A genome-wide screen for *Saccharomyces cerevisiae* deletion mutants that affect telomere length

Syed H. Askree*[†], Tal Yehuda[†‡], Sarit Smolikov[‡], Raya Gurevich[‡], Joshua Hawk*, Carrie Coker*, Anat Krauskopf[‡§], Martin Kupiec[‡], and Michael J. McEachern*[¶]

*Department of Genetics, University of Georgia, Athens, GA 30602; and [‡]Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Ramat Aviv 69978, Israel

Telomeres are nucleoprotein structures present at the ends of eukaryotic chromosomes that play a central role in guarding the integrity of the genome by protecting chromosome ends from degradation and fusion. Length regulation is central to telomere function. To broaden our knowledge about the mechanisms that control telomere length, we have carried out a systematic examination of ≈4,800 haploid deletion mutants of *Saccharomyces cerevisiae* for telomere-length alterations. By using this screen, we have identified >150 candidate genes not previously known to affect telomere length. In two-thirds of the identified mutants, short telomeres were observed; whereas in one-third, telomeres were lengthened. The genes identified are very diverse in their functions, but certain categories, including DNA and RNA metabolism, chromatin modification, and vacuolar traffic, are overrepresented. Our results greatly enlarge the number of known genes that affect telomere metabolism and will provide insights into how telomere function is linked to many other cellular processes.

In most eukaryotes, telomeres consist of tandem arrays of a short G-rich repeat that protects chromosome ends from being recognized as double-strand breaks (1). Telomeres are prone to shortening at each replication event because of an inherent inability of the replication machinery to fully replicate them (2, 3). This sequence loss is normally prevented by the action of the ribonucleoprotein enzyme telomerase, which reverse-transcribes telomeric repeats onto telomeric ends (4). Addition of new sequences by telomerase is typically tightly regulated, resulting in the telomeres of many organisms being kept within particular size ranges.

Most human somatic cells do not express telomerase, and their telomeres shorten with each cell division. This shortening can eventually trigger replicative senescence by means of the activation of growth inhibition pathways dependent on Rb and p53 (5). Bypass of replicative senescence produces further telomere erosion, telomere–telomere fusions, and the eventual cell death known as crisis (6–8). Exogenous expression of the catalytic subunit of telomerase prevents both replicative senescence and crisis (9). More than 90% of human cancers display cellular immortality because of the expression of telomerase activity and the resulting stabilization of telomere lengths (10, 11). Determining the mechanisms by which telomeres and telomerase are regulated could lead to better understanding of both carcinogenesis and aging.

Much of our basic knowledge of telomere biology has come from studies of the yeast *Saccharomyces cerevisiae.* Telomeres in this organism are composed of tracts of heterogeneous TG_{1-3} sequences that normally total a few hundred base pairs in length. A large number of proteins are already known to be involved in various aspects of yeast telomere function. In addition to telomerase, these include dedicated telomere binding proteins (1, 12, 13), the Ku (Yku70/Yku80) and MRX (Mre11/Rad50/Xrs2) DNA repair complexes (reviewed in ref. 14), and certain checkpoint (15) and replication proteins (16). Genes for most of these proteins alter telomere length when mutated. Average telomere length can therefore be a very sensitive sensor of telomere

function. Many genes related to telomere function in yeast have been found to have similar roles in other organisms, including humans. It is therefore reasonable to predict that identifying genes that alter yeast telomere will lead to useful insights into human telomere biology. In this work, we have used a collection of deletion mutants of all nonessential genes to systematically search for genes affecting telomere length. We report >150 genes that were not previously known to alter telomere length. These genes affect several different cell processes, including DNA and RNA metabolism, chromatin modification, and vacuolar traffic.

Materials and Methods

Strains. A collection of 4,852 haploid *Saccharomyces* strains (17) was used in which each strain has a single ORF replaced with the KanMX4 module, which confers G418 resistance. These strains are in the BY4741 background (MATa *his3Δ leu2Δ met15Δ ura3Δ*). The isogenic strain BY4742 (MATα *his3Δ leu2Δ lys2Δ ura3Δ*) was used for genetic analysis.

