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Review

Budding yeast with human telomeres: A puzzling structure

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Abstract

Telomeres share some common features among eukaryotes, with few exceptions such as the fruit fly *Drosophila* that uses transposons as telomeres, they consist of G-rich repetitive DNA that is elongated by telomerase and/or alternative pathways depending on recombination. Telomere structure comprises both cis-acting satellite DNA (telomeric DNA) and proteins that interact directly and/or indirectly with the underlying DNA. Telomeric DNAs are surprisingly conserved among the vertebrates and very similar in most eukaryotes, but present some differences in yeast such as *Saccharomyces cerevisiae*. The telomeric proteins are more variable although the basic mechanisms which control telomere lengthening and capping are very similar, in fact orthologues of the yeast telomeric proteins, which have been studied first, have been identified in other organisms. Here we describe the structure of human telomeres in budding yeast as compared to canonical yeast and mammalian telomeres taking into consideration the more recent findings highlighting the mechanisms that are responsible for chromosome end protection and lengthening, and the role of chromatin organization in telomere function. This yeast represents a model for the study of mammalian telomeres that could be reconstituted step-by-step in all their components, moreover it could be useful for the assembly of mammalian artificial chromosome.

Keywords: Budding yeast; Telomeric chromatin; Telomerase; Mammalian telomeres; Humanized telomeres

1. Introduction

The yeast *S. cerevisiae* has been used for many years as a model to isolate and study telomeres from different organisms including humans, more recently budding yeasts with human telomeres have been engineered. In fact, although telomeres were first isolated in ciliates [1], telomeres from different sources were successfully isolated in yeast by means of a functional assay based on the cloning of fragments that allow replication of a linear DNA molecule [2]; moreover by changing the telomerase telomeric template telomeres with variant telomeric repeats have been produced [3]. This was made possible by the ability of the telomerase, the reverse transcriptase that elongate telomeres, to tolerate mutations in the telomeric template and to add telomeric repeats to a number of different telomeric substrates ranging from protozoa to vertebrate. The sequencing of telomeric DNA from different organisms showed some common features: telomeres consist of a string of G-rich repeats of variable length, from few nucleotides in ciliates to many kb in mammals; the G-rich strand is orientated 5′-3′ toward the end of the molecule and terminates with a 3′ single-stranded overhang. But telomeres are not simply DNA, they are organized in a complex nucleoprotein structure in which both the DNA and the protein components are essential for proper functioning. In fact, telomere dysfunctions can be ascribed either to mutations in the telomeric sequence repeats [3], or to mutations in telomeric proteins as demonstrated by the uncontrolled telomere lengthening in rap1p$^{\Delta C}$ (C-term deletion) mutants [4]. Overall the structure of telomeres in yeast and mammals is conserved (Fig. 1) and can be divided in two subdomains: (I) a domain consisting of double-stranded telomeric DNA and telomere-repeat-binding proteins which recruit additional proteins; and (II) a distal G-strand overhang, specifically bound by ssDNA-binding proteins, that participate to telomere capping; it may adopt particular conformations, such as t-loops or G-quadruplexes. In the t-loop formation

