The Role of Mms22p in DNA Damage Response in Candida albicans

Lan Yan,*1 Juan Xiong,*+,1 Hui Lu,‡ Quan-zhen Lv,* Qian-yao Ma,§ Pierre Côte,† Malcolm Whiteway,†,2 and Yuan-ying Jiang*,2
*Center for New Drug Research, School of Pharmacy, Second Military Medical University, Shanghai 200433, P. R. China,
†Department of Biology, Concordia University, Montreal, Quebec, H4B 1R6, Canada, ‡Key Laboratory of the Plateau of the
Environmental Damage Control, Lanzhou General Hospital of Lanzhou Military Command, Lanzhou, 730050, China, and §School
of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning Province 110016, P.R. China
ORCID IDs: 0000-0002-2617-4418 (L.Y.); 0000-0001-6619-7983 (M.W.)

ABSTRACT To ensure correct DNA replication, eukaryotes have signaling pathways that respond to
replication-associated DNA damage and trigger repair. In both Saccharomyces cerevisiae and Schizosaccharomyces pombe, a complex of proteins, including the cullin protein Rtt101p and two adapter proteins Mms22p and
Mms1p, is important for proper response to replication stress. We have investigated this system in
Candida albicans. In this pathogen, Mms22p is important for recovery from DNA replication damage induced
by agents including methylmethane sulfonate, camptothecin, and ionizing radiation. Although no clear ortho-
log of Mms1p has been identified in C. albicans, loss of either Mms22p or Rtt101p generates similar damage
sensitivity, consistent with a common function. In S. cerevisiae, the Mrc1p–Csm3p–Tof1p complex stabilizes
stalled replication forks and activates a replication checkpoint and interacts with Mms22p. A similar complex in
S. pombe, consisting of the Tof1p and Csm3p orthologs Swi1p and Swi3p, along with the fission yeast Mrc1p,
genetically also interacts with Mms22p. Intriguingly in C. albicans only Mrc1p and Csm3p appear involved in
damage repair, and Mms22p is required for responding to DNA damage agents in MRC1 or CSM3 condi-
tional mutants. In C. albicans, although the loss of RAD57 greatly impairs response in the pathogen to many
DNA-damaging agents, lethality due to camptothecin damage requires concomitant loss of Rad57p and
Mms22p, suggesting that Mms22p is only essential for homologous recombination induced by camptothecin.
These results establish that although C. albicans uses conserved cellular modules to respond to DNA damage
and replication blocks, the specific details of these modules differ significantly from the S. cerevisiae model.

KEYWORDS genomic stability DNA repair replication fork homologous recombination Candida albicans

Accurate transmission of the genome from one generation to the next requires the faithful replication of the DNA. In eukaryotic organisms, the process of DNA replication is challenged by replication stresses, such as dNTP depletion caused by hydroxyurea (HU), alkylated DNA template bases induced by methylmethane sulfonate (MMS), replication fork blockage caused by the topoisomerase I inhibitor camptothecin (CPT) (Vaisica et al. 2011), and single-strand or double-strand breaks due to ionizing radiation (IR) (Ward 1990). To ensure the fidelity and coordinate the progression of DNA replication, this challenging process is regulated by a DNA damage response network that includes S-phase checkpoints that sense stalled replication forks and DNA damage and facilitate DNA repair processes (Harper and Elledge 2007). Mechanisms of DNA repair primarily involve homologous recombination (HR), nonhomologous end-joining, and nucleotide excision repair (Wu and Hickson 2006).

In both the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe, there is an ubiquitin-conjugating complex consisting of orthologs of the budding yeast Rtt101p,
Mms22p, and Mms1p that function in the damage repair process. Loss of ScMms22p or SpMms22p increases cellular sensitivity to a range of DNA-damaging drugs that generate lesions specifically in S-phase or that directly impede DNA replication (Chang et al. 2002; Araki et al. 2003; Baldwin et al. 2005; Dovey and Russell 2007; Duro et al. 2008; Vaisica et al. 2011). In S. cerevisiae, genetic epistasis between Mms22p and Mms1p suggests that Mms22p interacts with Rtt101p via Mms1p to form a protein complex (Rtt101p–Mms1p–Mms22p) required to promote recombinational repair at stalled replication forks and that this complex is required for replication of damaged DNA (Ho et al. 2002; Dovey and Russell 2007; Tourriere and Pasero 2007; Duro et al. 2008; Zaidi et al. 2008; Vaisica et al. 2011). ScMms22p is involved in sensing replication intermediates or in the prevention of DNA damage caused by blocked replication forks.

