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An Interlocked Dimer of the Protelomerase TelK Distorts DNA Structure for the Formation of Hairpin Telomeres

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SUMMARY

The termini of linear chromosomes are protected by specialized DNA structures known as telomeres that also facilitate the complete replication of DNA ends. The simplest type of telomere is a covalently closed DNA hairpin structure found in linear chromosomes of prokaryotes and viruses. Bidirectional replication of a chromosome with hairpin telomeres produces a catenated circular dimer that is subsequently resolved into unit-length chromosomes by a dedicated DNA cleavage-rejoining enzyme known as a hairpin telomere resolvase (protelomerase). Here we report a crystal structure of the protelomerase TelK from *Klebsiella oxytoca* phage ϕKO2, in complex with the palindromic target DNA. The structure shows the TelK dimer destabilizes base pairing interactions to promote the refolding of cleaved DNA ends into two hairpin ends. We propose that the hairpinning reaction is made effectively irreversible by a unique protein-induced distortion of the DNA substrate that prevents religation of the cleaved DNA substrate.

INTRODUCTION

The duplication of chromosome ends during replication is essential for genomic stability and, consequently, specialized replicative processes have evolved for this purpose. The termini of linear chromosomes pose challenges during replication because incomplete synthesis of DNA 5′ ends by the lagging strand of the replication fork could lead to progressive shortening of a chromosome over generations (Olovnikov, 1973; Watson, 1972). Also, the exposed ends of double-stranded DNA are substrates for aberrant recombination or repair, leading to chromosomal rearrangements and fusions (McClintock, 1941; Müller, 1938).

Several effective strategies have evolved to overcome the chromosome end replication problem. Bacterial and archaeal species with circular genomes have eliminated issues of exposed DNA ends, but instead must resolve interlinked (concatenated) chromosomes following replication by the action of topoisomerases and site-specific recombinases. The linear chromosomes of eukaryotes have guanine-rich repetitive DNA sequences near the ends (Szostak and Blackburn, 1982) that bind to protective proteins and adopt a compact structure that is resistant to degradation. GT-rich terminal sequences with a 3′ overhang can fold into structures such as the T loop (Griffith et al., 1999) and possibly the G quadruplex (Parkinson et al., 2002; Smith and Feigon, 1992) that together with site-specific binding proteins form specialized nucleoprotein complexes constituting telomeres (Baumann and Cech, 2001; Bilaud et al., 1997; Cooper et al., 1997; Smith and de Lange, 1997). The telomere provides physical protection of the DNA ends (van Steensel et al., 1998) while permitting extension of the telomeric sequence by the activity of a specialized reverse transcriptase to prevent chromosome shortening (Blackburn, 1991; Greider and Blackburn, 1985). Other types of terminal structures have also been identified in linear chromosomes. *Drosophila* telomeres are maintained by the insertion of a transposon sequence at chromosomal termini (Levis et al., 1993; Pardue and DeBaryshe, 1999). In *Streptomyces* (Bao and Cohen, 2001; Hirochika and Sakaguchi, 1982; Lin et al., 1993), adenoviruses (Rekosh et al., 1977), and bacteriophages such as ϕ29 (Mellado et al., 1980), DNA synthesis is primed by a protein that remains covalently attached to the 5′ ends of the genome and protects the DNA from degradation.

An elegantly simple solution to the chromosome end problem is widespread among bacteria and viruses, including the plant pathogen *Agrobacterium tumefaciens* (Goodner et al., 2001), eukaryotic brown algae viruses (Delaroque et al., 2001), linear plasmid prophage of several bacteriophages from *Vibrio*, *Yersinia*, *Klebsiella*, and *Escherichia coli* (Casjens et al., 2004; Hertwig et al., 2003; Lobocka et al., 1996; Oakey et al., 2002; Rybchin and Svarchevsky, 1999), and genus *Borrelia*, the

