this species, allowing most molecular approaches to be developed in this species from insertional mutagenesis to gene tagging and two-hybrid screens as well as the production of mutant collections. The recent identification of stable haploids allows new approaches to be developed. CRISPR-cas9-based gene editing allows one-step generation of mutants in the diploid background, speeding up their construction [99–112].



and Rad52, which are shown to interact with CENP-A in *C. albicans* [52]. As a consequence, CENP-A is deposited onto the CENs. Consistent with this, in a CEN-deleted strain, the neocentromere becomes the earliest replicating region [51].

A telomere, at the termini of a eukaryotic chromosome, ensures chromosome end replication and protects the chromosome from degradation or end-to-end chromosome fusion. *C. albicans* telomeres are unique in containing tandem copies of unusually long 23-bp repeating units [53] (Figure 3C). However, they are assembled into heterochromatin via the classical Sir2-mediated pathway [53,54]. The subtelomeric regions of *C. albicans* consist of the telomere-associated (*TLO*) family of genes, which encode for the subunits of the mediator complex; a crucial component for transcription initiation [55]. There are 15 *TLO* genes (including one pseudogene) in *C. albicans* but other non-*C. albicans* species have either one or two *TLO* genes [56]. In addition, overexpression of *TLO* genes in *C. albicans* influences many growth- and virulence-related properties [57]. The expansion in the number of *TLO* genes could thus explain the ability of *C. albicans* to adapt in various host niches.

A special feature of the *C. albicans* genome is the **major repeat sequence (MRS)**. The MRS is a long tract (10–100 kb) of repetitive DNA that is present on all chromosomes, except chromosome 3. Structurally, an MRS is composed of three subunits: the repetitive RPS

Key Figure

Major Defining Features of *Candida albicans* and Its Genome.

