Telomerase and DNA End Replication: No Longer a Lagging Strand Problem?

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Telomerase, a ribonucleoprotein enzyme that extends DNA termini by virtue of its internal RNA template, is critical for the replication of chromosomal termini, or telomeres. Indeed, in the absence of telomerase, chromosomes shrink from the ends (*1*). Recently there have been a number of breakthroughs in telomerase research: the identification of the RNA subunits from yeast (*1*), mouse (*2*), and human (*2*) and of two protein subunits from *Tetrahymena* (*3*), the organism in which the enzyme was first discovered (*4*). All this progress makes it a good time to reconsider exactly how and when telomerase acts during chromosome replication.

The problem solved by telomerase is commonly described as the inability of conventional DNA polymerases to complete the replication of the lagging (discontinuous) strand, the one for which DNA Okazaki fragments are synthesized starting with RNA primers. Here we argue that the real problem instead lies in the inability of DNA polymerase to complete synthesis of the leading strand, a conclusion that reveals a new dilemma concerning the mechanism of telomerase action.

DNA polymerases replicate only in the 5′ to 3′ direction; they can only extend existing polynucleotide chains (RNA or DNA); and they need a complementary strand to provide a template. Therefore, conventional DNA polymerases cannot synthesize the extreme 5′ ends of a blunt end DNA molecule: Even if an RNA primer were paired with the extreme 3′ end of its DNA template, this last RNA primer would soon be removed, giving rise to daughter molecules with a 5′-terminal gap. Thus, the "end-replication problem" was early on considered to be a problem of incomplete lagging strand synthesis due to RNA primer removal (*5*, *6*).

However, the structures of telomeres determined so far have revealed that they are not blunt ended but instead have a single-stranded 3′ overhang of 12 nucleotides or more. This was first demonstrated for the macronuclear DNA molecules of the hypotrichous ciliates *Oxytricha*, *Stylonychia*, and *Euplotes* (*7*) and later for the linear extrachromosomal ribosomal DNAs of the holotrichous ciliate *Tetrahymena* and the evolutionarily distant slime mold *Didymium* (*8*). Furthermore, *Saccharomyces cerevisiae* chromosomal telomeres acquire single-stranded 3′ overhangs late in S phase (*9*), suggesting that 3′ overhangs are a general feature of eukaryotic chromosomes.

How does the view of DNA end replication change if telomeres are not blunt but instead are maintained as 3′ overhangs? As illustrated in Figs. 1 and 2, lagging strand synthesis and RNA primer removal are not necessarily problems for the conventional DNA replication machinery. As long as the RNA primer (typically 8 to 14 nucleotides) is laid down on the 3′ overhang only, no information will be lost upon DNA replication followed by RNA primer removal. However, the leading strand will lose its 3′ overhang upon replication. If the 3′ overhang is not reestablished, then there would be a lagging strand problem in the next round of replication. As shown in Fig. 1, overextension of the parental telomeres would not solve the leading strand problem of telomere replication.

We envisage two ways in which telomerase could reestablish the 3′ overhang, thereby ensuring that the lagging strand problem never arises. In the first model, telomerase would act after semiconservative DNA replication (Fig. 2, model I). The freshly replicated leading strand would be extended by telomerase, reestablishing a 3′ overhang. This model is in agreement with that proposed by Zahler and Prescott (*10*) for the replication of *Oxytricha* telomeres.