Telomere Length Measurement. DNA was prepared from cells taken directly from yeast extract/peptone/dextrose plates. In most cases, cells had been freshly stamped from thawed microtiter plate stocks. DNA from each strain was digested overnight with *Xho*I and run on agarose gels for 16–20 h at 1.4 V/cm. Southern blotting was performed by using Hybond-N+ membrane (Amersham Biosciences), and probed with radiolabeled telomeric probe 26G (5′-TGTGGGTGTGGTGTGTGGGTGT-GGTG-3′) labeled with T4 polynucleotide kinase. All hybridizations were done in 200 mM Na_2HPO_4 and 2% SDS (18).

For the Southern blots shown, internal control fragments of sizes 1,865 and 644 bp, containing *S. cerevisiae* telomeric repeats were generated by *Bsm*AI and *Taq*I digests, respectively, of the plasmid pYt103 (19). We added ≈10 ng of each digested DNA to each lane together with the *Xho*I digested genomic DNA. For better resolution of size differences, 25-cm-long gels were run at 1.4 V/cm for 600 min and an additional 600 min at 2 V/cm. The average telomeric length for each lane was estimated by plotting the peak of signal intensity of the shortest telomere band (Y′ telomeres) against the position of the added internal telomere size standards.

PCR. To confirm ORF deletion identity, a general primer from inside the KanMX cassette (GCCATCAAAATGTATG-GATGC) and a specific primer from the 5′ UTR of each ORF (20) were used. When necessary, the reaction was repeated with the KanMX primer and a primer complementary to the UPTAG

primer that was used to create the deletion strains (GATGTC-CACGAGGTCTCT). This PCR product was sequenced to identify the strain by its unique tag.

Cosegregation Test. Telomere length phenotypes of 27 candidate strains were checked by testing cosegregation of the length phenotype with the deletion (G418ʳ). The candidate mutant strains were mated with BY4742 wild type, and the resulting heterozygous diploids were sporulated by the recommended method (17). Tetrads picked were scored for G418 resistance on yeast extract/peptone/dextrose plates supplemented with 400 mM G418 (Amersham Biosciences). Telomere length was estimated by Southern blot analysis. The presence of the KanMX cassette was confirmed by reprobing the Southern blots with a labeled ≈800-bp *Sca*I/*Nco*I fragment of pFA6 KanMX4 plasmid (21). All hybridizations with 26G and G418 were done at 55°C and 65°C, respectively.

Results

We have carried out a genome-wide screen for mutants affecting telomere length. A collection of 4,852 haploid-viable yeast strains, each deleted for a single ORF, was used. DNA from each strain in the collection was digested with *Xho*I, separated by gel electrophoresis under standardized conditions and subjected to Southern blot analysis with a telomere-specific probe. The telomeric probe produces a complex pattern, derived from hybridization to many telomeric fragments and to several subtelomeric repeats (22). The latter served as internal controls, because their electrophoretic mobility was usually not affected by the various mutations. The shortest fragments (≈1.3 kb in wild-type cells), resulting from Y′-containing telomeres, were the most reliable in determining telomeric length differences between strains, both because they represent multiple telomeres and because length differences are most pronounced in the shortest fragments. The next few bands, corresponding to telomeres containing an adjacent X element and no Y′ element (X′ telomeres), were also observed carefully to identify mutants with length alterations.

After the first round of screening, ≈600 mutants exhibiting possible short or long telomere phenotypes were retested by at least two additional rounds of fresh DNA preparations and Southern blotting. DNA from wild-type cells was run interspersed amongst the mutants in these gels to maximize the chances of correctly identifying mutants with modest effects on telomere length. By this very stringent criterion, 173 strains were identified that consistently exhibited either shorter or longer telomeres than wild type (Table 1). In addition, 26 other mutants were labeled as questionable gene candidates for telomere length phenotypes because they showed very mild telomere length phenotypes that failed to be detected in at least one repeat Southern blot (see Table 2, which is published as supporting information on the PNAS web site).