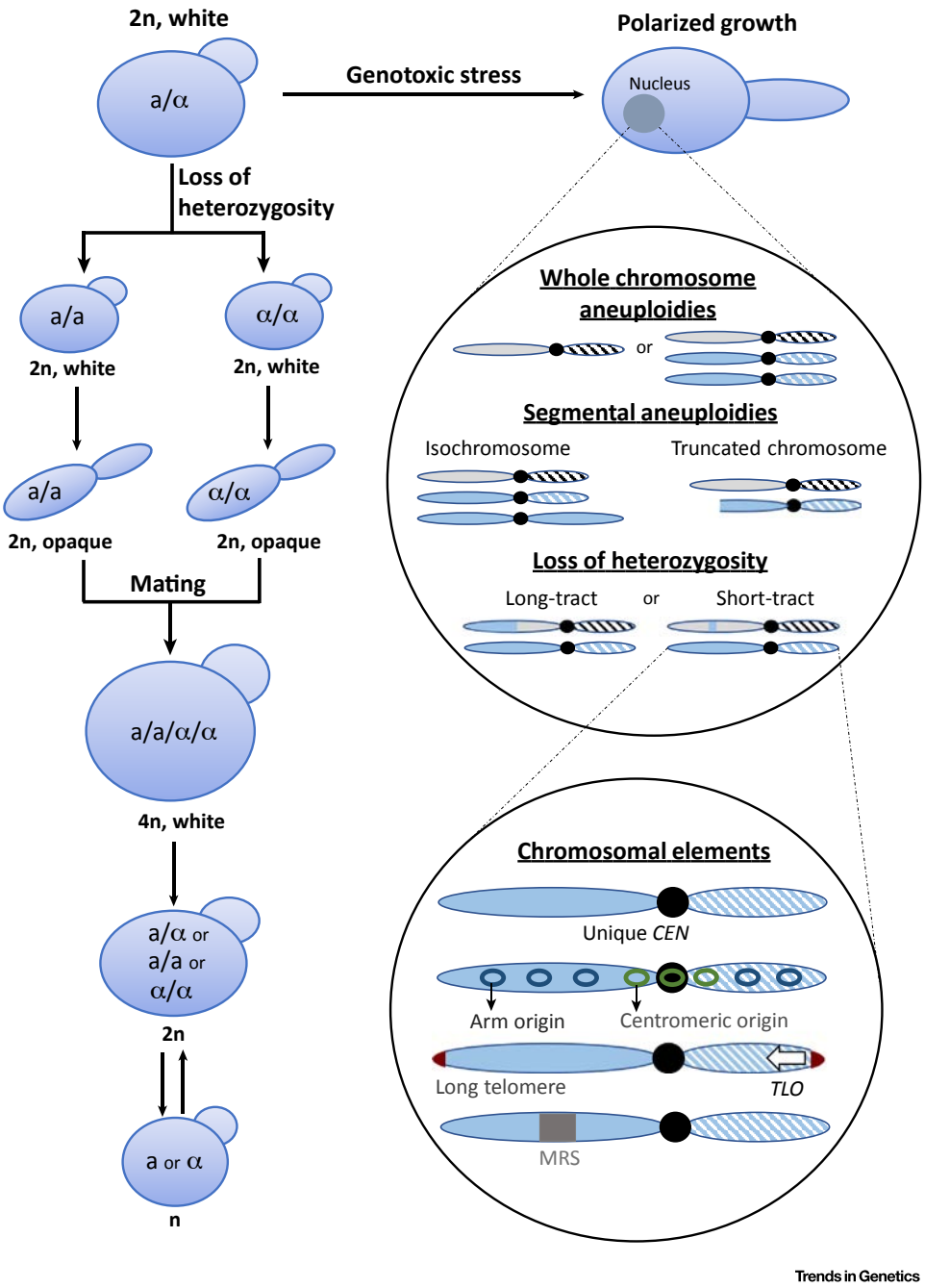


Figure 1. *C. albicans* undergoes a unique haploid–diploid–tetraploid life cycle. A phenotypic switch from white to opaque form due to homozygosis of the *MTL* locus is the initial step of this cycle. Opaque cells of opposite mating-type then fuse together to form tetraploids. These tetraploids undergo a nonmeiotic parasexual cycle to return to the diploid state. Diploid

(Figure legend continued on the bottom of the next page.)

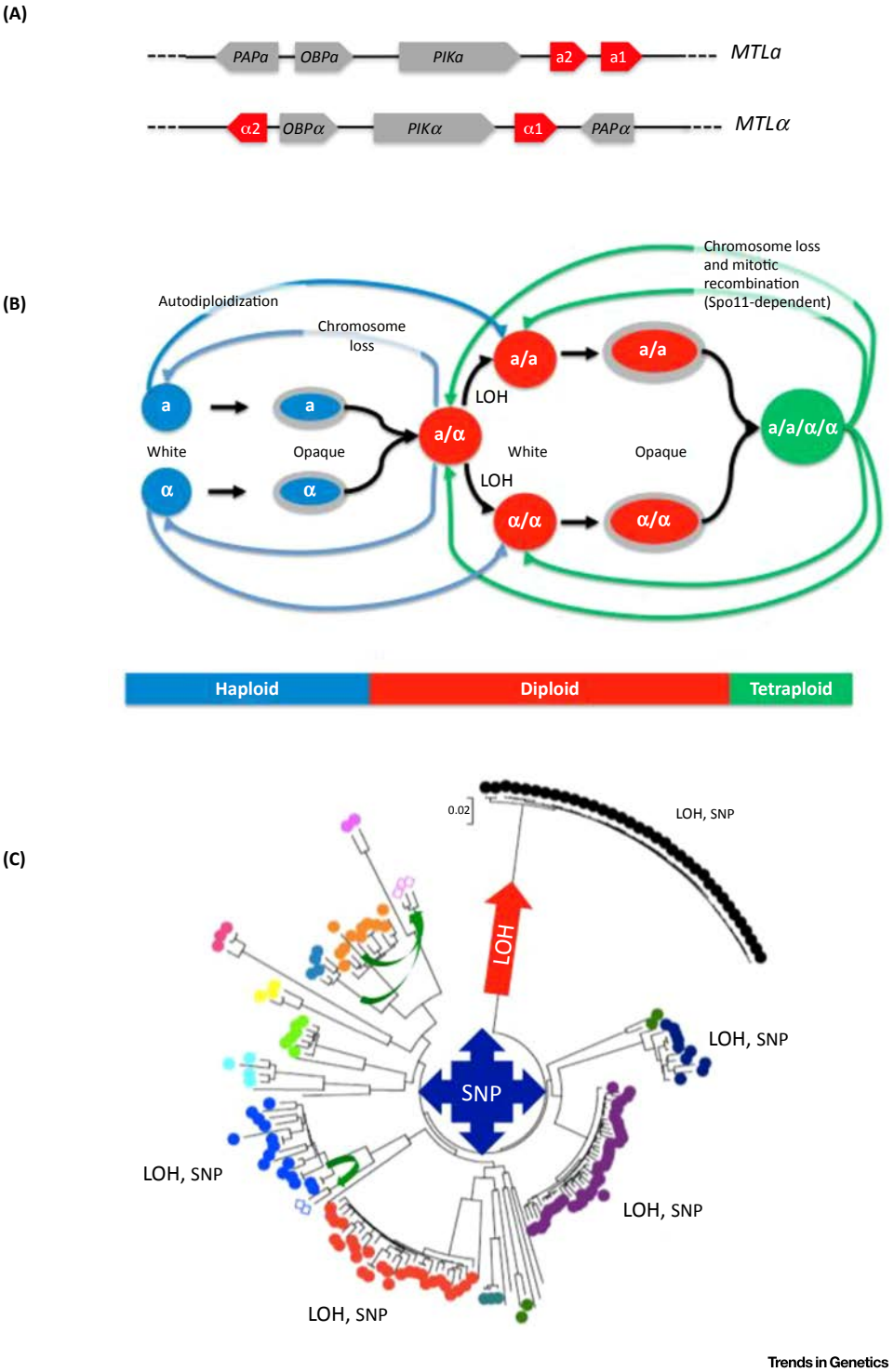
subunit flanked by nonrepetitive elements RB2 and HOK (Figure 3D). Chromosome 3 contains only the RB2 element without the RPS or HOK unit [58]. Surprisingly, the MRS, being a repetitive region, is not assembled into classical heterochromatin but carries marks of both euchromatin and heterochromatin [54]. The MRS covers about 3% of the total genome, yet its function remains elusive, except that it is considered to be a hotspot for genome rearrangements in *C. albicans* [59]. The MRS is a preferred site for chromosomal translocations [58], and the expansion and contraction of its RPS region give rise to chromosome length polymorphism [60]. Furthermore, the presence of the MRS affects the frequency of **nondisjunction**, whereby a homologue bearing a larger MRS is more likely to be lost at the time of chromosome segregation [59]. Thus, the MRS serves as an important means of generating karyotypic diversity in *C. albicans* and needs to be studied in greater detail for its function and origin.

