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# Nature vs nurture

## Interplay between the genetic control of telomere length and environmental factors

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**Telomeres are nucleoprotein structures that cap the ends of the linear eukaryotic chromosomes, thus protecting their stability and integrity. They play important roles in DNA replication and repair and are central to our understanding of aging and cancer development. In rapidly dividing cells, telomere length is maintained by the activity of telomerase. About 400 *TLM* (telomere length maintenance) genes have been identified in yeast, as participants of an intricate homeostasis network that keeps telomere length constant. Two papers have recently shown that despite this extremely complex control, telomere length can be manipulated by external stimuli. These results have profound implications for our understanding of cellular homeostatic systems in general and of telomere length maintenance in particular. In addition, they point to the possibility of developing aging and cancer therapies based on telomere length manipulation.**

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Telomeres are the specialized nucleoprotein structures present at the ends of eukaryotic chromosomes. Telomeres play a central role in maintaining the stability of the genome: they serve to differentiate the natural chromosomal ends, which should not be repaired, from double-stranded DNA breaks (DSBs), which may occur by accident and need to be repaired immediately to prevent loss of genomic information.[1] Protection of the chromosomal ends is conferred by the special structure of telomeres, created by specific telomeric proteins. In addition, telomeres provide a solution to the end-replication problem: the regular DNA replication machinery is unable to fully replicate the chromosomal ends;[2] as a consequence, information is lost with each cell division, eventually resulting in senescence and cell death.[3]

Highly proliferative cells, such as mammalian embryonic cells and unicellular organisms, solve this problem by expressing telomerase, a specialized reverse transcriptase[4] able to use an internal RNA molecule as a template to extend the telomeres. Indeed, it is enough to express active telomerase to overcome cellular senescence in somatic cells.[5] Cancer cells also require functional telomeres: in about 80% of tumors, the telomerase gene is expressed;[6] in the rest, an alternative mechanism, ALT, based on homologous recombination, allows telomere length extension.[7] Moreover, experiments have shown that replenishing telomeres is one of the few essential and earliest steps that a normal mammalian fibroblast must take in order to become cancerous.[8] Mutations that affect telomere function result in human diseases, such as dyskeratosis congenita, idiopathic pulmonary fibrosis, and others.[9-12] Expression of telomerase is downregulated in many somatic tissues in mammals. Accordingly, telomere length was found to decrease with age in human individuals,[13] suggesting a link between telomere length and aging. Indeed, in human fibroblasts in culture, a lack of telomerase expression leads to progressive telomere shortening, and cells cease to divide in a process called replicative senescence.[14] Thus, our understanding of the biology of telomeres has significant medical implications and is especially relevant to the fields of aging and cancer.

Although some differences exist between the organization of telomeres

in yeast and mammals, many basic rules are universal. The yeast *Saccharomyces cerevisiae*, with its sophisticated genetics and molecular biology tools, has been instrumental in providing basic information about telomere biology (recently reviewed in ref. 15). The yeast genome has close to 6000 recognized genes. A collection of 4700 mutants was constructed by systematically deleting each individual non-essential gene in yeast (non-essential yeast mutant collection[16]). This collection was later complemented with 2 additional libraries of mutants encompassing all the essential genes (yeast has ~1300 essential genes). In one of these, each essential gene was replaced by a hypomorphic allele;[17] in the other, temperature-sensitive alleles were used.[18]

The mutant collections allow researchers to carry out systematic mutant screens even if the phenotype of interest is not selectable. For example, 3 publications reported the systematic screening of the mutant collections, looking for those mutants that affect telomere length (telomere length maintenance or *tlm* mutants). In these publications, DNA was extracted from each individual yeast strain and telomere length was measured by Southern blot.[19-21] Together, these papers identified ~400 genes affecting telomere length. This list starkly contrasts with the 30 or so genes known to do so at the time the screens were performed,[19] and, in addition, it underscores the central role played by telomere biology in the yeast life cycle, as ~7% of the genome participates in telomere biology. Moreover, it also demonstrates the complexity of the challenge: a mutation in any of the *TLM* genes changes the final telomere length, as this length is determined by mechanisms that elongate (e.g., telomerase) or shorten (e.g., replication-related shortening, nucleases) telomeres (which are themselves positively and negatively regulated); this means that each of the ~400 genes participates in determining an equilibrium between the 2 types of activity. Remarkably, however, each wild-type yeast strain always exhibits telomeres of the same size; thus, in the tag-of-war between elongating and shortening mechanisms, the equilibrium is always attained (in a wt cell under optimal conditions) at the same telomere length. Researchers thus concluded that a very tight homeostatic mechanism involving hundreds of genes is at play.[22] The genes uncovered in these screens, as expected, include those affecting DNA and chromatin metabolism, but almost all functions in the cell are also represented, including RNA and protein synthesis, traffic and modification, metabolic pathways, mitochondrial functions, etc. A large number of these genes is evolutionarily conserved and present in the human genome. The challenge ahead, of course, is to determine how all these genes impinge on the telomere length determination. The fact that a near-complete list of *TLM* genes is available opens the door for further exploration of telomere biology. For example, using computational approaches and the vast amount of information about protein–protein and genetic interactions in yeast, initial network models of telomere biology have been established, allowing their study.[22-24]

