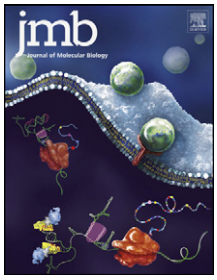

Spatial Organization and Molecular Interactions of the *Schizosaccharomyces pombe* Ccq1–Tpz1–Poz1 Shelterin Complex

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Abstract

The shelterin complex is a macromolecular assembly of proteins that binds to and protects telomeric DNA, which composes the ends of all linear chromosomes. Shelterin proteins prevent chromosome ends from fusing together and from eliciting erroneous induction of DNA damage response pathways. In addition, shelterin proteins play key roles in regulating the recruitment and activation of telomerase, an enzyme that extends telomeric DNA. In fission yeast, *Schizosaccharomyces pombe*, interactions between the shelterin proteins Ccq1, Tpz1, and Poz1 are important for regulating telomerase-mediated telomere synthesis and thus telomere length homeostasis. Here, we used electron microscopy combined with genetic labeling to define the three-dimensional arrangement of the *S. pombe* Ccq1–Tpz1–Poz1 (CTP) complex. Crosslinking mass spectrometry was used to identify individual residues that are in proximity to the protein–protein interfaces of the assembled CTP complex. Together, our data provide a first glimpse into the architectural design of the CTP complex and reveals unique interactions that are important in maintaining the *S. pombe* telomere in a non-extendible state.

Telomeres are nucleoprotein complexes that cap the ends of eukaryotic chromosomes [1]. The DNA component of telomeres consists of repetitive G-rich sequences that extend for thousands of bases of double-stranded (ds) DNA before ending in short, single-stranded (ss) DNA overhangs [1,2]. Because DNA polymerases are unable to synthesize the extreme end of the lagging strand, linear chromosomes become progressively shorter after each round of DNA replication and cell division [3]. Thus, one function of the repetitive telomeric DNA is to absorb this shortening event without incurring the loss of genetic information [4]. In both mammalian somatic cells and single-celled eukaryotes (such as yeast) with genetically inactivated telomerase, continuous telomere erosion upon cell propagations results in critically short telomeres, making genomic DNA more susceptible to alterations and chromosome ends more prone to end-to-end fusions [5–7]. To avoid this catastrophic event, cells initiate replicative senescence to halt cell division when telomeres reach a critically short length [8]. Conversely, cells that propagate indefinitely, such as human embryonic stem cells, the majority of cancer cells, and wild-type single-celled eukaryotes including ciliated protozoa and yeasts, avoid replicative senescence by sustaining elevated levels of telomerase activity [9,10]. Telomerase is a specialized ribonucleoprotein enzyme with reverse transcriptase activity and an intrinsic long noncoding RNA molecule that serves as a template to extend the telomeric DNA [11]. Telomere extension by telomerase allows for continuous proliferation of these cells, as it serves to protect against telomere attrition and thus the deleterious effects triggered by extremely short telomeres. In fission

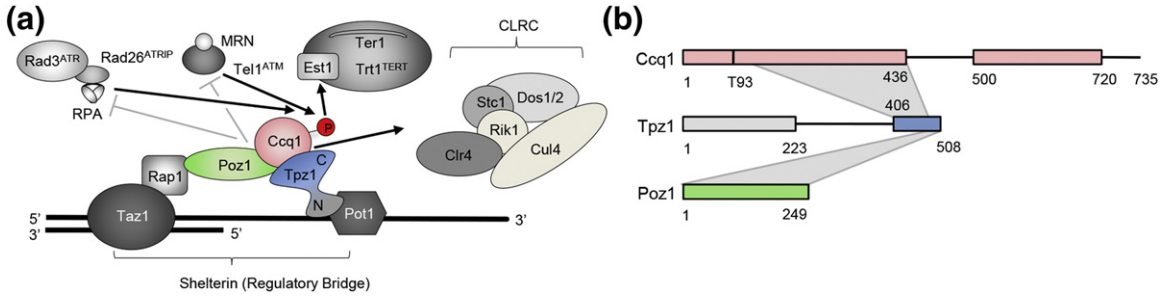

Fig. 1. Schematic representation depicting the diverse roles of the CTP complex in telomere homeostasis. (a) The intact shelterin complex interconnects telomeric ssDNA and dsDNA to regulate different cellular pathways. Destabilization of CTP interactions affects Rad3/Tel1-dependent phosphorylation of Ccq1 Thr93, which directs telomerase recruitment and telomere extension. Similarly, Ccq1–Tpz1 interactions are important for the recruitment of the CLRC complex and silencing of nearby marker genes. (b) Cartoon diagram depicting the interactions between the individual proteins that comprise the CTP complex.

yeast, *Schizosaccharomyces pombe*, telomerase-mediated telomere elongation functions to maintain telomere length homeostasis throughout successive cell divisions [12].