Candida albicans, a Heterozygous Diploid with a Dynamic Genome

Although genome variation has so far been explored primarily either in haploid or homozygous diploid genomes, it is now being tackled in diploid organisms having **heterozygosity**, with a hope of better understanding the genomics of adaptation in various environmental or host niches. Population genomics studies have revealed a number of aspects regarding heterozygosity in the *C. albicans* genome [19,42,61–63] (Figure 4A). Natural heterozygosity is observed across the *C. albicans* genome with an average rate of one SNP every 237–283 bases. By comparing a large number of strains, a higher level of heterozygosity is found to correlate with faster growth rates. These observations likely reflect the loss of alleles that influence fitness in strains that have undergone partial or complete homozygosis. Genome sequencing data have identified about 3600 open reading frames (ORFs) with high-confidence SNPs leading to changes in the amino acid sequence. In addition, SNPs found in regulatory regions, or even in ORFs, can also affect transcription levels and/or translation efficiency between the alleles [63]. Thus, extensive allelic differences may function to increase genetic and phenotypic diversity in an organism devoid of a true sexual cycle and contribute to the acquisition of new phenotypic traits such as drug resistance.

Population genomics studies in *C. albicans* have revealed that genome heterozygosity can vary from 48% to 89% – heterozygous and homozygous regions being defined as such based on the number of heterozygous SNPs within 5-kb windows [19,23,42,61]. The levels of heterozygosity are primarily influenced by large LOH events encompassing whole chromosomes or extending from a specific chromosomal locus to the telomere. These LOH events have been shown to be pervasive in *C. albicans* isolates and can be detected on all chromosomes. LOH can involve an entire chromosome upon chromosome loss due to chromosome nondisjunction during mitosis. Depletion of the centromeric histone H3, Cse4/CENP-A at centromeres has been reported in response to changes in ploidy and the environment, and is associated with chromosome instability [64]. The genome of *C. albicans* contains two homologous histone H2A-encoding genes, *HTA1* on chromosome 3 encoding Hta1p and *HTA2* on chromosome 1

C. albicans can become haploid or vice versa by chromosome loss and autodiploidisation, respectively. Several host factors or environmental stress can lead to karyotypic variations in *C. albicans*. The genome of *C. albicans* is remarkably plastic and can tolerate segmental aneuploidies, whole chromosome aneuploidies, and loss-of-heterozygosity events. Accumulation of such DNA alterations can be associated to polarised growth and drug resistance in *C. albicans*. Several chromosomal elements influence the organisation and stability of the *C. albicans* genome. These include centromeres (CEN), which are all unique and different in *C. albicans*; replication origins, which are of two types, arm origins and centromeric origins; telomeres, which are unusually long and associated with a family of telomere-associated genes (TLO); and the major repeat sequence (MRS), a feature unique to *C. albicans*.



Trends in Genetics

Figure 2. *Candida albicans* Mating Type, Parasexuality, and Population Structure. (A) Schematic of the *MTLa* and *MTLα* idiotypes. The genes encoding transcription factors responsible for cellular identity are shown in red, while the *OBP*, *PAP*, and *PIK* genes that are not involved in cellular identity but differ between the two idiotypes are shown in grey. (B) Schematic of the haploid–diploid–tetraploid life cycle of *C. albicans*. *C. albicans* is predominantly existing in the diploid

(Figure legend continued on the bottom of the next page.)

encoding Hta2p. Unlike Hta2p, Hta1p has lost the conserved phosphorylation site for the Bub1 kinase, a key regulator of chromosome segregation, and has been shown to facilitate chromosome gain and loss events in *C. albicans* [64]. Another frequent cause of LOH is somatic HR used to repair DNA DSBs. DSBs are the consequence of DNA replication defects or R loop formation but can also arise due to external stresses [65]. The extent of the LOH event can reflect the molecular mechanisms involved in DSB repair in *C. albicans.* Short-range LOH can occur by GC without crossover and long-range LOH including whole chromosome arms can occur by either GC with crossover, break-induced replication (BIR), or mitotic crossover (MCO). If left unrepaired, a DSB may result in chromosome truncation or chromosome loss, characterized by LOH events that span an entire chromosome or an arm of it. Several tools have been developed to study LOH events in *C. albicans* (Box 2). They have in particular revealed that DNA DSBs are predominantly repaired by GC but other repair events such as BIR/MCO and GC with crossover (CO) can also be observed at a significant frequency [66]. They have also revealed that the frequency at which LOH events arise and the nature of these LOH are influenced by environmental parameters (see below).

