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# Mechanisms and Controls of DNA Replication in Bacteria

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## 1. Introduction

DNA is the polymeric molecule that contains all the genetic information in a cell. This genetic information encodes the instructions to make a copy of itself, for the cellular structure, for the operative cellular machinery and also contains the regulatory signals, which determine when parts of this machinery should be *on* or *off*. The operative machinery in turn, is responsible for the cells functions either metabolically or in interactions with the environment. Part of this cellular machinery devoted to DNA metabolism is responsible for DNA replication, DNA-repair and for the regulation of gene expression. In this chapter we will focus our discussion on the mechanisms and controls that conduct DNA replication in bacteria, including the components, functions and regulation of replication machinery. Most of our discourse will consider this biological process in *Escherichia coli* but when possible we will compare it to other bacterial models, mainly *Bacillus subtilis* and *Caulobacter crescentus* as examples of organisms with asymmetrical cell division.

In order to maintain a bacterial population it is necessary that cells divide, but before the physical division of a daughter cell from its mother, it is necessary among other check points, that the DNA has been replicated accurately. This is done by the universal semi-conservative replication process of DNA-strands, which generates two identical strand copies from their parent templates. To better understand this process it has been divided into three phases: initiation, elongation and termination of DNA replication. In each of these steps, multiple stable and transient interactions are involved and we have summarized them below.

## 2. Components and mechanisms of the general process of DNA replication

Bacteria are subject to sudden changes in their surroundings, so they have adapted diverse strategies to allow them to persist through time. One of the adaptive changes consists in modifying growth rates, which is accompanied by adjusting mechanisms that control the timing of the cell-cycle. This adjustment ensures that the process of cell division is coordinated with the doubling of cell-mass and with the proper replication and segregation of the chromosome. The study of the cell-cycle in bacteria is usually divided into three stages: the period between cell-division (cell birth) and the initiation of chromosome replication, the period required to complete DNA replication (elongation of DNA) and, the

final phase, which goes from the end of DNA replication until the completion of cell-division (Wang & Levine, 2009).
Under the best growing-conditions, DNA replication starts immediately after cell division in most cells (Wang et al., 2005). Since replication of the chromosome takes more time than that the necessary for cell division under optimal culture conditions, such as *E. coli* growing in rich media, at 37ºC with good aeration, it can happen that more than one event of DNA replication can occur per cell cycle (Zakrzewska-Czerwinska et al., 2007). For the purposes of this work we shall divide the DNA replication process in bacteria into three steps: initiation, elongation and termination as follows.

### 2.1 Initiation of DNA replication

In bacteria, the process of DNA replication initiates in a specific DNA region called "origin of replication" (*ori*) where multi-protein complexes are positioned and recruits additional initiator proteins to form the Pre-Replicative complex (pre-RC) whose main function is to facilitate the aperture of duplex DNA to permit the loading of the replicative DNA helicase. The activity of this DNA helicase assists the entrance and assembly of a large multi-subunit molecular machine, the replisome (Zakrzewska-Czerwinska et al., 2007; Ozaki & Katayama, 2009).

#### 2.1.1 *oriC* and its *cis* regulatory regions

The origin of replication in *E. coli* (*oriC*) is a small DNA sequence of about 245 bp (Figure 1), which contains three AT-rich repeats named L, M, and R for left, middle and right positions respectively, each 13 bp long (Hwang & Kornberg, 1992). The *oriC* region also contains multiple boxes of 9 bp each where DnaA (replication initiation factor) proteins bind. These DnaA boxes recruit DnaA in two forms; DnaA-ATP and DnaA-ADP, although they show more affinity for the first form, which is the active replication initiation complex of DnaA. There are three DnaA-boxes of high affinity named R1, R2 and R4 and seven of low affinity (I1, I2, I3, τ1, τ2, R5M and R3), (Katayama et al., 2010; Ozaki & Katayama, 2009). The *oriC* region also contains GATC DNA motifs dispersed throughout, the GATC motif is recognized as a target for DNA-methylation by the Dam enzyme (DNA adenine methyltransferase). Finally, the *oriC* region also has DNA-binding sites for the union of several regulatory proteins such as Fis (Factor for inversion stimulation) and IHF (integration host factor), which assist in bending the DNA at this region (Leonard & Grimwade, 2009).
The comparison of the DNA sequence used as origin of replication in *E. coli* versus genomes of other sequenced bacteria indicates that the nucleotide composition and size of these regions is similar (Bramhill & Kornberg, 1988). A database of *ori* regions in bacterial genomes, the DoriC database, which contains a compilation of known and predicted DNA origins of replication in bacteria has been developed (Gao & Zhang, 2007).

#### 2.1.2 DnaA is the key protein required to form the pre-RC

The critical step for the successful replication of DNA is the unfolding of the DNA strands at the *oriC* region, action that is assisted by the orisome (proteins-oriC complex) (Leonard & Grimwade, 2005). This complex mainly comprises of the activity of the initiator protein DnaA. This protein belongs to the ubiquitous $AAA^+$ superfamily of ATPases (ATPases

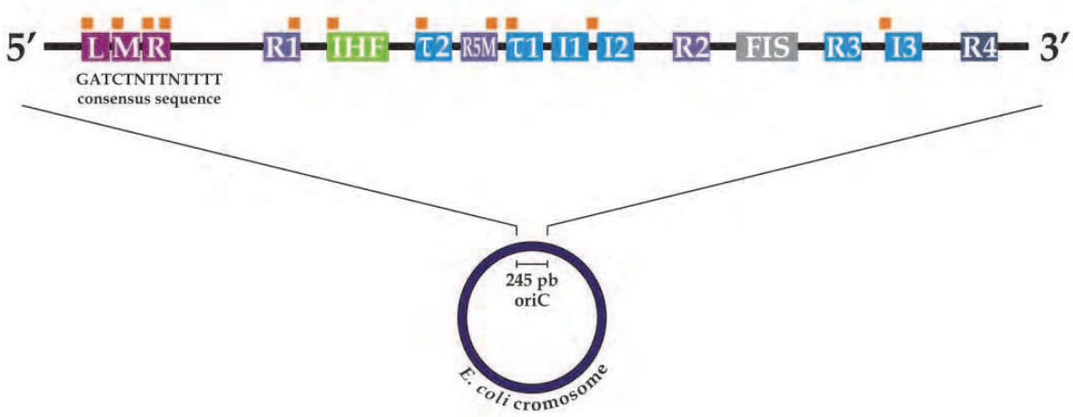


Fig. 1. Description of the origin of replication in the *E. coli* chromosome. The origin of chromosomal replication (oriC) contains three AT-rich repeats (L, M and R), each 13 nucleotide residues long and multiple DnaA-binding sites. There are three higher-affinity DnaA-boxes R1, R2 an R4 (dark blue) and seven lower-affinity sites τ1, τ2, I1, I2, I3, R5M and R3 (light blue). All the DnaA-boxes preferentially bind DnaA-ATP rather than DnaA-ADP complexes. *oriC* also contains one site where IHF binds (green), one for Fis (gray) and GATC sites (orange) which are recognized by the Dam enzyme.

