Chapter 3
Fate of DNA Replisome upon Encountering DNA Damage

Abstract The real DNA replication in cell is performed by DNA replisome. Herein, we will discuss the *Escherichia coli*, T4 and T7 DNA replisome. When DNA replisome encounters DNA damage, the replisome will have different fates. Some DNA replisome bypass DNA damage with the aid of protein interactions or other assistant proteins in the replisome. Other replisome will dissociate according to some specific pathways.

Keywords DNA replisome · DNA damage · T7 DNA replisome · T4 DNA replisome · *E. coli* replisome

3.1 The DNA Replisome of *E. coli*, T7 and T4

DNA replication in vivo is performed by the DNA replisome. DNA replisomes mediate assembly of proteins at the replication fork, unwinding of DNA, template-directed polymerization of nucleotides, and synthesis of RNA primers. A major role of the replisome is to coordinate DNA polymerization mediated by its protein constituents, so that leading- and lagging-strand DNA synthesis proceed at the same rates. The replisome is a dynamic structure, involving the release and recruitment of proteins as the replisome proceeds. The complexity of the DNA replisome results from protein interactions and various functions of accessory proteins within the complex. The DNA replisomes of *Escherichia coli* and bacteriophages T7 and T4 have been constructed in vitro.

The T7 DNA replisome contains gene 5 DNA polymerase (gp5), gene 4 helicase–primase (gp4), the *E. coli* processivity factor thioredoxin (trx), and gene 2.5 ssDNA binding protein (gp2.5). Gp5 and trx form a high-affinity complex (gp5/trx) to increase the processivity of nucleotide polymerization. The helicase at the C-terminal domain of gp4 assembles as a hexamer and unwinds dsDNA to yield two ssDNA templates for leading- and lagging-strand DNA synthesis. Gp2.5 coats ssDNA to remove secondary structures and also physically interacts with gp5/trx, interactions essential for coordination of leading- and lagging-strand DNA synthesis.

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The T4 DNA replisome consists of DNA polymerase, helicase, ssDNA binding protein, a trimetric clamp processivity factor, a pentameric clamp-loader complex, and six monomers of the primase. The DNA polymerase has polymerase and exonuclease activities. A clamp-loading complex contains four molecules of the gene 44 protein and one molecule of the gene 62 protein. T4 DNA polymerase is monomeric, but it forms a dimer (via a disulfide bond) when bound to primer–template.

The *E. coli* DNA replisome comprises DNA polymerase III holoenzyme, DnaB helicase, DnaG primase, ssDNA binding protein, and multiple accessory proteins. The Pol III holoenzyme consists of a three-subunit catalytic core (α-polymerase, ε-exonuclease, and θ), a homodimeric β processivity clamp, and a $\delta\delta'\tau_2\gamma\chi\varphi$-clamp-loader complex. The β clamp consists of ring-shaped dimeric proteins that encircle DNA and bind to the Pol III core, thereby giving the DNA polymerase high processivity. The clamp-loader complex opens and loads the β clamp onto a primed site. Each τ subunit binds to the DnaB helicase and one Pol III core polymerase, coupling the helicase and the polymerase.

The T7 DNA replisome contains only four proteins, but the replisomes of *E. coli* and bacteriophage T4 contain thirteen and eight proteins, respectively, most of which are accessory proteins. These accessory proteins have roles in protein interaction in the bacteriophage T7 system. In mammals, including humans, the DNA replisome is too complicated to be constructed at this point, and only simple replication complexes have been reported in vitro.

DNA replisomes have shown different patterns of DNA synthesis from systems using a single DNA polymerase. The differences mainly result from two major reasons: protein–protein interactions and accessory proteins in DNA replisome that facilitate DNA polymerase to bypass damage as described below.