In S. cerevisiae, Mms22p is important for the stable association of the fork-pausing complex (Mrc1p–Tof1p–Csm3p) when replication stress is present (Vaisica et al. 2011). ScMrc1p acts as a primary mediator for transducing replication fork-pausing checkpoint signals and

### Table 1: Strains used in this study

| Strain Name | Strain Ref | Parental Strain | Key Genotype | Reference |
|-------------|-----------|----------------|--------------|-----------|
| SN152       | WT        | SC5314          | arg4/arg4 leu2/leu2 his1/his1 URA3/ura3::imm434;IRO1/iro1::imm434 | (Noble and Johnson 2005) |
| CaLY8       | SN152     |                | orf19.7494::HIS1/ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MMS22} | CalY226 | CalY8          | orf19.2174::SAT1-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY219     | SN152     |                | orf19.4136::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-TOF1} | CalY337 | CalY219        | orf19.4136::LEU2/ARG-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY220     | SN152     |                | orf19.4105::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-CSM3} | CalY249  | CalY220        | orf19.4105::LEU2/ARG4-MET3p;ORF19.4105 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY222     | SN152     |                | ORF19.7494::ARG4/SAT1-MET3p;ORF19.4105 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MRC1} | CalY316  | CalY222        | orf19.658::LEU2/ARG4-MET3p;CaORF19.658 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY223     | SN152     |                | orf19.2174::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| Δrad57      | CalY235   | CalY223        | orf19.2174::LEU2/ARG4-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY224     | SN152     |                | orf19.2440::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| Δrtt101     | CalY226   | CalY224        | orf19.2440::LEU2/ARG4-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY228     | SN152     |                | ORF19.4105::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MMS22}/P_{M MET3-MRC1} | CalY251   | CalY228        | orf19.658::LEU2/ARG4-MET3p;CaORF19.658 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY234     | CalY226   |                | orf19.4105::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MMS22}/P_{M MET3-CSM3} | CalY246   | CalY234        | orf19.4105::LEU2/ARG4-MET3p;ORF19.4105 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY238     | CalY226   |                | orf19.2174::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MMS22}/Δrad57 | CalY242   | CalY238        | orf19.2174::LEU2/ARG4-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |
| CalY240     | CalY226   |                | orf19.2440::LEU2 arg4/arg4 leu2/leu2 his1/his1 | This study |
| P_{M MET3-MMS22}/Δrtt101 | CalY244   | CalY240        | orf19.2440::LEU2/ARG4-MET3p;ORF19.7494 arg4/arg4 leu2/leu2 his1/his1 | This study |

WT, wild type.
forms a stable complex with Cam3p and Tof1p to promote sister chromatid cohesion after DNA damage (Nedelcheva et al. 2005). S. pombe Mms22p also has been confirmed to interact genetically with components of the replication fork, such as the Swi1p–ScMRC1 complex, to restart DNA replication at stalled forks. S. pombe Mms22p functions in the stabilization of paused replication forks as well (Dovey and Russell 2007).

HR is a high-fidelity DNA repair pathway. Besides playing a critical role in accurate chromosome segregation during meiosis, HR functions in DNA repair and in the recovery of stalled or broken replication forks to ensure genomic stability. In S. cerevisiae, HR proceeds through either Rad51p-dependent or Rad51p-independent pathways. The Rad51p-dependent pathway of recombination, also requiring Rad52p, Rad55p, Rad57p and Rad54p, is the most efficient pathway for gene conversion and is also required for repair of most double-strand DNA breaks in mitotic cells (Johnson and Symington 1995). The Rad51p-independent pathway depends on Rad59p (Sakofske et al. 2012). Budding yeast Mms22p is required for HR-mediated repair of stalled or broken DNA replication forks (Duro et al. 2008), whereas S. pombe Mms22p might block the action of HR (Dovey and Russell 2007).

Although the central role of Mms22p in the maintenance of genome integrity is well characterized in S. cerevisiae and in S. pombe (Dovey and Russell 2007; Duro et al. 2008; Vaisica et al. 2011), the orthologous protein in Candida albicans has not been investigated extensively. Here we reported the identification and initial characterization of the MMS22 gene in C. albicans as the putative ortholog of the MMS22 gene in S. cerevisiae, and we identified C1_06040W_A, a putative ortholog of ScRtt101 in C. albicans, as the Mms22p-interacting protein in the ubiquitin-conjugating complex. To test the function of Mms22p and its potential partner proteins in the fork-pausing complex in C. albicans, we identified C1_11440C_A (a putative ortholog of ScMRCl) (Shi et al. 2007), C2_06130W_A (a putative ortholog of ScCSM3), and C5_01460W_A (a putative ortholog of ScTOF1) in C. albicans. To further explore the involvement of Mms22p in HR repair, we characterized C2_08110W_A (a putative ortholog of ScRAD57 in C. albicans), as previous studies have revealed that the conserved Rad51p, Rad52p, Rad54p, and Rad59p play important role in the HR in C. albicans (Ciudad et al. 2004; Garcia-Prieto et al. 2010; Hoot et al. 2011). We constructed a set of single-gene and double-gene mutants, including the conditional single-gene mutants of P_MET5-MMS22, P_MET5-MRC1, P_MET5-TOF1, and P_MET5-CSM3, as well as the double-gene mutants of the aforementioned genes repressed together with MMS22. We also constructed the null mutants of Δrtt101 and Δrad57, as well as the double-gene mutants of the genes deleted together with MMS22. Our present study shows that CaMms22p plays a vital role in preserving genome integrity during DNA replication and is important for viability after DNA replication-associated damage.