associated with a variety of cellular activities). The X-ray structure of crystals of this protein from *Aquifex aeolicus* shows that the protein has four distinctive domains (Erzberger et al., 2002). Domain I serves for the interaction with other proteins, among those identified are: the replicative DnaB helicase and the DnaA-binding assistance protein DiaA (DnaA-initiator association). Domain II is a flexible linker, which provides free rotation for the adjacent domains III and I. Domain III has typical motifs that are characteristic of the $AAA^+$ protein superfamily of ATPases characterized by a conserved nucleotide phosphate-binding motif, named Walker A (GxxxxGK[S/T]), where x is any amino acid residue). This domain serves in protein binding to either ATP or ADP. When DnaA binds ATP it can form multimeric structures each consisting of 5–7 protomers (DnaA-ATP) by interactions of one subunit with the ATP of the anterior subunit through their "arginine fingers" as shown in Figure 2. It is suggested that the DnaA-*oriC* complex forms a circular pentamer, which is stabilized by interactions between each DnaA unit as mentioned before. The formation of these complexes promotes the unwinding of DNA strands on the initiation of replication. Finally, domain IV of DnaA has a helix-turn-helix motif that allows it to interact with the DnaA-box of *oriC* (Figure 2), (Erzberger et al., 2002; Ozaki & Katayama, 2009).

### 2.1.3 Additional components of the orisome

There are additional components of the orisome that may increase or impede the further unfolding of DNA at the origin of DNA replication. Some of these proteins in *E. coli* include the histone-like DNA-binding proteins IHF and Fis. IHF is a protein that binds to DNA at a poorly defined sequence. It stimulates the initiation of replication *in vivo* and *in vitro*. IHF assists the binding of DnaA to the low-affinity DnaA-boxes during the formation of the pre-replicative-complex. Contrarily, Fis seems to act as a repressor of initiation of DNA replication by inhibiting the binding of DnaA and IHF to their targets sites on DNA. This is achieved because Fis binds to *oriC* in a specific region of 13 nucleotides from position 87 to

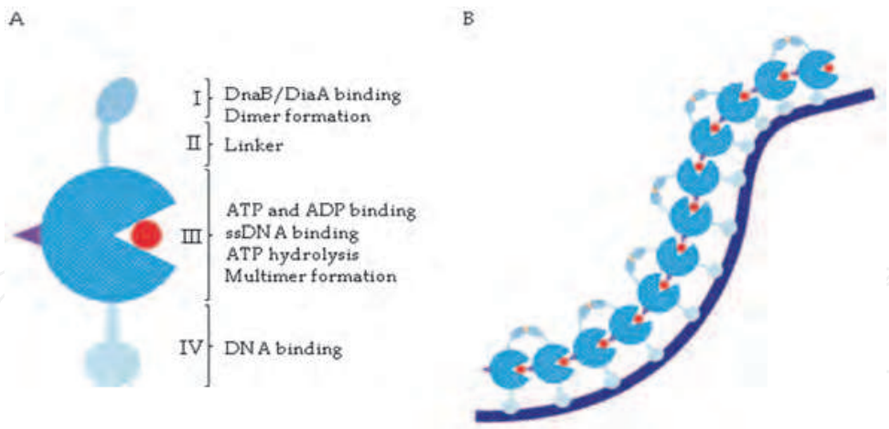


Fig. 2. DnaA as the main protein for the unfolding of DNA strands at *oriC*. A) The DnaA protein family is part of the AAA+ ATPases. In *E. coli* DnaA contains four functional domains as shown in the diagram. The ATP molecule is shown in red, and the arginine finger in purple. B) Domain III binds preferentially ATP over ADP, in addition it has an "arginine finger" which permits the multimerization of these protomers over the DNA.

119 (Figure 1), (Cassler et al., 1995; Ryan et al., 2004). Additional proteins such as DiaA and HU (Heat unstable protein) bind to domain I of DnaA, contributing to the stabilization of the joining of their protomers to *oriC* (Ishida et al., 2004; Chodavarapu et al., 2008). Another protein, ArgP (arginine protein, also called IciA) binds to the AT-rich regions in L, M and R boxes blocking the opening of DNA by DnaA (Hwang et al., 1992), this protein binds in the order of 10-20 monomers per *oriC*. Mutants in this gene however have no a clear defective phenotype of DNA replication and possibly this protein is functioning as an additional mechanism to maintain the robustness of this process. ArgP is also a transcriptional regulator which counts *dnaA* among its target genes. The activity of ArgP is regulated by arginine as its alosteric ligand and the protein is degraded by a specific protease. Another protein that inhibits the binding of DnaA to its target sequences is CNU (*oriC*-binding nucleoid-associated). CNU is a small protein composed of 71 amino acids (8.4 kDa) that binds to a sequence of 26 bp (named *cnb*), which overlaps with the binding sites for DnaA, thereby preventing its binding to *oriC* (Kim et al., 2005). When DnaA-ATP binds to *oriC* it twists the DNA and promotes the separation of DNA-strands in the AT-rich region to produce a single-stranded bubble or "open complex" (Figure 3). The next step is the recruitment of the $(DnaBC)_6$ complex to DnaA to obtain the pre-Replicative Complex preRC.

Four or five DnaA-ATP molecules interact with the $(DnaBC)_6$ complex via the N-terminal of the replicative DnaB helicase and their common binding to *oriC* (Seitz et al., 2000). $DnaB_6$ is a monohexameric helicase with a ring shape. Its function is the unwind of double-stranded DNA employing the hydrolysis of ATP, this activity is maintained as the elongation phase proceeds. $DnaB_6$ in its inactive form is found associated with the small protein DnaC (also of the $AAA^+$ superfamily) forming a closed complex $DnaB_6$-$(DnaC\text{-}ATP)_6$, (Biswas & Biswas-Fiss, 2006).

The DnaB protein should be loaded onto each of the single-stranded DNA (ssDNA) molecules. For this to happen, the pre-RC needs to release the DnaC from the complex $(DnaBC)_6$. It has been suggested that the DNA helicase translocates between parental templates of DNA and interacts via its N-terminal domain with the DnaG primase. The

formation of the DnaB-DnaG complex is known as the "primosome". Since replication is bidirectional in most bacterial chromosomes, one primosome is loaded on each single stranded parental (Figure 3). DnaB is responsible for the unwinding of the double stranded DNA (dsDNA) in the 5′-3′ direction and the primase synthesizes a small fragment of RNA complementary to the parental DNA-strand, not shorter than 12 and up to 29 ribonucleotides (Figure 3), (Swart & Griep, 1995; Rowen & Kornberg, 1978). The interaction of the primase with DnaB and the use of these primers trigger the release of DnaC. This action defines discrete events in the transition from initiation to the elongation phase of DNA replication (Makowska-Grzyska & Kaguni, 2010).

### 2.2 Elongation of DNA

Since the holoenzyme DNA polymerase III (Pol III, see below for components) cannot initiate DNA polymerization *de novo*, the strands are extended from the RNAs synthesized by the DnaG primase (Figure 4).

Pol III is positioned at the 3´ end of the first RNA primer complementary to the leading strand of DNA and extends it continuously. In contrast on the lagging strand the new DNA-strand is synthesized discontinuously producing Okazaki fragments of about 1 kb in length. The RNA primers are removed and substituted by DNA by DNA polymerase I (Pol I). Pol I uses 5´-3´exonuclease activity to remove these primers and fill out the gaps with its 3′-5′ DNA polymerase activity. Then DNA-ligase joins adjacent DNA fragments by catalyzing the formation of phosphodiester bonds between the 5′ phosphate of a hydrogen-bonded nucleotide and an adjacent 3′ OH of the nucleotide of the following Okazaki fragment.

