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Dynamics of replication proteins during lagging strand synthesis: A crossroads for genomic instability and cancer

Amit Laxmikant Deshmukh, Chandan Kumar, Deependra Kumar Singh, Pooja Maurya, Dibyendu Banerjee*

Molecular and Structural Biology Division, CSIR-Central Drug Research Institute, B.S. 10/1, Janakipuram Extension, Sitapur Road, Lucknow, 226031, India

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ABSTRACT

DNA replication is a complex phenomenon that requires the concerted action of several enzymes, together with their protein and non-protein cofactors. In the nucleus, the two DNA strands are duplicated by two completely independent methods due to their anti-parallel orientation and the restrictive nature of DNA polymerases that allow DNA synthesis in the 5'–3' direction only. In this review, we focus on the proteins that are involved in the more complex and discontinuous process of lagging strand DNA synthesis by the formation of small DNA fragments called Okazaki fragments which are later sealed to form a continuous strand of DNA. We try and connect all the protein-protein interactions important for lagging strand synthesis in the S-phase of the cell cycle, describe the dynamics of these interactions and go on to discuss the post-translational modifications that affect them. We also look at how mutations in any of the players of the lagging strand synthesis can cause genomic instability leading to cancer and discuss if any of the players may be targeted for cancer therapy.

Contents

1. Introduction

In eukaryotes, the process of cell division is a complex phenomenon and is preceded by the division of the nuclear DNA. Nuclear DNA replication is carried out by a network of proteins and enzymes that work rapidly in a well coordinated manner to duplicate the all-important genetic information. Several components of the replication apparatus perform their exact roles in a complex called the replisome that are located in so called nuclear "replication factories". For replication to begin, the chromosomes must unwind and the synthesis of both new strands must occur simultaneously. Several models proposing the spatial arrangement of the replication apparatus are available [1,2]. They are based on the idea that one of the anti-parallel DNA strands must loop around

* Corresponding author.
E-mail address: d.banerjee@cdri.res.in (D. Banerjee).

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Table 1
Post translational modifications in replication proteins.

Protein	Subunits	Modifications	Modifiers	Effect on DNA synthesis	Refrences
RFC	P140	Phosphorylation	CamkII	PCNA binding is blocked	[23]
			Cdc2-cyclinB	RFC complex dissociates	[24]
PCNA		Acetylation	P300	Interaction with Pol δ stimulated	[22,25]
		Phosphorylation	EGFR tyrosine kinase	Increases PCNA stability	[18]
RPA	P32	Phosphorylation	Cdk2-cyclinA	Dissociation of RPA	[20]
			Cdk1-cyclin A	complex	
			DNA-PK		
	P70	Phosphorylation	ATR	No effect on DNA binding	[26]
			Chk1	Reduced ss DNA binding activity	
FEN 1		Phosphorylation	Cdk2-cyclin A	Inhibit PCNA binding	[27]
		Methylation	PRMT	Requires for PCNA binding	[21,28]
		Acetylation	p300	Decreases flap cleavage activity	[28–30]
Pol δ	P125	Phosphorylation	Cdk2-cyclins	Unknown	[31]
	P68	Phosphorylation	PKA	PCNA binding blocked	[32]
	P12	Acetylation	P300	Improves strand displacement function	[28]
Lig I		Phosphorylation	Cdk2-cyclin A	Controlling association with replication machinery	[33,34]

A brief description of these proteins and their post-translational modifications in the context of lagging strand synthesis follows.

in such a manner that the DNA polymerases (Pols) that can synthesize only in the 5'–3' direction, are able to carry out DNA synthesis simultaneously for both the strands [3,4] (Fig. 1). To achieve this, the leading strand is synthesized continuously by polymerase ε (Pol ε) [5] or polymerase δ (Pol δ) [6] from a single initiation event at the replication origin, while the lagging strand synthesis is initiated repeatedly at multiple sites and extended by (Pol δ) [7] as a series of discrete fragments that are named after their discoverer Okazaki et al. [8].