A link between LOH, aneuploidy, and the elevation of antifungal resistance by studying drug-resistant *C. albicans* isolates has been precisely established (Figure 4B). Gain-of-function alleles of genes involved in the resistance to azole antifungals have been shown to be codominant with wild-type alleles and therefore high levels of resistance cannot be achieved in the presence of the wild-type allele. It is only upon homozygosis of the gain-of-function allele, as a result of LOH, that high levels of azole resistance can be achieved [67–69]. In addition, the appearance of aneuploidy and, in particular, the formation of an isochromosome composed of the two left arms of chromosome 5 is often associated with the acquisition of azole resistance [70]. Increased copy number of *ERG11* and *TAC1*, both located on the left arm of chromosome 5, accounts for the majority of drug resistance associated with the chromosome 5 isochromosome [71]. Large-scale genome changes have been characterized in *S. cerevisiae* as a means of adaptation in response to stress [72,73]. Similarly, *in vitro* exposure to oxidative stress, elevated temperature, and antifungal drugs [74,75], as well as passaging through an animal model of infection result in increased genomic rearrangements in *C. albicans* [76,77]. The large-scale changes described above provide *C. albicans* with the ability to rapidly generate genetic and phenotypic diversity within the host environment.

Although LOH events are frequent and widespread in the genome of *C. albicans*, several studies have observed that, for some chromosomes, one haplotype or even a part of it is never found in the homozygous state in the *C. albicans* laboratory strain SC5314. This homozygosis bias has been observed in haploids, in parasexual derivatives, and in *rad52* mutant derivatives [35,37,78]. These observations suggest that recessive lethal and deleterious alleles can be found in the heterozygous state in the genome of *C. albicans*. Several groups, including ours, have identified such recessive lethal or deleterious alleles on chromosome 3A and chromosome 4B [66,79]. Overall, consistent with clonal reproduction, *C. albicans* strains harbour

state with heterozygosity at the *MTL* locus. Homozygosis at the *MTL* locus allows occasional white–opaque phenotypic switching and more efficient mating between opaque cells. Transition from tetraploidy to diploidy or diploidy to haploidy is independent of meiosis and involves random concerted chromosome loss with the intermediate aneuploidy progeny cells. Haploids are shown in blue, diploids in red, and tetraploids in green. (C) Genome sequencing of 182 *C. albicans* isolates confirms a predominantly clonal population structure and reveals, for the first time, footprints of admixtures in two genetic clusters (green arrows), demonstrating the occurrence of (para)sexuality in the *C. albicans* natural environment. LOH (BIR/MCO and GC) is a major driver of intraclade evolution and major LOH events can result in the emergence of clusters with altered virulence/niche restriction, possibly due to pseudogenization [42]. Abbreviations: BIR, break-induced replication; GC, gene conversion; LOH, loss of heterozygosity; MCO, mitotic crossover.