The Pol III holoenzyme is composed of three subassemblies: the core polymerase, the β-sliding clamp and the clamp-loader complex. The core DNA polymerase is in turn, composed of three subunits α, θ and ε. The α-subunit is that which really has the activity of DNA polymerase whereas the small subunit ε has proofreading 3′-5′ exonuclease activity and its function is to remove nucleotides that have been misincorporated by the core-polymerase. The ε-subunit is stabilized by the θ-subunit, which as yet has not been assigned additional functions (Schaeffer et al., 2005).

The clamp-loader or DnaX complex consists of six different subunits (δ′, δ, γ, τ, ψ, χ). γ and τ subunits are encoded by the same *dnaX* gene. The full sequence of *dnaX* encodes the protein τ. However when the mRNA is being translated the ribosome sometimes undergoes a frame shift and a shorter product (only two-thirds) results. The frameshift occurs in a poly(A) tract and yields a new stop codon immediately following the frameshift signal. This truncated form of τ corresponds to the γ protein. In this way, the first three domains of γ and τ are identical. These different protein versions bind to the δ and δ′ subunits forming a complex composed of $\delta'\gamma\delta\tau_2$ subunits. The χ-ψ dimer binds either γ or τ subunits via the amino-terminus of ψ constituting the clamp-loader (Gao & McHenry, 2001; Reyes-Lamothe et al., 2010). τ proteins have two defined interactions; on one side they attach to the α-subunit of the core and on the other, interact with the $DnaB_6$ helicase on the lagging strand, so that this complex forms a bridge between the replicase and helicase proteins (Lee et al., 1996).

The single strands of DNA (ssDNA) are stabilized by a protein called single-stranded DNA-binding protein (SSB). SSB binds to single DNA-strands as a tetramer through its N-terminal domain, which makes contact with the DNA. The clamp-loader recognizes ssDNA coated by SSB4, interacting with the χ subunit of $SSB_4$. χ forms a heterodimeric complex with ψ, which in turn, interacts with the γ and τ subunits. In this way χ senses the presence (or absence) of ssDNA, facilitating the recognition of the terminal parts of RNA primers by τ (Schaefffer, 2005).

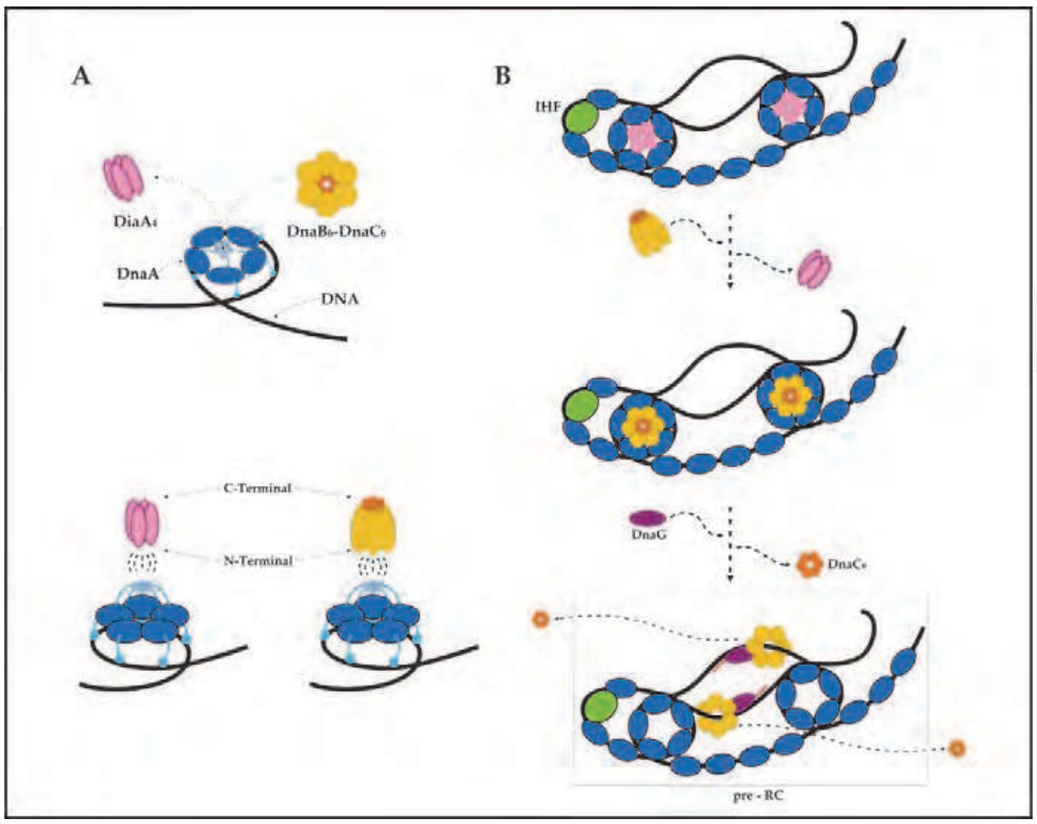


Fig. 3. Formation of the pre-RC. A) Binding of DnaA-ATP to *oriC* to form multimeric structures in conjunction with DiaA and $(DnaBC)_6$ via domain I, these interactions are important in order to form the pre-RC. B) The binding of DnaA-ATP to this region of DNA is favored when the protein IHF also binds to *oriC*, about 20 molecules of DnaA-ATP bind to OriC simultaneously. This DnaA-ATP complex is stabilized by DiaA and finally leads to the unfolding of the DNA at the AT-rich region. At this stage the $(DnaBC)_6$ complex is attached to domain I of the DnaA-ATP, forming the pre-RC. Subsequently, DnaB releases DnaC and loads to each single stranded DNA in direction 5'-3' with the assistance of the DnaG primase.

The sliding-clamp (β2) is a dimer of DnaN proteins, which binds to the hybrid DNA-RNA and serves to direct Pol III to this position for the synthesis of Okasaki fragments. During the elongation phase Pol III can hop from one clamp to another without leaving the replication fork. So Pol III overcomes possible delays due to blockage of DNA by the activity of transcription factors or DNA damage (Georgescu et al., 2010).

### 2.3 Termination of DNA replication

The end of DNA replication takes place when the replisome helicase $DnaB_6$ on the leading strands collides with a protein called Tus. Tus recognizes and is bound to sites for termination of DNA replication (*ter*). These sites are physically arranged in positions opposite to the *oriC* (Figure 5). In the collision of Tus with the helicase a trap is formed that prevents the further advancement of the replicative machinery in the leading strand and remains arrested until the replicative machinery on the lagging strand reaches this position (Neylon et al., 2005).