3.2 The *E. coli* DNA Replisome Bypassing DNA Damage

DNA replication machinery constantly encounters DNA lesions under normal growth conditions. Cox et al. have estimated that 10–50 % of all replication forks may be subjected to collapse in one generation of a single cell. Translesion DNA synthesis by single DNA polymerases may not reflect the accurate situation in vivo. Therefore, translesion DNA synthesis using DNA replisomes should be the object of more studies.

Addition of ddTTP can selectively block leading-strand DNA synthesis. The fate of *E. coli* DNA replisome was studied after the leading-strand DNA synthesis is blocked. The leading-strand polymerase remains stably bound to the helicase at the replication fork. The helicase continues to unwind DNA for ~1 kb ahead of the blocked leading-strand polymerase. The lagging-strand polymerase is connected to the stalled leading-strand polymerase and remains active in converting the lagging ssDNA to duplex DNA. When the lagging-strand DNA polymerase is blocked by a DNA lesion on the lagging strand, the leading- and lagging-stand DNA polymerases remain physically coupled, but functionally uncoupled. The

leading-strand polymerase continues unabatedly, allowing the replication fork to continue. This action causes a large loop of ssDNA to accumulate on the lagging-strand template. This loop grows until the supply of ssDNA binding protein is depleted. At that point, the naked ssDNA triggers the release of the stalled lagging-strand polymerase from the blocked site and resumes the synthesis of a new Okazaki fragment on a newly primed site.

After encountering a CPD DNA lesion at leading-strand template, the *E. coli* DNA replisome is only transiently blocked, which is then reinitiated downstream of the damage, dependent on the assistance of primase DnaG in the DNA replisome but not on any other known replication-restart proteins. Therefore, the *E. coli* DNA replisome can tolerate leading-strand template lesions and synthesize the leading-strand template discontinuously. However, the single *E. coli* DNA polymerase alone cannot bypass this DNA damage. Additionally, polymerases can transiently dissociate upon encountering the CPD lesion and allow repair enzymes or translesion polymerases to repair or bypass this lesion. The helicase–primase complex remains bound to the template DNA and serves to maintain the integrity of the replication fork, directing the reassembled replisome to the correct location.

3.3 The T4 DNA Replisome Bypassing DNA Damage

Bypass of a noncoding abasic site lesion in either a leading- or lagging-strand template has been studied by T4 DNA replisome. Lesion at the lagging strand blocks the lagging-strand DNA polymerase but does not block the helicase, primase, or leading-strand polymerase. When the primase synthesizes another RNA primer and the clamp is loaded, the stalled lagging-strand polymerase recycles from the DNA lesion and initiates the synthesis of a new Okazaki fragment. Therefore, this lesion does not affect the movement of the DNA replisome, with only a ssDNA gap left behind. In contrast, when a blocking lesion is at the leading-strand template, the leading-strand polymerase is blocked, but the bound helicase continues to travel, causing the leading-strand template to loop out. The leading-strand template is then rapidly coated with ssDNA binding protein. The replication fork travels about 1 kb beyond the DNA lesion before the replication fork completely collapses. The primase and lagging-strand polymerase remain active, and Okazaki fragments are synthesized beyond the leading-strand lesion.

Single-molecule magnetic tweezers were used to study the mechanism of restarting the T4 replication fork that has been blocked by blocked DNA. The T4 DNA holoenzyme, in cooperation with UvsW helicase, can overcome a leading-strand lesion by periodical formation and migration of a four-way Holliday junction. The initiation of the repair process requires partial disassembly of the replisome through the departure of the replicative helicase. With the assistance of other accessory proteins, T4 DNA holoenzyme can bypass this leading-strand lesion.

3.4 The T7 DNA Replisome Bypassing DNA Damage

Single-phosphodiester bond interruptions (nicks) can be introduced by endonucleases, recombination, repair, and the presence of two adjacent Okazaki fragments. Either T7 helicase or DNA polymerase alone was blocked upon encountering a nick in duplex DNA. However, the helicase–polymerase complex can bypass this nick. In dsDNA unwinding, helicase contacts both DNA strands. However, a nick does not provide these contacts, and the helicase itself cannot unwind a nick. When helicase is associated with a DNA polymerase, protein interactions allow the helicase to bind to the template and to encircle helicase onto the 5′-end ssDNA of a nick, thus bypassing the nick. Addition of ssDNA binding protein gp2.5 to the complex further facilitates nick bypass by ~twofold.