One additional DNA preparation and Southern blot analysis was then carried out to measure telomere length in each of the 173 mutants identified in the screen. The average telomeric length for the shortest telomeric fragment (Y′ telomeres) was then estimated by using fragments generated by restriction digests of a plasmid containing a cloned *Saccharomyces* telomere that were added to each genomic DNA sample. These telomeric fragments ran at positions above and below the Y′ telomeric bands and served as internal controls to measure size and to assure uniform migration of different samples (see *Materials and Methods*). Fig. 1 shows an example of one of these Southern blots, each of which was run on long gels (25 cm) to maximize resolution of telomeric length differences. The rest of these Southern blots are shown in Fig. 2, which is published as supporting information on the PNAS web site. Average Y′ telomere length for the wild-type strain was
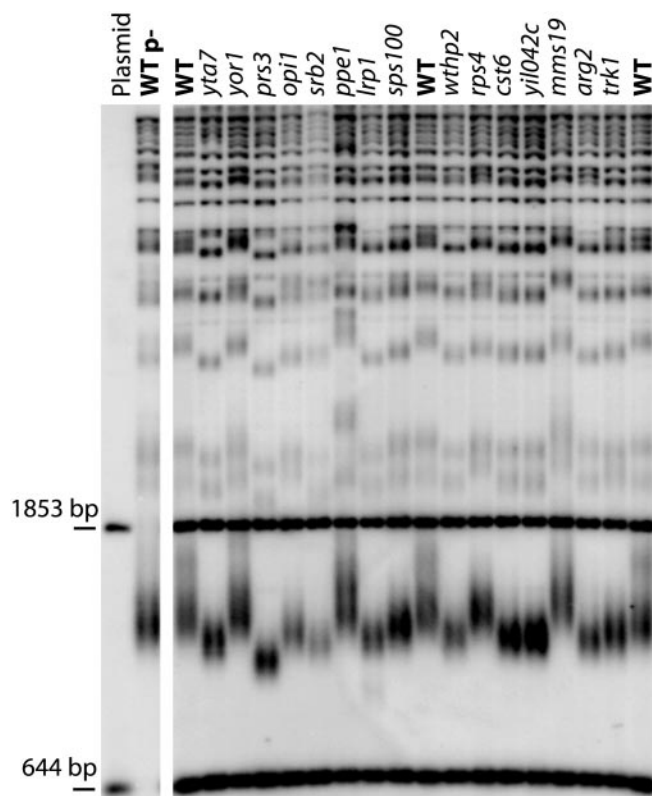
Fig. 1. Representative Southern blot of mutants that alter *S. cerevisiae* telomere length. Southern blot of *Xho*I-digested DNA of single gene knockout mutants of *S. cerevisiae* probed with telomeric sequence. Gene or ORF names are indicated as are strain BY4741 controls (WT). Samples of DNA from mutants were combined with restriction-digested plasmid DNA to provide internal size standards. The two internal control bands are fragments of the plasmid pYt103 containing telomeric sequences that were generated by mixing separate digests done with *Bsm*AI and with *Taq*I (producing the 1,835- and 644-bp fragments, respectively). The panel on the left shows a strain BY4741 control (WT p−) as well as the size standards without added yeast DNA.

estimated from multiple samples run on the same gels. The change in Y′ telomere length relative to the wild type was then estimated for each of the 173 mutants. These values are presented in Table 3, which is published as supporting information on the PNAS web site. Of the 173 mutants in this last Southern blot analysis, 25 showed Y′ telomeres that had only slight length differences with the wild type control (<25 bp). We have not excluded them from our list because they had reproducibly shown slight length differences in all other Southern blots carried out. These mutants are marked with an asterisk in Table 1.

The identity of each of the 173 mutants shown in Table 1 was confirmed by PCR analysis (see *Materials and Methods*). The sizes of the Y′ telomeres were grouped into the following categories: slightly short (<50 bp shorter than wild type), short (50–150 bp shorter than wild type), very short (>150 bp shorter than wild type), and equivalent categories for long telomere mutants (Table 1). Although consistently showing either long or short telomere length, many of the deletion strains exhibit some variation in the degree of their length phenotype when observed over repeated Southern blot analyses. We have considered the length phenotypes from all Southern blot analyses before assigning a gene to one of the phenotypic groups shown in Table 1. Of the mutants identified, 123 exhibited shorter and 50 showed longer telomeres than

