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Composition and conservation of the telomeric complex

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Abstract. The telomere is composed of telomeric DNA and telomere-associated proteins. Recently, many telomere-associated proteins have been identified, and various telomere functions have been uncovered. In budding yeast, scRap1 binds directly to telomeric DNA, and other telomere regulators (Sir proteins and Rif proteins) are recruited to the telomeres by interacting with scRap1. Cdc13 binds to the most distal end of the chromosome and recruits telomerase to the telomeres. In fission yeast and humans, TTAGGG repeat binding factor (TRF) family proteins bind directly to telomeric DNA, and Rap1 proteins and other telomere regulators are recruited to the telomeres by interacting with the TRF family proteins. Both organisms have Pot1 proteins at the most distal end of the telomere instead of a budding-yeast Cdc13-like protein. Therefore, fission yeast and humans have in part common telomeric compositions that differ from that of budding yeast, a result that suggests budding yeast has lost some telomere components during the course of evolution.

Key words. Rap1; TRF; Myb motif; Pot1; Ku.

Introduction

The telomere is a specialized heterochromatin composed of telomeric DNA and various telomere-associated proteins. Telomeric DNA contains specific G-rich repeat sequences, such as $TG_{2-3}(TG)_{1-6}$ in budding yeast, $TTACAG_{2-5}$ in fission yeast, TTTAGGG in many plants and TTAGGG in humans [1–4]. Most of the telomeric DNA is duplex, but the most distal end of the telomere contains a single-stranded 3′-overhang called the G tail. Because protein-free DNA ends are vulnerable to degradation by nucleases and are the target of DNA end fusion by DNA repair, telomere-associated proteins provide a sort of protective cap to the ends of linear chromosomes. In addition to this role, telomeres also function at meiosis [see Bass, this issue]. For example, telomeres cluster toward the SPB (spindle pole body) or in the vicinity of the centrosome at a specific stage in meiosis, and at least in fission yeast, this clustering is prerequisite for the normal pairing of homologous chromosomes [5–9]. This dynamic change of the nuclear localization of chromosomes is realized by interactions between telomere-associated proteins and components of the nuclear structure. Furthermore, the telomere is a specific heterochromatin, and therefore a gene located near the telomere is transcriptionally repressed by the heterochromatin structure (telomere silencing or telomere position effect) [see Perrod and Gasser, this issue]. Recent studies have revealed that the composition of telomere is conserved among eukaryotes to some extent. Here, the functions of telomere-associated proteins in budding yeast, fission yeast and humans will be described.

Telomere-associated proteins in budding yeast

The budding yeast, *Saccharomyces cerevisiae*, is a well-characterized organism for the study of telomeres (fig. 1). *S. cerevisiae* Rap1 (scRap1, repressor/activator protein 1) was identified as a protein that binds to the UAS (upstream activation sequence) of genes for ribosomal proteins, translational components and glycolytic enzymes, as well as to mating-type silencer elements and telomeres [10–14]. scRap1 is essential for normal cell growth. It binds directly to DNA through its DNA binding domain, which comprises two Myb motifs located in the central region. scRap1 regulates the transcription of genes through its

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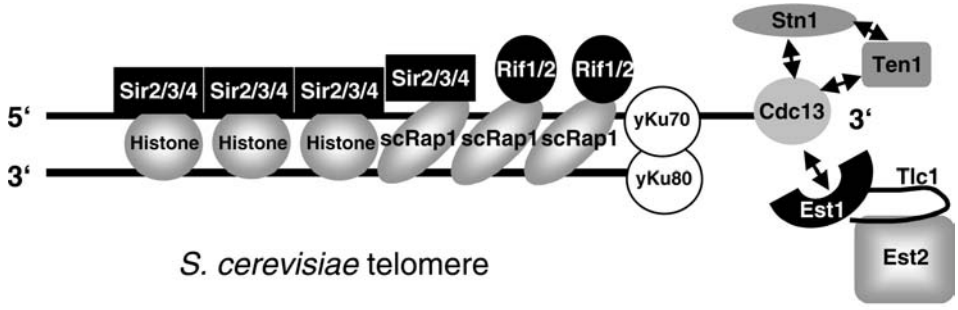

