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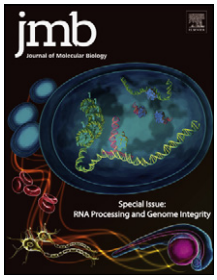

Life and Death of Yeast Telomerase RNA

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Abstract

Telomerase reverse transcriptase elongates telomeres to overcome their natural attrition and allow unlimited cellular proliferation, a characteristic shared by stem cells and the majority of malignant cancerous cells. The telomerase holoenzyme comprises a core RNA molecule, a catalytic protein subunit, and other accessory proteins. Malfunction of certain telomerase components can cause serious genetic disorders including dyskeratosis congenita and aplastic anaemia. A hierarchy of tightly regulated steps constitutes the process of telomerase biogenesis, which, if interrupted or misregulated, can impede the production of a functional enzyme and severely affect telomere maintenance. Here, we take a closer look at the budding yeast telomerase RNA component, TLC1, in its long lifetime journey around the cell. We review the extensive knowledge on TLC1 transcription and processing. We focus on exciting recent studies on telomerase assembly, trafficking, and nuclear dynamics, which for the first time unveil striking similarities between the yeast and human telomerase ribonucleoproteins. Finally, we identify questions yet to be answered and new directions to be followed, which, in the future, might improve our knowledge of telomerase biology and trigger the development of new therapies against cancer and other telomerase-related diseases.

Telomeres are essential nucleoprotein structures that cap the ends of linear chromosomes and shield them from Double Strand Break (DSB) repair activities, which could otherwise lead to chromosome end-to-end fusions and massive genome rearrangements [1,2]. In addition, telomeres are designed to protect chromosome ends from successive erosion and loss of genetic information that would inevitably arise due to the natural limitation of the DNA replication machinery to completely duplicate the ends of chromosomes [3]. In most eukaryotic cells, this "end-replication problem" is solved by telomerase, a specialised reverse transcriptase that adds additional telomeric repeats to preexisting telomeres [4,5]. Telomerase is expressed in constantly dividing cells such as germ and stem cells, most cancer cells, and unicellular eukaryotes [6,7]. In contrast, in human somatic cells, telomerase expression is downregulated, limiting the replicative cell life span, thus acting as a major tumour-suppressive mechanism [8,9].

The telomerase ribonucleoprotein (RNP) complex minimally requires a catalytic protein subunit and an RNA moiety [10–13]. In budding yeast, the telomerase RNA component [called *tel*omerase *c*omponent 1 (TLC1 RNA)], is a large non-coding RNA (1158 nt), which provides a template for the synthesis of telomeric repeats [11,14]. This telomerase RNA has been proposed to act as a flexible scaffold for other telomerase components and accessory proteins [15]. The Est2 catalytic protein subunit binds to a central domain of TLC1, which includes the template region, a pseudo-knot, and a template boundary element [11,16–18]. Est1 and Est3, accessory telomerase subunits, which are dispensable for telomerase activity *in vitro* but essential for its *in vivo* function, also associate with TLC1 [12,13,19]. Est1 directly binds to a specific stem-bulge region of TLC1, whereas the Est3 interaction with telomerase RNA might be mediated by other protein subunits [19–21]. Recently, Pop1, Pop6, and Pop7 were identified as essential components of the telomerase holoenzyme [22]. All three are important for the stabilisation of Est1 and Est2 on the telomerase RNP *in vivo* and hence are absolutely required for its

function. These newly identified telomerase components are well-known subunits of RNA processing RNases P and Mitochondrial RNase P (MRP) [23]. Strikingly, the domain of TLC1 that serves as a docking site for the Pop proteins is highly similar to the Pop-binding domain of RNase P/MRP RNA moieties [22]. TLC1 is also bound by the Sm_7 protein complex, which stabilises telomerase RNA, and the yKu70/80 heterodimer, which appears crucial for the import and/or retention of telomerase in the nucleus (see below) [24–27].