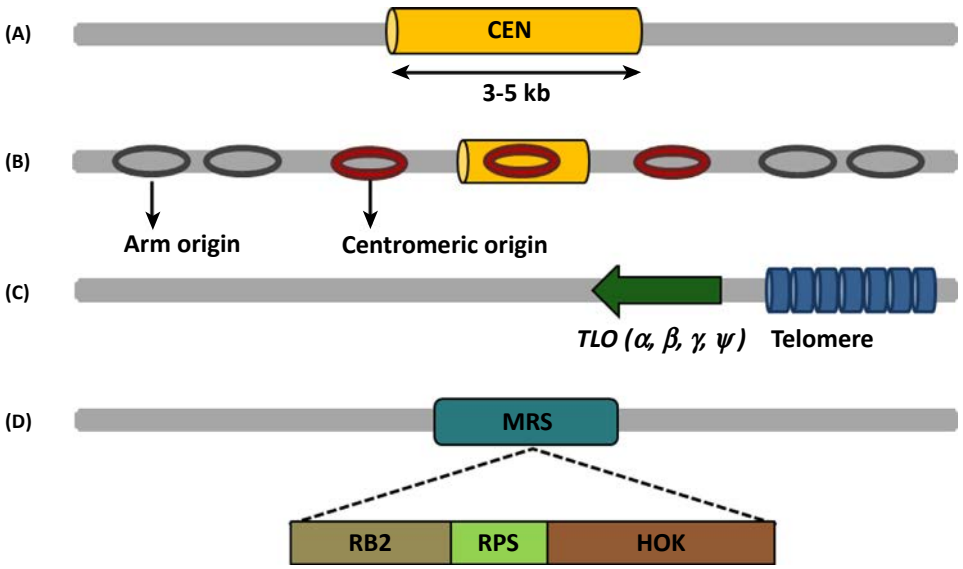


Figure 3. Schematic of Essential Chromosomal Elements in *Candida albicans*. (A) A 3–5-kb long CENP-A-rich centromere (CEN) that lacks any common DNA sequence elements or pericentric repeats. The AT% of CEN regions is not significantly different from the rest of the genome and the CEN DNA sequence does not show any DNA methylation. The CEN function is dependent on the chromosomal context rather than DNA sequence, and thus epigenetically regulated. (B) DNA replication origins (*ORI*s), identified as ORC-bound regions, are categorised into arm *ORI*s and centromere *ORI*s. Like centromeres, origins do not show any strong common DNA sequence motif. (C) Telomere repeats in *C. albicans* are unusually long and subtelomeric regions have an unusually high number of *TLO* genes in this organism. The high copy number of *TLO* genes in *C. albicans* is expected to be the result of subtelomeric recombination, mediated positively by TLO recombination element and negatively by Sir2 [98]. (D) The MRS, which further consists of three sequence elements, namely, RB2, RPS, and HOK. The RB2 (~6 kb) and HOK (~8 kb) elements are nonrepetitive sequences that occur only once per MRS, flanking the RPS element. The repeated sequence (RPS) is a ~2-kb-long repetitive sequence whose number can vary in an MRS. Each RPS unit carries an *Sfi*I restriction enzyme site. *Sfi*I mapping of the *C. albicans* genome served as a valuable tool to study chromosomal rearrangements before the whole genome sequence was available.

recessive lethal and deleterious alleles that constrain the outcome of LOH events. Although **homozygosity** of some alleles has been linked to fitness advantage in a specific host niche, overall genome-wide heterozygosity remains prevalent in the *C. albicans* population. For these reasons, the fate of cells having undergone LOH still needs to be addressed in *C. albicans*.

Specificities of DNA Repair Mechanisms in *Candida albicans*

DNA DSBs, where both strands of the double helix are severed, are the most serious forms of DNA damage. Indeed, failure to repair a DSB leads to loss of the CEN-lacking chromosome fragment, while improper repair of a DSB can lead to gross chromosomal rearrangements such as translocations, inversions, and deletions. Two major pathways of DSB repair are known: HR and **nonhomologous end joining (NHEJ).** Characterisation of deletion mutants of genes involved in HR (*RAD52*, *RAD51*, *RAD59*, *RAD54,* and *RDH54*) have shown that HR plays a crucial role in DNA damage repair in *C. albicans* [78,80–83]. In contrast, the characterisation of mutants impaired for NHEJ has suggested that this process is not efficient in *C. albicans* [78,82]. Recent results on the mode of repair of **CRISPR-cas9**-induced DSBs are in agreement with this observation but suggest that NHEJ could occur in *C. albicans*, albeit rarely. Indeed, it has been shown that HR is the predominant repair pathway of CRISPR-Cas9-induced DSBs in *S. cerevisiae* and *C. albicans*, in contrast to other yeast species such as