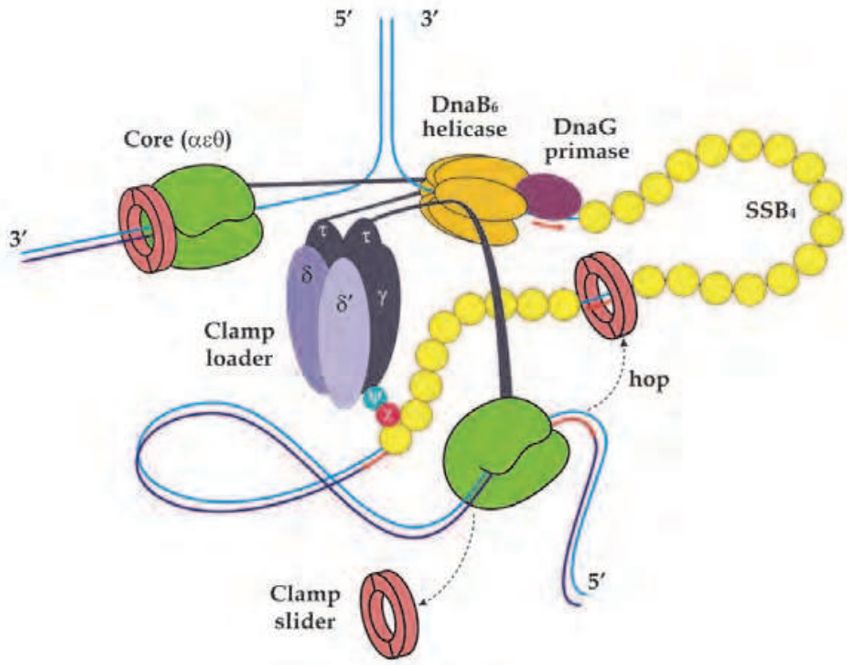


Fig. 4. Elongation of DNA by the replisome machinery.
The elongation of DNA in the *E. coli* chromosome is carried out in both directions of the fork by a multisubunit machinery called the replisome. Each replisome is located in both directions of the fork. The helicase $DnaB_6$ is loaded on the (3′-5′) lagging strand to unfold the DNA duplex in the 5′-3′ direction, at this time the primase synthesizes RNA primers complementary to each ssDNA. These primers are extended by Pol III, forming the Okasaki fragments on the lagging strand. When Pol III extends a new Okasaki fragment and reaches a previously synthesized one, it gives a hop, joining to another slider clamp (β-subunit), which recognizes DNA-RNA hybrids. DNA polymerases working on both parent strands are coordinately driven by the clamp-loader, which also binds to the helicase. SSB stabilizes the ssDNA. For the recognition of ssDNA by Pol III, the clamp-loader makes contact with SSB4-DNA via its χ-subunit.

The resolution of chromosomes is produced by the activity of several proteins which act together to separate the two daughter chromosomes. In this process the FtsK protein is very important as it acts by coordinating cell division with chromosome segregation through the activities of its N-transmembranal domain ($FtsK_N$) and its C-cytosolic domain ($FtsK_C$), respectively. $FtsK_N$ is the target for the division protein that forms the septum FtsZ, which stabilizes the interactions of FtsK with the other components of the divisome FtsQ, FtsI and FtsL (Aussel et al., 2002; Dubarry et al., 2010). FtsK also contains a linker, $FtsK_L$, localized between the $FtsK_N$ and $FtsK_C$ domains (Bigot et al., 2004). Recently two distinct regions within $FtsK_L$, have been identified (FtsK179–331 and FtsK332–641), which together with $FtsK_N$, are required for normal septation in *E. coli* (Dubarry et al., 2010). $FtsK_C$ can lead to the dimerization of circular chromosomes, thereby compromising their segregation (Figure 5). $FtsK_C$ activates events of recombination at the *dif* site (localized beside the replication termination region), which are mediated by two proteins with activities of tyrosine recombinases, XerC and XerD to resolve chromosome dimers to monomers and at the same time promote DNA translocation (Bigot et al., 2004; Kennedy et al., 2008). $FtsK_C$ is part of the

AAA+ superfamily and therefore can form a ring-shaped multimer that wraps the DNA and moves along it at the expense of ATP. When a chromosome dimer is present, a site-specific recombination event by XerCD introduces an additional cross over at *dif*, resolving thus the dimer into two monomers, all this is under the control of FtsK (Aussel et al., 2002).

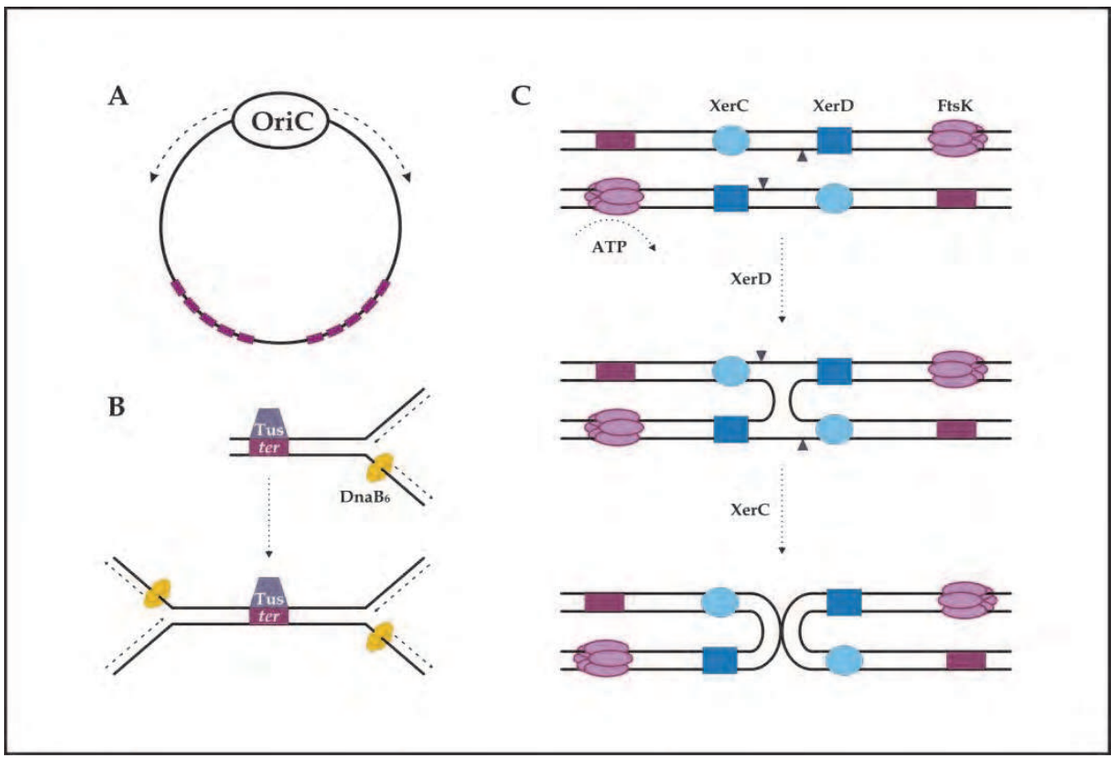


Fig. 5. Termination of DNA replication. A) The site of termination of replication in *E. coli* is opposite to *oriC*, where there are specific. *ter* sequences which are recognized by the Tus protein (purple boxes). B) Tus protein-terminator sequence (Tus-*ter*) is a barrier that pauses the leading fork until the lagging fork arrives from the opposite direction and induces termination, which occurs when the helicase touches Tus. The helicase dissociates from DNA and Pol III synthesizes the complementary strand on both sides of the forks. C) Near to the Tus-*ter* sites is found a sequence named *dif*, where site-specific recombination mediated by the XerC and XerD recombinases assisted by the translocase FtsK takes place. Figure taken and modified from Aussel et al. (2010).

