The topoisomerase I gene from *Candida albicans*

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We report here the cloning of the *Candida albicans* genomic topoisomerase I gene (TOP1) by use of PCR and subsequent hybridization. The predicted protein sequence shared 58.8% identity with the Saccharomyces cerevisiae topoisomerase I and 30–50% identity with other eukaryotic topoisomerase I proteins. A conditional gene disruption strain (CWJ477) was constructed so that one copy of TOP1 was deleted and the other copy of TOP1 was placed under a regulatable promoter. Under repressed conditions, cells grew slowly and cell morphology was abnormal. The virulence of CWJ477 was markedly reduced in a mouse model system, and that of the single gene knockout strain was slightly attenuated, indicating that TOP1 might play a role in the infection of *C. albicans* in mice in a dose-dependent manner. Despite the reduced virulence of both the single and double knockout strains, viable cells of the pathogen were recovered from the kidneys as late as 22 d post-infection.

**Keywords**: *Candida albicans*, TOP1, null mutant, virulence

INTRODUCTION

DNA topoisomerases are key enzymes that resolve topological problems incurred during the cellular processes of replication, transcription, recombination and chromosome segregation. Based on their mechanism of action, they are classified into two types: type I DNA topoisomerases can break and rejoin one DNA strand at a time, whereas type II enzymes work by making reversible breaks in both strands, in a somewhat concerted manner, and by passing another segment of DNA duplex through this transient break (reviewed by Roca, 1995). Type I topoisomerases work as monomeric and ATP-independent enzymes. Based on their common enzymic properties and protein sequence analysis, the type I topoisomerases can be divided into two subtypes: type 1-5', and type 1-3'. Type 1-5' topoisomerases bind, cleave and open transient gates in single-stranded DNA segments, in order to allow the passage of another single-stranded or double-stranded DNA segment. At the DNA cleavage stage, a protein-DNA covalent intermediate is formed between a tyrosyl residue and the 5'-phosphate at the DNA break site. Examples of type I-5' topoisomerases are bacterial DNA topoisomerase I and III and eukaryotic DNA topoisomerase III. In contrast to type 1-5', type 1-3' topoisomerases bind preferentially to double-stranded DNA, and cleave one of the DNA strands of the duplex by forming a protein-DNA covalent intermediate between a tyrosyl residue and the 3'-phosphate at the break site. This family includes all eukaryotic DNA topoisomerase I enzymes and prokaryotic DNA topoisomerase IV.

The *Saccharomyces cerevisiae* TOP1 gene is a member of the highly conserved group of type 1-3' topoisomerases (Goto & Wang, 1985; Roca, 1995). Despite the evidence for involvement of topoisomerase I in essential functions such as transcription and DNA replication, TOP1 is not required for growth, and deletion of this gene results in only a modest growth defect. Deletion of TOP1 does, however, cause a large increase in mitotic recombination in the tandemly repeated ribosomal DNA arrays, a chromosomal domain that is normally suppressed for recombination (Christman et al., 1988). Null mutations in the topoisomerase I gene are also not lethal in *Schizosaccharomyces pombe* (Uemura et al., 1987) or *Ustilago maydis* (Gerhold et al., 1994), but are lethal to a developing *Drosophila melanogaster* embryo (Lee et al., 1993).

The GenBank accession number for the nucleotide sequence reported in this paper is U41342.

