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Review

Mechanism and physiological significance of programmed replication termination

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ABSTRACT

Replication forks in both prokaryotic and eukaryotic systems pause at random sites due to depletion of dNTP pools, DNA damage, tight binding nonhistone proteins or unusual DNA sequences and/or structures, in a mostly non-polar fashion. However, there is also physiologically programmed replication termination at sequence-specific authentic replication termini. Here, the structure and functions of programmed replication termini, their mechanism of action and their diverse physiological functions in prokaryotes and eukaryotes have been reviewed.

Contents

1. Introduction

The replicon hypothesis introduced the concepts of a unit of replication consisting of a *cis*-acting replication origin (*ori*) and a *trans*-acting initiator protein which interacted with the *ori* to control replication initiation. However, the model did not take into consideration whether there might also be defined replication termini (*Ter*) at which replication was completed [1]. The prevailing notion at the time was that replication stopped wherever the two divergent forks, initiated from a fixed *ori*, met each other on the circular chromosome. While bacterial and certain plasmid chromosomes (e.g. of R6K) contain sequence-specific replication termini, some other plasmids (e.g. ColE1) and all phage chromosomes, that have been studied so far, do not seem to have specific *Ter* sites (see reviews in [2–7]).

DNA replication forks can stall randomly because of various reasons such as depletion of the dNTP pool, DNA damage, at barriers formed by certain DNA sequences or strong DNA binding nonhistone proteins, etc. [8,9]. Forks are also arrested at physiologically programmed authentic replication termini, usually in a polar mode, to facilitate certain DNA transactions. This latter class is the subject of this review. The first part of this review is focused on prokaryotic replication termination (of *Escherichia coli*, *Bacillus subtilis* and the plasmid R6K). It discusses the *Ter* sequences, crystal structures

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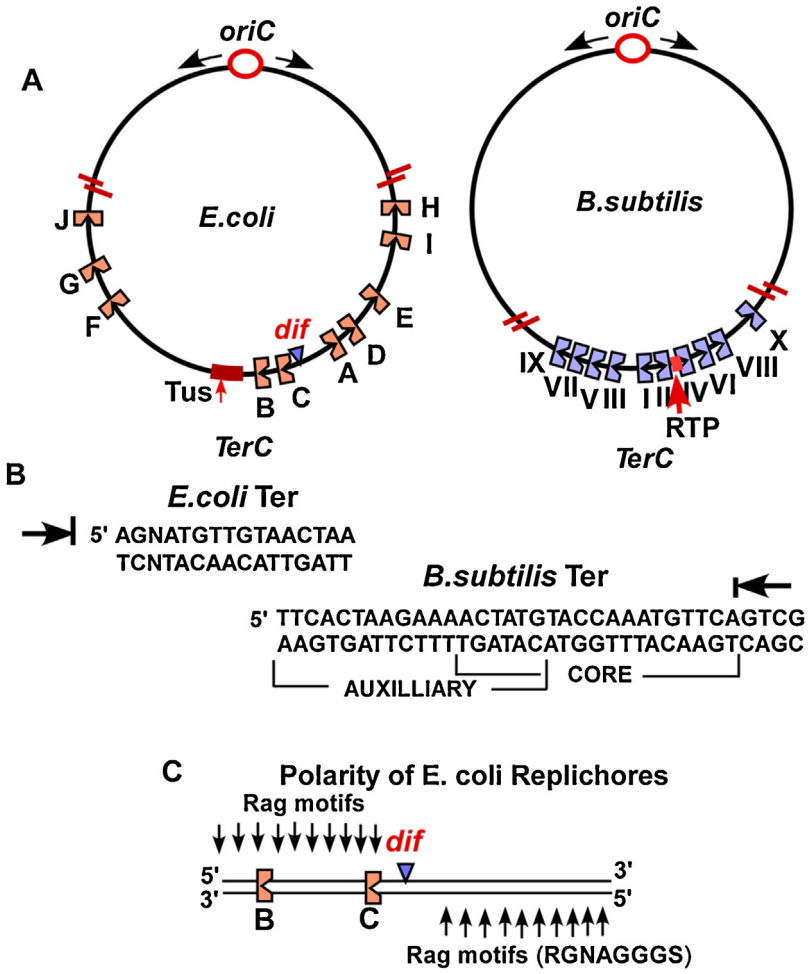

Fig. 1. The *Ter*C regions of *E. coli* and *B. subtilis* and the terminator sequences. (A) The relative locations of the *Ter* sites in the two *Ter*C regions are shown including the regions encoding the terminator proteins Tus and RTP; the *Ter*C regions are expanded and not drawn to scale; (B) the rag sequence motifs and the polarized replicores in the *Ter*C region of *E. coli*; (C) the sequences of the *Ter* sites of *E. coli* and *B. subtilis* with the arrows showing the ends that arrest forks; the Ter site of *B. subtilis* shows the overlapping core and auxiliary sites.

of the corresponding terminator proteins, their structure–function analysis, a critical evaluation of the models of polar fork arrest and its physiological significance. The second part discusses similar points mostly from two model eukaryotic systems namely *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, along with brief references to fork arrest in mammalian and other systems.

2. Replication termination in prokaryotes

2.1. *E. coli* and plasmid Ter systems

The first clear evidence for a site specific terminator site(s) was uncovered in the plasmid R6K. The plasmid has 3 *ori* sequences called α, β and γ that are located within a kb of each other. The origins initiate unidirectional replication with one *ori* firing per molecule. From the *ori*(s) located at 12 o'clock in the circular DNA, fork travel unidirectionally until reaching the 2 Ter sites (present as inverted repeats) at 2 o'clock. Then the second fork at the *ori* fires unidirectionally in the opposite direction until meeting the first one stalled at the *Ter* sites. Thus, the topology of replication is sequentially bidirectional due to an asymmetrically located Ter region with respect to the origins [10,11]. The host replication termini were discovered shortly thereafter and were found to be located at the antipode (Fig. 1A) with respect to the *ori*, in the circular chromosomes [12,13]. The replication terminus regions of R6K and of the host *E. coli* were cloned and sequenced [14,15]. The activity of the replication termini in vitro was first investigated using partially fractionated cell extracts [16] and then reconstituted with a system of 26 purified proteins [17].

Hill cloned the Tus gene of *E. coli* that encodes the host terminator protein [18–20]. The Tus protein was independently identified and purified by other groups [21,22]. The protein was shown to bind specifically to *Ter* sites [18,22,23]. *E. coli* chromosome contains two groups of 5 *Ter* sites of opposite polarity that are arranged in such a way that they form a replication trap that restricts fork arrest to the antipodal *Ter*C region. Each cluster of 5 *Ter* sites of the same polarity located on one replichore (also called chirochore). Replichore refers to each arm of the chromosome from *ori* to *Ter* traced clockwise and anticlockwise that have sequence polarity (see Fig. 1A and [24]). Each replichore is marked by G rich sequences called Rag motifs that switch to polarity at the antipode (Fig. 1C).

3. Crystal structure of the Tus–*Ter* system and structure-guided mutagenesis

How does a Tus–*Ter* complex arrest a replication fork in a polar mode? At least a partial understanding of the mechanism of polar fork arrest came from in vitro replication work using purified Tus supplementing a partially fractionated cell extract and a plasmid DNA substrate containing a unidirectionally replicating *ori* and a *Ter*

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