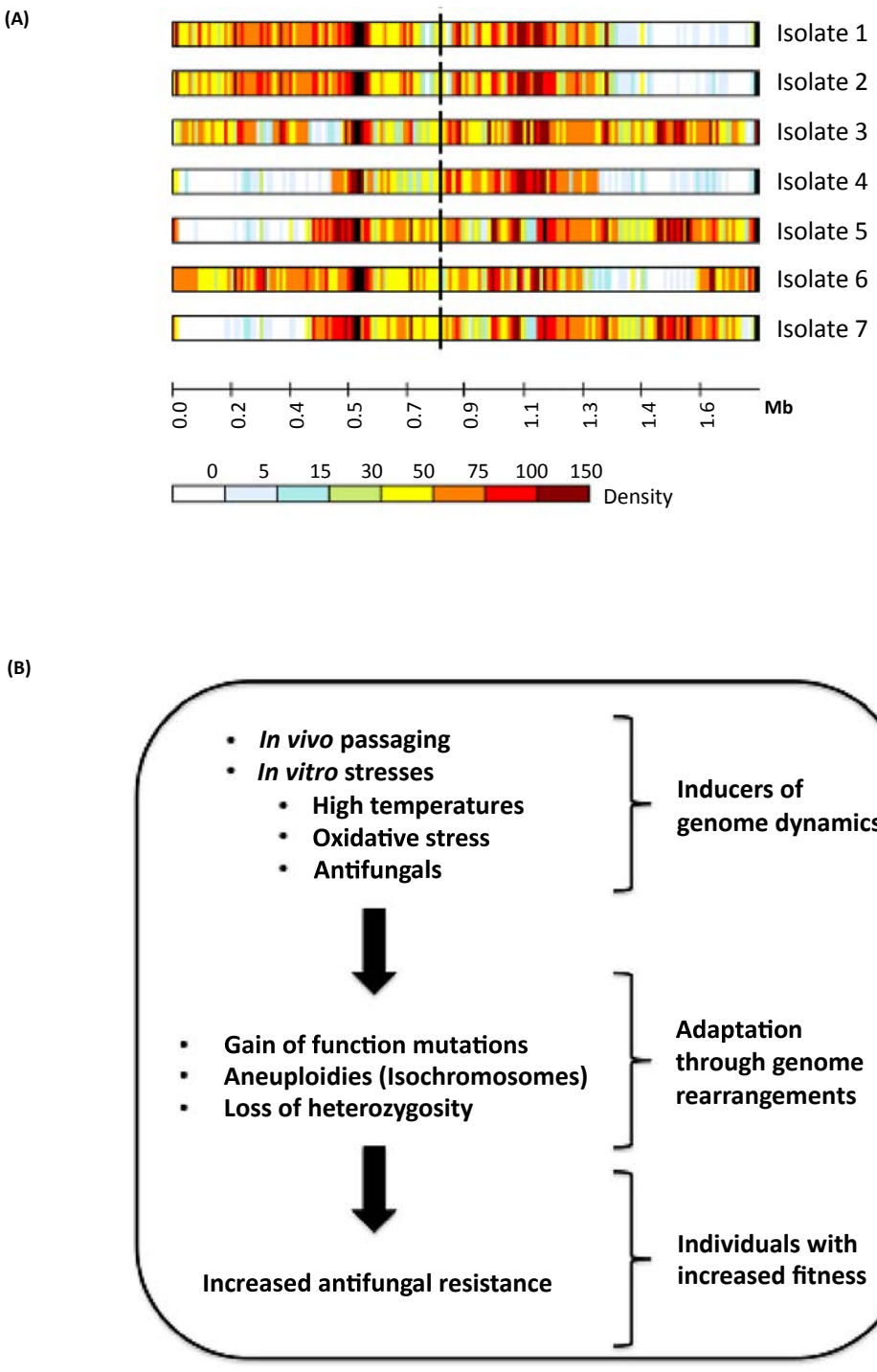
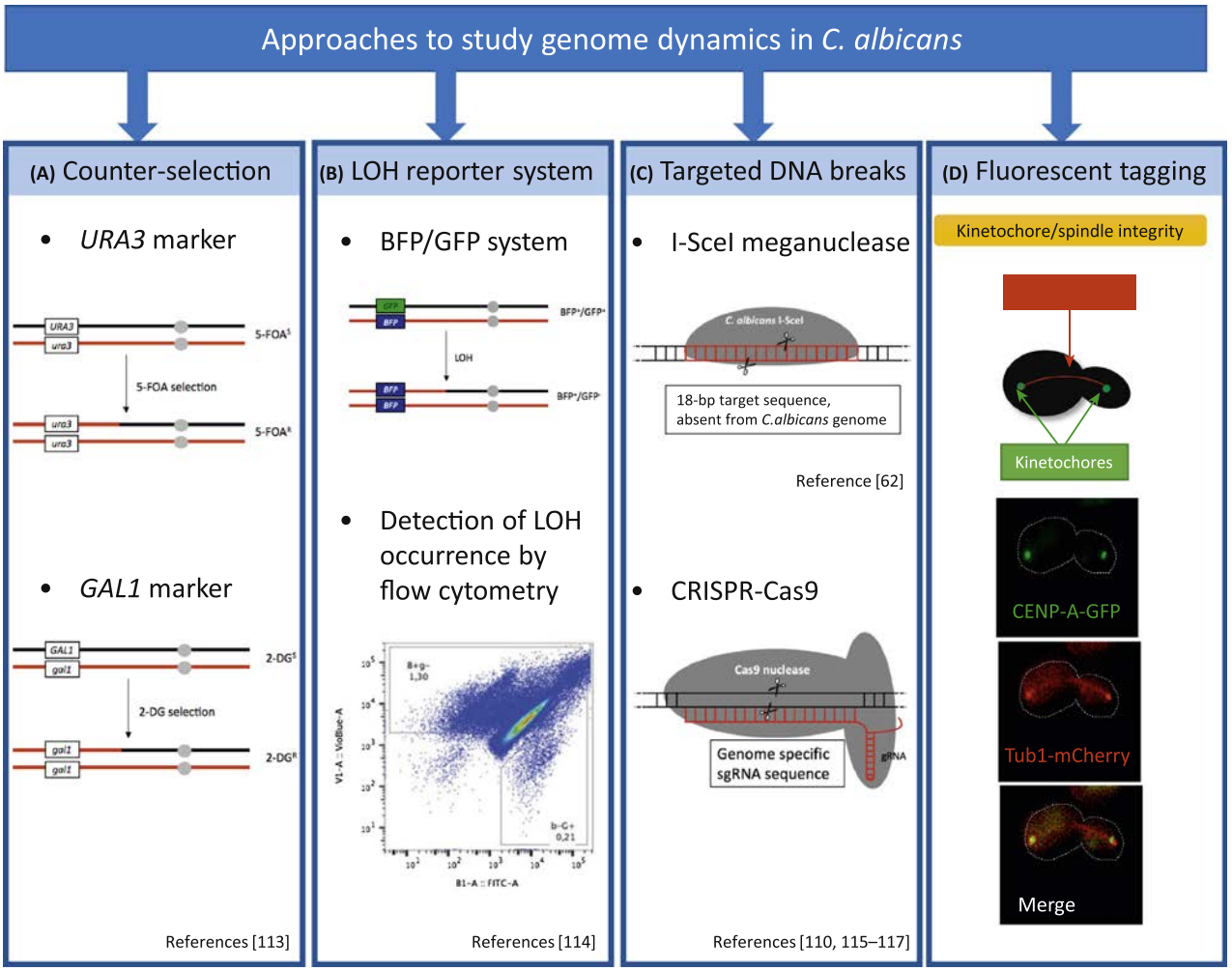