A summary of the key enzymes involved in DNA replication known to date in *Escherichia coli*, are shown in table I.

## 3. Regulation of DNA replication

The regulation of DNA replication is a vital cellular process. In a general view, DNA replication is controlled by a series of mechanisms that are centered on the control of cellular DnaA levels, its availability as a free protein and modulation of its activity by binding the small-molecule ligand ATP (Leonard & Grimwade, 2009); the other point of control is by modulating the accessibility of replisome components to the *oriC* region on the DNA. We discuss some aspects of these regulatory mechanisms below.

| Protein name | Gene name | Function | Gene length (bp) | MW[a] (kDa) | Essentiality[b] |
|---|---|---|---|---|---|
| DnaA | *dnaA* | Initiator of DNA synthesis by binding to the origin of replication and also acts as a transcriptional regulator. It binds to DnaA boxes, and binds ATP. Around 20 to 30 DnaA monomers bind to the *oriC* region. It is calculated that around of 1000 molecules per cell are bound reaching up to 70% DnaA-ATP. | 1404 | 52.551 | E |
| DnaB | *dnaB* | A hexameric DNA helicase, it progressively unwinds DNA strands ahead of replication forks. About 100 DnaB molecules are calculated to be present per cell. | 1416 | 52.39 | E |
| DnaC | *dnaC* | DnaC is an accessory protein that assists the loading of DnaB onto DNA duplex to initiate replication and onto ssDNA to assist primer formation by the primase. Six DnaC monomers bind to the hexameric DnaB | 738 | 27.935 | E |
| DnaG | *dnaG* | DNA primase, it catalyzes the synthesis of RNA primers on ssDNA. These primers are necessary for DNA synthesis by DNA polymerase III. A DnaB–DnaG complex was observed by mixing DnaB with a six molar excess of DnaG (hexamers of DnaB and monomers of DnaG). Log-phase cells contain 50 to 100 molecules of primase. | 1746 | 65.565 | E |
| DNA polymerase III holoenzyme (Pol III) | | DNA polymerase III holoenzyme is the primary enzyme for DNA synthesis in *E. coli*. It carries out 5′ to 3' DNA polymerization using ssDNA as a template; it also carries out 3'-5' exonuclease edition of mispaired nucleotides. There are estimated to be 10 holoenzymes of DNA polymerase III per cell.<br>Pol III holoenzyme is made up of the following components $[(DnaE)(DnaQ)(HolE)]_3[(DnaX)_3(HolB)(HolA)][(DnaN)_2]_2[(DnaX)_2][(HolC)(HolD)]_4$. | | | |
| DNA polymerase III (core) | | The DNA polymerase III core enzyme can carry out the basic polymerase and exonuclease activities of polymerase III. | | | |
| α | *dnaE* | α subunit catalyzes DNA polymerization from 5' - 3'. | 3483 | 129.9 | E |
| ε | *dnaQ* | ε subunit catalyzes the 3' - 5' proofreading activity | 732 | 27.099 | E |
| θ | *holE* | θ subunit allows stabilization of α and ε subunits | 231 | 8.846 | NE |
| β | *dnaN* | The β subunit dimerizes to form the sliding clamp which positions the core polymerase onto the DNA. | 1101 | 40.587 | E |
| Clamp loader | | It catalyzes ATP-driven assembly of the sliding clamp onto primer-template DNA. Clamp loader = $\delta\delta'\tau_2\gamma\psi\chi$ | | | |
| δ | *holA* | δ subunit acts as a wrench to open the sliding clamp probably using ATP. Some δ units exist independently of the preinitiation complex, possibly playing a role in stripping β clamps from DNA in the absence of replication initiation. | 1032 | 38.704 | E |

| | | | | | |
|---|---|---|---|---|---|
| δ ' | *holB* | δ' subunit is part of the clamp loader complex. | 1005 | 36.937 | E |
| τ | *dnaX* | τ subunit binds to the alpha subunit dimerizing the core alpha-epsilon-theta polymerase subunits. This is required for synthesis on the lagging strand. | 1932 | 71.138 | E |
| γ | *dnaX* | γ subunit is part of the clamp loader complex. | 1932 | 47.545 | E |
| χ | *holC* | χ subunit allows the binding of the clamp loader to SSB. ψ-χ also acts in multiple ways improving the binding of DNA polymerase to DNA templates. | 444 | 16.633 | E |
| ψ | *holD* | ψ subunit allows the interactions between γ and X subunits | 414 | 15.174 | NE |
| Fis | *fis* | Fis for "factor for inversion stimulation" allows the organization and maintenance of the nucleoid structure through direct DNA bending and by modulating the production of gyrase and topoisomerase I as well as regulating the expression of other proteins that modulate the nucleoid structure, such as HNS, and HU. It reaches a cell concentration of 40,000-60,000 molecules/cell at the beginning of the exponential phase | 297 | 11.24 | E |
| Dam | *dam* | The DNA adenine methyltransferase is responsible for methylation of GATC sequences in *E. coli*. A wild-type, rapidly growing *E. coli* cell (doubling time = 30 min) was found to contain about 130 molecules of Dam methyltransferase. | 837 | 32.1 | NE |
| DiaA | *diaA* | DiaA interacts with DnaA, it is required for the timely initiation of chromosomal replication and stimulates the replication of minichromosomes *in vitro*. | 591 | 21.106 | NE |
| ArgP/IciA | *argP* | The ArgP transcriptional activator or inhibitor of chromosome initiation (IciA) regulates DNA replication by binding to three 13-mers located in the origin of replication (OriC), blocking the DNA opening by DnaA. It is also a transcriptional repressor of *dnaA*. There are about 800 molecules/cell of IciA in the exponential phase and the level decreases to about 500 molecules per cell in the early stationary phase. | 894 | 33.472 | NE |
| IHF | | "Integration host factor", is a global regulatory protein that helps to maintain the DNA architecture. It binds and bends DNA. IHF plays a role in DNA supercoiling and DNA duplex destabilization and affects processes such as DNA replication, recombination, and the expression of many genes. Consisting of two subunits α and β. IHF reaches 6,000-15,000 complexes in the exponential phase and up to 30,000-55,000 in the stationary phase. | | | |
| IHF-α | *ihfA* | α subunit of IHF | 300 | 11.354 | NE |
| IHF-β | *ihfB* | β subunit of IHF | 285 | 10.651 | NE |