Figure 1. Telomere-associated proteins in budding yeast. Arrows indicate physical interactions.

transactivation domain, located C-terminal to its Myb motifs [15]. scRap1 also has a BRCT (BRCA1 C-terminal) domain in the N-terminal region, which is well conserved among Rap1-related proteins in other organisms.

scRap1 regulates telomere length and the formation of telomeric heterochromatin by recruiting two groups of proteins to its RCT (Rap1 C terminus) domain [16, 17]. The first group, scRif1 (Rap1-interacting factor 1) and scRif2, primarily regulates telomere length [18, 19]. Deletion of either scRif1 or scRif2 causes moderate elongation (200–600 bp in the *scrif1* mutant, ~100 bp in the *scrif2* mutant) of telomeric DNA, and the deletion of both causes marked elongation (600 bp–2.5 kb) of telomeric DNA, which is the same as that observed in the *rap1-17* mutant. According to the "protein-counting model," cells count the number of scRif1 and scRif2 molecules at the telomeres to regulate telomere length [19, 20]. The second group, Sir3 (silent information regulator 3) and Sir4, is mainly involved in heterochromatin formation [21, 22]. Sir2, a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase, forms a complex with Sir3 and Sir4 by interacting mainly with Sir4 [23]. Sir3 and Sir4 interact not only with scRap1 but also with the N-termini of histones H3 and H4, which are hypoacetylated by Sir2 [24, 25]. Thus, the Sir2/Sir3/Sir4 complex spreads over the nucleosomes proximal to the telomeres from the nucleation sites with scRap1 at the telomeres. The Sir complex is also localized at the silencer of the *HM* mating-type loci with scRap1 to repress the expression of *HM* genes [21] [see also Perrod and Gasser, in this issue]. Furthermore, the Sir complex is recruited to DSB (double-strand break) sites produced by DNA damage and is involved in the Ku-dependent nonhomologous end-joining (NHEJ) pathway [26, 27]. Sir2 also forms a complex with Net1 and Cdc14, and the Sir2/Net1/Cdc14 complex is required for transcriptional repression at ribosomal DNA (rDNA) repeats [28, 29].

Cdc13 protein binds to the single-stranded G tail and interacts with Est1 (ever shorter telomere 1), which associates with Tlc1 (the RNA template subunit of telomerase), to recruit the catalytic subunit of telomerase (Est2) to the telomeres [30–35]. Furthermore, Cdc13 prevents the degradation of the C-rich strand at the chromosome end [see Wei and Price, this issue]. The function of Cdc13 is regulated by two interacting proteins, Stn1 and Ten1 [36–39]. The Cdc13/Stn1/Ten1 protein complex is required for telomere end protection and telomere length control. All the temperature-sensitive mutants (*cdc13, stn1* and *ten1*) accumulate single-stranded DNA in telometric regions and exhibit abnormally elongated telomeres.

Budding yeast Ku heterodimer (yKu70/yKu80) binds to the distal end of the telomere. Generally, Ku binds to the DNA ends of DSBs with the Sir2/Sir3/Sir4 complex and mediates NHEJ [27]. Therefore, yKu70/yKu80 at the telomere should be regulated so as not to ligate telomere ends for telomere maintenance. yKu regulates telomere structure, telomere length, telomeric silencing and telomere localization to the nuclear periphery by interacting with other regulatory factors [27, 40–43]. Recently, it has been suggested that yKu helps to recruit or activate telomerase by interacting with the stem-loop of Tlc1 [44]. Furthermore, yKu interacts with Mlp2 protein, which forms a complex with Mlp1 that associates with Nup60, a component of the nuclear pore complex [45]. These interactions might permit the localization of telomeres near the nuclear pore, but recent study has shown that yKu, but not Mlp1 and Mlp2, is necessary for anchoring telomeres at the nuclear envelope (NE) in interphase and that increased telomeric repression of transcription is correlated with increased anchoring of telomeres to NE [46]. Sir proteins also contribute significantly to telomere anchoring specifically in S phase [46].

