Regulation of telomere dynamics in *C. albicans*

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Telomeres are specialized nucleoprotein structures that maintain the integrity of eukaryotic chromosomal termini by protecting them from fusion and recombination, and promoting their replication. In most organisms, telomeric DNA consists of short repetitive sequences which terminate in a 3' overhang (named G-tail because the strand is typically G-rich). The telomere repeats are maintained by a ribonucleoprotein (RNP) known as telomerase, which acts as an unusual reverse transcriptase (RT). Both telomere binding proteins and telomerase are critical for the maintenance of telomere integrity through multiple cell divisions, which in turn is pivotal in supporting genome stability and promoting cellular lifespan. Remarkably, both telomeric and telomerase components are evolutionarily diverse, suggesting that their regulatory mechanisms are malleable. We have undertaken a detailed investigation of telomerase and telomeric proteins in *C. albicans*, which carries an unusual telomere repeat unit (long, regular, and non G-rich). By comparing findings in *C. albicans* to those from *S. cerevisiae*, we hope to gain a broader understanding of the essential and plastic aspects of telomere regulation. Here I report our preliminary functional analysis of *C. albicans* double and single strand telomere binding proteins.

Rap1 is the major double strand telomere binding protein in budding yeasts. It has been shown to prevent chromosome fusion and regulates telomere lengths in *S. cerevisiae*. It is also an important transcription factor and is essential for cell viability. Interestingly, we found that the *C. albicans* Rap1 homologue is substantially smaller than those in other budding yeasts, and is not essential for cell viability. The *rap1* null strain, however, does exhibit significant growth defects, extremely long telomeres, and elevated levels of extra-chromosomal telomeric circles (t-circles) consistent with loss of regulation. Purified recombinant *C. albicans* Rap1 binds the cognate telomere repeat with high affinity and sequence specificity. These results suggest that the Rap1 protein can readily evolve alternative sequence specificity to accommodate diverse telomere repeats in budding yeasts. They also point to a more exclusive and dedicated role for *C. albicans* Rap1 at telomeres.

The single stranded telomere G-tails in *S. cerevisiae* are thought to be bound by an RPA-like trimeric complex named CST (Cdc13-Stn1-Ten1). Each subunit of the complex is essential for cell viability and hypomorphic alleles often result in aberrant telomere degradation. We identified homologues of all three subunits in the *C. albicans* genome. In-depth bioinformatic analysis suggests that all three proteins consist of variable number of RPA-like OB-fold, as proposed before. Remarkably, the Cdc13 homologues in many *Candida* and related species are less than 1/2 of the size of *S. cerevisiae* Cdc13, and lack the putative recruitment domain that presumably interacts with the Est1 subunit of telomerase. Full length *C. albicans* Cdc13 can be expressed and purified from *E. coli*. As expected, the purified protein binds with high affinity and sequence specificity to the unusual *Candida* telomere repeat. Attempts to generate a *cdc13* null strain were unsuccessful, suggesting that the gene is essential in *C. albicans*. In contrast, *stn1* null and *ten1* null strains can be readily obtained. Both mutant strains exhibit grossly elongated telomeres and elevated levels of t-circles. Further studies revealed that the telomere elongation was due to increased telomerase action as well as increased recombination. Our data thus reveal further diversity within the RPA-like family of G-tail binding complex, underscore the versatility of the OB fold in the recognition of different telomere repeat sequences, and indicate a role for the CST complex in suppressing both excessive telomerase action and recombination at telomere ends.