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Single-stranded DNA repeat synthesis by telomerase

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Abstract

The eukaryotic ribonucleoprotein reverse transcriptase (RT) telomerase uses a template within its integral RNA subunit to extend chromosome ends by synthesis of single-stranded telomeric repeats. Telomerase is adapted to its unique cellular role by an ability to release product DNA in single-stranded form, regenerating free template from the product-template hybrid. Furthermore, by retaining a template-independent grip on the single-stranded product, telomerase can catalyze processive repeat synthesis. These specialized nucleic acid handling properties are dependent on the protein and RNA domain network within an active RNP. RNP domain architecture and mechanisms for single-stranded DNA handling have been a focus of recent studies highlighted here.

Introduction

Eukaryotic genome maintenance requires *de novo* sequence addition to chromosome ends to balance sequence loss with replication and damage. Telomerase elongates chromosome 3' ends by the synthesis of single-stranded telomeric DNA repeats, using an active site within telomerase reverse transcriptase (TERT) and a template within the integral telomerase RNA (TER). Other reviews summarize the current state of knowledge about TERT and TER structure [1–3], which at high resolution is limited to regions of TERT or TER alone.

Heterologous expression of TERT and TER can reconstitute a minimal active RNP. The surprising extent of phylogenetic divergence in TERT and TER sequences has functional consequences for RNP assembly and activity, with combinations of even closely related TERTs and TERs often reconstituting enzymes with distinct profiles of product synthesis *in vitro*. Whether this variation reflects evolutionary drive or drift, it underlies dramatic cross-species differences in telomerase repeat synthesis fidelity and processivity [4].

Biologically active telomerase holoenzymes harbor numerous additional proteins (Figure 1), as reviewed in depth elsewhere [1,5–7]. One universal requirement for telomerase holoenzyme subunits is to promote RNP assembly and accumulation *in vivo*. Another shared function for telomerase holoenzyme subunits and holoenzyme-interacting factors is in the coordination of single-stranded DNA handling. This perspective aims to integrate recent insights into a working model to describe how the telomerase active site, the network of TERT and TER domain interactions, and additional DNA binding subunits create a machine for single-stranded repeat synthesis.

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Template copying in the active site

Telomerase differs from other polymerases in its use of a single-stranded template to generate single-stranded product. Also, in addition to the nucleotide addition processivity of copying across the template, telomerase shows repeat addition processivity (RAP) accomplished by retaining hold of the template-dissociated product while the template repositions and reestablishes an elongation-competent hybrid (Figure 2). It is proposed that some telomerase enzymes recruit a helicase to enhance the displacement of substrate and/or product from the template [8,9]. However, for most telomerases, the thermodynamically challenging activity of single-stranded product synthesis is an inherent enzyme property.

RNA is the optimal template for telomerase, which is logical considering both its biological function and its evolutionary relationship with other RTs [10]. RNA oligonucleotides physically separate from full-length TER can be positioned in the active site and copied [11]. However, a DNA template ligated into full-length TER supported only weak primer extension activity with limited nucleotide addition processivity [12]. A template-independent single-nucleotide terminal transferase activity of TERT has also been detected, but only at high manganese concentration *in vitro* [13]. Like the preference for RNA as template, telomerase has an expected preference for DNA as primer [14]. A primer with an RNA 3' end can be efficiently elongated, suggesting that the template hybrid can be RNA:DNA or RNA:RNA duplex. Replacement of more 5' regions of primer with RNA results in substrates with high affinity of association but inefficient and non-processive elongation. Finally, telomerase also shows substantial selectivity for dNTP rather than NTP incorporation [14]. The mechanism for nucleotide discrimination by the telomerase active site is the same as in other RT enzymes, involving an active-site side chain that introduces steric clash with a ribonucleotide 2' hydroxyl group [15]. For TERT to accomplish processive synthesis of long double-stranded RNA [16], the active site would have to subvert the structural determinants that adapt it to its cellular task of short, single-stranded DNA repeat synthesis.