Without ssDNA binding protein gp2.5, helicase and DNA polymerase cannot initiate strand-displacement DNA synthesis from a nick. Gp2.5 can bind to the displaced ssDNA, and its acidic C-terminal tail can interact with DNA polymerase. The helicase replaces gp2.5 and catalyzes strand-displacement DNA synthesis with gp5/trx at this nick. Therefore, protein interactions within the T7 DNA replisome may alter the ability of the DNA polymerase to bypass DNA damage and may produce completely different results from those obtained from isolated T7 DNA polymerase (or the trx complex).

Further Reading

Zhang H, Lee SJ, Richardson CC (2011) Essential protein interactions within the replisome regulate DNA replication. Cell Cycle 10:3413–3414

Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ (2000) The importance of repairing stalled replication forks. Nature 404:37–41

Zhang H, Lee SJ, Zhu B, Tran NQ, Tabor S, Richardson CC (2011) Helicase-DNA polymerase interaction is critical to initiate leading-strand DNA synthesis. Proc Natl Acad Sci USA 108:9372–9377

Tabor S, Huber HE, Richardson CC (1987) Escherichia-Coli Thioredoxin Confers Processivity on the DNA-Polymerase-Activity of the Gene-5 Protein of Bacteriophage-T7. J Biol Chem 262:16212–16223

Marintcheva B, Hamdan SM, Lee SJ, Richardson CC (2006) Essential residues in the C terminus of the bacteriophage T7 gene 2.5 single-stranded DNA-binding protein. J Biol Chem 281:25831–25840

Kuzminov A (1995) Collapse and repair of replication forks in Escherichia coli. Mol Microbio 16:373–384

Zhu B, Lee SJ, Richardson CC (2011) Bypass of a nick by the replisome of bacteriophage T7. J Biol Chem 286:28488–28497

Kaplan DL (2000) The 3′-tail of a forked-duplex sterically determines whether one or two DNA strands pass through the central channel of a replication-fork helicase. J Mol Biol 301:285–299

Ghosh S, Marintcheva B, Takahashi M, Richardson CC (2009) C-terminal Phenylalanine of Bacteriophage T7 Single-stranded DNA-binding Protein Is Essential for Strand Displacement Synthesis by T7 DNA Polymerase at a Nick in DNA. J Biol Chem 284:30339–30349

Hamdan SM, Richardson CC (2009) Motors, Switches, and Contacts in the Replisome. Annu Rev Biochem 78:205–243

McInerney P, Donnell MO (2007) Replisome fate upon encountering a leading strand block and clearance from DNA by recombination proteins. J Biol Chem 282:25903–25916

McInerney P, Donnell MO (2004) Functional uncoupling of twin polymerases - Mechanism of polymerase dissociation from a lagging-strand block. J Biol Chem 279:21543–21551

Yeeles JTP, Marians KJ (2011) The Escherichia coli Replisome Is Inherently DNA Damage Tolerant. Science 334:235–238

Jeiranian HA, Schalow BJ, Courcelle CT, Courcelle J (2013) Fate of the replisome following arrest by UV-induced DNA damage in Escherichia coli. Proc Natl Acad Sci USA 110:11421–11426

Nelson SW, Benkovic SJ (2010) Response of the Bacteriophage T4 Replisome to Noncoding Lesions and Regression of a Stalled Replication Fork. J Mol Biol 401:743–756

Manosas M, Perumal SK, Croquette V, Benkovic SJ (2012) Direct Observation of Stalled Fork Restart via Fork Regression in the T4 Replication System. Science 338:1217–1220