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Drugs against DNA topoisomerase I, such as camptothecin, have been developed as potential anticancer agents. The cytotoxicity of the drug is due to the inhibition of an essential function (reviewed by Chen & Liu, 1994). Therefore, although TOP1 is not essential in many fungi, fungal topoisomerase I could still be considered as a potential antifungal drug target. The Candida albicans DNA topoisomerase I enzyme has been recently isolated and characterized (Fostel et al., 1992; Fostel & Montgomery, 1995). In vitro experiments showed that stabilization by camptothecin of the fungal topoisomerase I cleavage complex requires a concentration 10-fold greater than the minimum concentration of camptothecin needed to enhance nicking by the human topoisomerase I (Fostel et al., 1992). Conversely, the fungal topoisomerase I is more susceptible to the aminocolechol A-3253 than the human topoisomerase I (Fostel & Montgomery, 1995). This evidence suggests that there are structural differences between the two enzymes which may allow for the identification of new antifungal agents which target the fungal topoisomerase I and not the human enzyme. To begin to verify the structural differences of topoisomerase I from fungal pathogens and humans, we characterized TOP1 from C. albicans. Here we report the cloning and sequencing of TOP1, and the use of an inducible/repressible promoter to study the phenotype of gene shut-off. We also demonstrate here that the TOP1 gene disruption strain has reduced virulence in a mouse model.

METHODS

Strains. C. albicans strains CAF2-1 (ura3Δ::imm434/URA3) and CA14 (ura3Δ::imm434/ura3Δ::imm434) were obtained from W. Fonzi (Fonzi & Irwin, 1993). DNA cloning was carried out in Escherichia coli XL1-Blue [recA1 endA1 gyrA96 thi-1 hsdR17 supE44 thi-1 gyrA96 relA1 lacI43 X114 leuB6 proA+ lacY1 F' lacZM15 tnl1]; Stratagene) or DH5α [F- M15 su1ΔlacZΔM15 ΔlacZΔargF169 deoR recA1 endA1 p(o) ha1 lysS::Tn10(oriT)].

Gene cloning and sequencing. The same conditions and primers were used here to amplify a TOP1 gene fragment from C. albicans genomic DNA as were described for cloning of the U. maydis TOP1 gene by PCR (Gerhold et al., 1994).

The amplified PCR fragment was subcloned into pT7Blue vector (Novagen), forming plasmid pCaT1. The clone was verified by sequencing. Using this PCR fragment, genomic DNA of pCaT1 was obtained from W. Fonzi (Fonzi & Irwin, 1993) was cloned into the BamHI site of pBluescript SK(+) (Stratagene), resulting in plasmid pUMT1. The same conditions and primers were used to amplify a 5′-ATGGATCCATGAGTTCATCAGACGAA fragment from plasmid pKW16 for cloning of the C. albicans promoter induced by maltose and repressed by glucose (MRPI) and cloned into the pT7Blue vector. A. C. albicans promoter fragment was then subcloned from the pT7Blue vector into the same sites of plasmid pKM183, which is based on a pBluescript plasmid, pSKII, with a URA3 marker at the SacI site, resulting in plasmid pUM2. The BamHI–HindIII fragment of TOP1 was then subcloned from the pT7Blue vector into the same sites of pUM2, forming plasmid pUMT2. AspI-linearized pUMT2 was transformed into the heterozygous TOP1 gene disruption strain CWJ431. Transformants were selected on a minimal medium containing maltose as sole carbon source. Again transformants were verified by Southern blot hybridization. After verification by Southern analysis, one transformant was designated CWJ477. The strain contained one disrupted, nonfunctional copy of TOP1 and one copy of TOP1 under the control of the maltose-inducible promoter.

DNA topoisomerase I assays. Glass beads were used to prepare C. albicans cell extracts as described for S. cerevisiae (Levin et al., 1993). Cells from a 50 ml culture at late-exponential phase were collected, washed once with protein buffer (30 mM Tris/HCl, pH 7.5; 1 mM EDTA; 0.3 M KCl; 10%, v/v, glycerol; 2 mM PMSF), and resuspended in the same buffer with a volume equal to the volume of the cell pellet. Acid-washed glass beads (2 vols) were added to the suspension. The suspension was vortexed at 4°C for 60 s three times. Supernatant was collected after an additional wash of beads with 1 vol. of the protein buffer and centrifuged at maximum microfuge speed for 20 min. The volume of the final clear protein extract was about 1 ml, which was stored at −70°C for later use. Protein extract was diluted 1:10 with protein buffer and 1 μl was used in each reaction containing 200 ng supercoiled pUC19 in 20 μl of the topoisomerase assay buffer (20 mM Tris, pH 7.5; 100 mM KCl; 10 mM EDTA; 50 μg gelatin ml−1). After 30 min incubation at 37°C, reactions were stopped with 1 μl 20 mg proteinase K ml−1 and 2 μl 20% (w/v) SDS. DNA was examined in a 0.8% agarose gel containing ethidium bromide after incubation at 37°C for 30 min and extraction with phenol/chloroform.