In the second model (Fig. 2, model II), telomerase would act before telomere replication by extending the 3′ overhang. This would provide a template for gap-filling synthesis on the complementary strand, giving rise to overextended parental telo-

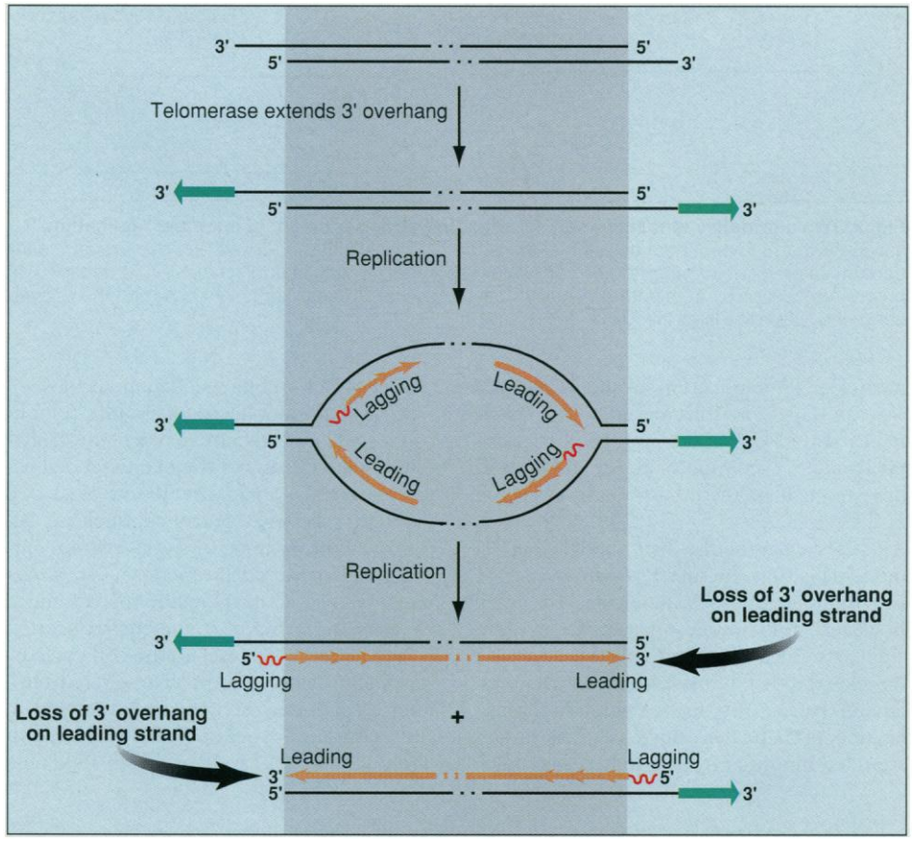

Fig. 1. The leading strand problem of telomere replication. Overextension of parental telomeres by telomerase followed by semiconservative DNA replication does not regenerate a 3′ overhang on the leading strand. Sequence added by telomerase is shown by the thick arrow (green). RNA primers are indicated by wavy lines (red). Parental DNA is indicated by thin lines (black) and replicated DNA by thicker lines (orange). Arrows indicate the polarity of DNA 5′ to 3′.

