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A possible mechanism of processive nucleotide and repeat additions by the telomerase

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ABSTRACT

Telomerase is a specialized cellular ribonucleoprotein complex that can synthesize long stretches of a DNA primer by using an intrinsic RNA template sequence. This requires that the telomerase must be able to carry out both nucleotide and repeat additions. Here, based on available structures and experimental data, a model is presented to describe these two addition activities. In the model, the forward movement of the polymerase active site along the template during the processive nucleotide addition is rectified through the incorporation of a matched base, via the Brownian ratchet mechanism. The unpairing of the DNA:RNA hybrid and then repositioning of product 3′-end after each round of repeat synthesis, which are prerequisites for the processive repeat addition, are caused by a force acting on the primer. The force results from the conformational transition of the stem III pseudoknot, which is mechanically induced by the rotation of TERT fingers together with stem IV loop towards the polymerase active site upon a nucleotide binding. Based on the model, the dynamics of processive nucleotide and repeat additions by recombinant *Tetrahymena* telomerase is studied analytically, which gives good quantitative explanations to the previous experimental results. Moreover, some predicted results are presented. In particular, it is shown that the repeat addition processivity is mainly determined by the difference between the free-energy change required to disrupt the DNA:RNA hybrid and that required to unfold the stem III pseudoknot. A large difference in free energy corresponds to a low repeat addition processivity while a small difference in free energy corresponds to a high repeat addition processivity.

1. Introduction

Telomerase is a specialized cellular ribonucleoprotein (RNP) complex that uses an intrinsic RNA to template the de novo synthesis of telomeres onto the 3′ ends of linear chromosomes (Greider and Blackburn, 1985; Cech, 2004; Collins, 2006; Lue, 2004; Harrington, 2003). The telomerase activity was shown first in the ciliate *Tetrahymena thermophila* (Greider and Blackburn, 1989) and subsequently in many other eukaryotes (Shippen-Lentz and Blackburn, 1990; Romero and Blackburn, 1991; Lingner et al., 1994; McCormick-Graham and Romero, 1995, 1996; Singer and Gottschling, 1994; McEachern and Blackburn, 1995; Blasco et al., 1995; Chen et al., 2000; Feng et al., 1995). Telomerase RNP functions as a multisubunit holoenzyme that contains an RNA component (telomerase RNA), a catalytic protein component telomerase reverse transcriptase (TERT) and other associated proteins (Witkin and Collins, 2004). *Tetrahymena* telomerase RNA is a 159 nucleotide transcript, which contains the sequence 3′-AACCCCAAC-5′ that serves as a template for synthesis of the telomeric repeat TTGGGG (Greider and Blackburn, 1989). Immediately located 5′ of the template is a template boundary element (TBE) and 3′ of the template is a template recognition element (TRE) (Lai et al., 2002; Miller and Collins, 2002). In addition to these template-adjacent elements, pseudoknot III and stem-loop IV have also been shown to play important roles in telomerase function (Lai et al., 2002, 2003; Mason et al., 2003; Sperger and Cech, 2001). The TERT protein contains polymerase active site motifs shared among all reverse transcriptases (Lingner et al., 1997) as well as unique N- and C-terminal extensions that harbor both phylogenetically conserved and variable motifs (Kelleher et al., 2002). In addition, it has been shown that the TERT protein alone provide the anchor site for the telomerase that interacts with the primer (Greider, 1991; Morin, 1991; Hammond et al., 1997). A recently solved structure of the *Tribolium castaneum* telomerase catalytic subunit TERT (without telomerase RNA) revealed that it consists of a RNA-binding domain (TRBD) (N-terminal extension), a putative thumb domain (C-terminal extension) and a reverse transcriptase domain that includes a palm subdomain and a finger subdomain that is simply called “fingers” (Gillis et al., 2008). The reverse transcriptase domain is most similar to those of HIV reverse transcriptase, DNA polymerase (DNAP) and RNA polymerase (RNAP). Of the accessory proteins, p65 has been shown to form a complex with telomerase

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RNA, playing the role in assembly of telomerase RNP (Prathapam et al., 2005; O'Connor and Collins, 2006; Stone et al., 2007).

Compared to other polymerase enzymes such as DNAP, retroviral reverse transcriptase and RNAP, the most peculiar feature of the telomerase is its ability to synthesize long stretches of a primer by using the short template sequence within telomerase RNA (Lue, 2004; Greider and Blackburn, 1989; Greider, 1991; Morin, 1989). This requires that the telomerase must be able to carry out two types of translocation events: (i) simultaneous translocation of DNA:RNA hybrid relative to the polymerase active site or translocation of the polymerase active site along the template after each nucleotide incorporation (nucleotide addition processivity) and (ii) the disruption of the DNA:RNA hybrid and then repositioning of the product at the 3′ end to the beginning of the template after each round of repeat synthesis (repeat addition processivity). It was shown that the recombinant telomerases that are composed of only the two subunits telomerase RNA and TERT suffice to give the telomerase activity (Weinrich et al., 1997; Harrington et al., 1997), but the recombinant enzyme demonstrates a more limited repeat addition processivity than the endogenous one (Collins and Gandhi, 1998).