Virulence test of TOP1-deleted C. albicans strains. Three
strains were used to determine virulence in the mouse model system. CAF2-1 is a heterozygous URA3 knockout strain, which is used as a control for wild-type TOP1. CWJ429 is a heterozygous TOP1 knockout strain with one copy of TOP1 replaced by a hisG-URA3-hisG cassette and one wild-type copy of TOP1. CWJ477 is derived from CWJ429, in which one copy of TOP1 was disrupted and the second copy of TOP1 was placed under the regulation of a maltose-inducible and glucose-repressible promoter. Both CWJ429 and CWJ477 have one copy of URA3, which is similar to the parental strain, CAF2-1. Single colonies from strains CWJ429 and CWJ477 were inoculated into the liquid culture media YEPD (1%, w/v, yeast extract; 2%, w/v, peptone; 2%, w/v, dextrose) and YEPM (1%, w/v, yeast extract; 2%, w/v, peptone; 2%, w/v, maltose) and grown overnight (12 h) at 30°C. The parental strain (CAF2-1) was cultured only in YEPD. The virulence of these *C. albicans* strains was tested in a normal mouse model system as described previously (Becker et al., 1995). Male ICR mice (22–25 g; Harlan Sprague–Dawley) were inoculated with 100 μl of a suspension of 10⁷ cells ml⁻¹ via the lateral tail veins. Kidneys were excised on the indicated days post-infection, homogenized and plated onto YEPD or YEPM to determine c.f.u. (g tissue)⁻¹. For histological examination, paraffin sections were prepared from infected kidneys preserved in neutral-buffered formalin. Sections were stained using a commercially available silver stain kit (Sigma) to detect the presence of *Candida*, and counterstained with haematoxylin and eosin.

**Germ-tube formation test.** *C. albicans* cells were grown in either YEPD or YEPM and washed once with water. Germ-tube formation was induced in 10% (v/v) foetal bovine serum (Gibco-BRL), RPMI 1640 medium (Mediatech) or 50% serum/50% Sabouraud Dextrose Broth (Difco) at 37°C (Bulawa et al., 1995). After 1, 3, or 4 h incubation, cells were examined microscopically.

**RESULTS**

**Cloning of TOP1**

Our strategy for cloning the *C. albicans* TOP1 gene was to identify peptide domains conserved among eukaryotic topoisomerase I genes, to design oligonucleotide primers based on these domains, and to use PCR to amplify the intervening TOP1 DNA fragment from genomic template DNA. The gene fragment recovered was then used to select a full-length gene from a genomic DNA library. We have successfully used this approach to clone the *U. maydis* TOP1 gene (Gerhold et al., 1994). The same oligonucleotide primers were used in the amplification of a fragment of TOP1 from the *C. albicans* genomic DNA. The amplified DNA fragment was ligated into plasmid pT7Blue (Novagen). The DNA sequence of this fragment predicted an amino acid sequence showing strong homology to other topoisomerase I proteins. Using this fragment as a probe, we screened a genomic DNA library based on plasmid YEpl3 (Rosenbluh et al., 1985) by hybridization and obtained five positive clones. DNA sequence analysis demonstrated that the largest clone, pCaT1, contained the entire coding region of the *C. albicans* TOP1 gene. A 3145 bp EcoRI fragment from pCaT1 was subcloned into pBC KS(−) (Stratagene), forming plasmid pCaT1-R12. The complete sequence from both DNA strands revealed the complete C. **Fig. 1.** Nucleotide sequence of the genomic TOP1 from *C. albicans*. (a) EcoRI DNA fragment (3143 bp) containing the TOP1 gene. The amino acid sequence of the ORF is shown under the corresponding nucleotide sequence. The codon CUG (CTG in the DNA) encodes tyrosine in yeast, Candida (Gerhold et al., 1993). The essential tyrosine residue in the catalytic domain and the nearby methionine residue which is different from those in most other eukaryotic cells (usually leucine or isoleucine) are indicated as bold letters. (b) DNA strider pattern of the EcoRI fragment. EcoRV and NcoI sites were used for gene disruption and their relative positions to the ORF (547–2889) are indicated.
albicans TOP1 ORF with highly significant homology to other TOP1 genes (Fig. 1a).