Table 1. List of *S. cerevisiae* genes that affect telomere length when deleted

Gene	Telomere phenotype	Function
DNA metabolism		
EST1	VS	Telomerase holoenzyme complex
EST2	VS	Telomerase reverse transcriptase
EST3	VS	Telomerase holoenzyme complex
TEL1	VS	DNA damage response kinase
YKU70	VS	DNA repair, Ku70/Ku80 complex
YKU80	VS	DNA repair, Ku70/Ku80 complex
MRE11	VS	DNA repair, MRX complex
RAD50	VS	DNA repair, MRX complex
XRS2	VS	DNA repair, MRX complex
RNH35*	VS	RNaseH, DNA replication; Int./Rif2
DCC1	S	Sister chromatid cohesion
HUR1	S	DNA replication; Int./Mec3
LRP1	S	C1D ortholog, double-strand break repair
YPL205C	S	Overlaps with *HRR25*
RIF1	VL	Telomere maintenance, silencing
RIF2	VL	Negative telomere regulator
ELG1	VL	Genome stability
PIF1	VL	Telomere maintenance, recombination
OGG1	L	Base excision repair, shares *PIF1* promoter
POL32*	sl	DNA polymerase Delta complex
MLH1*	sl	Mismatch DNA repair
CSM1	sl	Meiotic chromosome segregation. Int./Zds2
YML035C-A*	sl	Antisense to *SRC1*
Chromatin, silencing, PolII transcription		
HST1	S	*SIR2* homolog, histone deacetylase complex
SUM1	S	Suppressor of *sir* mutants
RFM1	S	Part of Hst1 histone deacetylase complex
SIN3	S	Part of Rpd3 histone deacetylase complex
SAP30	S	Part of Rpd3 histone deacetylase complex
OPI1	S	Interacts with Sin3
DEP1	S	Part of the Rpd3 histone deacetylase complex
HDA2	S	Part of the HDA histone deacetylase complex
CDC73	S	Part of the Paf1 complex
RTF1	S	Part of the Paf1 complex
BRE2	S	Part of the SET1 histone methylase complex
MFT1**	ss	Tho and Paf1 complexes
THP2	S	Tho and Paf1 complexes
SOH1**	S	Suppressor of *hpr1* mutants (Tho and Paf1)
RPB9	S	RNA polymerase II subunit
RPB4, CTF15	S	RNA polymerase II subunit
SRB2	S	Transcription, mediator complex
SRB5**	S	Transcription, mediator complex
RSC2	S	RSC complex, chromatin modelling
CTK1	S	PolII transcription regulation, protein kinase
SPT21**	S	PolII transcription, chromatin
CST6	ss	Transcriptional activator, chromosome stability
NUP60*	ss	Silencing, part of the nuclear pore
HTL1**	VL	DNA replication and chromosome cycle
HPR1	L	Tho and Paf1 complexes
HCM1	L	Transcription factor (forkhead2)
MMS19**	L	PolII transcription (TFIIH) and repair
YDJ1*	sl	Forms a complex with Mms19

Table 1. (continued)