A specialized multi-protein complex called shelterin binds to the telomeric DNA to protect it from eliciting aberrant DNA damage signaling and for regulating telomerase activity [1,5,7,13]. The shelterin complex is well conserved, particularly between mammals and fission yeast, and consists of proteins that recognize both ds and ss telomere DNA specifically (Fig. S1). In the mammalian complex, TIN2 and adrenocortical dysplasia (ACD; previously known as TPP1) form a protein linker connecting ssDNA and dsDNA-binding proteins, an action that is important for regulating telomere length [14,15]. In *S. pombe*, shelterin components Ccq1, Tpz1 (ACD ortholog), and Poz1 (TIN2 ortholog) form a similar sub-complex (CTP complex) that fills this role [16].

In addition to contributing to telomere length homeostasis, the protein–protein interactions of the *S. pombe* CTP complex are known to regulate other cellular processes as well (Fig. 1a). For example, Ccq1 mediates the physical interaction between the shelterin complex and the histone H3K9 methyltransferase Clr4 complex (CLRC) to establish telomeric heterochromatin [17]. The CTP complex interactions, which are coordinated by the Tpz1 C-terminal domain (CTD) [18,19] (Fig. 1b), are additionally important for regulating telomere protection *versus* telomerase-mediated telomere extension. The Ccq1–Tpz1 interaction promotes telomere extension by controlling telomere switching from a non-extendible to an extendible state and by participating in the cell-cycle regulated telomerase recruitment [20,21]. Recruitment of telomerase is mediated through Ccq1–Est1 interactions coupled with Tpz1–Trt1 (catalytic subunit of telomerase) interactions that are initiated by phosphorylation of Ccq1 [20–23] (Fig. 1a). In contrast, mutants that disrupt the Tpz1–Poz1 interaction of the CTP complex result in uncontrolled telomerase-mediated telomere extension [16]. Together, these data indicate that the CTP sub-complex exhibits both positive and negative regulatory roles in telomerase-mediated telomere elongation.

Currently, published structural information regarding the CTP complex or any of its individual proteins is limited to that inferred from biochemical experiments

Fig. 2. The three-dimensional structure of the CTP complex reveals a cage-like complex assembled as a dimer of heterotrimers. (a) Size-exclusion profile of the assembled C_fTP complex. (b) SDS-PAGE analysis of the C_fTP with and without DSSO crosslinker. (c) Representative two-dimensional class averages of the C_fTP complex generated from EM images. (d) Angular distribution of the EM projections of the C_fTP complex that contributed to the three-dimensional reconstruction. (e) Surface rendering of the three-dimensional structure of the C_fTP complex filtered to 34-Å resolution. Labels identify areas of density described as triskelion (T) and L1, L2, and L3 lobes. The panel includes different views of the structure at the defined angles of rotation. For bacterial expression of *S. pombe* proteins, cDNAs for Ccq1 NTD (2–439), Ccq1 CTD (504–719), Ccq1 (2–716; full-length), and Poz1 (2–249)/Tpz1(406–508) were cloned into a modified pET28-6His-SUMO vector with Ulp1-cleavage site following the 6His-SUMO tag (Champion™ pET SUMO protein expression system; Invitrogen, Carlsbad, CA) and subsequently transformed into Rosetta-BL21 (DE3) cells. Protein expression was induced with 0.4 mM IPTG for 5 h at 30 °C. The complex was purified with affinity purification using the 6His-tag followed by anion exchange on a HiTrap Q column. To stabilize complexes for EM imaging, C_fTP was chemically crosslinked using 10 mM DSSO for 1 h on ice before quenching with 2 µl of 1 M Tris–HCl at pH 8.0 for 15 min. The crosslinked complexes were further purified on a G200 10/300GL column (GE Healthcare). EM grids were prepared using 3.5 µl of 200 nM purified complex on glow-discharged carbon-coated, copper grids. The grids were stained in 2% uranyl acetate and data were collected at 200 keV on a FEI TF-20 transmission electron microscope at 42.67 kX (3.654 Å/pixel) equipped with a 4k × 4k TVIPS CCD camera. After selection of 1006 particle pairs in EMAN2.0 [25], particles were CTF-corrected and subjected to reference-free alignment to produce class averages and to generate an initial model using random conical tilt procedures. Particles were imported into RELION 1.3 [26] for further three-dimensional refinement.