| | | | | | |
|---|---|---|---|---|---|
| HU | | HU for heat unstable protein, is a global regulatory protein and shares properties with histones for nucleoid organization and regulation. It is a heterodimer formed by an α- and a β-subunit. HU reaches 30,000-55,000 dimers in the exponential phase and 10,000-17,000 in the stationary phase. | | | |
| HU-α | *hupA* | α-subunit of HU | 273 | 9.535 | NE |
| HU-β | *hup B* | β-subunit of HU | 273 | 9.226 | NE |
| DNA Pol I | *polA* | In addition to polymerase activity, this DNA polymerase exhibits 3'→5' and 5'→3' exonuclease activities. It is able to utilize nicked circular duplex DNA as a template and can unwind the parental DNA strand from its template. Its cellular abundance is of around 400 molecules per cell. | 2787 | 103.12 | NE |
| LigA | *ligA* | LigA is one of two known NAD(+)-dependent DNA ligases, it catalyzes the formation of phosphodiester bonds on duplex DNA. | 2016 | 73.606 | E |
| SSB | *ssb* | Single-stranded DNA-binding protein acts as a tetramer when binding to DNA. Each *E. coli* cell has about 800 monomers of *SSB*. | 537 | 18.975 | E |
| Tus | *tus* | Tus, also known as *ter*-binding protein (TBP), binds to *ter* sites, blocking the progress of DNA replication in a polar like form. | 930 | 35.783 | E |
| FtsK | *ftsK* | FtsK is an essential cell division protein linking cell division with chromosome segregation | 3990 | 146.66 | E |
| Hda | *hda* | Regulator of DnaA that prevents premature initiation of DNA replication. Around 100 molecules/cell are found. | 702 | 28.37 | E |
| RapA | *hepA* | A RNA Polymerase-binding ATPase and RNAP recycling factor. | 2907 | 109.77 | NE |
| SeqA | *seqA* | Sequesters newly replicated hemimethylated *oriC* to prevent re-initiation; it also binds hemimethylated GATC sequences. | 546 | 20.315 | E |
| Xer site-specific recombination system | | Two lambda integrases of the family of recombinases involved in converting chromosome dimers of into monomers so that segregation of the chromosomes can occur during cell division | | | |
| XerC | *xerC* | XerC is part of the Xer site-specific recombination system | 897 | 33.868 | E |
| XerD | *xerD* | XerD is part of the Xer site-specific recombination system | 897 | 34.246 | NE |

[a]MW: Molecular weight of the polypeptide product.
[b]Essential gene (E)/ non essential gene (NE).

Table 1. Description of major proteins for replication in *E. coli*

### 3.1 Regulatory mechanisms of DNA replication in *E. coli*

One of the main mechanisms associated with DNA replication is the so-called RIDA system (Regulatory Inactivation of DnaA). The elements of this system are the sliding-clamp of DNA polymerase III and Hda (Homologous to DnaA). This mechanism takes place when DnaA is activated by its binding to ATP. The accumulation of DnaA in this active form leads to the initiation of chromosomal replication since it facilitates its binding to the *oriC* on the DNA. DnaA reverts to its inactive form DnaA-ADP by hydrolysis of ATP (Katayama et al., 1998). Hda-ADP is the monomeric active form for promoting the hydrolysis of DNA-ATP, a process which is mediated by the slider-loader clamp (Su'etsugu et al., 2008). This inactivating regulation of DnaA is key for preventing the over-initiation of replicative events during the cell cycle (Katayama & Sekimizu, 1999). The free-living bacteria *C. crescentus* also presents this regulatory mechanism, as it has HdaA, a protein similar to the *E. coli* Hda. In *C. crescentus* HdaA also inactivates DnaA in a replication-coordinated manner, if DNA replication is successfully initiated then HdaA and the β-sliding clamp promote the hydrolysis of DnaA-ATP to DnaA-ADP and force DnaA to leaves the *oriC* (Collier & Shapiro, 2009). A conserved bacterial protein, YabA, has been found in *B. subtilis* and other Gram-positive bacteria where it acts as a repressor for initiation of DNA replication. This is achieved by forming a complex with DnaA and the β-sliding clamp independently of the DNA, a common activity shared between Hda and YabA (Mott & Berger, 2007). Thus the RIDA system is present in *B. subtilis* and is also the primary mechanism for regulation of DNA replication in this bacterium (Noirot-Gros et al., 2006). The formation of the *oriC* and DnaA complex is assisted by the protein DiaA, which forms homo-tetramers and binds various DnaA molecules, especially in the active form of DnaA-ATP but it can also stimulate the formation of the DnaA-ADP-*oriC* complex, this is an inactive complex for initiation of replication (Ishida et al., 2004).

Another mechanism that regulates the initiation of DNA replication is by controlling the availability of free DnaA to bind to DnaA boxes on the *oriC* (Figure 1). Here the role of the 1kb *dat*A locus, which is localized near (downstream) from the *oriC* is important. The *datA* locus shows high affinity for DnaA, even more than the DnaA boxes on the *oriC*. Thus the *dat*A region is able to bind over 300 DnaA molecules whereas *oriC* binds to 45 DnaA monomers (Kitagawa et al., 1998). The operability of this mechanism is facilitated by the fact that the *oriC* had only few DnaA boxes compared to the *datA* locus and by the close proximity of data in respect to *oriC* on the DNA molecule (Figure 6).

One related control system depends on the property of DnaA to act as a transcription factor and to the presence of DnaA boxes in the promoter regions of several genes. In most cases DnaA represses the expression of the associated gene but in some cases it can activates certain genes (Messer & Weigel, 1997). DnaA regulates around 10 genes in *E. coli* as documented in RegulonDB (Gama-Castro et al., 2010). The transcription of DnaA is one of the most important regulatory mechanisms that directly affect the replication of DNA and one of the proteins that negatively regulate the expression of *dnaA* is DnaA itself (Figure 6). At high levels DnaA binds to the DnaA boxes in the promoter region and impedes transcription. This auto-repressive process directly affects the amount of DnaA-ATP available and controls the efficiency of initiation of DNA replication (Mott & Berger, 2007). In *C. crescentus*, it was found that DnaA also auto-represses the transcription of its own gene but additionally DnaA is highly unstable in this organism and gradually degrades after initiating a replication event (Gorbatyuk & Marczynski, 2005).

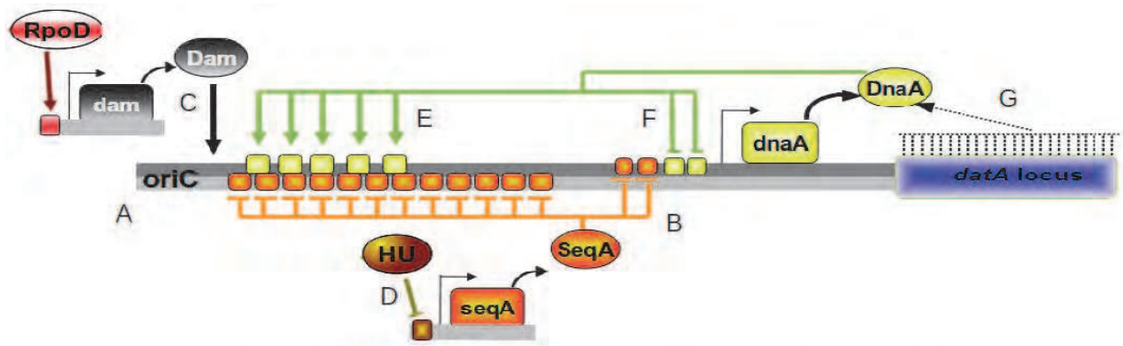


Fig. 6. Mechanisms that regulate DNA replication in *E. coli*. A) The newly replicated DNA duplex is in a hemimethylated state. B) SeqA binds to the hemimethylated GATC sites immediately after they are replicated. C) RpoD activates the transcription of *dam* and Dam methylates GATC sites of the newly synthesized strand. D) HU represses the transcription of SeqA. E) DnaA binds to the DnaA boxes on the *oriC* region. F) when there are many DnaA molecules they repress the transcription of the *dnaA* gene. G) *datA* locus binds many DnaA molecules.