Telomere-associated proteins in fission yeast

Recent studies have shown that the components of the telomere in fission yeast, *Schizosaccharomyces pombe*, differ from those in budding yeast (fig. 2). First, the *S. pombe* protein Taz1, which contains a Myb motif but is not a homologue of scRap1, binds directly to telomeric DNA [47]. Taz1 is bound to telomeres both in mitotically growing cells and in meiotic cells. Taz1 contains a single Myb motif at its C terminus and a TRFH (TRF homol-

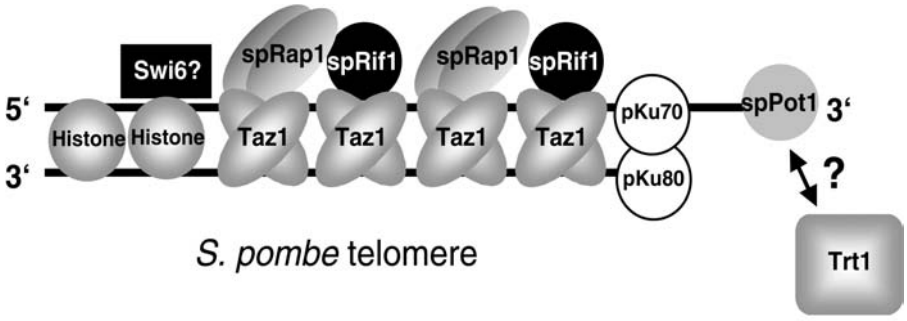

Figure 2. Telomere-associated proteins in fission yeast. Localization of Swi6 is speculative. spPot1 may regulate the access of telomerase (Trt11) to the telomeres.

ogy) domain of approximately 200 amino acids in the central region. The deletion of *taz1*$^+$ was found not to be lethal but to cause severe defects in telomere function [47, 48]. The *taz1* mutant has extensively elongated telomeric DNA (up to ~5 kb) and is defective in telomere silencing like that in the *swi6* mutant, in which the heterochromatin protein Swi6 (HP1 homologue) is lost. Furthermore, the *taz1* mutant has a defect in telomere clustering toward SPB in the premeiotic horsetail stage, which causes abnormal spore formation. Taz1 therefore plays important roles in at least three major telomere functions: regulation of telomeric DNA length, telomere silencing and telomere clustering toward SPB in meiosis.

S. pombe Rap1 (spRap1) has a BRCT domain and a Myb motif [49, 50]. It also has a Myb-like motif that has similarity with the second Myb motif in scRap1 but contains different residues at the positions of the hydrophobic core of the three-helix bundle. The physiological significance of this motif is not known. spRap1 contains no apparent RCT domain, so the mechanism of protein-protein interaction between spRap1 and putative spRap1-binding proteins may be different from that between scRap1 and Rif or Sir proteins. spRap1 interacts with Taz1 in a two-hybrid system and is localized at the telomeres by its binding to Taz1 in mitotically growing cells, so spRap1 does not bind directly to telomeric DNA and is basically bound to telomeres by interaction with Taz1. In the premeiotic horsetail stage, however, spRap1 is localized at the telomeres in about 30% of the cells in the absence of Taz1, so spRap1 may have an intrinsic tendency to be localized near SPB, which is colocalized with telomeres at the horsetail stage, or spRap1 may bind inefficiently to telomeres in a Taz1-independent manner. The deletion of *sprap1*$^+$ causes telomere-deficient phenotypes as severe as those observed in the *taz1* mutant. Among them, the frequency of telomere clustering toward SPB at the horsetail stage in the *sprap1* mutant is much lower than that in the *taz1* mutant, so spRap1 plays a major role in telomere clustering.