**MATERIALS AND METHODS**

**Media and culture conditions**

Unless otherwise indicated, all the strains were grown routinely in YPD medium (i.e., 1% yeast extract, 2% peptone, and 2% dextrose) at 30°C with shaking overnight, diluted to an OD600 of 0.1–0.2, grown to logarithmic phase, and used for subsequent experiments. As indicated, synthetic complete (SC) medium (0.67% yeast nitrogen base and 2% dextrose) was supplemented with histidine (20 μg/mL), leucine (60 μg/mL), or arginine (40 μg/mL) as appropriate. For
repressing conditions for the \textit{C. albicans} MET3 promoter, mutants were cultured in SC medium with 2.5 mM methionine (Met) and 2.5 mM cysteine (Cys) (SC-Met+/Cys+). To induce the MET3 promoter, mutants were grown in SC medium without Met and Cys (SC-Met−/Cys−) (Care et al. 1999).

**Strain constructions**

The \textit{C. albicans} strains used in this study are listed in Table 1. The oligonucleotides used in this study are listed in supporting information, Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the Table S1. A detailed version of all the strain constructions is provided as supplementary information (File S1). In summary, to generate the

The double-gene mutants of \textit{P}_{M}{M}_{E}T_{3}M{M}_{S}22/\Delta c{s}m_{3}, \textit{P}_{M}{M}_{E}T_{3}M{M}_{S}22/\Delta r{t}t_{1}0{1}, and \textit{P}_{M}{M}_{E}T_{3}M{M}_{S}22/\Delta r{t}t_{1}0{1} were generated by transforming the conditional M{M}_{S}22 mutant (strain CaLY226) with csm3Δ::c.m. LEU2 (creating CaLY234), \textit{rtt101Δ::c.m. LEU2} (creating CaLY235), \textit{or rad57Δ::c.m. LEU2} (creating CaLY236), followed by csm3Δ::c.m. AR4G (creating CaLY246), \textit{rtt101Δ::c.d. AR4G} (creating CaLY244), \textit{or rad57Δ::c.d. AR4G} (creating CaLY242), respectively. The double mutant of \textit{P}_{M}{M}_{E}T_{3}M{M}_{S}22/\textit{P}_{M}{M}_{E}T_{3}M{M}_{S}22/\Delta c{s}m_{3} was generated by transforming strain CaLY226 with mrc1Δ::c.m. LEU2 (creating CaLY228), followed by AR4G-\textit{MET3p-MRC1} cassette (creating CaLY251). All transformants were checked for correct genome integration by genomic PCR.

**Flow cytometry**

\textit{C. albicans} cells derived from an exponentially growing of culture in SC-Met+/Cys+ medium were arrested with 0.01% MMS or 20 mM HU for 4 hr at 30°C with sampling every 2 hr. Cells were then washed to remove MMS or HU, resuspended in SC-Met−/Cys− medium, and incubated for an additional 4 hr with sampling every 2 hr. Samples of 3 mL were fixed with 70% ethanol overnight at 4°C, washed with phosphate-buffered saline (pH = 7.4) and digested with 1 mg/mL RNase A for 1 hr at 37°C to remove RNA. Cells were then stained with 50 mg/mL of propidium iodide for at least 4 hr at room temperature. The DNA content of 5 × 10^6 cells was monitored by fluorescence-activated cell sorting (FACS) analysis using a flow cytometer (FACSCALIBUR; BD Bioscience) and analyzed by Cellquest software (BD Bioscience). The vertical axis is cell counts and the horizontal axis is nuclear fluorescence. Three independent experiments were performed.

**DNA damage sensitivity assays**

Mid-log phase cultures were adjusted to 5 × 10^6 cells/mL, fivefold serially diluted, and spotted onto solid SC-Met+/Cys+ or SC-Met−/Cys− medium, which contains the indicated concentration of MMS, CPT, or HU. Alternatively, serial dilutions of cells were spotted onto solid SC-Met+/Cys+ or SC-Met−/Cys− medium for irradiating with the indicated dose of IR. Growth of cells was detected after a 48-hr incubation period at 30°C.