### 3.2 Regulation of DNA replication by DNA methylation

A requirement for initiation of DNA replication is that both DNA strands are methylated, principally the adenine nucleotide in the GATC motifs, this process is mediated by Dam (DNA adenine methyltranferase), (Wion & Casadésus, 2006). Dam binds to the DNA nonspecifically, and methylates the GATC motifs (Figure 6). On DNA strands recently synthesized these motifs are rapidly methylated and exist in the hemimethylated state only during a fraction of the time needed for the replication of the entire DNA (Casadésus & Low, 2006).

The methylation process occurs asynchronously on the newly synthesized strands; i. e. methylation on the lagging arm occurs only after the ligation of the Okazaki fragments. It is postulated that Dam is always present in a complex bound near the replication origin, thus the methylation of nascent DNA strands occurs as soon as polymerization begins. In summary, the presence of hemimethylated GATC sites provides a cue to indicate that DNA replication has just occurred (Stancheva et al., 1999).

Another way to repress the transcription of *dnaA* is that which occurs immediately after the initiation of DNA replication. Here, SeqA binds to the hemimethylated GATC sequences in the regulatory regions of the *dnaA* gene (Lu et al., 1994; Brendler et al., 2000). Similarly, SeqA also represses the replication of DNA by binding to the hemimethylated GATC sequence at the *oriC*, this is possible because SeqA DNA-binding sites overlap with those of low affinity for DnaA (DnaA boxes) on the *oriC*. This overlap impedes the complete access of DnaA-ATP to the *oriC* (Han et al., 2004).

This prevention of replication, dependant of DNA methylation, has been considered as an epigenetic regulatory mechanism because it depends on the chemical modification of the nucleotide residues of the DNA and not in its sequence.

### 3.3 Regulation of DNA replication in *Bacillus subtilis*

*B. subtilis* shares some orthologous genes to the regulators that are involved in DNA replication in *E. coli*, but particular regulatory mechanisms must occur in this organism, as it

lacks some important components of the regulatory machinery found in *E. coli* such as the *seqA* and *dam* genes. In their place other players are present in *B. subtilis* such as Spo0A (Figure 7) and SirA (sporulation inhibitor of replication) (Katayama et al., 2010). Spo0A is the master regulator for sporulation and, at the same time, is an inhibitor of DNA replication. Spo0A is activated by a multicomponent phosphorelay process, this is initiated by a histidine kinase (KinA), that autophosphorylates, and transfers the phosphate to Spo0A through two intermediate phosphotransferases (Spo0F and Spo0B), (Burbulys et al., 1991). Spo0A-P (the active form) binds to specific sites on the *oriC* region and blocks the unwinding of the DNA duplex. Spo0A-P activates SirA, and SirA binds to DnaA in Domain I inhibiting the ability of DnaA to bind to the *oriC* (Wagner et al, 2009). Sda maintains the cellular levels of Spo0A-P low when a new round of replication has initiated (Veening et al., 2009), by inhibiting the accumulation of the autophosphorylated form of KinA (Cunningham & Burkholder, 2008).

Other regulators also implicated in DNA replication in *B. subtilis* are Soj and Spo0J (Figure 7), both components are required for proper chromosome segregation and for the repression of DNA replication. Soj exerts its activity in repressing replication by interacting with DnaA at the *oriC*, thereby preventing DnaA from initiating DNA replication (Murray & Errington, 2008). Otherwise Spo0J produces the complex Soj-Spo0J at the *parS* locus (Autret et al., 2001), promoting the release of Soj from the DNA strands, and allowing DNA replication to be initiated (Lee et al., 2003).

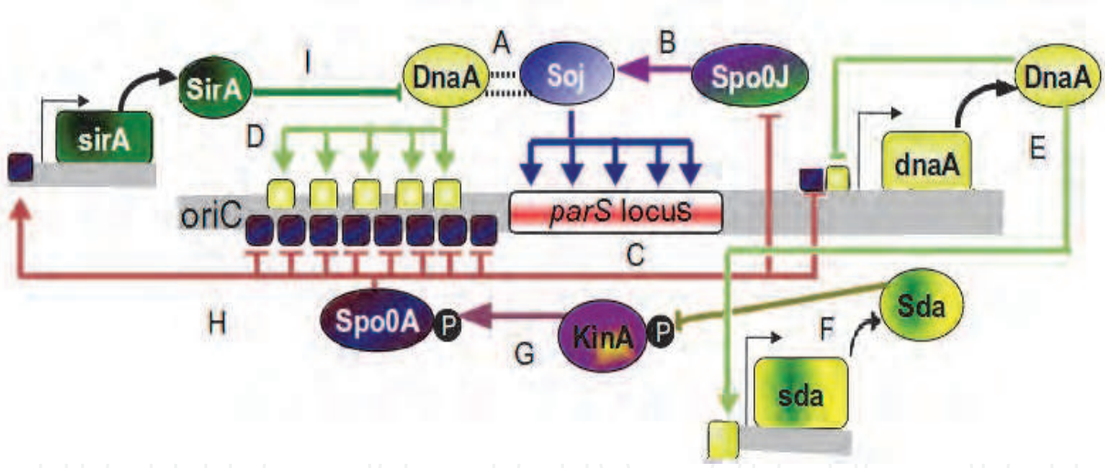


Fig. 7. Mechanisms that regulate DNA replication in *B. subtilis*. A) Soj represses DnaA activity. B) Spo0J stimulates Soj binding to the *parS* locus. C) The complex of Soj at the *parS* locus promotes the separation of Soj from the DNA. D) DnaA binds the DnaA boxes in the *oriC* initiating DNA replication. E) DnaA represses *dnaA* itself and activates the transcription of *sda*. F) Sda inhibits the accumulation of KinA-P. G) KinA activates Spo0A by transferring a phosphate group to Spo0A. H) Spo0A binds to specific sites in the *oriC* and represses replication, it also represses *dnaA* and *spo0J* and activates *sirA*. I) SirA, in turn, binds to DnaA and represses its binding to the *oriC*.

### 3.4 Regulation of DNA replication in *Caulobacter crescentus*

An interesting mechanism for control of DNA replication takes place in the cell cycle of *C. crescentus*, this aquatic, free-living bacteria, divides asymmetrically and this process is

regulated by a complex circuit of master regulatory proteins (Figure 8) coupled to a two-component system.

One of these regulators is the master regulator of cell cycle CtrA (Cell cycle transcriptional regulator), which is transcriptionally regulated by methylation of the GANTC motif on the first of the two of *ctrA* promoters (P1). Transcription initiation at P1 is repressed when the GANTC motifs are fully methylated while in the hemimethylated state transcription takes place. This mechanism ensures that *ctrA* is transcribed only while replication is in progress, producing enough protein to block and prevent the reinitiating of another round of DNA replication during this time (Reisenauer & Shapiro, 2002). In the hemimethylated form the production and accumulation of CtrA occurs, this protein binds to the regulatory region of *ccrM* and activates the transcription of a DNA-methylase encoded by this gene. Once synthesized, this enzyme proceeds to complete the methylation of both DNA strands. CtrA ceases its repressing activity when it is degraded by a Lon-type protease. The transcription of *ccrM* mediated by CtrA is inhibited when the two GANTC regulatory motifs are methylated. This complex machinery determines that when DNA is fully methylated, the transcription of *ctrA* and *ccrM* genes turns off (Stephens et al., 1995). This regulatory mechanism ensures that the synthesis of CcrM remains *off* and takes place only when the replication fork reaches the position of the *ccrM* gene preventing its premature transcription (Reisenauer et al., 1999).

