A genome-wide telomere screen in yeast: The long and short of it all

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Following the identification of the first *Saccharomyces cerevisiae* gene whose mutation leads to ever-shorter telomeres (EST1) (1), several dozen genes have been identified that play critical roles in telomere length regulation. Their functions can be loosely categorized into those that affect the action of the telomerase enzyme (e.g., EST1, EST2, EST3, TLC1, KU70/KU80, and the MRX complex) (reviewed in ref. 2); those that affect stability of telomerase components such as the telomerase RNA TLC1 (e.g., Sm proteins, MTR10) (3, 4); and those that play a role in the regulation of telomeric heterochromatin, replication, or end protection (e.g., CDC13, STN1, TEN1, RAP1, RIF1, RIF2, MEC1, and TEL1) (reviewed in ref. 2). Although these studies have vastly increased our understanding of telomere integrity and replication, a comprehensive telomere length analysis of the collection of haploid yeast strains containing a marked deletion at each known nonessential ORF has not yet been reported (5).

In an article in this issue of PNAS, Askree et al. (6) are the first authors to publish such a genome-wide screen for deletions that affect average telomere length in *S. cerevisiae*. In an admirable feat, DNA was prepared from 4,852 strains comprising the haploid yeast deletion set (6) and subjected to enzymatic digestion and Southern blotting using a probe specific for telomeric DNA. Strains that exhibited a difference in average telomere length were subjected to further confirmatory Southern blots, and the identity of each deletion was verified by using PCR-based methods (5). A subset of the positively identified strains was also tested by regenerating the corresponding haploid strains from heterozygous diploid parents to determine whether the telomere phenotype cosegregated two-to-two with G418 resistance (used to mark the deletion).

A large number of deletions with perturbations in telomere length were identified; 172 strains possessed either consistently shorter (123 strains) or consistently longer (50 strains) telomeres, representing 3% of the almost 6,000 postulated genes in the budding yeast genome and encoding proteins involved in numerous cellular functions (7). Not surprisingly, 32 genes involved in DNA or RNA metabolism were identified (including 16 genes previously implicated in normal telomere length maintenance) and 35 genes involved in chromatin structure and silencing (6, 8). In addition, 30 genes involved in vesicular trafficking, 13 genes involved in ribosome function and translation, and 10 mitochondrial genes were also identified. However, even these links might be rationalized upon further inspection, particularly when hits are displayed by using a network visualization tool called OSPREY (9) (Fig. 1). For example, a gene encoding a protein important for vesicular trafficking, VPS9, shows genetic associations with genes whose products play a role in silencing and thus can be rationalized to play a direct or indirect role in telomere length regulation (6) (Fig. 1).

Several genome-wide screens using the haploid yeast deletion set (5) have been published to date. Previous studies reinforce, as does this one, the notion that such screens are by nature prone to error, including both “false-negative” and “false-positive” hits. For example, Askree et al. (6) failed to identify 14 of the 32 genes known to affect telomere length when singly deleted, suggesting that ~40% of genes that affect telomere length either are not represented in the haploid yeast deletion set (due to invia-

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bility or other factors) or their phenotype was missed for various reasons (6). Another factor that may contribute to the inability to identify the complete set of single gene deletions that affect telomere length is that continued propagation of the deletion set can select for suppressors that can mask telomere-related phenotypes, such as the generation of “survivors” that maintain telomeres via homologous recombination (10–12). In addition to the inability to identify all regulators of telomere length, a fraction of the genes identified will likely not show reproducible effects on telomere length when further analyzed. For example, Askree et al. (6) showed that 1 in 27 strains tested did not recapitulate a 2:2 segregation of the telomere phenotype with the G418 resistance marker, as expected if the telomere phenotype is linked to the disrupted gene.

Regardless of the caveats to genome-wide screens in general, this study (6) is an important and exciting point of departure. Dissecting the roles of the newly identified genes will allow for a more sophisticated understanding of the fundamental processes affecting telomere length maintenance. In addition, network-based tools to view these interactions on a larger scale (such as OSPREY, http://biodata.mshri.on.ca/osprey) will assist in deciphering networks and pathways not previously appreciated to play a role in telomere homeostasis. Research in S. cerevisiae has proven pivotal to the identification of homologues in other eukaryotes that are important for telomere and telomerase function, including the identification of the telomerase reverse transcriptase in fission yeast and mammals (13) and the recently identified homologues of the telomerase recruitment factor Est1 (14). Because telomerase activity is prevalent in many human cancers, this study might also lead to new insights into how telomere replication contributes to genome integrity during cell proliferation in humans as well as yeast and ultimately to the development of therapeutic interventions effective against cancer.