**Predicted TOP1 peptide**

The predicted *C. albicans* TOP1 protein (Toplp) contains most of the peptide motifs conserved in Toplp from other eukaryotes (Fig. 2a). Peptide sequence comparison of Toplp revealed that *C. albicans* Toplp is most closely related to *S. cerevisiae* Toplp over the entire sequence (58.8% identity; see Fig. 2b). In the conserved region, in close proximity to the tyrosine (residue 738; Figs 1a and 2b) known to attach transiently to the DNA, the *C. albicans* enzyme is typified by a methionine rather than the isoleucine or leucine that is found in seven other eukaryotic enzymes. It is unclear whether this difference may lead to differential specificity of the *C. albicans* enzyme.

**Conditional gene disruption of TOP1**

The essentiality of TOP1 has been tested in a variety of organisms. Thus far, it appears not to be essential in some organisms and can be considered a target for poisons rather than inhibitors (Chen & Liu, 1994). In *Drosophila* it is an essential enzyme that affects embryo development (Lee et al., 1993). To test whether TOP1 is essential in *C. albicans*, we initiated the disruption of *C. albicans* TOP2 using the technology and procedure developed by Fonzi & Irwin (1993). A gene disruption construct was made by multi-step cloning so that a 1630 bp internal coding sequence (encoding 543 amino acids; 70% of the ORF; Fig. 1b) of TOP1 was replaced by a hisG-URA3-hisG cassette (Fig. 3; also see Methods). Linearized plasmid pUC19TAU was transformed into *C. albicans* strain CA14. Transformants were selected on minimal SD (0.67% Yeast Nitrogen base; 2%, w/v, glucose) plates. To identify
the looping out of URA3 the transformants were replated on 5-fluoroorotic acid plates supplemented with uridine. Transformants resistant to 5-fluoroorotic acid expected to be heterozygous TOP1 disruptants (CWJ431; TOP1/top1Δ::hisG) were recovered. Both the primary transformant (CWJ429) and the loopout strain (CWJ431) were verified by Southern hybridization of the EcoRI-digested genomic DNA using a 900 bp EcoRI–NcoI fragment of the 5'-end of TOP1 as a probe (data not shown). The same linearized plasmid DNA was used for the second round transformation of strain CWJ431 to obtain a homozygous knockout. No transformants were obtained after repeated transformation. This result suggests that TOP1 may be essential or that TOP1 deletion homozygosity may impair the growth of transformants. Therefore, we attempted another approach, i.e. using a regulatable promoter (Fig. 3) to test the essentiality of the gene.