For testing the survival of cells with exposure to MMS or HU, mid-log phase \textit{C. albicans} cells were cultured in SC-Met+/Cys+ medium containing 0.005% MMS or 20 mM HU for 12 hr. At the indicated time points, samples were pooled, washed, and a range of 500–1000 cells
| Species | \(P_{\text{MET}}\)-MMS22 | \(RTT101\)Δ/Δ | \(P_{\text{MET}}\)-MMS22 | \(RTT101\)Δ/Δ | \(P_{\text{MET}}\)-MRC1 | \(P_{\text{MET}}\)-MRC1 | \(P_{\text{MET}}\)-CSM3 | \(P_{\text{MET}}\)-CSM3 | \(P_{\text{MET}}\)-TOF1 | \(P_{\text{MET}}\)-MMS22 | \(P_{\text{MET}}\)-MMS22 | \(RAD57\)Δ/Δ | \(RAD57\)Δ/Δ |
|---------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| C. albicans | | | | | | | | | | | | | |
| Morphology | Elongated | Wild type | Elongated | Elongated | Elongated | Elongated | Elongated | Elongated | Wild type | Elongated | Elongated | Elongated | Elongated |
| MMS sensitivity | Increased | Increased | More increased | Less increased | Increased | Less increased | Less increased | Less increased | No change | Increased | Increased | Increased | Increased |
| HU sensitivity | No change | No change | No change | No change | No change | No change | No change | No change | No change | Increased | Increased | Increased | Increased |
| CPT sensitivity | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | No change | Increased | Increased | Increased |
| IR sensitivity | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | No change | Increased | Increased | Increased |
| S. cerevisiae | mms22Δ | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased | Increased |
| Morphology | | | | | | | | | | | | | |
| MMS sensitivity | | | | | | | | | | | | | |
| HU sensitivity | Increased | | | | | | | | | | | | |
| CPT sensitivity | Increased | | | | | | | | | | | | |
| IR sensitivity | | | | | | | | | | | | | |
| S. pombe | mms22Δ | mrc1Δ | mms22Δ | mrc1Δ | mrc1Δ | mms22Δ | swi3Δ | mms22Δ | swi1Δ | mms22Δ | rhp57Δ | mms22Δ | rhp57Δ |
| Morphology | Elongated | Increased | No change | No change | No change | No change | No change | No change | No change | No change | No change | No change | No change |
| MMS sensitivity | Increased | Increased | More increased | More increased | No change | No change | No change | No change | No change | No change | No change | No change | No change |
| HU sensitivity | Increased | Increased | More increased | More increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased |
| CPT sensitivity | Increased | Increased | More increased | More increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased | Less increased |
| IR sensitivity | Resistant | | | | | | | | | | | | |
were spread onto solid SC-Met+/Cys+ medium in triplicate. The number of colonies was counted following incubation at 30°C for 2 d. The percentage of survival with untreated normalized to 100% at the indicated time points were calculated. Three independent experiments were performed.

**Morphogenesis analysis**

Mid-log phase *C. albicans* cells were adjusted to 1 × 10^3 cells/mL. A total of 100 μL of each strain culture were spread onto solid SC-Met+/Cys+ or SC-Met-/Cys− medium, incubated at 30°C for 3–4 d, and photographed. Meanwhile, mid-log cultures adjusted to 1 × 10^5 cells/mL were grown for another 9 hr in liquid SC-Met+/Cys+ or SC-Met-/Cys− medium with shaking at 30°C, then photographed with an EVOS X1 microscope (Life Technologies).

**Alignments**

We aligned Mms22p, Tof1p, Csm3p, Mrc1p, plus Rad57p primary amino sequences in *S. cerevisiae*, *C. albicans*, and *S. pombe*. Rtt101p sequences were aligned in *S. cerevisiae* and *C. albicans*. Multiple protein sequence alignments were performed with the MAFFT
A web application (http://mafft.cbrc.jp/alignment/server/) and visualized with Jalview (Version 2.8). The primary amino sequences of the S. cerevisiae, C. albicans, and S. pombe proteins were downloaded from the Fungal Orthogroups Repository (http://www.broadinstitute.org/cgi-bin/regev/orthogroups) hosted by the Broad Institute, MIT.

Data availability
Strains are available upon request. File S1 contains detailed descriptions of all supplemental files.

RESULTS
Identification of Mms22p and its partner proteins in C. albicans
We used the Fungal Orthogroups Repository (Wapinski et al. 2007) to identify that the C. albicans CR_00390W_A gene is orthologous to both the S. cerevisiae MMS22 gene and the S. pombe mms22 gene. C. albicans CR_00390W_A encodes a protein with 1704 amino acids (molecular weight 196.9 kDa). When this protein is aligned with ScMms22p, it showed 5% identity and 19% similarity (Table 2; Figure S1A). We predicted that CaCR_00390W_A is a functional ortholog of ScMMS22 and classified CaCR_00390W_A as CaMMS22.

Similarly, we identified C. albicans C5_01460W_A and C2_06130W_A, as orthologous to ScTOF1 and ScCSM3, as well as Spswi1 and Spswi3, respectively, by using the Fungal Orthogroups Repository. Alignment of ScTOF1 and C. albicans C5_01460W_A indicated 6% identity and 20% similarity over their full-length sequences (Table 2; Figure S1B), and 9 and 22% between ScCSM3 and C. albicans C2_06130W_A (Table 2; Figure S1C), respectively. We named C. albicans C5_01460W_A and C2_06130W_A as CaTOF1 and CaCSM3. We also identified C2_08110W_A/CaRAD57 as the ortholog to ScRAD57 with 5% identity and 16% similarity, and to Sprph57 with 5% identity and 15% similarity (Table 2; Figure S1E). However, we failed to find a gene homologous to ScRAD55 or Sprph55 in C. albicans. To address the function of possible Rtt101p and Mms1p paralogs in C. albicans, the Fungal Orthogroups Repository was used to identify CI_06040W_A as an ortholog to ScRTT101 with 5% identity and 18% similarity (Table 2; Figure S1F), whereas no homologous gene
some during replication stress, we constructed conditional single-gene mutants of regulated expression of TOF1, MRC1, or CSM3, and the conditional double-gene mutants of P_{MET3}-MMS22/P_{MET3}-MRC1 and P_{MET3}-MMS22/P_{MET3}-CSM3 (Figure S3) to explore a potential link between Mms22p and Tof1p, Mrc1p, or Csm3p in *C. albicans*. The regulated expression of the target genes was confirmed by quantitative real-time PCR (data not shown).