S. pombe Rif1 (spRif1) shares homology with scRif1 throughout the amino acid sequence, although it does not contain any currently characterized protein motifs [50]. Database searches reveal that some proteins from *Drosophila melanogaster* and *Homo sapiens* show significant homology with the limited N-terminal region of spRif1, but the functions of these proteins are not known. Like spRap1, spRif1 is localized at the telomeres by interaction with Taz1. Furthermore, spRif1 does not interact with spRap1, and they are bound to the telomeres independent of each other, so protein interactions in the two yeasts are quite different. spRif1 is not essential for normal vegetative growth. The *sprif1* mutant has slightly longer telomeric DNA than the wild type, indicating that spRif1 plays a role in the regulation of telomeric DNA length. The *sprif1* mutant does not exhibit any other significant defects in telomere function. It is not known whether there is a functional homologue for Rif2 in fission yeast. Neither spRap1 nor spRif1 is required for the telomere localization of Taz1.

Collectively, three telomere functions, namely telomere length control, telomere silencing and telomere clustering toward SPB at the horsetail stage, are severely impaired in *sprap1* cells, in which Taz1 remains associated with the telomeres. Therefore, telomere-associated Taz1 by itself cannot fulfill these telomere functions, and the primary role of Taz1 is to recruit telomere regulators to the telomeres.

Sir3 and Sir4 seem to have no homologue in fission yeast, but an as-yet uncharacterized protein, spSir2, shares homology with budding yeast Sir2 [51]. In place of Sir3 and Sir4, fission yeast has the Swi6 protein, which is responsible for the heterochromatin structure. Swi6 contains a chromo domain and a chromo-shadow domain that mediate the formation of protein complexes and their association with chromatin [52, 53]. Swi6 has been shown to play important roles at the centromeres and the silent mating-type loci. Swi6 binds to the outer repeat sequences (*otr*) in the fission yeast centromeres and acts to recruit cohesin to the centromeres. In the *swi6* mutant, the centromeres lag on the spindle during anaphase, and the chromosomes are lost at high rates [54–57]. These results

indicate that Swi6 plays a role in realizing the proper function of the kinetochore. At the silent mating-type loci, Swi6 is localized at the K region containing the repeat sequences called 'dg' or 'dh' that also exist in the *otr* of the centromere, and it plays a critical role in the inheritance of silencing and the regulation of the efficiency of mating-type switching [58–60]. Although Swi6 is well studied in terms of its functions at the centromeres and at the mating-type loci, little is known about its function at the telomeres. The *swi6* mutant shows a defect in telomere silencing, and microscopic studies have showed that Swi6 is localized at the telomeres in vivo [61, 62]. Therefore, Swi6 plays a role at least in telomeric heterochromatin, although the physiological significance of telomeric heterochromatin is not well characterized in fission yeast, as in humans.

Similar to yKu70/yKu80 in budding yeast, the fission yeast yKu70/yKu80 homologues, pKu70 and pKu80, are involved in telomere maintenance [63–66]. The *pku70*$^+$- or *pku80*$^+$-deleted strain has slightly shorter telomeric repeat DNA than the wild type, and deletion of pKu70 or pKu80 causes rearrangement of telomere-associated sequences (TASs) that are located proximal to the telomeric repeat sequences [67], so pKu inhibits the degradation and recombination of chromosome ends. Taz1 has been shown to protect pKu-dependent telomere fusion under nitrogen depletion intended to induce sexual development [68]. pKu is not involved in telomere silencing, in contrast to yKu [64, 66].

S. pombe Pot1 (protection of telomeres 1) protein is thought to be associated with telomeric DNA at the most distal end of the telomere [69]. Recombinant spPot1 protein binds to single-stranded telomeric DNA (G strand) in vitro, and the deletion of spPot1 causes immediate loss of telomeric DNA, indicating that spPot1 plays a critical role in protecting chromosome ends. spPot1 may also play a role in recruiting telomerase to the chromosome ends, like Cdc13 in budding yeast [reviewed by Wei and Price, this issue].