In biological systems, homeostasis usually works to isolate the organism or some of its cells from the effects created by the environment. Thus, warm-blooded organisms maintain body temperature despite external fluctuations, and cells maintain constant levels of ions and cofactors irrespective of their environmental level.[25,26] Any change in steady state introduced into the system by the external signals is immediately "corrected" by the interacting proteins, in order to restore the state at which the system is equilibrated. However, our understanding of complex homeostatic mechanisms has been impaired by our lack of knowledge about the genes involved. Although a small number of genes that, when mutated, led to a noticeable change in the "equilibrium point" were described for several biological systems, it is seldom that a complete regulatory circuit comprising hundreds of genes, such as the *TLM* system, is characterized.

It, therefore, may be surprising to see that the telomere length maintenance system, while normally maintaining telomere length at a fixed size, is able to respond to external signals. Here we summarize 2 recent studies that characterize the response of this highly homeostatic system to external cues and the mechanisms involved.[27,28]

One study explored the effect of cellular starvation on telomere length.[27] Cells regulate their growth according to the availability of nutrients from their surroundings. Tor1 and Tor2 (targets of Rapamycin[29]) are 2 serine/threonine kinases that regulate cell growth in response to nutrients and stress. A single TOR protein exists in mammalian cells (mTOR[30]). The drug Rapamycin inhibits TOR by forming an inhibitory complex with FKBP12 (Fpr1p protein in *Saccharomyces cerevisiae*).[31]

The Tor kinases can be found as part of the TOR complex 1 (TORC1), which controls many cellular processes, including protein synthesis,[32] ribosome biogenesis,[33] autophagy,[34] and sorting and turnover of nutrient permeases.[35] TORC1 represses the transcription of specific genes induced by nutrient starvation and is sensitive to Rapamycin.[36] A second Tor-containing complex, TOR complex 2 (TORC2), is Rapamycin-insensitive, because the rapamycin–Fpr1p complex does not bind to Tor2 in this complex.[37] TORC2 is involved in the regulation of actin cytoskeleton polarization during cell cycle progression.[38]

Ungar et al. grew yeast cells either under starving conditions, or in the presence of sublethal Rapamycin concentrations. Strikingly, in this situation, yeast cells respond by dramatically shortening their telomeres. Mutations in *FPR1* or in other components of TORC1 resulted in a lack of telomeric response to Rapamycin, confirming that Tor complex 1 is the target for the Fpr1–Rapamycin silencing. Among the various growth-related metabolic pathways controlled by TORC1, the authors identified the nitrogen response pathway as the one involved in the signal transduction to the telomeres. Upon nitrogen starvation, the Gln3 and Gat1 transcription factors (TFs) enter the nucleus and promote transcription from a battery of nitrogen catabolite repression (NCR) genes.[39,40] As long as nutrients are available, the activity of TORC1 prevents the TFs from entering the nucleus: they are kept in a phosphorylated form, bound to the Ure2 protein. Inhibition of TORC1 by the absence of nitrogen (mimicked by Rapamycin) leads to the dephosphorylation of Gln3 and Gat1, their dissociation from the cytoplasmatic Ure2p and their import to the nucleus, where they promote gene expression.[39,41]

The existence of an almost complete list of *TLM* genes involved in regulating telomere length allowed the authors[27] to ask how was the starvation signal transmitted from the environment to telomeres. They performed a screen for *tlm* mutants that fail to shorten their telomeres in the presence of Rapamycin. This screen identified the Ku complex as the main responder to starvation: no change in telomere length was observed in *yku70*Δ or *yku80*Δ mutants. The *YKU70* and *YKU80* genes encode the 2 components of the Ku heterodimer, which plays important roles in telomere biology[42,43] as well as being pivotal for the repair of DSBs through non-homologous end joining (NHEJ).[44] Further investigation showed that, indeed, under nutrient-poor conditions or Rapamycin treatment, the release of Gln3p and Gat1p from Ure2p and their entrance into the nucleus leads to a reduction in Yku70 protein levels. Degradation of Yku70 results in rapid telomere shortening. Since Yku70 is also involved in DNA double-strand break (DSB) repair via NHEJ,[43,44] the authors tested a possible role of the cascade described in this process, and found that the *ure2* strain (in which the TFs are constitutively nuclear and Ku levels are low) is also deficient in NHEJ repair, and that this defect can be suppressed by preventing the entrance of the Gln3 and Gat1 transcription factors to the nucleus.[27]