Figure 4. Heterozygosity, LOH, Genome Dynamics, and Antifungal Resistance. (A) Heat map illustrating the density of heterozygous SNPs across chromosome 3 for seven sequenced isolates. Regions that have undergone LOH appear white and are most often extending towards the telomere, indicating that they are the result of either mitotic recombination, break-induced replication, or gene conversion with crossover. (B) Schematic view of the impact of environmental stresses on genome dynamics and adaptation in *C. albicans*. Abbreviation: LOH, loss-of-heterozygosity.

Box 2. A Molecular Toolkit to Study *Candida albicans* Genome Dynamics

Molecular tools have been developed and successfully used to identify and study genome dynamics in *C. albicans*. (A) Identification of LOH events using the *URA3* or *GAL1* markers. In strains that have been engineered to be heterozygous for the *URA3* or *GAL1* gene at a given locus, LOH at this locus leads to resistance to 5-fluoroorotic acid (5-FOA) or 2-deoxygalacatose (2-DG), respectively. (B) Identification of LOH events using a combination of *GFP* (green fluorescent protein-coding gene) and *BFP* (blue fluorescent protein-coding gene). In strains that have been engineered to be heterozygous at a given locus through insertion of *GFP* on one chromosome and *BFP* on its homologue, LOH at this locus results in loss of one of the *FP* genes and therefore results in monofluorescence, which can be revealed by flow cytometry. The monofluorescent cells are localised in the side gates and the double fluorescent cells are found in the middle gate. (C) Genome editing systems have been developed in *C. albicans*. A DNA DSB-inducing system was developed through conditional expression of the *S. cerevisiae* I-SceI meganuclease in a *C. albicans* strain engineered to harbour a unique I-SceI cleavage site. Characterisation of repair events at this I-SceI site show that they almost always correspond to GC events but some instances of BIR/MCO or WCL were also observed, as well as combinations of independent events. A method based on the CRISPR/CRISPR-Cas9 system has emerged for genome editing in *C. albicans*. By combining several guiding RNAs, multiple sites can be targeted simultaneously allowing simultaneous editing of multiple chromosomal sites. (D) Live cell fluorescence imaging of chromosome segregation. CENP-A-GFP strains with Tub1-mCherry allows one to follow kinetochore integrity and spindle morphology [110,113–117].



Candida glabrata and *Naumovia castelli* [84]. Moreover, while HR-defective *S. cerevisiae* mutants could still repair a CRISPR-Cas9-induced DSB in the absence of a repair template, likely via NHEJ, the same is not true in *C. albicans*, consistent with limited efficiency of NHEJ in this species [84]. Nevertheless, NHEJ is likely to occur in *C. albicans* as scars typical of this repair process have been reported at repaired CRISPR-Cas9-induced DSBs [85]. Notably, all of these studies have been performed in diploid isolates of *C. albicans* that are heterozygous at the *MTL* locus. In *S. cerevisiae*, NHEJ is downregulated in *a*/α diploid cells when compared with homozygous diploid or haploid cells only expressing *MATa* or *MAT*α. This is shown to be accomplished by transcriptional repression of specific genes by the a1/α2 repressor [86]. Therefore, NHEJ studies in *MTL* homozygous diploid cells or haploid cells should address the absence or presence of NHEJ in *C. albicans*.

Several studies have shown that treatment of *C. albicans* with DNA-damaging agents triggers polarized growth [87,88]. Mutants with altered expression of genes coding for proteins involved in DNA damage response and cell cycle regulation also display aberrant filamentous morphology [48,49,78,87,89–93] (Figure 5). Strikingly, these data suggest that genotoxic-stress-induced polarised growth involves but does not require the expression of hyphal-specific genes. It is possible that stress-induced polarised growth is different from standard hyphal growth. Another key aspect of DNA repair is the accessibility of the DNA lesion to the DNA repair machinery. Proteins involved in chromatin assembly and remodelling have been shown to be important for efficient DNA repair in *C. albicans* as they change chromatin structure and allow repair proteins to access damaged DNA through the acetylation of histone H4 [94]. Similarly, involvement of HR in the kinetochore assembly has been demonstrated in *C. albicans* [52]. In mammalian cells, acetylation of histone H4 has been shown to also play a critical role in directing changes both in chromatin organisation and in promoting recruitment of DSB repair proteins to sites of DNA damage [95]. Moreover, different chromatin signatures associated with HR or NHEJ repair have been recently defined [96]. It has also been demonstrated that, apart from rapidly accumulating DNA damage, *C. albicans* cells lacking the histone acetyltransferase Hat1 also switch from yeast-like to polarized growth [94]. Altogether, these data corroborate