Evolution has specialized TERT for use of a template within TER. Much of the specificity of template use depends on TERT-TER interactions beyond the active site, which constrain the positioning of the intended template region [1]. TERT-TER interactions and TER secondary structure enforce a template 5' boundary by limiting the length of template that can be pulled into the active site. Unlike the template 5' boundary, principles that determine the template 3' boundary are not well defined. At least for some telomerases, a default template position in the active site is evident from the sequence added to substrates that lack template complementarity [17]. Use of mid-template positions requires formation of template-substrate hybrid. Because telomeric repeats can have up to 4 consecutive guanines, up to 4 bp of template hybrid would be required to discriminate the correct positioning of a permuted-repeat substrate 3' end with the template. Curiously, the maximum hybrid length appears to be only 7 bp for the *Saccharomyces cerevisiae* enzyme [18], despite the copying of a template more than twice that length.

It seems likely that the active site of different telomerase enzymes limits hybrid length to 4–7 bp, forcing hybrid in the template 3' region to denature before the template 5' region can enter the active site (Figure 2). This has an obvious advantage for the ultimate release of a single-stranded product, but it would require compensating interactions that stabilize duplex melting. Loss of RNA-protein interactions that favor unpairing of the 3' template region could account for the exaggerated mid-template pause induced by substitutions of a ciliate-conserved TER motif 3' of the template [19]. Sequence-specific recognition of the template itself may play a role, based on mid-template pauses in synthesis induced by template sequence substitutions [20]. From the protein perspective, TERT-specific motifs are

excellent candidates for contributing telomerase-specific innovations of hybrid dynamics [21,22]. Also, template and product trapping within the larger TERT and TER domain network (described in the next section) could constrain the distension and rotation of template and product.

Beyond the active site: a network of telomerase-specific domain interactions

TERTs include up to 5 regions designated as domains [1], not all of which are necessarily autonomous in their folding. All of these regions are present in the relatively well-characterized human protein (Figure 3), which encompasses an N-terminal domain (TEN), a vertebrate-expanded linker, the high-affinity TERT RNA binding domain (TRBD), the RT domain with the active site, and the C-terminal extension (CTE). An intramolecular interaction brings together the TRBD and CTE of the physiologically folded TERT monomer [23–26]. As a consequence of TRBD-CTE interaction, the active site of the RT domain is constrained to occupy a surface cavity of the ring-like TRBD-RT-CTE TERT core [24,25].

Two conserved TER domains required for enzyme activity both associate with the TRBD (Figure 3). *In vivo*, the two TER sites of TRBD interaction are constrained prior to TERT assembly by holoenzyme RNP biogenesis proteins that fold and orient the intervening region of TER [26–29]. The network of protein-RNA and protein-protein domain interactions made by the TERT core creates an active RNP capable of complete single-repeat synthesis [26]. Association of the TEN domain with the TERT core RNP converts single-repeat synthesis to high-RAP activity by trapping substrate and product within the RNP domain architecture (Figure 3).

Unlike TERT interaction with TER, TERT interaction with DNA does not rely on a protein domain for high-affinity nucleic acid interaction. Instead, other telomerase holoenzyme subunits provide the telomeric DNA binding affinity and specificity necessary for telomerase recruitment to its telomere substrates *in vivo*, as discussed in the following section. Nonetheless, TERT and TER alone do support some RAP, implying mechanism(s) of single-stranded DNA retention in the RNP catalytic core. As one possible mechanism, TERT surfaces that contact DNA may channel the product released from the template hybrid, functioning as a sliding clamp or cleft for single-stranded DNA. This clamp or cleft could be prearranged, or it could form stably only after chromosome 3' end engagement in the active site.