For the nucleotide addition processivity, the following issues are still unclear. (1) How does the polymerase active site make a forward translocation by one base along the template after a correct nucleotide incorporation? (2) Why does the nucleotide addition become stalled after a misincorporation? For the repeat addition processivity, it has been proposed that distortion or conformational transition of the pseudoknot III may provide the energy necessary for the unpairing of the DNA:RNA duplex and then repositioning of product 3′-end (Lai et al., 2003; Tzfati et al., 2003). However, two related and important issues are not clear. (3) How does the energy that is provided by the distortion or conformational transition of the pseudoknot III induce the unpairing of the DNA:RNA duplex and then repositioning of the product at the 3′-end? (4) Why only when all of the bases on the template are paired with the primer to form the DNA:RNA duplex can the distortion or conformational transition of the pseudoknot III induce the unpairing of the DNA:RNA duplex and then repositioning of the product at the 3′-end? Moreover, it has been observed that, for some recombinant telomerases, optimal repeat addition processivity requires high dGTP concentration, a much higher dGTP concentration than required for processive nucleotide addition within a repeat, and the repeat addition processivity retains the same dGTP-dependence for different alterations of the template sequence (Hardy et al., 2001; Hammond and Cech, 1997; Maine et al., 1999). To explain this phenomenon, it has been proposed that dGTP either promotes the interaction of nascent product with enzyme or promotes a subsequent conformational change required to reposition the product at the 3′-end (Hardy et al., 2001). However, how dGTP promotes either of the two effects is not clear. Alternatively, (5) how is the repeat addition processivity stimulated allosterically by dGTP?

The purpose of this paper is to present a model capable of explaining quantitatively all these important but still elusive issues on the nucleotide addition and repeat addition processivities by recombinant telomerase. The model is based on available biochemical results and structural studies of TERT and telomerase RNA. In the model, during the processive nucleotide addition the forward translocation of the polymerase active site along the template is rectified through the incorporation of a matched base, via the Brownian ratchet mechanism, as proposed previously for DNAP (Xie, 2007) and RNAP (Xie, 2008). After each round of repeat synthesis, the unpairing of the DNA:RNA hybrid and then repositioning of the product at the 3′-end on the template are caused by a force acting on the primer. The force results from the unfolding of stem III pseudoknot that is automatically induced by the rotation of the fingers together with the stem IV loop towards the nucleotide-bound polymerase active site. The energy that is generated by the distortion or conformational transition of the pseudoknot III is transferred into the force acting on the primer. Before the incorporation of the nucleotide paired with the last base on the template the force mainly acts on the template rather than on the primer and, thus, the DNA:RNA hybrid cannot be disrupted. Moreover, the model also gives a reasonable explanation to the last issue that, for a high repeat addition processivity, a much higher dGTP concentration is required than for processive nucleotide addition within a repeat.

2. Model

Before presenting our model for the processive nucleotide and repeat additions by recombinant telomerase, based on available structures and experimental data we present three basic hypotheses.

2.1. Three hypotheses

2.1.1. Hypothesis I

In the reverse transcriptase domain of TERT, there exists an ssRNA-binding site A adjacent to the polymerase active site. The binding site A has a high affinity for the unpaired base (*or the sugar-phosphate backbone of the unpaired base*) *on the template in the RNA component*.

This hypothesis is supported by the co-immunoprecipitation assay showing that the template region of *Tetrahymena* telomerase is also important for optimal TERT binding besides other regions such as TBE element, TRE element and stem IV loop in the RNA component (Lai et al., 2003). From the available structure of the *T. castaneum* telomerase catalytic subunit TERT it was implicated that the polymerase active site is located on the palm subdomain and adjacent to the finger subdomain (Gillis et al., 2008). Thus, the ssRNA-binding site *A*, which is adjacent to the polymerase active site, should be also located in the reverse transcriptase domain. Note that this hypothesis is similar to that adopted in other polymerase enzymes. For example, in DNAP, retroviral reverse transcriptase and RNAP, it was also hypothesized that there exists ssDNA-binding site in the vicinity of the polymerase active site that has a high affinity for the unpaired base (or the sugar-phosphate backbone of the unpaired base) on template DNA (Xie, 2007, 2008).

In DNAP and retroviral reverse transcriptase (Xie, 2007) and RNAP (Xie, 2008), the strong binding of site *A* to ssDNA can be used to explain the induced-fit mechanism. Similarly, we have the following anticipation for the telomerase. The strong interaction of the site *A* with an unpaired base on the template RNA induces the conformational change in residues in the vicinity of the polymerase active site that is adjacent to the site *A*. This unpaired-base-related conformational change thus results in the polymerase active site having a much higher affinity for the structurally compatible nucleotide than structurally incompatible nucleotides.

2.1.2. Hypothesis II

It was structurally determined that, in bacteriophage T7 DNAP (Doublie et al., 1998; Doublie and Ellenberger, 1998) and HIV reverse transcriptase (Huang et al., 1998), the nucleotide binding to (releasing from) the polymerase active site induces the rotation of the finger subdomain from open (closed) conformation to closed (open) conformation, with the nucleotide being able to bind to the polymerase active site in the open-finger conformation while the closed-finger conformation activating the chemical reaction of nucleotide incorporation. In single-subunit phage T7 RNAP, the nucleotide binding (releasing) induces the rotation of an α helix, termed the O helix, from open (closed) conformation to closed (open) conformation, with the nucleotide being able to bind