Since DNA polymerases cannot synthesize *de novo*, primers are synthesized by an enzyme called Primase (a heterotetramer of RNA polymerase and DNA polymerase α) that synthesize an RNA primer and a short DNA segment on both the leading and lagging strands [9]. The polymerase α is later displaced by the combined action of replication factor C (RFC), proliferating cell nuclear antigen (PCNA) and Pol ε on the leading strand resulting in the assembly of a highly processive complex carrying out DNA synthesis [10]. On the lagging strand, the DNA Pol α/Primase synthesize 9–10 nucleotide RNA primers [11]. Pol δ then displaces Pol α *via* a RFC/PCNA-dependent polymerase switching mechanism which results in the processive synthesis of segments of nascent lagging strand DNA, 100–200 nt in length [1,12,13]. The RNA primers in these nascent nucleotide segments are then removed by nucleases such as RNase H, Flap endonuclease 1 (FEN1) and Dna2 to generate the Okazaki fragments that are joined together by DNA ligase I into an intact lagging DNA strand [2,14,15]. In this review, we focus on the protein-protein interactions involved in lagging strand synthesis and maturation and the post-translational modifications and mutations that are known to affect these processes. The proteins such as DNA helicases and topoisomerases, although very essential, are not discussed in this review.

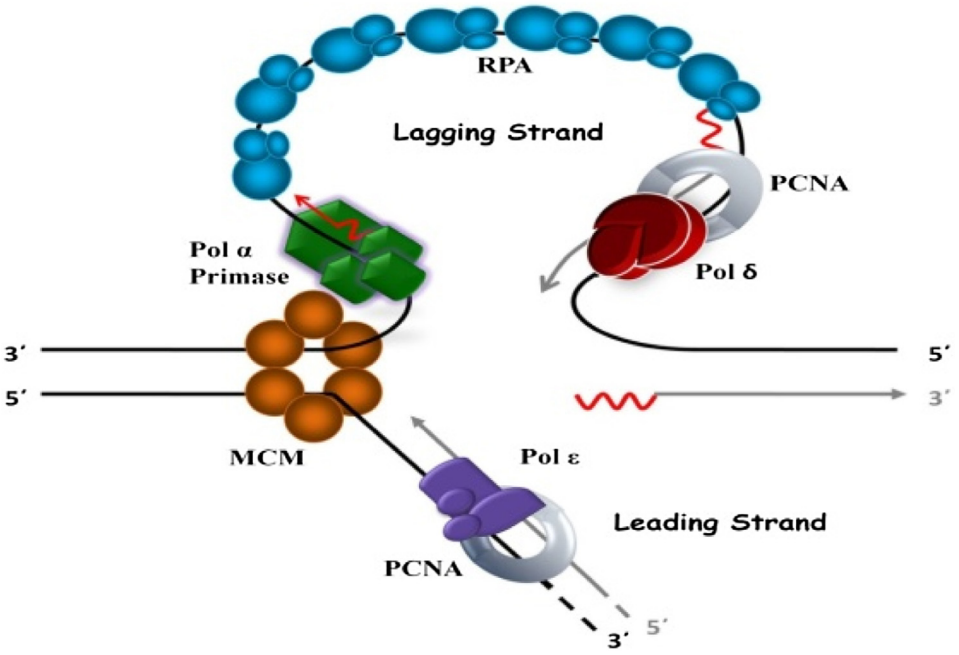

Fig. 1. Proteins involved at a typical DNA replication fork.MCM (mini-Chromosome maintenance), is a homo-hexameric protein with helicase activity that opens up the DNA duplex to initiate DNA replication. RPA, a hetero-trimeric single-stranded DNA binding protein binds ss DNA and protectsit from nuclease cleavage. Primase (a complex of RNA polymerase and Pol α) synthesizes RNA primers and a short DNA fragment to initiate Okazaki fragments. Pol δ, a hetero-tetrameric DNA polymerase synthesizes the majorportions of Okazaki fragments. Pol ε, another DNA polymerase drives leading strand DNA synthesis while PCNA, which is a homo-trimeric DNAclamp, is needed for the processivity of DNA polymerases and coordination of the Okazaki fragment maturation processes.