A maltose-inducible/glucose-repressible promoter (MRPI) was cloned into an integrative URA3 plasmid, forming plasmid pUM2. A DNA fragment encoding the N-terminal 198 amino acids of Toplp was placed under this promoter (see Methods), resulting in plasmid pUMT2. Integration of the AspI-linearized pUMT2 into a wild-type copy of TOP1 is expected to create a truncated Toplp with only a 54 N-terminal amino acid peptide and a full-length TOP1 under the regulatable promoter. Transformants were selected on a maltose minimal medium and then streaked onto both maltose and glucose media. All the transformants grew on both media. However, some of them grew more slowly than others. The slower growing cells did not develop hyphae on rich medium after extended incubation (data not shown). These cells were subcultured and their morphology was further examined microscopically. Cells cultured in glucose medium were elongated and tended to lyse (Fig. 4a, lower panel) while cells from the maltose medium grew normally (Fig. 4a, upper panel). To determine whether the slow-growing cells lacked topoisomerase I activity, crude protein extracts were prepared from cells growing in both media and tested for DNA topoisomerase I activity using a supercoiled DNA relaxation assay (Gerhold et al., 1994). In order to determine the sensitivity of this assay, protein extracts were diluted tenfold prior to use. Under these conditions, topoisomerase I activity was still detectable in cells grown in maltose, suggesting that the assay has a broad range of sensitivity (data not shown). No topoisomerase I activity was detectable in the slow-growing cells growing in medium containing glucose, while the activity was detectable in all cells grown with maltose (Fig. 4b). Each of the three strains demonstrating maltose-inducible topoisomerase I activity was characterized to verify their genetic construction by Southern hybridization using a 600 bp fragment of the 5'-end of TOP1 (Fig. 4c). To further verify that the disruption of TOP1 was specific, Southern hybridization performed using a URA3 probe yielded a band of the expected size thereby ruling out additional integration events (data not shown). The results indicate that C. albicans TOP1 is not essential for cell growth, but depletion of the protein leads to slow growth and a change in cell morphology.

Virulence test of the TOP1-deleted C. albicans strains

The phenotypic change of the Toplp-depleted C. albicans strain indicates that TOP1 may affect the infectious phase of the organism. To test the effect of TOP1 deletion on virulence in an animal model, normal mice were infected with the parental strain (CAF2-1), TOP1 heterozygous knockout strain (CWJ429), and the strain (CWJ477) with the only existing copy of TOP1 under the maltose-inducible and glucose-repressible promoter. Prior experiments demonstrated that the maltose promoter used for gene deletions was not induced in vivo in the mouse model, presumably due to the absence of maltose in tissues and serum. Confirmation of the absence of induction of the maltose promoter was seen by placing URA3 under control of the maltose promoter. This resulted in strains that were rapidly cleared from infected mice and as such were avirulent (data not shown). This outcome is similar to the lack of pathogenicity described for ura3 homozygous knockout strains (Kirsch & Whitney, 1991).
Strains were cultured in both glucose and maltose media prior to injection. Since strain CWJ477 clumps and is fragile in glucose medium (Fig. 4a), it was difficult to determine cell density accurately. The suspension was vigorously vortexed for 30 s, which was sufficient to disrupt the cell clumps but not to lyse the cells, thus allowing accurate counting in the haemocytometer.

The survival curve for mice infected with the three strains indicated that inactivation of one or two copies of \( \text{TOP1} \) leads to dramatic attenuation of virulence (Fig. 5). There were no survivors after 7 d among mice infected with the parental strain, whereas about 50% of the mice survived infection with the heterozygous \( \text{TOP1} \) knockout strain, CWJ429, at day 11 from maltose-cultured cells and at day 13 from glucose-cultured cells. However, only one of nine mice infected with maltose-cultured CWJ477 cells had died by day 22, while about 50% mice infected with glucose-cultured CWJ477 cells survived.