Gene	Telomere phenotype	Function
SSN8	L	Transcription, mediator complex
NUT1*	sl	Transcription, mediator complex
NFI1	sl	SUMO ligase, chromatin protein
FMP26*	sl	Int./SAGA, reported mitochondrial protein
VPS65*	sl	Deletion affects *SFH1* (RSC complex)
HMO1*	sl	ssDNA binding, HMG-box protein
NPL6	sl	Protein–nucleus import
Vesicular traffic (vacuole, Golgi, ER, membrane synthesis)		
CAX4	VS	ER, *N*-glycosylation, phosphatase
VPS3	S	Vacuolar sorting protein
VPS9	S	Vacuolar sorting protein
VPS15**	S	Vacuolar sorting protein
VPS18	S	Vacuolar sorting protein
VPS23**	S	Vacuolar sorting protein, ESCRT-I
VPS28**	S	Vacuolar sorting protein, ESCRT-I
VPS22**	S	Vacuolar sorting protein, ESCRT-II
VPS25**	ss	Vacuolar sorting protein, ESCRT-II
VPS36**	S	Vacuolar sorting protein, ESCRT-II
VPS32	S	Vacuolar sorting protein, ESCRT III
YEL057C	S	Vacuolar sorting protein, ESCRT-III
BRO1	S	Vacuolar sorting protein, after ESCRT-III
VPS34	S	Vacuolar sorting protein
VPS39	S	Vacuolar sorting protein
VPS75	S	Vacuolar sorting protein
VPS43	ss	Vacuolar sorting protein
APE3	S	Vacuolar protein degradation
ATG11	S	Vacuolar targeting
MOT3	S	Suppressor of *spt3*, increased sterol levels
ARV1	S	Sterol metabolism and transport
AGP2	S	Carnitine transporter, fatty acid metabolism
YTA7	S	Affects ergosterol and dolicol synthesis
PDX3**	S	Pyridoxine phosphate oxidase
ERJ5	S	Golgi transport to ER
LST7	S	Golgi-to-surface traffic protein
SUR4**	S	Fatty acid synthesis, transport
RPN4	S	Ubiquitin degradation pathway
PMT3*	ss	Protein-*O*-mannosyl-transferase, ER
YSP3	ss	Subtilisin-like peptidase
RNA metabolism		
UPF1	VS	Nonsense-mediated mRNA catabolism
UPF2	VS	Nonsense-mediated mRNA catabolism
UPF3	VS	Nonsense-mediated mRNA catabolism
KEM1	VS	Exonuclease, mRNA degradation
RRP8	sl	Methyltransferase, pre-rRNA processing
STO1	sl	mRNA splicing and snRNA cap binding
LEA1	sl	mRNA splicing
YPL105C*	sl	Unknown, Int./Mud2, Msl5
YMR269W*	sl	Unknown, Int./eIF2B
Cell polarity, cell wall, bud site selection		
BEM4**	S	Cell polarity, actin organization
YOR322C	S	Clathrin-coated vesicles
BUD16**	S	Unknown, putative pyridoxal kinase
YPL041C	S	Int./cell wall mutants, phosphoglucomutase

wild type. None of the newly identified mutants appeared to have the ever shortening phenotype characteristic of telomerase deletion mutants (23, 24). Several other mutants, listed in Table 4, which is published as supporting information on the PNAS web site, repeatedly had telomeres that were heterogeneous in length. Some of these appear to have Y′ telomeres of normal length but have elongated X telomeres, others displayed Y′ telomeres with a bimodal length distribution. Conceivably, the former might be altered in the uncharacterized mechanism that leads to size differences between X and Y′ telomeres (25).

We tested 27 of the mutants (marked with a double asterisk in Table 1) for cosegregation between the telomere phenotype, and we inserted the G418 resistance determinant in place of the deleted gene. All of these except one showed clear segregation of telomeric phenotype with G418 resistance. The single exception, *yel033w*, showed a short telomere phenotype that segregated independently from the antibiotic resistance. From our analysis, we conclude that the *yel033w* deletion segregates with a very slow growth phenotype and the short telomeres segregate with a suppressor mutation responsible for restored growth. Thus, *YEL033W*, a dubious ORF, appears to genetically interact with an unidentified gene with telomere function. Our results imply that in the vast majority of our strains, the telomere phenotype was caused by the relevant deletion.

Table 1. (continued)

Gene	Telomere phenotype	Function
YPL144W	S	Cell wall, phospholipids
SMI1	S	Cell wall synthesis, chromatin binding
CCW14	S	Cell wall structural protein
CSR2*	ss	Cell wall organization
GPB2	ss	Pseudohyphal growth
SPS100	ss	Spore wall assembly
LDB7	L	Cell wall organization
BUD30	sl	Bud site selection
BUD23*	sl	Bud site selection
BEM2*	sl	Polarity, cytoskeleton, budding
Protein modification and heat shock proteins		
SSE1**	S	Antioxidative response, cochaperone
XDJ1**	S	Chaperone regulator
HSC82	S	Heat shock protein
HCH1	ss	Chaperone activator, Hsp90 suppressor
MAK10**	L	N-acetyltransferase
MAK31**	L	N-acetyltransferase
MAK3**	L	N-acetyltransferase
HSP104	sl	Heat shock protein
CDH1	sl	APC/cyclosome regulator
Ribosome and translation		
RPP1A	VS	Large ribosomal subunit
RPL12B	S	Large ribosomal subunit
RPL13B	S	Large ribosomal subunit
RPL1B	S	Large ribosomal subunit
MRT4	S	Ribosomal large subunit biogenesis
EAP1	S	Translation regulation
RPS17A	VL	Small ribosomal subunit
RPS10A*	L	Small ribosomal subunit
RPS14A	L	Small ribosomal subunit
ASC1	L	Ribosomal subunit
RPS16A*	sl	Small ribosomal subunit
RPS4B	sl	Small ribosomal subunit
HIT1*,**	sl	Ribosome assembly
Mitochondria		
MRPL44	S	Mitochondrial ribosomal subunit
MRPL38	S	Mitochondrial ribosomal protein
MMM1	S	Mitochondrial organization
ISA1	S	Mitochondrial Fe/S protein maturation
PTC1	S	Mitochondrial inheritance
TOM5*	ss	Mitochondrial outer membrane transport
PCP1	ss	Mitochondrial protease
YIL042C	ss	Mitochondrial kinase
YDR115W	ss	Mitochondrial ribosomal protein
UGO1*	sl	Mitochondrial fusion
Nucleotide metabolism		
PRS3	VS	Ribose-phosphate pyrophosphokinase
MET7**	VS	Folylpolyglutamate synthetase
ADO1	S	Adenosine kinase
ADE12 BRA9	S	Adenylosuccinate synthetase
GCV3	S	Folate production