To probe the function of the *RTT101* gene and the relationship between the *RTT101* gene and the MMS22 gene in *C. albicans*, both alleles of *RTT101* were deleted in the wild-type SN152 and P_{MET3}-MMS22 strains, to generate Δrtt101 and P_{MET3}-MMS22/Δrtt101 mutants (Figure S4). Similarly, to explore whether *CaMMS22* is involved in HR repair, both alleles of *CaRAD57* in either the wild-type SN152 or the P_{MET3}-MMS22 mutant were deleted, respectively, to obtain Δrad57 and P_{MET3}-MMS22/Δrad57 mutants (Figure S4).

**Mms22p is important for the recovery from a disturbed DNA replication in *C. albicans***

In contrast to the wild-type strain, in which yeast cells formed smooth, domed colonies and separated readily after cytokinesis, we found that the P_{MET3}-MMS22 strain grew normally under non-repressing conditions but formed rough, flattened colonies (Figure 1A) and elongated cells (Figure 1B) when the *MET3* promoter was repressed in SC-Met^{+}/Cys^{+} medium, even in the absence of any genotoxic stress. In general, in response to cell-cycle arrest in *C. albicans*, a filamentous cell type with characteristics of both pseudohyphae and true hyphae appears (Berman 2006). The elongated cells suggested that the repressed MMS22 mutant could be defective in DNA replication or were unable to repair DNA breaks appearing spontaneously during replication.

*S. cerevisiae mms22Δ* and *S. pombe mms22Δ* mutants are sensitive to MMS, HU, and CPT but less sensitive (or resistant) to IR (Table 3) (Chang et al. 2002; Araki et al. 2003; Dovey and Russell 2007). We assessed the sensitivity of the P_{MET3}-MMS22 mutant strain to various DNA damaging agents. The P_{MET3}-MMS22 strain showed increased sensitivity to MMS and CPT, and intriguingly showed enhanced sensitivity to IR, but not to HU, when the gene is shut off by growth in SC-Met^{+}/Cys^{+} medium (Figure 2A). To further confirm the differential sensitivity to MMS and HU in the P_{MET3}-MMS22 strain in the repressed condition, we checked the viability of the strains during a 12-hr period of MMS or HU exposure (Figure 2B). MMS began to significantly inhibit growth of the P_{MET3}-MMS22 strain relative to wild type after 4 hr of treatment, whereas HU affected viability in both the wild-type and P_{MET3}-MMS22 strains similarly. These results indicated that the MMS22 gene is required for MMS resistance even during short-term MMS exposure in *C. albicans*, but is not needed for HU resistance, in contrast to *S. cerevisiae MMS22* and *S. pombe mms22*, which are required for both MMS and HU resistance (Bennett et al. 2001; Dovey and Russell 2007).

We then used flow cytometry (FACS) to examine the changes in cell-cycle progression in the P_{MET3}-MMS22 strain during treatment with MMS or HU for 4 hr, and during a following 4-hr recovery period. The wild-type and P_{MET3}-MMS22 strains showed similar behavior during the whole cell cycle of the treatment with MMS or HU, and arrested in S phase (Figure 2C, S-phase arrested arrows). After removal of either agent followed by culturing in fresh media, the wild-type strain progressed through the cell cycle within 2 hr, as evidenced by the re-emergence of cells in the G2 phase (Figure 2C, recovery arrows) and the increase in the percentage of G2 cells (Figure 2D). By contrast, the P_{MET3}-MMS22 strain treated with MMS remained in S phase with only

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**Figure 4** DNA content analyzed by flow cytometry. Cells derived from an exponentially growing of culture in SC-Met^{+}/Cys^{+} medium for 12 hr. Samples were collected every 3 hr. (A) One represented cell-cycle progress in the mutants is shown. The vertical axis is cell counts and the horizontal axis is nuclear fluorescence. Blue and red curves indicate DNA contents of aggregated cells which have not been analyzed. (B) Percentage of G1/G1+G2 and G2/G1+G2 of three independent experiments are shown. **P < 0.01 when compared with the counterpart phase of the wild type.**

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**Generation of conditional mutants of mms22 and its partner genes in *C. albicans***

To test the function of MMS22 in *C. albicans*, the first allele was replaced with the mms22Δ::C.d.HIS1 cassette (strain CaLY8) using the fusion–PCR-based strategy in strain SN152 (Noble and Johnson 2005). A conditional MMS22 mutant was constructed from CaLY8 in which the single remaining copy of MMS22 was placed under the control of the *MET3* promoter (strain CaLY226; Figure S2). MMS22 mRNA levels in the wild-type SN152 and the P_{MET3}-MMS22 strain grown under nonrepressed and repressed conditions were analyzed by relative quantitative real-time PCR. The transcription level of CaACT1 was used as a standard for normalization. MMS22 mRNA levels in the repressed P_{MET3}-MMS22 strain were 10-fold lower than in the wild-type strain after 12 hr of growth in SC-Met^{+}/Cys^{+} medium, whereas the transcription level of MMS22 is similar to the wild type strain in SC-Met^{−}/Cys^{−} medium. The conditional expression of the MMS22 mutant allowed us to study the function of this gene in *C. albicans*.