To test whether the reduced virulence of \( \text{C. albicans} \) cells was due to either attenuation or clearance from the mice, c.f.u. (g tissue)\(^{-1}\) was determined from homogenized kidney tissue on various days post-infection in survivors from each group, and on day 22 from all
Fig. 5. Virulence test of *C. albicans* TOP1 disruptants. Mice were inoculated with approximately $1 \times 10^6$ cells of three different *Candida* strains grown in YEPM (upper panel) and YEPD (lower panel). The percentage survival of mice was plotted against the days of observation. The experiment was repeated and the results are summarized in the plot. ●, CAF2-1 (control; $n = 10$); ■, CWJ429 (TOP1/top1; $n = 9$); ▲, CWJ477 (top1/top1; $n = 9$).

remaining mice. Large numbers of c.f.u. were recovered from the kidneys of mice infected with either TOP1/top1 or top1/top1 strains (Table 1). Results of a repeat experiment were also similar to the primary experiment (data not shown). Therefore, the survival rate of the host did not reflect the clearance of the pathogen or a significant decrease of c.f.u. in the kidneys of the infected host but rather reflected attenuated virulence of the strains.

**TOP1-deleted cells have reduced capability to form germ tubes**

Although TOP1-deleted *C. albicans* cells have reduced virulence, they were recovered from the kidneys of the infected mice. We tested the germ-tube formation of these strains under three different conditions. When cells were grown in maltose medium (induction conditions) and then tested for germ-tube formation, both heterozygous and homozygous *top1* strains (CWJ429 and CWJ477) were able to form germ tubes like the wild-type strain (CAF2-1) (data not shown). However, when cells were pre-grown in glucose medium, the homozygous *top1* strain (CWJ477) formed germ tubes with decreased frequency at the beginning of the serum induction (1.5 h) (Fig. 6, left panel). After extended incubation at 37 °C with serum, few germ tubes could be observed in the homozygous *top1* strain (Fig. 6, right panel). Similar results were also obtained from cells induced with 10% dialysed serum supplemented with 2% maltose or glucose. All strains could form germ tubes almost equally well in the 10% dialysed serum with maltose; however, the homozygous disruptant strain formed germ tubes poorly in the 10% dialysed serum with glucose. These results indicate that

<table>
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<th>Strain* (growth conditions)</th>
<th>Days post-infection</th>
<th>Kidney mass (g)</th>
<th>c.f.u. (g tissue)$^{-1}$</th>
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<td></td>
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* CWJ429 = *top1:*hisG–URA3–hisG/TOP1; CWJ477 = *top1:*hisG/MRPl–TOP1–URA3.
Fig. 6. Germ-tube formation of *C. albicans* TOP1 disruptants. *C. albicans* cells were grown in YEPD glucose medium overnight and inoculated into 50% serum/50% Sabouraud Dextrose Broth at 37 °C. Cells were examined under the microscope after 1.5 h (left panel) and 14 h (right panel) incubation. Top, CAF2-1 (wild-type TOP1); middle, CWJ429 (TOP1/top1); bottom, CWJ477 (top1/top1).

**Fig. 6.** Germ-tube formation of *C. albicans* TOP1 disruptants. *C. albicans* cells were grown in YEPD glucose medium overnight and inoculated into 50% serum/50% Sabouraud Dextrose Broth at 37 °C. Cells were examined under the microscope after 1.5 h (left panel) and 14 h (right panel) incubation. Top, CAF2-1 (wild-type TOP1); middle, CWJ429 (TOP1/top1); bottom, CWJ477 (top1/top1).

topoisoerase 1 plays an important role in germ-tube formation.

**TOP1-deleted cells form hyphae in the kidney**

Histological examination of the kidneys of mice infected with the homozygous deletion strain (CWJ477) revealed that *Candida* was found in a hyphal form (Fig. 7). The characteristics of infection observed in kidneys containing the heterozygous knockout strain (CWJ429) were indistinguishable from CWJ477. At days 7–10 post-infection, kidneys obtained from mice infected with the wild-type *Candida* (CAF2-1) contained well-defined focal lesions, comprised of hyphal forms, primarily within the renal cortex (data not shown; see Kwon-Chung & Bennett, 1992). The kidneys infected with the deletion strains exhibited a very different pattern of infection. Renal tissue obtained 10 d post-infection revealed a dilated renal pelvis (Fig. 7a). Contained within the urinary space of the renal pelvis was a large mass of *Candida* hyphae, commonly referred to as a ‘fungus ball’ (Fig. 7b). The lack of lymphocytes in the region surrounding the inflammation suggests that this is an acute, suppurative response. Although there were indications of inflammation in the remainder of the renal tissue, no *Candida* (either yeast or hyphal forms) was observed in these regions.