Table 1. (continued)

Gene	Telomere phenotype	Function
Phosphate metabolism		
PHO85**	S	Phosphate metabolism
PHO80**	S	Phosphate metabolism
GTR1	S	Phosphate transport
PHO87**	L	Phosphate uptake
TAT2	sl	Int./Pho23, synthetic lethality with pho85
Nitrogen metabolism		
URE2	S	Nitrogen catabolite repression regulator
ARG2	S	Glutamate acetyl transferase
Glycerol uptake		
GUP1	S	Glycerol uptake
GUP2*	sl	Glycerol uptake
Potassium transport		
TRK1	S	Potassium transporter
Killer toxin-related		
FYV12*	sl	Killer toxin sensitive
KRE21	sl	K1 killer toxin resistant
KRE28	sl	Killer toxin resistant
PP2A-related		
TPD3*	S	Phosphatase type 2A subunit
SIT4	S	Phosphatase type 2A subunit
YOR1	L	Multidrug resistant transporter, Int./PP2A
PPE1	L	Protein modification, Int./PP2A
Unknown		
YDL118W	VS	Unknown
YEL033W**	VS	Unknown (failed cosegregation test)
YGR042W	S	Unknown
YOL138C	S	Unknown
YMR031W-A	S	Unknown, overlaps YMR031c
YGL039W	sl	NADPH-related oxidoreductase
YOR008C-A	sl	Diepoxybutane and mitomycin C resistance

Single knockout mutants of the listed 173 genes screened positive for telomere length defects in our analysis. Genes are grouped according to broad cellular function. The second column shows estimated average Y′ telomere lengths relative to wild-type length: ss, slightly short (<50 bp shorter than wild type); S, short (50–150 bp); VS, very short (>150 bp); sl, slightly long (<50 bp longer than wild type); L, long (50–150 bp); and VL, very long (>150 bp). Telomeric length was measured by plotting the peak signal of the shortest telomeric band (Y′ telomeres) against the positions of the added internal controls of the gels shown in Figs. 1 and 2 by using a PhosphoImager and IMAGEQUANT 4.1 software. Final telomeric length classification shown here is additionally based on data from other gels. Single asterisks indicate mutants that either showed average measured Y′ telomere length <25 bp different in size from those of wild-type cells in the gels shown in Figs. 1 and 2, or they showed length phenotypes inconsistent with reproducible phenotypes noted in all previous Southern blots. Because these mutants showed altered telomere lengths in all previous Southern blots, they are included in this table. Cosegregation test was carried out with the deletion mutants marked with a double asterisk. Int., interaction known with. Genes previously known to affect telomere length are indicated in bold. ER, endoplastic reticulum; snRNA, small nuclear RNA; ssDNA, single-stranded DNA; APC, antigen-presenting cell; SAGA, Spt/Ada/Gcn5 acetyltransferase; SUMO, small ubiquitin-related modifier.