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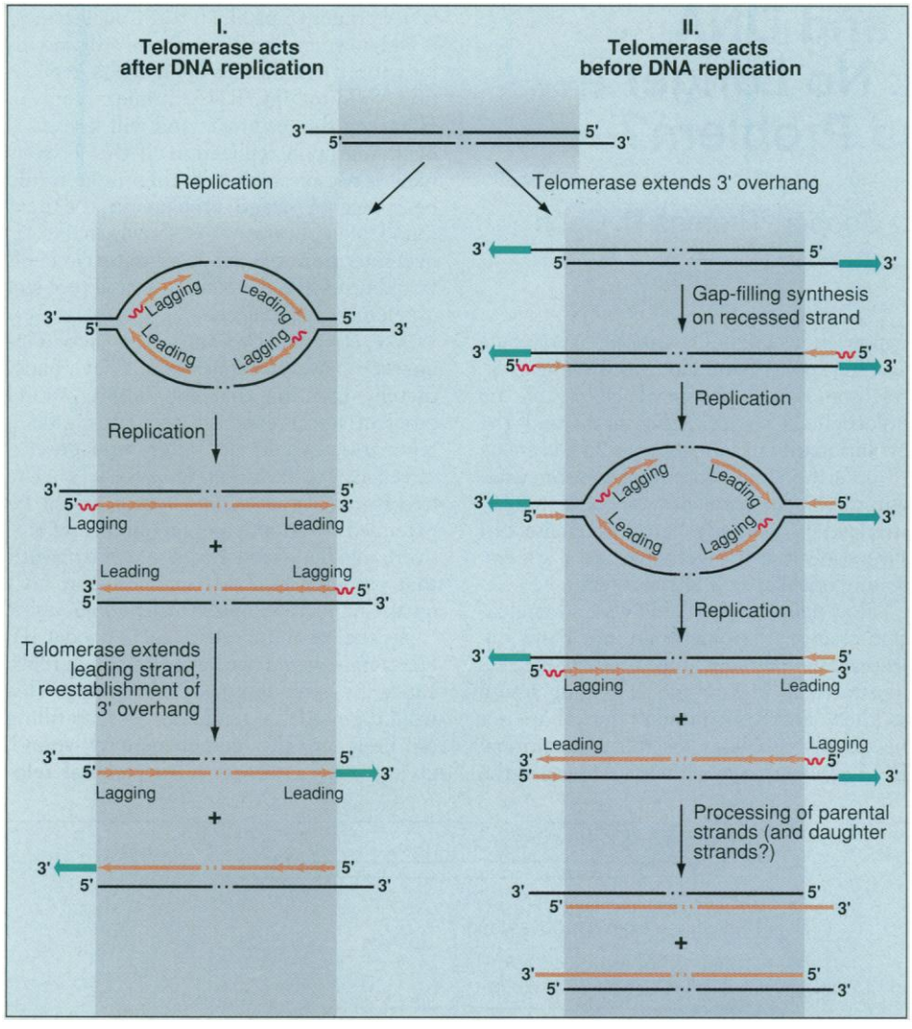

Fig. 2. Two alternative models to solve the leading strand problem of telomere replication. It is assumed that 3′ overhangs are maintained at both chromosomal ends, as has been most clearly demonstrated for macronuclear DNA of ciliates. Note that lagging strand synthesis followed by RNA primer removal gives complete replication as long as the RNA primer is laid down on the 3′ overhang only. Labeling is as in Fig. 1.

meres. DNA replication would then give rise to daughter chromosomes that each have extra DNA at both ends on the parental strands. Trimming of these ends would have to occur to reestablish the original size of the telomeres.

The caveat of the first model (Fig. 2, model I) is that the substrate for telomerase is a blunt-end DNA molecule. In vitro, however, telomerase appears to require single-stranded ends and will not extend a double-stranded blunt-end molecule (*11*). Therefore, the first model predicts the existence of helicases, nucleases, or single-stranded binding proteins that permit telomerase to act on blunt-ended molecules.

The second model invokes more complicated and unprecedented biochemical machinery. Processing of the overextended parental strands would involve at least two distinct, precisely controlled nuclease activities. Furthermore, two observations argue against the second model. First, as depicted in Fig. 2, all telomeric repeats added by telomerase before telomere replication would be removed later in the cell cycle by processing. However, in vivo–altered telomeric sequences are stably incorporated in cells containing mutant telomerases with altered templates (*1*, *12*). This model could still hold if we envision that telomeres are much more dynamic structures than depicted and that telomeric repeats are removed and added throughout the cell cycle or shuffled by recombination. More strongly arguing against the second model is the conclusion of Wellinger *et al.* (*13*) that long 3′-overhanging telomeric repeats are added after the replication fork reaches the end of a linear plasmid. Assuming that the overhangs are added by telomerase, this observation suggests that telomerase acts on the replicated daughter telomeres rather than on parental telomeres, favoring the first model.

In summary, if eukaryotic chromosomes are to maintain a 3′-terminal extension, their replication problem would appear to be the inability of leading strand DNA synthesis to produce the 3′ overhang, not a problem with the lagging strand or with the gap left by removal of an RNA primer. Biochemical fractionation and yeast genetics should make it possible to test specific models for chromosome end replication and to identify additional components involved in this essential process.

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