These experiments thus clearly show that Rapamycin treatment, or starvation conditions, lead to telomere shortening. What advantage can a massive loss of telomeres provide to the starving cell? Telomere maintenance, with its complex regulation, may be energy-intensive and therefore highly costly with respect to nutrient consumption. It is possible that telomere length regulation is compromised in starved stress in order to reallocate cell resources to more essential processes. At the same time, telomere degradation may supply much-needed nitrogen and carbon sources.[27]

The ability of cells under a complex telomere length homeostasis to respond to environmental clues could be advantageous and increase cellular fitness under certain circumstances. In order to find conditions at which yeast cells respond to the environment by modifying their telomere length, Romano et al.[28] recently subjected yeast cells to a battery of stressful environments, including high and low temperature, changes in pH, exposure to different drugs, etc. Interestingly, most of the stresses, including oxidative stress, did not significantly alter telomere length, indicating that telomere length homeostasis is robust under many, probably most, environmental conditions. Some stresses, however, such as high temperature, the addition of caffeine, and low levels of the drug hydroxyurea, resulted in telomere shortening, while others, such as added acetic acid and alcohols including ethanol, methanol, and isopropanol, caused a significant increase in telomere length. Strikingly, under alcohol stress, telomeres were not only longer, but also exhibited length heterogeneity, indicating that the mechanism(s) that preferentially elongates short but not long telomeres,[45] thus ensuring a narrow telomere length distribution in the cell, was disrupted. The effect of alcohols on telomere length was independent of the ability of these cells to metabolize the alcohol: telomeres elongated upon ethanol treatment in petite yeast strains lacking mitochondrial function, which are unable to utilize ethanol. Telomere elongation by ethanol was telomerase-dependent, rather than ALT-like. In all cases, removal of the stressing agent resulted in a gradual restoration of wild-type telomere length, demonstrating that the changes in telomere length were physiological rather than genetic, and thus may have been mediated by altered gene expression and protein activity.[28]

By analyzing the genome-wide transcription levels in yeast cells under no stress, or exposed to ethanol (elongating conditions), high temperature and caffeine (shortening conditions), or peroxide (no effect on telomere length), the authors identified genes that showed differential response. They then integrated transcript abundance data with the in silico *TLM* network[22] created by using protein–protein interactions data to connect all *TLM* genes to the telomere maintenance machinery (telomerase and nucleases). The pairwise distances between stress-specific differentially expressed *TLM* genes were compared with pairwise distances of other *TLM* genes. This revealed that stress-specific, differentially expressed *TLM* genes lie significantly closer to each other for ethanol, caffeine, and 37 °C but not for hydrogen peroxide stress, which does not affect telomere length. These results suggested that the differentially expressed *TLM* genes may be involved in transducing the external signals and disrupting telomere length homeostasis.

Based on this analysis, a list of candidate mutants was generated, which were grown under the various stress conditions in order to look for those defective in transmitting the stress signal to the telomeres. Strikingly, they found a strong correlation between the rate of change in telomere length and the initial length of the mutant: in ethanol, long *tlm* mutants elongate more rapidly and short *tlm* mutants elongate more slowly than the wild type. Similarly, in caffeine and at 37°C, long *tlm* mutants shorten more rapidly and short *tlm* mutants shorten more slowly than the wild type. This correlation between abnormal telomere length and response magnitude to the stresses suggests that telomere elongation/shortening in the presence of external cues is performed by the same basic mechanism(s) that maintain telomere length under unperturbed conditions.[28]

As in the case of the response to Rapamycin (described above), the authors concentrated on *tlm* mutants that were unresponsive to the external signals. Among these, *rif1*Δ was the most striking. The Rif1 and Rif2 proteins are negative regulators of telomerase that interact with the C terminus of Rap1, an essential protein that binds to the telomeric repeats.[46] Downregulation of the *RAP1* gene or mutations in the C terminus of *RAP1* led to extreme telomere elongation and to an increase in telomere length variability, similar to that observed in the presence of ethanol.[47,48] The genome-wide transcriptional analysis revealed a reduction in the level of Rap1 expression in cells grown in the presence of ethanol, suggesting a model in which alcohol leads to a reduction in the levels of Rap1, reducing Rif1 recruitment to telomeres and leading to telomere elongation. Indeed, telomere did not respond to ethanol when the *RAP1* promoter was replaced by a tetracycline-inducible one (which does not respond to