Discussion

S. cerevisiae is the best understood system for studying telomere biology and many genes with roles in telomere function are already known in this organism. Nonetheless, our efforts have resulted in a wealth of additional candidate genes that alter telomere length when deleted. Our results indicate that a surprisingly large percentage of the yeast genome is in some way linked to telomere metabolism. The 173 genes listed in Table 1 represent ≈3.2% of the estimated 5,538 genes of *S. cerevisiae*. This number is certainly an underestimate of the total, given that >1,000 essential genes were not examined and because many genes known to mildly affect telomere length in other strains were not found in our screen.

Of the 32 genes previously known to exhibit a telomeric phenotype when singly deleted, our screen identified 18 (bold in Table 1). Genes not identified in our screen include some with reported phenotypes that were very mild. These include *FOB1* (26), *SWD1* and *SWD3* (27), *RRM3* (28), *EBS1* (29), *MLP1* and *MLP2* (30), *SIR3* (31), and *DDC1* and *RAD17* (32). We have not determined whether these genes were missed in our screen or whether they simply behave differently in our strain background. Of the four additional genes not identified in our screen, one (*TOP3*) (33) was not present in the collection. Two of the three others [*GAL11* (34) and *SIR4* (31) but not *CTF18* (35)] showed mild telomere length phenotypes when individually reexamined (data not shown). It is not

unexpected that mutants with slight telomere length alterations could be difficult to distinguish from wild type or even overlooked by our screen. Yeast telomeres are not only heterogeneous in size, but they are subject to size fluctuations even within clonal lineages (36). Although it is clear that our screen failed to detect all mutants with slight telomere length phenotypes, we are confident that it successfully identified the great majority of the nonessential genes that appreciably affect telomere length when deleted. Although absolute confidence that individual genes identified in this study affect telomere length will require additional experimentation, it is unlikely that our screen identified many false positives. Each mutant has had its telomere length examined several times and the identity of all has been confirmed through PCR testing. Moreover, 26 of 27 randomly sampled mutants have shown the expected meiotic cosegregation between the KanMX marker and the telomeric phenotype.

The genes identified in our screen have very diverse functions. Although some of them are probably directly involved in telomere metabolism, most are likely to affect telomere length indirectly either by altering the activity of proteins directly involved in telomere maintenance, or by eliciting cellular mechanisms that lead to changes in telomere length. The genes most likely to be directly involved in telomere size maintenance are those affecting DNA metabolism. In addition to the known DNA repair genes involved in telomere size control, such as components of the MRX (Mre11/Rad50/Xrs2) and Ku (Yku70/Yku80) complexes, we have identified *LRP1*, the yeast homolog of the human protein C1D (37). In mammalian cells, this is a γ-irradiation-inducible nuclear matrix protein that activates DNA PK (38). The yeast homologue has roles both in homologous recombination and in nonhomologous end-joining (37).

DCC1 encodes a component of an replication factor C-like clamp (RLC) loader complex that works with the Trf4 DNA polymerase to ensure proper sister chromatid cohesion during DNA replication (35). Although both Δ*dcc1* and Δ*trf4* were identified among the short telomere strains, mutations in *ELG1*, the main component of an alternative RLC, lead to elongated telomeres (Table 1) (39, 40) and the Δ*rad24* strain, defective in a third RLC (41), exhibited no telomeric phenotype (data not shown). Another mutant with links to DNA replication, the deletion of which causes telomere shortening, is *RNH35*. This gene encodes a RNase H required for RNA primer removal during DNA synthesis (42). Its activity may be required for the coordination between replication of subtelomeric regions by the DNA polymerases and telomeric elongation by the telomerase. A similar role has been proposed for Elg1p (40).

Genes located in the proximity of the chromosomal ends are often subjected to epigenetic silencing, also known as telomeric position effect (43). Although many mutants that affect telomeric silencing have been isolated, not all of them exhibit changes in telomere length. Our screen has identified components of several complexes previously known to affect silencing that produce short telomeres. These components include the *HST1-SUM1-RFM1* histone deacetylase (44) and the *SIN3, SAP30, OPI1,* and *DEP1* genes, encoding components of the Rpd3 histone deacetylase complex (45, 46). In addition, we have identified several components of the Paf1, Set1, and Tho complexes, which seem to interact both in chromatin remodeling and during transcription elongation (reviewed in ref. 47). Moreover, mutations in certain components of the RSC, Mediator, and CTD phosphorylation complexes, which are located at the interphase of chromatin remodeling and RNA polymerase activation, also caused shortening of the telomeres. Not all of the nonessential members of these complexes reduced telomere length, suggesting that the link to telomere homeostasis may be due to the individual proteins in these complexes. The isolation of so many mutants that lead to shortened telomeres by interfering with chromatin remodeling functions suggests that chromatin