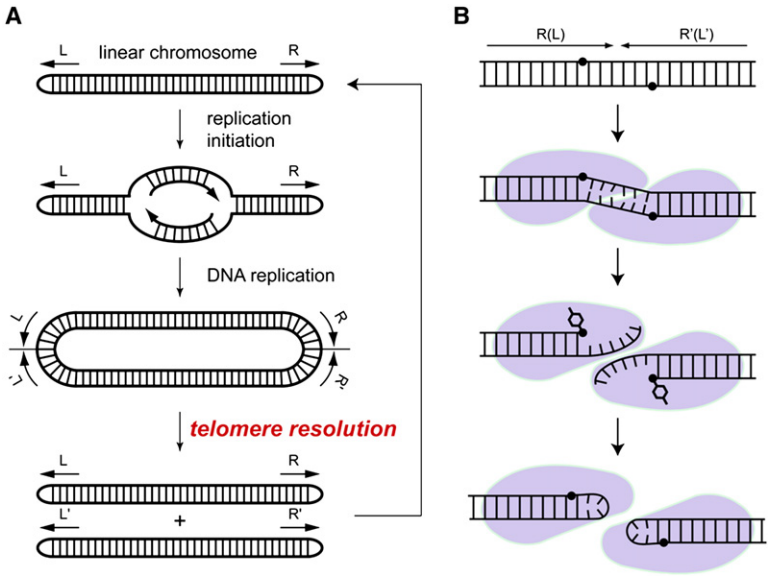

Figure 1. Protelomerase Resolves Replicated Hairpin Telomeres

(A) Replication of a linear chromosome with hairpin telomeres produces a dimeric circular intermediate that is resolved into unit-length chromosomes by the activity of protelomerase.

(B) A model for the hairpin formation reaction by the protelomerase TelK, proposed based on the crystal structure presented in this study.

causative agent of Lyme disease and relapsing fever (Casjens et al., 1997; Hinnebusch and Barbour, 1991; Hinnebusch et al., 1990). These organisms have linear chromosomes or replicons with covalently closed hairpin termini (telomeres). Here, bidirectional DNA replication starts from an internal origin (Picardeau et al., 1999; Ravin et al., 2003) and produces a circular dimer with inverted telomeric repeat sequences at the junctions between two copies of the replicated chromosome. This circular replication intermediate is then resolved into two unit-length linear chromosomes with covalently closed DNA hairpin ends by a specialized telomere resolvase/protelomerase (Casjens, 1999; Kobryn and Chaconas, 2001; Ravin et al., 2001) (Figure 1A). Y-shaped intermediates that have been observed in electron micrographs of these chromosomes result from telomere resolution at one end of the chromosome dimer before the replication fork reaches the other end (Ravin et al., 2003).

Protelomerases share limited sequence homology with the λ integrase family of site-specific recombinases (also known as tyrosine recombinases) and with type IB topoisomerases (Deneke et al., 2000; Kobryn and Chaconas, 2002; Rybchin and Svarchevsky, 1999). These enzymes have similar catalytic mechanisms for DNA cleavage and ligation, generating a 3′-phosphotyrosine DNA intermediate that enables the covalent rejoining of cleaved DNA strands without the use of a high-energy cofactor (Champoux, 1981; Craig and Nash, 1983; Huang et al., 2004; Pargellis et al., 1988). However, each family of enzymes performs a different DNA remodeling task using a monomer, dimer, or tetramer bound to one or two DNA molecules. The monomeric type IB topoisomerases cleave and rejoin a single strand of DNA to relax supercoils (Champoux, 2001). Protelomerases bind as dimers to a double-stranded DNA and generate a staggered cut that is converted into two DNA hairpin ends (Figures 1B and 2). Site-specific recombinases catalyze the pairwise exchange of four DNA strands in the context of a tetrameric recombinase bound to two recombining DNAs (Biswas et al., 2005; Chen and Rice, 2003; Van Duyne, 2001). Using a common reaction chemistry, these enzymes perform specialized DNA remodeling functions to achieve different biological outcomes.

DNA hairpins are generated following an endonucleolytic strand cleavage as transient reaction intermediates during the remodeling of immunoglobulin and T cell receptor genes, and during the transposition of some mobile genetic elements (van Gent et al., 1996). Enzymes catalyzing these reactions have specialized DNA-binding elements that stabilize a hairpin fold during the direct attack of a nicked 3′-OH end on a phosphodiester bond (Davies et al., 2000; Rice and Baker, 2001). Protelomerases are unique in converting a doubly nicked DNA duplex into two stable hairpins via a transient enzyme-DNA covalent intermediate. This reaction sequence suggests a different mechanism from that of DNA transposases that form a hairpin starting from DNA with one nicked strand.

A key question concerning the mechanism of DNA hairpin formation by protelomerases is how the canonical double-helical conformation of the DNA substrate is efficiently rearranged into a seemingly higher-energy (enthalpically unfavorable) hairpin structure. To better understand the mechanism of this unique DNA cleavage and rejoining reaction, we have determined crystal structures of the protelomerase TelK from *Klebsiella oxytoca* phage ϕKO2 (Casjens et al., 2004; Huang et al., 2004), an essential factor for the maintenance of a linear plasmid prophage episome during lysogenic growth of ϕKO2. Our crystallographic and biochemical data suggest that TelK breaks the duplex structure of DNA to allow the spontaneous folding of individual DNA strands into hairpins.