the ethanol signal).[49] Similarly, no changes were observed upon ethanol exposure in mutants lacking the Rap1 C-terminal region or the Rif1 protein. These results single out the Rap1–Rif1 pathway as central to telomere elongation in response to ethanol. Consistent with this hypothesis, chromatin immunoprecipitation (ChIP) experiments showed that upon exposure to ethanol there is a 2-fold reduction in the level of Rif1 at telomeres. Rif1 activity was also required to shorten telomeres in the presence of caffeine. Therefore, Rif1 may play a general sensing/structural/regulatory role, rather than a catalytic one, in the telomeric response to environmental signals. This is consistent with recent studies that found a role for Rif1 in the regulation of chromatin structure and of DNA replication origin firing.[50,51] Rif1 and Rif2 were thought to form a complex. Remarkably, however, *rif2*Δ cells exhibited a stronger-than-expected response to ethanol, underscoring the different roles of Rif1 and Rif2 in telomere length maintenance.[52-54] Surprisingly, a strain deleted for the *TEL1* gene, which encodes the yeast ortholog of the mammalian ATM protein kinase or strains lacking components of the MRX complex (Mre11, Rad50, Xrs2), which work in the same pathway, also exhibited an over-reaction to ethanol. This result suggests that in addition to its positive role in promoting elongation of the shortest telomeres in the cell, the Tel1/ATM pathway also functions to restrain elongation of telomeres by telomerase.

Deletion of Rif1 and mutations in Rap1 also significantly decreased the telomeric response to caffeine, indicating that Rif1-Rap1 is not only involved in telomere elongation under ethanol stress, but also in telomere shortening under caffeine. Caffeine is a known inhibitor of phosphatydyl inositol-3 kinase-related kinases (PI3K-like kinases), such as human ATR and ATM[55] and their yeast counterparts, Tel1 and Mec1.[56] Indeed, deletion of either *TEL1* or *MEC1* individually did not prevent the response to caffeine, but a double mutant *tel1*Δ *mec1*Δ was completely insensitive to the telomeric effect of caffeine, consistent with the known redundant function that these 2 kinases play in telomere biology.[57] Thus, caffeine causes telomere shortening by inhibiting the ATM/ATR-like regulatory kinases.

Interestingly, mutations in Rap1 and the deletion of Rif1 affected only the shortening rate in the presence of caffeine but did not change the response to high temperature. High temperature has a broad, pleiotropic effect and may alter telomere length via several mechanisms. No single deletion mutant failed to shorten its telomere length at the high temperature, suggesting that either there are redundant temperature-responding functions, or, more likely, some telomerase component(s) is intrinsically thermolabile, as has been recently suggested.[58] Consistent with this idea, cells treated with caffeine, which exhibited telomere shortening, elongated their telomeres back to normal length once the caffeine was removed, but only if grown at a permissive temperature; no elongation was observed at 37 °C.

Telomere length and telomerase activity are important factors in the pathobiology of human disease. Age-related diseases and premature aging syndromes, for example, are characterized by the shortening of telomeres.[59] Tumor cells, on the other hand, prevent telomere shortening and telomere loss by upregulating telomerase, thereby perpetuating cells with short telomeres and high chromosomal instability.[60] Thus, although the mechanisms at work differ, changes in telomere length fuel disease pathology in cancer and other premature aging syndromes. Interestingly, previous studies identified correlations between telomere length and environmental conditions, such as mental stress,[61] socioeconomic status,[62] and health-related behavior in adults.[63] Although intriguing, these studies presented only a correlation between 2 phenomena, and lacked a demonstration of causality. By using controlled experimental approaches in a model organism amenable to manipulations, the 2 papers reviewed demonstrate a direct causality between environmental cues and changes in telomere length. The identification of mechanisms by which external signals modify telomere length significantly advances our understanding of the complex interplay of genes and environment. More critically, however, these findings also suggest the possibility of strategic manipulations of telomere length that may well have important therapeutic implications in the treatment of human disease.[64] Studies to assess the effect of different environmental stresses on telomere length in human cells are under way.

One of the best immediate candidates for such therapy is Rapamycin. Due to its central role in cell growth and metabolism, inappropriate upregulation of the TOR pathway has been implicated in various malignancies, including cancers of the colon, breast, liver, brain, stomach, lung, and ovary (reviewed in ref. 65). Several Rapamycin derivatives are currently undergoing clinical trials for the treatment of carcinomas, lymphomas, and other types of cancers. If the results presented hold true for human cells, they have potential implications for the use of Rapamycin as a therapeutic agent against cancer, as telomere maintenance is essential for maintaining the immortality in cancer cells.[5] Moreover, drugs directed against mTOR have shown synergy with DNA damaging agents.[66] In yeast cells, starvation leads to reduced levels of Ku heterodimer, resulting in NHEJ deficiency. The effect of Rapamycin on NHEJ in mammals is currently being tested, as NHEJ is one of the main double-strand break repair mechanisms in mammals, and Rapamycin may thus render the cells hypersensitive to DNA damaging agents.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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