Expression of telomerase components is very low in both yeast and human cells [28,29]. In *Saccharomyces cerevisiae*, there are only about 25 TLC1 molecules per haploid cell, with the protein subunits being slightly more abundant (approximately 40 molecules of Est2, and 70 to 80 molecules of each Est1 and Est3) [28,30]. Hence, in budding yeast, the RNA moiety is the limiting factor for telomerase holoenzyme assembly and activity. In immortalised human cells, 1150 molecules of human telomerase RNA (hTR) and 500 of human telomerase reverse transcriptase (hTERT) molecules were detected, suggesting that unlike in budding yeast, hTERT is the limiting component of human telomerase [29]. Notably, there are only about 240 assembled telomerase molecules per human cell, which approximately equals the number of telomeres after replication. This also indicates that a pool of free telomerase subunits is maintained in human cells.

A low abundance of the TLC1 RNA implies that its expression is strictly downregulated. Nevertheless, the amount of telomerase RNA has to be kept at a certain minimal threshold level to sustain normal telomere maintenance. Indeed, losing just one copy of the *TLC1* gene from a diploid cell leads to telomere shortening [28]. Furthermore, "additive haploinsufficiency" of the genes encoding telomerase RNA and the catalytic subunit in *TLC1/tlc1Δ EST1/est1Δ* double mutants results in even shorter telomeres than in single mutants [12]. Moreover, haploinsufficiency of the gene encoding human telomerase RNA is associated with dyskeratosis congenita and aplastic anaemia [31–33]. Surprisingly, too high of a level of telomerase RNA is also dangerous for a cell. For example, in yeast, overexpression of *TLC1* may impede telomerase function and lead to telomere shortening due to the sequestering of yKu [11,34]. Unregulated expression of telomerase components may also increase the incidence of *de novo* telomere addition at DNA double-strand breaks that would lead to the loss of genes and chromosome rearrangements [35]. Therefore, a delicate balance in telomerase RNA level must be maintained to achieve the desired telomerase activity without jeopardising genome stability.

The abundance and quality of telomerase RNA are controlled at multiple levels including its transcription, processing, and subcellular trafficking. Therefore, accurate performance of a number of cellular machineries involved in telomerase RNP biogenesis is crucial for optimal telomerase activity and telomere maintenance [36–41]. Here, we focus on the main stages of the telomerase RNA life cycle in *S. cerevisiae*, highlighting recent studies, identifying open questions, and drawing parallels with the human telomerase RNA or that of other species where appropriate.

The Birth of the Telomerase RNA: Transcription and 3′-End Processing

Transcription

The *TLC1* gene is transcribed by RNA polymerase II at the transition between the G1 and S phases of the cell cycle (Fig. 1-1) [42,43]. In budding yeast, G1/S-specific gene expression is largely controlled either by Swi4/6 cell Cycle Box (SCB)-binding factor or the Mbp1/Swi6 cell Cycle Box (MCB)-binding factor complex [44]. SBF is composed of Swi4 and Swi6 factors, which activate transcription at the G1/S transition. The MBF complex is represented by the Mbp1/Swi6 heterodimer, which represses transcription outside the G1 phase. The *TLC1* promoter contains both enhancer and repressor elements: the SCB consensus binding site for the SBF complex overlapping with the MCB binding site for MBF and a separate MCB element [43]. This complex architecture of the *TLC1* promoter controls the cell cycle specificity of TLC1 RNA synthesis [43]. In addition, it allows the tight regulation of *TLC1* transcription, thus ensuring that the level of TLC1 as the limiting component of telomerase stays low.

The level of another telomerase component, Est1, is also cell-cycle-regulated. The control mechanisms act mostly at the protein level via proteasome-dependent Est1 degradation in G1 [45]. However, Est1 regulation at the mRNA level also occurs; similar to *TLC1*, transcription of the *EST1* gene also peaks at the G1/S transition [46]. In addition, the *EST1* promoter region includes a complex of the SCB and MBF consensus binding sites, suggesting that Est1 and TLC1 might be co-regulated during the cell cycle [43].

3′-end processing

Originally, two distinct TLC1 RNA species were identified in yeast: a minor short-lived polyadenylated fraction, which constitutes 5–10% of the total TLC1, and a major non-polyadenylated population, representing the mature TLC1 associated with the active telomerase RNP [14,42]. Polyadenylation is a characteristic of the mRNA transcription termination pathway, whereby the nascent RNA molecule undergoes endoribonucleolytic cleavage at the 3′-end, followed by polyadenylation of the upstream fragment and degradation of the downstream cleavage product [47]. Indeed, the cleavage of the nascent TLC1 RNA and its polyadenylation is mediated by the same machinery