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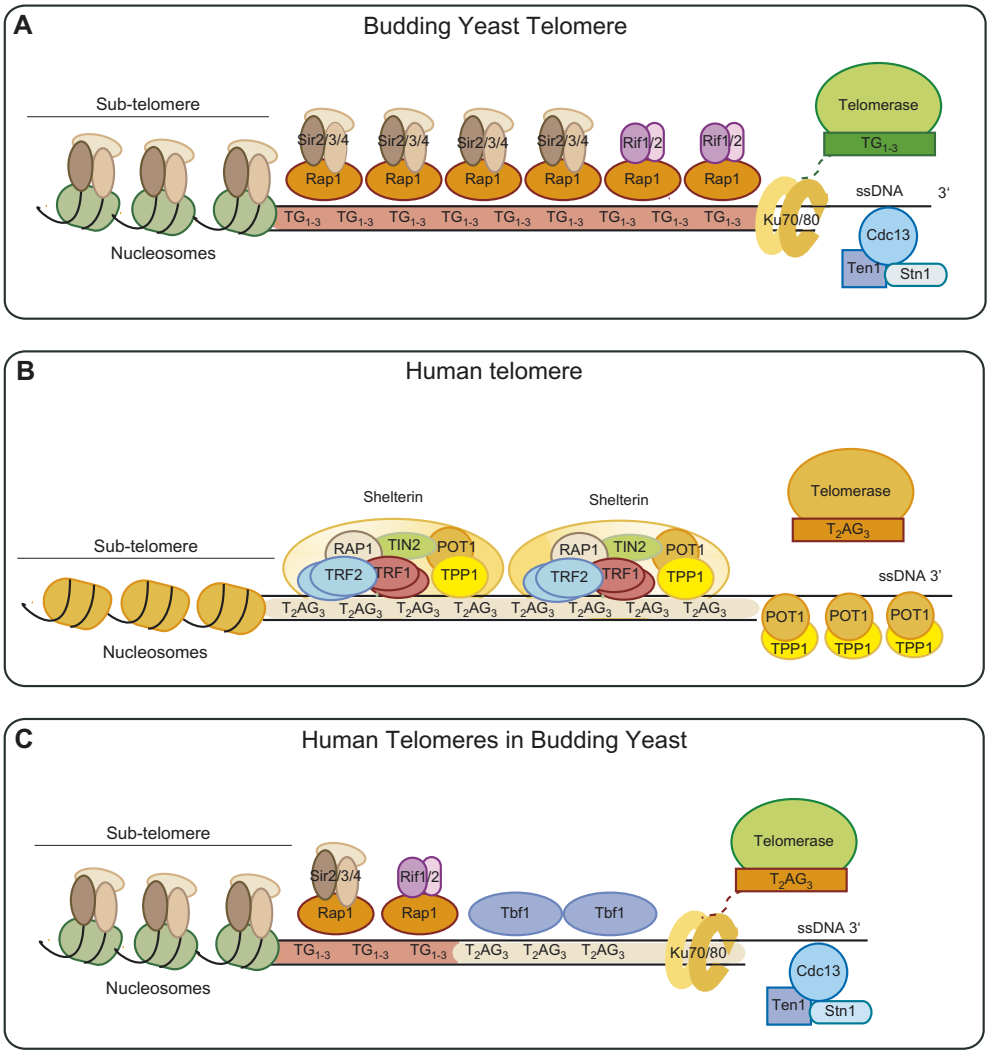

Fig. 1. Structure of telomeres in yeast and human. (A) Budding yeast telomeres and sub-telomeres. Rap1 (Repressor Activator Protein 1) binds the double-stranded telomeric sequences and recruits the Sir2, Sir3 and Sir4 proteins (Silent Information Regulator Protein) that spread over the subtelomeric region; it also binds Rif1 and Rif2 (Rap1 Interacting Factors) which regulate telomere length. The heterodimeric Ku complex (Ku70/80) interacts with the terminal part of the telomere and, depending on the cell cycle, with the ku-loop of the telomerase RNA; Cdc13 (Cell Division Control Protein 13) and the Cdc13-interacting factors Ten1 and Stn1 bind to the single strand overhang. (B) Human telomeres. Double-stranded telomeric DNA are bounded by Shelterin complex which is constituted by six subunits: TRF1 and TRF2 which bind directly the double-stranded telomeric DNA through the myb-like domain, POT1–TPP1 bind single-stranded DNA; hRap1 binds the telomere by interacting with TRF1 and TRF2; TIN2 tethers POT1–TPP1 to TRF1 and TRF2. Human telomeric chromatin is nucleosome-based but the interaction between nucleosomes and shelterin is at the present unknown. (C) Human telomere in budding yeast. Mixed yeast-human telomeres are produced by the human RNA template telomerase that adds human-like T2AG3 repeats to yeast canonical telomeres. Binding of Rap1/Sir and Rap1/Rif complexes is impaired by the reduced amounts of yeast telomeric repeats that remain confined in the internal yeast core region. The sub-telomeric protein Tbf1, which binds T2AG3, moved to the terminal part of the telomeres where it may compensate for Rap1 lost. Cdc13 and the interaction Stn1 and Ten1 bind to the G-tail as in the canonical yeast telomere. The T2AG3-only telomere is the same as the mixed telomere except for the absence of the internal TG1–3 core.

the telomere folds back on itself forming a large telomere loop (t-loop) and the 3′ overhang invades the adjacent duplex telomeric repeat, forming the D-loop [5]. The G-rich 3′ overhang can also form a variety of quadruplex structures based on four Hoogsteen-paired coplanar guanines; these structures can be assembled in either intramolecular or intermolecular configurations, which has been proposed to participate to telomere capping and to mediate chromosome alignment during meiotic prophase I respectively (reviewed in Ref. [6]). Adjacent to telomere a subtelomeric domain consisting of blocks of recent genomic duplications is thought to function as a chromatin insulator that blocks heterochromatin spreading along the chromosome arms (reviewed in Ref. [7]).

2. Yeast telomere structure

Yeast telomeres have the unique feature of being composed of heterogeneous repeats abbreviated as TG1–3, 250–400 bp long and terminating with a 3′ short (12–14 nt) single-stranded G-overhang that is longer (50–100 nt) in late S-phase (Fig. 1A) [8]. The double-stranded part of the telomere is bound by Rap1 (repressor/activator-site binding protein)