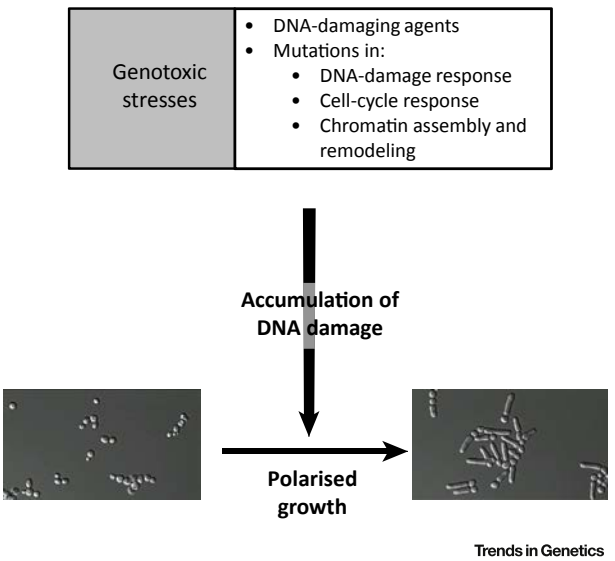


Figure 5. DNA-Damage-Induced Morphogenesis in *Candida albicans*. Schematic of the link between accumulation of DNA damage induced by various genotoxic stresses and polarised growth in *C. albicans*.

that perturbations of cell-cycle progression, a direct consequence of DNA damage, induce filamentous growth in *C. albicans*, in a manner dependent on the DNA damage/replication checkpoint kinase Rad53.

Concluding Remarks

In this review, we have highlighted some of the distinctive features uncovered from recent studies on the genome biology of *C. albicans* that make this yeast a model complementary to *S. cerevisiae* and *S. pombe*; two model species that are at the two extremes of yeast evolution. *C. albicans* exhibits specificities with respect to: (i) its life cycle whereby haploid, diploid, and tetraploid alternate through means of a meiosis-independent, recombinogenic parasexual cycle; (ii) chromosomal landmarks, especially the CENs that have unique organisation and epigenetic properties; (iii) an unusual genome plasticity, that frequently generates aneuploidies and LOH events; (iv) an almost obligate usage of HR for DSB repair; and (v) a coupling of the DNA damage response to morphogenetic shifts. A unique combination of these attributes in one organism allows investigators to address a variety of questions in genome biology that are not generally studied using the conventional yeast models. For instance, as most studies on recombination in *S. cerevisiae* and *S. pombe* employ laboratory haploid or homozygous diploid strains, little is known about the impact of heterozygosity on the biology of yeasts. Results from studies in *C. albicans* clearly demonstrate that heterozygosity is often advantageous, yet allows the propagation of recessive deleterious or lethal alleles that are detrimental upon homozygosis.

Despite changes in epidemiology, *C. albicans* remains responsible for a majority of yeast infections and the treatment of these infections is still a challenge in specific cases, such as systemic or recurrent vulvovaginal candidiasis. One of the key questions is whether *C. albicans* genome plasticity and hallmarks indeed contribute to making this species such an important pathogen. There is no doubt that LOH, ploidy changes, and isochromosome formation contribute to the elevation of antifungal resistance and treatment failure. Moreover, exposure of *C. albicans* to a variety of stresses – antifungals, oxidative stress, high temperature – has been shown to promote recombination events that could help *C. albicans* permanently adapt to changes in environment. Residence of *C. albicans* in animals including in healthy humans, whether as a commensal or pathogen, is accompanied by mutation and recombination events, predominantly short-range LOH (76; Sitterlé *et al.*, personal communication), that could not only reflect adaptation to the host but also help in repairing DNA alterations caused by constant exposure to DNA damaging agents (e.g., reactive oxygen species produced by immune cells). A recent study reports that a combination of mutations and LOH events acquired during serial passaging in the gastrointestinal tract of mice, where *C. albicans* does not normally reside, allows *C. albicans* to shift from a poor gastrointestinal commensal and harmful bloodstream-borne pathogen to an efficient commensal and poor systemic pathogen [97]. Therefore, the high genomic plasticity of *C. albicans* can bear long-term impact on its biology and gaining further insights into the genome biology of this species will certainly impact not only on our understanding of eukaryotic genome biology in general but also the mechanisms that make *C. albicans* such a successful opportunistic pathogen (see Outstanding Questions).

Outstanding Questions

Is *C. albicans* completely devoid of meiosis?

How does Spo11 function during the parasexual cycle?

Can we utilise the parasexual cycle to understand recombination in organisms with cryptic meiosis?

Why is the MRS the hotspot for chromosomal rearrangements?

What is the contribution of the DNA sequence in replication origin and centromere function?

Is heterozygosity necessary for fitness of *C. albicans* in the host?

How is heterozygosity maintained in the largely clonal *C. albicans* population?

What are the mechanisms for tolerance of genome plasticity in *C. albicans*?

Is genome plasticity contributing to *C. albicans* success as a commensal and/or pathogen?

Is there an influence of the microbiota on *C. albicans* genome plasticity?

Is NHEJ active in *C. albicans*?

How is the link between DNA damage and cell polarity precisely established?

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