Among fungal pathogens responsible for opportunistic infections, species of the genus *Candida* have a major role (Mitchell, 1998). Various *Candida* species cause superficial infections which can be cured by the currently available antifungal arsenal (Noble and Johnson, 2007). However, species of the genus *Candida* are also responsible for life-threatening systemic infections, particularly in immunocompromised patients with weakened immune system. Among *Candida* species, *C. albicans*, which can also be a commensal of the skin and the gastrointestinal and genitourinary tracts, is responsible for the majority of *Candida* bloodstream infections. However, there is an increasing incidence of infections caused by *C. glabrata* because it is less susceptible to azoles. Other medically important *Candida* species include *C. parapsilosis*, *C. tropicalis* and *C. dubliniensis*. The problem has been further worsened by the emergence of many drug resistant isolates which pose a major hurdle during a given treatment regimen. Therefore, there is a dire need to identify novel drug targets and the current study focuses on one such protein found in *C. albicans* and related *Candida* species.

**CaGAL102 does not encode a functional galactose epimerase**

*CaGAL102* was previously identified in the lab as a paralog of *CaGAL10*. *CaGAL10* endoes a functional UDP-galactose 4-epimerase and it can complement a *Scgal10* null strain. Further, work on the Gal10 protein in the encapsulated yeast *Cryptococcus neoformans* identified two Gal10 paralogs in the genome, Uge1 and Uge2 with distinct functions (Moyrand et al., 2008). A similar scenario is found in *S. pombe* in which two Gal10 sequence homologs have been annotated. In the light of these observations, we wanted to test if *CaGAL102* also encodes a functional *ScGAL10* homolog. We found that *CaGAL102* could not complement *Scgal10* null
strain though there was a strong conservation in the cofactor and the catalytic motif in both the proteins. We found after a careful literature review that Gal10 belongs to a family of proteins called the short chain dehydratase/reductase family (SDR) (Jornvall et al., 1995), members of which are characterised by the presence of glycine rich cofactor binding motif at the N-terminus and an YXXXK catalytic motif. Proteins belonging to the SDR family have a residue level identity of 15-30% indicating early duplication and divergence. Based on our literature survey we carried out a BLAST search in the NCBI protein database using CaGal102 as the bait protein. We found that CaGal102 is 32% identical at the protein level to dTDP-glucose 4,6 dehydratase (RmlB), another member of the SDR family. RmlB is the second enzyme of the rhamnose biosynthetic pathway which gives rise to dTDP-rhamnose. This pathway is involved in cell wall biosynthesis in bacteria and it has been shown that rmlB is essential for growth of Mycobacterium smegmatis (Li et al., 2006). Interestingly rhamnose is not present in the cell wall of C. albicans.

**Biochemical characterisation of CaCaGal102**

A plant homolog of RmlB is found in A. thaliana which uses UDP-glucose as the substrate (Oka et al., 2007). Based on our alignment data we identified many critical residues in CaGal102. Most importantly we identified that lysine at position 159 lies in the YXXXK motif and could be important for activity. We therefore, mutated the lysine at position 159 to alanine. In order to find out the biochemical function of CaGal102 in vitro, we cloned expressed and purified recombinant wild type and catalytic mutant proteins from E. coli and used the purified proteins for our assays. We found that CaGal102 uses UDP-glucose as the preferred substrate. To further substantiate our data, we reintegrated the wild type or the mutant alleles in the native locus of CaGAL102 and checked for the rescue of morphology defects like filamentation and sensitivity to cell wall damaging agents. We also found that the
CaGAL102Δ/Δ strain is avirulent in a mouse model of systemic infection. We have also carried out infection studies with the null mutant and the wild type and the catalytic mutant reintegrant strains. Our observation suggests that reintegrating one copy of the wild type allele rescues the virulence defect. Interestingly the strain harbouring one copy of the mutant allele behaves like the null mutant in a mouse model of systemic infection.

We have also identified sequence homologs of CaGal102 in related Candida species. It is plausible to think that the homologs in related species also have similar effects and hence targeting this protein by a small molecule could help in treating candidiasis caused by related species.

**CaGAL102 is involved in cell wall architecture in C. albicans**

To elucidate the role of CaGal102 in C. albicans we generated a knockout out strain and studied various mutant phenotypes. The most striking observation was that the cells of the null mutant were filamentous as compared to the wild type control when grown in normal rich media. Further the cells were sensitive to various cell wall damaging agents and also to hygromycin B. We reasoned that lack of CaGal102 causes perturbation in the cell wall architecture rendering the cells sensitive to various cell wall damaging agents. To further strengthen this hypothesis, we decided to study the genetic interaction of CaGAL102 with genes known to be involved in cell wall biosynthesis in C. albicans. One of the candidate genes we chose for our study was GAL10, deletion of which in C. albicans renders the cells sensitive to various cell wall damaging agents. Loss of function of UGE1 in C. neoformans impaired biosynthesis of a cell wall component, galactoxylomannan. We found that cells lacking both Gal102 and Gal10 adhered to nylon membranes poorly as compared to single mutants or the wild type control. The second gene we chose was a P-type ATPase, PMR1
deletion of which causes increased sensitivity to cell wall damaging agents and hyper-
activation of the cell wall integrity pathway similar to Cagal102Δ/Δ strain. We found that
cells lacking both Pmr1 and Gal102 were more sensitive to hygromycin B as compared to the
single mutants. This confirmed our idea that CaGal102 is a novel gene involved in cell wall
biogenesis in C. albicans.

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