Since the budding yeast *Mms22p* and the fission yeast *SpMms22p* genetically interact with the fork-pausing complex to stabilize the repli-
one peak and did not proceed into the cell cycle within 2 hr (Figure 2C, no recovery arrow) and even 4 hr (data not shown) without the increased percentage of G2 cells (Figure 2D). However, the \( \text{P}_{\text{MET}}\text{-MMS22} \) strain treated with HU re-entered the cell cycle 2 hr upon removal of the HU, as evidenced by the re-emergence of G2-phase peak and the increase in the percentage of G2 cells, similar to the wild-type strain (Figure 2C, recovery arrows; Figure 2D). These results suggested that during decreased expression of MMS22, cells were unable to recover from arrest triggered by MMS. Thus, our data suggest that \( C. \text{albicans} \) Mms22p is essential for recovery from DNA replication damage induced by MMS (and potentially CPT and IR) and that the repression of MMS22 caused an abnormal cell cycle after recovery from replication stress.

Mms22p is required for responding to DNA damage agents in \( \text{MRC1} \) or \( \text{CSM3} \) conditional mutants’ fork-pausing complex

To explore a potential link between Mms22p and Tof1p (\( S. \text{pombe} \) Swi1p), Mrc1p or Csm3p (\( S. \text{pombe} \) Swi3p) in \( C. \text{albicans} \), the conditional single-gene mutants permitting regulated expression of \( \text{TOF1} \), \( \text{MRC1} \), or \( \text{CSM3} \), and the conditional double-gene mutants of \( \text{P}_{\text{MET}}\text{-MMS22} \)/\( \text{P}_{\text{MET}}\text{-MRC1} \), \( \text{P}_{\text{MET}}\text{-MMS22} \)/\( \text{P}_{\text{MET}}\text{-CSM3} \) were constructed.

In the absence of any genotoxic stresses, repression of \( C. \text{albicans} \) \( \text{CSM3} \) or \( \text{MRC1} \) produced viable colonies that were rough, large in size compared with the wild-type colonies (Figure 3A). The single cells also displayed an elongated phenotype (Figure 3C). These were consistent with the FAC5 results, which revealed an accumulation of \( \text{P}_{\text{MET}}\text{-MRC1} \) or \( \text{P}_{\text{MET}}\text{-MMS22} \) mutants arrested in the G2 phase that can’t complete mitosis within 12 hr in the repressive conditions (Figure 4). By contrast, the repression of \( \text{TOF1} \) generated similar colony and cellular morphology to the wild type (Figure 3, A and C), and cells progressed through the cell cycle and completed mitosis within 12 hr normally (Figure 4). Strikingly, combined repression of both MMS22 and \( \text{MRC1} \) led to significantly smaller colonies with wrinkled edges (Figure 3B) and generated elongated cells (Figure 3D), whereas combined repression of both MMS22 and \( \text{CSM3} \) generated similar morphological phenotypes to those in each independent shut off (Figure 3, B and D). Because the filamentous cells can be indicative of DNA replication defects, our results suggest that the loss of \( \text{MRC1} \) or \( \text{CSM3} \) may result in defects in either DNA replication or in the repair of DNA breaks that arise spontaneously during DNA replication. The absence of MMS22 exacerbated the defect in the \( \text{mrc1} \) mutant in \( C. \text{albicans} \), but interestingly not in the \( \text{csm3} \) mutant.

Repression of either \( \text{MRC1} \) or \( \text{CSM3} \) caused increased sensitivity to MMS, CPT, IR, and less sensitivity to HU, whereas the repression of \( \text{TOF1} \) did not affect cellular sensitivity to these agents (Figure 5; Table 3). These data suggest that these three proteins might have different function in checkpoint control or in DNA replication. By contrast, Mrc1p is essential in \( S. \text{cerevisiae} \) but dispensable in \( S. \text{pombe} \) (Bennett et al. 2001; Calzada et al. 2005; Dovey and Russell 2007). Moreover, the repression of MMS22 led to a partial rescue of the sensitivity of the \( \text{P}_{\text{MET}}\text{-CSM3} \) or \( \text{P}_{\text{MET}}\text{-MRC1} \) mutant to MMS and HU, whereas the repression of MMS22 caused increased the sensitivity to CPT and IR of the \( \text{P}_{\text{MET}}\text{-MRC1} \) mutant (Figure 2A; Table 3), supporting the idea that Mms22p is required for responding to paused replication forks.