Telomere-associated proteins in humans

The known telomere-associated proteins are summarized in fig. 3. In humans, Tazl has two homologues, TRF1 and TRF2, both of which bind directly to telomeric DNA [70–72]. Each has a TRFH domain in its central region and a single Myb-like motif in its C-terminal region. Like Tazl, they form homodimers by means of their TRFH domains to bind to duplex telomeric DNA by means of their Myb-like motifs [72–74], but TRF1 and TRF2 do not form a heterodimer, and their N-terminal regions are quite different: the N-terminus of TRF1 is acidic, whereas that of TRF2 is strongly basic. Overexpression of TRF1 or TRF2 results in a gradual decrease of telomere DNA length [75, 76]. The truncated forms of TRF1 induce abnormal elongation of telomeric DNA, whereas those of TRF2 induce loss of G tails and chromosome end fusions [75–78]. Electron microscopy has demonstrated that TRF2 acts to remodel linear telomeric DNA into duplex loops (T loops) in vitro [79, 80] and has also suggested that the T loops are formed by the invasion of the G tail into the duplex telomeric repeat array and that TRF2 is preferentially present at the loop-tail junction. Currently TRF1 is thought to be a negative regulator of telomeric DNA length and TRF2 is thought to protect telomeric DNA ends in the T loop by masking the G tail from cellular activities, such as nucleases and DNA repair.

Two proteins, tankyrase and TIN2, are localized at the telomeres by interaction with TRF1 [81, 82]. Tankyrase interacts with the acidic domain of TRF1 through its

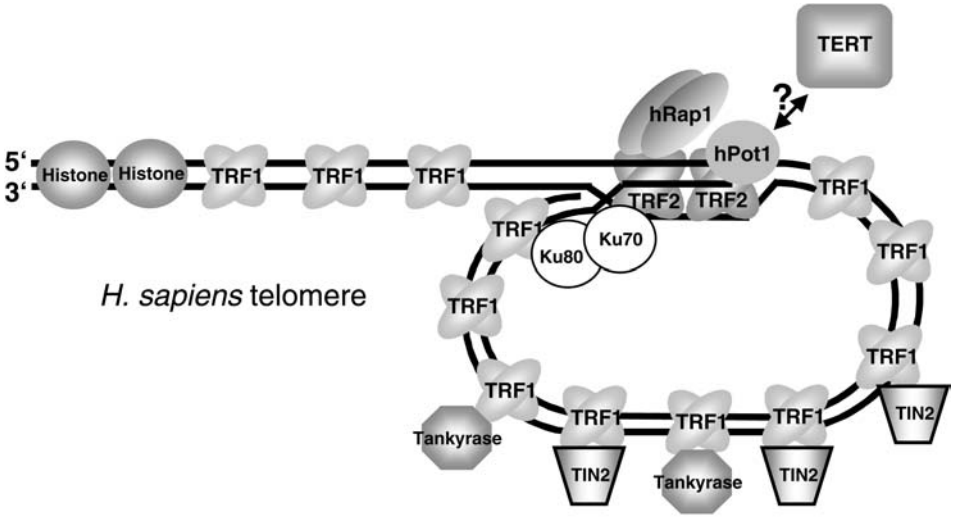

Figure 3. Telomere-associated proteins in human. Relationship between hPot1 and telomerase is not known.

ankyrin-related domain, which contains 24 ankyrin repeats. Tankyrase is a poly(ADP-ribose) polymerase. ADP ribosylation of TRF1 by tankyrase inhibits binding of TRF1 to telomeric DNA in vitro. Overexpression of tankyrase in the nucleus induces ADP ribosylation of TRF1 and releases TRF1 from the telomere. It also induces the gradual elongation of telomeric DNA [83]. Thus, tankyrase-mediated ADP ribosylation of TRF1 would give telomerase access to the telomeric complex. TIN2 interacts with the TRFH domain of TRF1, so the regions in TRF1 that bind to tankyrase and to TIN2 do not overlap. TIN2 has no protein motifs and shares no homology with known proteins. The truncated forms of TIN2 induce abnormal elongation of telomeric DNA in a telomerase-dependent manner, so TIN2 is an essential mediator of TRF1 function and may recruit telomerase inhibitors to telomeres.