Principles of engagement: telomeric DNA association, elongation, and release

Telomerase holoenzymes from ciliate, yeast, and vertebrate cells include numerous proteins essential for telomere maintenance but dispensable for RNP assembly *in vivo* (Figure 1). The roles of these proteins have been difficult to study due to recombinant expression challenges, with likely requirements for chaperones and other transiently acting regulators. Recent reconstitution of the *Tetrahymena* telomerase holoenzyme provides insight into how holoenzyme subunits negotiate the handling of single-stranded DNA [30,31]. The RNP core of *Tetrahymena* telomerase (p65-TER-TERT) is linked through a three-protein telomere adaptor subcomplex (TASC) to a high-affinity telomeric-repeat DNA binding subunit (Teb1), which together create a holoenzyme (Figure 1) with dramatically increased RAP compared to the recombinant catalytic core.

Surprisingly, high RAP activity by *Tetrahymena* telomerase requires only the Teb1 C-terminal domain without autonomously detectable DNA binding affinity [31]. In elongation mode (Figure 4, right), single-stranded DNA could slide across low-affinity contact surfaces within the active core RNP and TASC while maintaining a fixed register of repeat association with high-affinity Teb1 DNA-binding domains. Teb1 OB-fold domains A and B have a permutation-dependent telomeric-repeat binding affinity that suggests a requirement for a specific register of repeat association [31]. The ability of Teb1 A and B domains to suppress nascent product folding and their fast off-rate from DNA suggest that these domains thread nascent product rather than loop the DNA expelled from the catalytic core [30,31]. Future studies will be required to define the principles of single-stranded DNA handling during processive repeat synthesis, extending beyond analysis of mutagenesis-induced changes in product profile to direct physical mapping of the entire path of DNA transiting the holoenzyme.

Initial recruitment to and subsequent elongation of a chromosome end present different challenges to a telomerase complex. In most eukaryotes, the selectivity and efficiency of telomerase association with its biological substrates would benefit from mechanisms that increase the effective concentration of active enzyme near chromosome ends. By stable physical association with the telomere, telomerase would then be poised to capture a transiently accessible, single-stranded chromosome terminus. Conversion of the recruitment to elongation architecture may regulated by changes in the association and/or conformation of DNA binding domains with the RNP core (Figure 4).

Human TPP1 may play the role of *Tetrahymena* TASC as a bridge between the telomerase catalytic core and high-affinity telomeric-repeat DNA binding proteins (Figure 1). Direct physical association of TPP1 and active human telomerase has been suggested based on copurification [32]. Recent evidence pinpoints TPP1 as an essential bridge between human or mouse TERT and its telomere substrates [33,34]. *In vivo* this role of TPP1 requires its association with the TIN2 subunit of double-stranded telomeric-repeat binding complexes but not the single-stranded telomeric-repeat DNA binding protein POT1 [34]. On the other hand, current *in vitro* assays of DNA handling by human telomerase suggest a synergy of POT1 and TPP1 activities [35–37]. Future studies will benefit from the integration of *in vivo* and *in vitro* assays to explore in greater detail how telomere binding proteins promote telomerase-DNA association.

Conclusions and future directions

Telomerase-specific features of the active site and the active RNP domain network confer specialization for recycling the internal template and releasing product DNA in single-stranded form. Despite the conservation of TER motifs critical for catalytic activity, little is known about how these motifs function at a mechanistic level beyond their contributions to RNP assembly [1]. Defining RNA roles has been complicated by the evolutionary divergence in TER structure and holoenzyme catalytic activity, but it will be important for understanding telomerase evolution as an RNP enzyme.

The principles of single-stranded DNA recognition and handling will also be fascinating to dissect, enabled by recent progress in molecular-level definition of the telomerase-telomere connection. Single-molecule approaches have great promise for correlating enzyme dynamics with DNA synthesis [38], hopefully informed by snapshots of active RNP structure at high resolution. Another open question is whether the enzyme-product complex requires active remodeling to disengage from the telomere, potentially through the activity of chaperones [39].