integrity/modification plays an important role in elongating telomeres. We have also identified Δ*nup60* as a strain exhibiting short telomeres. Nup60 is required to anchor telomeres to the nuclear periphery, and a link between telomere position within the nucleus and chromatin remodeling affecting telomeric position effect has been shown (48). It is possible that telomere elongation also requires anchoring of telomeres to the nuclear periphery.

Many genes with known vacuolar functions showed telomere length alterations when individually deleted. The yeast vacuole is the functional analogue of the mammalian lysosome, the major site of degradation of both exogenous and endogenous macromolecules (reviewed in ref. 49). Prominent amongst these genes are components of the ESCRT (endosomal sorting complex required for transport) complexes. Three ESCRT complexes are known to bind in succession to ubiquinated cargos in late endosomes and function in the sorting of proteins to be degraded by vacuole/lysosome in the multivesicular bodies pathway, a well conserved process in eukaryotes (reviewed in ref. 50). The following 10 genes involved in this process display a short telomere phenotype when deleted. These genes encode components of the ESCRT complexes: Vps23; Vps28; Vps22; Vps25; Vps36; Vps32; Yel057c; a phosphatidylinositol 3-kinase, Vps34; its associated kinase, Vps15; and a downstream player in the process, Bro1 (51–53). Additional vacuolar genes identified include those that act in vacuolar targeting and fusion.

This connection between vacuolar targeting and telomere metabolism may be due to one or more telomeric proteins being regulated by degradation in the vacuole. Interfering with the vacuolar pathway could cause an increase in the level of these proteins, creating an imbalance in telomere size. At this moment there are no obvious telomeric proteins that are known to be degraded by means of this pathway.

In contrast to the >100 genes whose mutations led to short telomeres, only 50 deletion mutants exhibited a clear phenotype of elongated telomeres. The reason for this asymmetry is unclear. It likely indicates that there are more genes connected to telomerase-mediated sequence addition than there are to the negative regulation of that process. The mutants causing lengthening were more difficult to organize in clear functional categories. In addition to *ELG1*, discussed above, *POL32*, a nonessential subunit of DNA polymerase δ (54), is a candidate for having a direct link to telomere metabolism. Two additional genes causing telomere elongation affect chromosome segregation: *CSM1*, encoding a component of the kinetochore, and *SRC1*, which affects sister-chromatid segregation (55) and seems to be a target for cyclin-dependent kinase phosphorylation (56).

Deletion of only a few genes affecting chromatin/silencing caused telomere elongation. These genes include two components of the Mediator complex (*NUT1* and *SSN8*) (57, 58), two components of the RSC complex (*HTL1* and *SFH1*) (59, 60), and several less well characterized genes. For example, *NPL6* was defined as a nuclear pore component; however, it binds histone H2B and many chromatin-remodeling factors and localizes to the nucleus (61).

Among the genes with long telomeres identified in our screen, there is only one clear case for which deletion of each subunit of a known complex caused a similar telomere phenotype. Deletion of each subunit of the NatC N-terminal acetyltransferase led to elongated telomeres. N-terminal acetylation is one of the most common cotranslational modification processes in eukaryotes (reviewed in ref. 62) and is carried out by one of three complexes in a substrate-specific fashion. In several cases, genes affecting telomere length are physically next to one another. Deletion of one could potentially alter telomere length by changing the expression of its neighbor. Examples include one neighbor of *STN1* and both neighbors of *PIF1* (including the DNA repair gene *OGG1*). In addition, there are at least six more pairs of neighboring genes in our list of candidates that show telomere length phenotype when

individually deleted; they are listed in Table 5, which is published as supporting information on the PNAS web site.

Our genome-wide approach has allowed us to identify many genes that affect telomere length control. These reveal unexpected links to various aspects of the cellular metabolism. Given the conservation of telomere maintenance mechanisms throughout evolution, analysis of these genes will certainly be relevant to other eukaryotes and may have important consequences for therapeutic treatment of cancer.

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