Human Rap1 (hRap1) was identified in a screen for TRF2-interacting proteins [84]. hRap1 has a BRCT domain in its N-terminal region and a single Myb motif in its central region, as spRap1 does. The Myb motifs of scRap1, spRap1 and hRap1 constitute a subfamily with respect to the residues at the hydrophobic core of the three-helix bundle and the length of turns connecting the first and second helices [85]. Furthermore, hRap1 has an RCT domain at its C terminus, suggesting that other proteins interact with it. Neither spRap1 nor hRap1 has a transactivation domain, so they play no role as transcription factors. Gel-shift analysis suggested that hRap1 by itself has no telomeric DNA binding ability, but it binds to telomeric DNA when it is associated with TRF2. In fact, a microscopic study showed that hRap1 is localized at the telomeres in a TRF2-dependent manner [84]. Therefore, hRap1 is localized at the telomeres by interaction with TRF2. Overexpression of hRap1 causes gradual elongation of telomeric DNA, indicating that hRap1 plays a role in the regulation of telomere length.

Human Pot1 (hPot1) binds to single-stranded telomeric DNA (G strand) in vitro and is colocalized with TRF2 and hRap1 in vivo [69, 86]. Although the precise in vivo function of hPot1 remains to be determined, it may directly protect the telomere end and may recruit telomerase to the telomeric end [see Wei and Price, this issue].

The human Ku70/Ku80 heterodimer is involved in NHEJ and telomere maintenance as in yeast. It has been suggested that the Ku70/Ku80 heterodimer interacts with TRF1 or TRF2 in vivo [87, 88]. Because X-ray crystallographic analysis reveals that Ku is an end-loading ring, Ku70/Ku80 may be directly loaded onto the telomere end [89, 90]. Ku80-deficient mouse embryonic fibroblasts exhibit high levels of telomere fusion [87]. On the other hand, NHEJ is required for telomere fusion induced by the overexpression of the truncated form of TRF2 in mouse embryonic fibroblasts [78]. Although the in vivo telomere function of Ku70/Ku80 in human cells remains to be determined, Ku apparently plays dual roles at the telomeres: one is to prevent end fusion, and the other to fuse chromosome ends in the absence of functional TRF2. Furthermore, Ku70 may interact with HP1α (heterochromatin protein 1α) [91]. In humans, the telomere position effect (TPE) is observed, although proteins involved in TPE have not been identified so far [92]. The interaction of Ku70 with HP1α may provide a clue to the nature of telomeric heterochromatin in humans.

Conclusions

The mechanism of loading of telomere-associated proteins is well conserved among eukaryotes. First, the protein (scRap1, Taz1, TRF1 or TRF2) that recognizes telomeric repeat sequences binds directly to telomeric DNA. Next, telomere regulators are recruited to the telomeres by interaction with those telomeric DNA-binding proteins, but species differ in the nature of their telomere-associated proteins. scRap1, in budding yeast, has two Myb motifs and binds directly to telomeric DNA, whereas spRap1 and hRap1 are recruited to the telomeres by interaction with Taz1 or TRF2. Each of Taz1, TRF1 or TRF2 has a TRFH dimerizing motif and a single Myb motif. Thus, they bind to telomeric DNA as a homodimer. Fission yeast and humans have Pot1 proteins in common as well. Therefore, the compositions of telomere-associated proteins in fission yeast and in humans are similar but differ from those in budding yeast. Because spRap1 has relatively weak ability to associate with telomeric DNA in the absence of Taz1 in meiosis, we speculate that the common ancestor had both Rap1 and TRF proteins and that budding yeast has lost TRF proteins in the course of evolution. The telomeric repeat sequence has probably changed in budding yeast during the course of evolution and thereby eliminated the ability of TRF proteins to bind to telomeric DNA. In fact, the telomeric repeat sequence of budding yeast [$TG_{2-3}(TG)_{1-6}$] has a pattern different from those of fission yeast and humans. Telomeric repeats in plants are also known (TTAGGG), but the proteins there are not yet characterized.

Although many telomere regulators have been identified in eukaryotes, we do not know the molecular mechanisms underlying their roles. For example, spRap1 has been shown to play roles in at least three telomere functions: telomere length control, telomere silencing and telomere clustering toward SPB in meiosis. Like scRap1, spRap1 may have associating molecules that execute each telomere function. More telomere regulators are expected to be identified in eukaryotes in the future.

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