Mms22p is required for responding to CPT in the rad57-null mutants

The \( S. \text{cerevisiae} \) Rad55p-Rad57p complex and the \( S. \text{pombe} \) Rhp55p-Rhp57p complex have unique nonredundant functions in recombination, and mutations in any one of these components can lead to recombination defects, chromosomal instability, sensitivity to DNA damage, and meiotic defects (Khasanov et al. 2008). Because \( S. \text{pombe} \) mms22 is indispensable for replication-associated DNA damage that is repaired by HR, and the \( \text{mms22} \)/\( \text{rad57} \) null mutant was highly sensitive to the presence of DNA damaging agents such as MMS, HU, and IR (Figure 7, Figure 2A in \( \text{SC-Met}^+/\text{Cys}^+ \) medium). Intriguingly, the mutant showed only a slight sensitivity to CPT in comparison with the wild type, but the \( \text{P}_{\text{MET}}\text{-MMS22} \)/\( \text{Δrad57} \) strain showed high sensitivity to CPT after MMS22 promoter shut-off (Figure 2A in \( \text{SC-Met}^+/\text{Cys}^+ \) medium; Table 3). These data suggested that Rad57p is critical for responding to MMS, HU, or IR damage in \( C. \text{albicans} \) but is only essential for CPT damage repair in the absence of MMS22. This requirement of Rad57p for DNA repair in either the \( \text{P}_{\text{MET}}\text{-MMS22} \) mutant or the wild type strain indicated that in the absence or the presence of MMS22, cells could experience DNA damage that is repaired by HR.

Mms22p and Rtt101p promote replication through damaged DNA

In budding yeast, Mms22p interacts with Rtt101p, bridged by the DNA repair protein Mms1p, and is recruited to ubiquitinate a currently unidentified substrate (or substrates) in the DNA repair process (Zaidi et al. 2008). Cells lacking MMS22, RTT101, or MMS1 showed similarly increased sensitivities to MMS and HU. The importance of the Mms22p-Mms1p module in stabilizing the replisome during replication stress is conserved in both budding yeast and fission yeast (Dovey and Russell 2007; Zaidi et al. 2008; Vaissica et al. 2011). However, no clear ortholog to ScRtt101 has been identified in \( S. \text{pombe} \).

The \( C. \text{albicans} \) \( \text{rtt101} \) null mutant displayed wild-type colony growth on both solid media and liquid media, which was similar to the \( \text{rad57} \) null mutant (Figure 6). The \( \text{rtt101} \) null mutant was sensitive
to MMS but not to HU, CPT, or IR (Figure 7; Figure 2A in SC-Met−/Cys− medium). Compared with the P_MET3-MMS22 single mutant, the P_MET3-MMS22/Δrtt101 strain exhibited enhanced sensitivity to MMS, similar sensitivity to CPT and IR, and unchanged sensitivity to HU (Figure 2A; Table 3), suggesting that Rtt101p might work together with Mms22p in the same pathway in response to MMS.

**DISCUSSION**

In this study, we identified and characterized a DNA-repair protein, Mms22p, in *C. albicans*. In untreated cells, repression of the MMS22 gene resulted in elongated and deformed cells. Shut off of the P_MET3-MMS22 mutant on solid SC-Met−/Cys− medium caused hypersensitivity to the chemical agents MMS and CPT as well as IR. Moreover, after transient exposure to MMS, P_MET3-MMS22 mutants were unable to complete mitosis in a timely fashion and showed decreased viability, accumulating with an elongated morphology and arresting in S phase. In *C. albicans*, Mms22p likely participates in the DNA repair pathway that is important for the recovery from S-phase-specific DNA damage caused by MMS, CPT, or IR. As well, Mms22p is required for normal cell cycle progression after recovery from replication stress.

In *S. cerevisiae*, Mms22p has been proposed to be a substrate-specific adaptor of a DNA repair—specific Rtt101p-based cullin complex that is stimulated by MMS, works in an Mms1p-dependent manner, and is involved in the processing of stalled replication forks (Zaidi et al. 2008). Cullins are a family of proteins that act as scaffolds for the assembly of multisubunit ubiquitin ligases. Protein ubiquitination involves three enzymes: E1, E2, and a ubiquitin ligase E3, which can directly recognize specific substrates to perform different functions (Mellon et al. 1987; Dovey and Russell 2007; Daulny and Tansey 2009; Fujii et al. 2009). Rtt101p is a cullin-based protein that forms part of an E3 ubiquitin ligase complex required for replication fork progression through DNA lesions and naturally occurring pause sites in yeast (Luke et al. 2006). In response to DNA damage, Rtt101p is recruited to chromatin, in a process that depends on the histone H3 lysine-56 acetyltransferase Rtt109p and the BRCA1 C terminus repeat-containing protein Rtt107p (Roberts et al. 2008).

Each component of the Rtt101p–Mms1p–Mms22p complex is important for the stable association of the replisome with replication forks during replication stress (Vaisica et al. 2011). Moreover, an evolutionary conserved Mms1p–Mms22p module is required for replication of damaged DNA in fission yeast (Dovey et al. 2009). Although no clear ortholog of Mms1p has been identified in *C. albicans*, the Δrtt101 mutant displayed similar sensitivities to the chemical agents and IR, as did the P_MET3-MMS22 mutant, which suggests that Mms22p and Rtt101p may function in the same pathway in the presence of replication-associated DNA damage and is consistent with the Rtt101p–Mms22p complex (either without Mms1p or with a structurally highly divergent Mms1p) also being essential for the stabilization of the replisome during replication stress in *C. albicans*.

