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Mechanism of primase activity human primase polymerase PrimPol

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Motivation and Aim: Human primase and DNA polymerase PrimPol belongs to the archaeo-eukaryotic superfamily and synthesizes primers *de novo* using deoxyribonucleotides. PrimPol plays a role in replication both the nucleus and mitochondria by re-initiation of DNA synthesis at sites containing DNA damage or secondary structures that block high-fidelity DNA polymerases.

PrimPol is composed of two domains: an N-terminal AEP-like catalytic domain (NTD) harboring catalytic amino acid residues Asp114/Glu116 and Asp280 involved in the coordination of Me2+ ions and a C-terminal domain (CTD) containing a conserved C-H-C-C zinc finger (ZnF) motif that coordinates the Zn2+ ion (Cys419, His426, Cys446, Cys451). Deletion of ZnF and mutations of Cys419 and His426 disrupt PrimPol primase activity while retaining DNA polymerase activity. PrimPol prefers to initiate DNA synthesis on the 3'-GTC-5' sequence with cryptic G and its activity is primase activity is stimulated by Mn2+ ions. It is assumed that binding of the first two nucleotides occurs at the 3'-elongation site and the 5'-initiation site (preferably ATP), followed by catalysis and formation of the rA-dG dinucleotide.

The detailed mechanism of PrimPol primase activity is not fully understood. Structures of the full-length PrimPol and the initiation complex of PrimPol with a DNA template and a dinucleotide have not been deciphered. However important information on the mechanism of PrimPol primase activity has been obtained in biochemical studies.

Methods and Algorithms: We investigated the mechanism of PrimPol primase activity using its variants that selectively disrupt any catalytic activity (D114A) or only primase activity (ZnF mutations R417A and R424A), as well as individual NTD and CTD domains of PrimPol. To analyze protein interaction with DNA, a DNA oligonucleotide containing the 5′-terminal adenosine triphosphate was synthesized enzymatically *de novo* using the wild-type PrimPol.

Results: Due to bi-modal organization, primases can operate in the *cis*- or *trans*-orientation. We show that PrimPol performs primer synthesis in the *cis*-orientation, when the NTD catalytic and CTD regulatory domains of one molecule take part in catalysis. According to the suggested model, the catalytic NTD is responsible for catalysis and can initiate *de novo* DNA synthesis alone but the CTD plays a key regulatory role and is involved in template-primer binding by PrimPol. We showed that the interaction of ZnF of the CTD involves the terminal 5′-triphosphate of ATP. During *de novo* DNA synthesis, PrimPol makes pauses.

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