**DISCUSSION**

We report here the cloning and characterization of the *C. albicans* TOP1 gene. A conditional TOP1 disruption mutant strain was constructed to investigate the role of topoisoerase I in cellular processes and in pathogenesis. Our data show that this gene is not essential for *in vitro* growth of *C. albicans*, but morphological changes are obvious in minimal medium when the protein is depleted under promoter-repressed condi-
S. cerevisiae. We also showed that the growth defect and a large increase in mitotic recombination elevations, indicating that TOP1 plays an important role in cellular processes. A similar effect in TOP1-disrupted cells of S. cerevisiae and U. maydis has also been observed (Thrash et al., 1985; Gerhold et al., 1994). In S. cerevisiae, deletion of this gene results in a modest growth defect and a large increase in mitotic recombination in the tandemly repeated ribosomal DNA arrays (Christman et al., 1988). A top1 gene disruption mutant of U. maydis displayed a subtle colouration phenotype evident during cell senescence and a much lower level of mitotic recombination elevation (Gerhold et al., 1994).

We also showed that the TOP1 deletion strains were deficient in their ability to form germ tubes and to grow in the hyphal form after serum induction in vitro. It is possible that the inability of the conditional knockout to form hyphae in vitro might be due to a general effect on cell viability rather than specifically to the lack of topoisomerase I activity. However, in vivo, clear histological evidence indicated that these same strains grew in the hyphal form in the renal pelvis; at day 10 post-infection it appears that Candida harboured in the kidney is limited to the urinary space with little or no evidence of hyphal growth in the tissues. These in vivo and in vitro observations are difficult to resolve. It is possible that there are additional factors present in the microenvironment of the murine urinary space which promote hyphal growth of these Candida mutants in the kidney. This possibility warrants further investigation.

Injection of TOP1 deletion strains into mice indicates that deletion of one copy of TOP1 causes some attenuation of virulence, while deletion of both copies leads to a more pronounced degree of attenuation. It is possible that these results might be due to the result of a gross general defect in growth, but the combined histological and survival curve data suggest otherwise. Cells which cannot grow in a host (ura3/ura3 mutants; Kirsch & Whitney, 1991), for example, are simply cleared from the body before ever colonizing tissues. The TOP1 deletion strains are maintained in the kidney for a prolonged period of time (up to 22 d) at quite high levels [>10^9 c.f.u./g tissue], and are able, in this environment, to form hyphae. Despite the obvious maintenance of the mutant Candida in the kidney, these strains are very much reduced in their capacity to cause death in infected mice. Similar results were reported recently for other gene disruptions (Bulawa et al., 1993; Becker et al., 1995).

Gene knockout of DNA topoisomerase I in S. cerevisiae (Goto & Wang, 1983; Thrash et al., 1985; Schiz. pombe (Uemura et al., 1987) and Ustilago (Gerhold et al., 1994) shows that this enzyme is not essential for viability of these fungi. In contrast, topoisomerase I is essential in a developing D. melanogaster embryo (Lee et al., 1993). Most of the potential drugs against human DNA topoisomerase I are poisons which do not inhibit the enzyme activity. Rather, these drugs damage cellular processes by trapping the putative covalent intermediate of the reaction (reviewed by Chen & Liu, 1994). We show here that TOP1 is not essential for in vitro growth; however, its expression is important for normal cellular morphology, germ-tube formation and virulence in a mouse model.

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