During DNA synthesis, replication forks are exposed to various types of stress. Csm3p, Tof1p, and Mrc1p have been identified as checkpoint-specific mediators in budding yeast, and they have the overlapping role during activation the replication checkpoint (Foss 2001; Osborn and Elledge 2003; Tong et al. 2004). Recent studies suggested that Mrc1p was required to maintain the normal rate of replication fork progression, whereas Tof1p was critical for DNA replication forks to pause at diverse chromosomal sites where non-nucleosomal proteins bind very tightly to DNA (Bando et al. 2009). Swi1p and Swi3p of *S. pombe*, the homologs of *S. cerevisiae* Tof1p and Csm3p, form a complex and play important roles in the stabilization of stalled replication forks and DNA-damaging agents. Spot assays comparing the sensitivity of the wild-type SN152 (WT) and the mutant strains. Cells were grown as described in the section Materials and Methods, diluted, and spotted onto plates with the indicated concentrations of MMS, HU, CPT, and indicated dose of IR. The strains were cultured on solid SC-Met−/Cys− medium and photographed after 2 d growth at 30°.
activation of the DNA replication checkpoint (Noguchi et al. 2004). Our study suggests that although MrClp and Csm3p are involved in DNA replication and repair in C. albicans, Tof1p is apparently not required for these processes. In the absence of exogenous DNA damaging agents, when either MRCl or CSM3 was repressed, the cells exhibited a mitotic delay and were arrested in the G2 phase with a constitutively pseudohyphal morphology. Furthermore, these mutants had increased sensitivity to the agents MSS, HU, CPT, and IR, resulting in reduced viability compared with the wild-type strain; this occurred whether Mms22p was repressed or not. Usually, pseudohyphae and true hyphae emerge in response to cell-cycle arrest in C. albicans (Berman 2006). We speculate that the delayed cell cycle in either mutants or cells treated with reagents that alter cell-cycle progression can cause cell elongation in C. albicans. This point is consistent with the view that cell polarity during hyphal morphogenesis is regulated by a change in the cell cycle (Ahn et al. 1999; Loeb et al. 1999). Our results suggest that the mutants in which the replisome components MrClp or Csm3p were repressed were unable to recover from DNA damage, supporting an important role for MrClp and Csm3p in DNA repair in the fungal pathogen.

Mms22p, together with Mms1p, is indispensable for the stabilization of the S. cerevisiae MrClp–Csm3p–Tof1p component under conditions of replication stress. The deletion of MMS22 reduces either MrClp or Csm3p localization to stalled replication forks (Dovey and Russell 2007; Vaisica et al. 2011). In contrast, S. pombe has a negative relationship between Mms22p and Swi1p or Swi3p. The deletion of either swi1 or swi3 rescues the phenotypes in the mms22 mutant (Table 3) (Dovey and Russell 2007). Similarly to the situation in S. pombe, in this study, we observed that the repression of either CSM3 or MRCl led to a partial rescue of the sensitivity of the PMET5-MMS22 mutant to MMS, whereas the repression of MRCl caused increased sensitivity to CPT and IR of the PMET5-MMS22 mutant. This finding suggests that Mms22p is required for responding to DNA damage agents in MRCl or CSM3 conditional mutants.

In the budding yeast, Rad51p-mediated HR plays a central role in promoting repair of double-strand breaks generated during replication (Heyer et al. 2010; Holthausen et al. 2010). HR is initiated at regions of single-strand DNA that become coated by the evolutionarily conserved Rad51p recombinase to form nucleoprotein filaments. These filaments, assisted by Rad52p and Rad55p-Rad57p, facilitate the search for homologous sequences in an intact duplex that acts as a template for repair synthesis (Paques and Haber 1999; Herzberg et al. 2006; Wu and Hickson 2006). In this study, the Δrad57 mutant was more sensitive to MMS and IR and especially to HU compared with PMET5-MMS22 mutant. The Δrad57 mutant was hypersensitive to CPT in the absence of MMS22. Our results suggest that Mms22p is only essential for HR induced by CPT. This finding is in contrast to the requirement of Mms22p in budding yeast for HR-mediated repair (Duro et al. 2008), or the blockage action of S. pombe Mms22p for HR repair pathway (Dovey and Russell 2007).

In conclusion, our results show that although C. albicans orthologs of S. cerevisiae and S. pombe DNA damage repair pathway members are involved in DNA damage repair in the fungal pathogen, the details of their function show distinct characteristics. In the pathogen Mms22p has little role in protecting against HU-mediated damage, whereas Tof1p appears unimportant in response to any damage investigated, in sharp contrast to their importance in these roles in S. cerevisiae. Overall, in the pathogen it appears that Mms22p plays a critical role in preserving genome integrity during DNA replication; perhaps Mms22 functions to maintain genomic integrity by HR through coordination of DNA synthesis by interacting with Rtt101p in the rescue of paused replication forks after they confront a block.

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