It remains an important future goal to exploit advances in understanding telomerase structure and mechanism for clinical applications. Several recent reviews provide background and perspective on the role of telomerase and telomere length regulation in aging and cancer [40,41]. The unique features of the telomerase active site, RNP architecture, and biological regulation should increase the range of opportunities beyond the current strategies for telomerase-based therapeutics [42].

Highlights

- The telomerase catalytic cycle requires dynamic nucleic acid handling
- TERT and TER domain interactions trap DNA within the active RNP
- Telomerase holoenzyme subunits mediate telomere recruitment and elongation

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Figure 1. Telomerase holoenzyme subunits and holoenzyme-interacting proteins

In addition to TERT and TER, numerous holoenzyme subunits and holoenzyme-interacting proteins play key biological roles. Despite different molecular identities, holoenzymes from the three major model systems of telomerase characterization have subunits that promote RNP assembly and concentration within the nucleus (green and yellow). Telomerase- and telomere-associated proteins that have high affinity and sequence specificity for telomeric-repeat DNA (dark orange) or that bridge the DNA binding subunit(s) to TERT with potentially lower affinity DNA contacts (light orange) are crucial determinants of telomere elongation in cells. *Saccharomyces* enzyme is *S. cerevisiae*; note that Est1 and Ku may be

missing from the holoenzyme or not associated with TER in other yeasts [1]. A dashed line suggests an opportunity for additional factors or regulations to affect subunit interactions.

Figure 2. Nucleic acid mobility during the telomerase catalytic cycle

As illustrated for *Tetrahymena* telomerase, the TER template region (red) must move relative to the TERT RT domain (blue) including the active site (shown in yellow in the illustration at top and also in stages of the catalytic cycle with nucleic acid engaged in the active site). The template hybrid should reach a length of at least 4 bp for accurate synthesis of T₂G₄ repeats, but synthesis beyond mid-template is likely to require denaturation of the template 3' end due to a length limit on hybrid accumulation. At the 5' boundary of template that can be pulled into the active site, synthesis halts until product dissociates completely from the template. This could follow a slow step of hybrid release from the active site, or the product 3' end could remain in the active site while only template is released. Template repositioning would be accompanied by either product dissociation or reformation of hybrid at the template 3' end. Template-proximal DNA interactions are illustrated to slide independent of the telomeric repeat register, although a fixed register of single-stranded DNA interaction is possible as well.

Figure 3. TERT and TER domain architecture within an active RNP

In this model for human TER and TERT domain architecture, paired stem (P) and adjacent single-stranded regions of TER that mediate TRBD binding are shown in thick outlining. Other regions of TER dispensable for activity *in vitro* but important for RNP biogenesis *in vivo* are not drawn in full (thin outlining). Interaction of the TRBD and CTE organizes the TERT core, which is sufficient for single-repeat synthesis (left), while the vertebrate-expanded linker region (no fill in the TERT domain schematic) and TEN domain are involved in stable DNA capture (right). DNA substrate is shown as a dashed line. The TER template/pseudoknot region (including the template, P2, and P3) influences TEN domain positioning and may contact the TEN domain directly. Trapping of an elongation-competent

DNA substrate within the active RNP is stabilized by single-stranded DNA contact(s) with the TEN domain and/or other protein surfaces.

Figure 4. Telomerase recruitment and elongation of telomere substrates

Numerous contact surfaces for DNA are contained within holoenzyme subunits and their individual domains, as shown for *Tetrahymena* telomerase. Initial telomere recruitment should involve high-affinity telomeric-repeat DNA binding activity provided by Teb1 OB-fold domains A and B (dark shading). During elongation, single-stranded DNA could slide across additional lower-affinity contact surfaces in Teb1C and possibly TASC (lighter shading). Transition between the recruitment and elongation conformations could be triggered by capture of a chromosome 3' terminus in the active site.