The phosphorylated state of CtrA (CtrA-P) is the active form of this regulatory protein and this process is mediated by a cascade of phosphorylations which start with the activation of Divk, mediated by CtrA. DivK transfers the phosphate group to the CckA intermediate (Cell cycle histidine kinase) and CckA and ChpT finally transfer the phosphate group to CtrA. In the swarmer cell type of *C. crescentus*, CtrA-P binds to five DNA motifs on the *oriC* region, repressing the process of DNA replication (Marczynski & Shapiro, 2002). For the replication process to take place CtrA-P must be degraded by the ClpXP protease, which releases the origin of replication. ClpXP and CtrA are localized to each of the poles in stalked cells. This polar targeting of ClpXP is mediated by CpdR (a two component receiver protein), which is a dephosphorylating protein positioned at the pole where it recruits ClpXP (Jenal, 2009). Sometime after this happens, the proteolysis of CtrA ends and a positive transcriptional feedback loop generates the accumulation of CtrA, blocking again the access of DnaA to the *oriC* (Hung & Shapiro, 2002).

Another regulatory system for DNA replication in *C. crescentus*, is the regulatory circuit of DnaA, CtrA, GcrA and SciP. This genetic circuit regulates the transcription of multiple genes (DnaA alone controls the expression of approximately 40 genes in this bacterium) and many of these genes encode components of the replisome, in particular activating *gcrA*. On the other hand, CtrA regulates about 95 genes principally those involved in flagella biogenesis, cellular division and other regulators, and inhibits *gcrA*. GcrA in turn controls over 50 genes including the activation of *ctrA* and the repression of *dnaA* (Laub et al., 2007). Finally, Scip represses *ctrA*, and it is regulated in a feed forward loop manner; activated by CtrA and repressed by DnaA (Tan et al., 2010).

Some of the regulatory mechanisms concerning DNA replication are conserved in bacteria (as shown throughout this chapter) but specific mechanisms are also characteristic of each organism, table 2 shows the comparison of the regulators present in the three bacterial models described above.

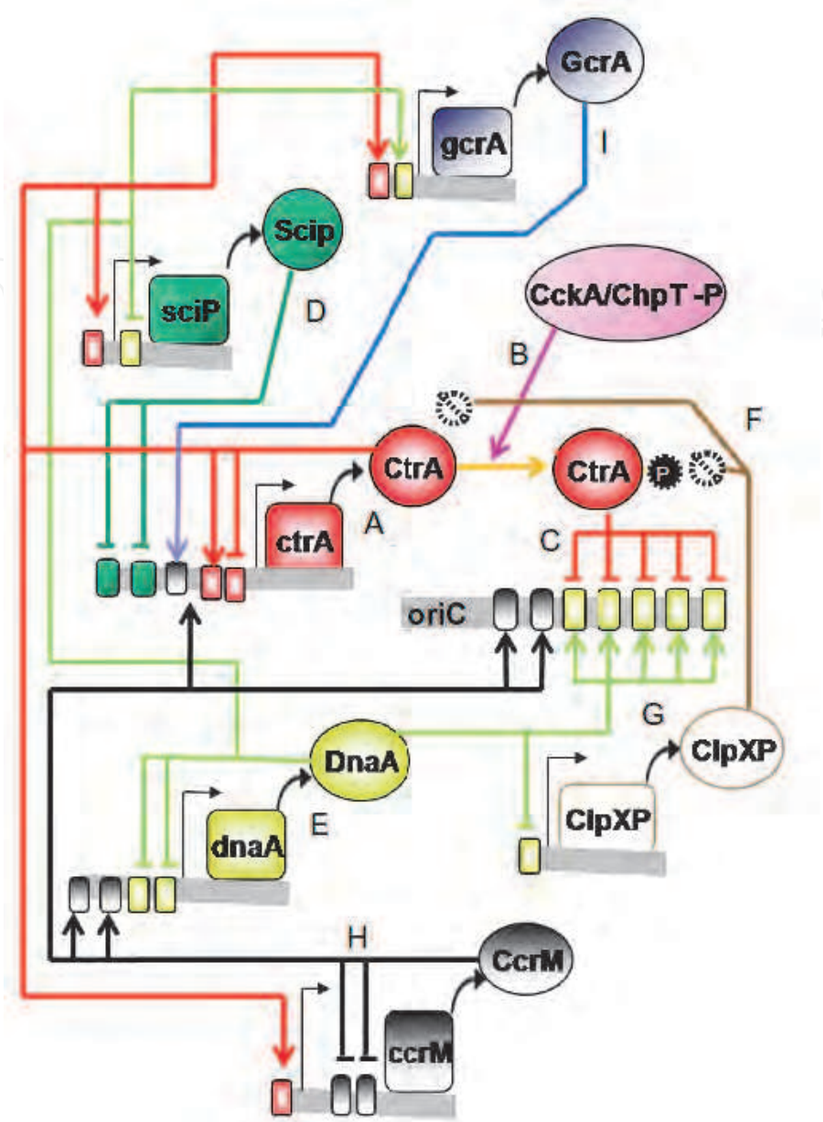


Fig. 8. Regulatory circuits that control the process of DNA-replication in *C. crescentus.* A) CtrA activates and represses the transcription of its own gene, additionally it activates *gcrA, ccrM* and *sciP.* B) CckA and ChpT transfer the phosphate group to CtrA. C) CtrA-P binds to *oriC* and inhibits the initiation of DNA replication. D) SciP represses the transcription of *ctrA*. E) DnaA auto-represses its own transcription in addition to the *clpXP* and *sciP* genes, it also actives *gcrA*. F) ClpXP degrades both CtrA and CtrA-P forms. G) DnaA binds to *oriC* to promote the initiation of DNA replication. H) CcrM methylates the GANTC sites on the regulatory regions of *dnaA, ctrA* and on its own gene, and also on the *oriC* region. I) GrcA activates the transcription of *ctrA*. Figure taken and modified from Tan et al. (2010).

## 4. The stringent response arrests DNA replication in bacteria

When bacteria are under metabolic stress, mainly in starvation conditions, they activate a regulatory mechanism called the stringent response. This response usually corresponds to

| **Regulatory systems of DNA replication** | **Regulatory genes present in the organisms** | | |
|---|---|---|---|
| | *E coli* | *B. subtilis* | *C. crescentus* |
| RIDA | Hda | YbaA * | HdaA* |
| *dnaA* gene regulation | *DnaA autoregulation, promoter methylation* | DnaA, Soj, SirA | DnaA, CcrM |
| DnaA regulation | DnaA-ATP/ADP, *datA* sequestration | DnaA-ATP/ADP | DnaA-ATP/ADP |
| *oriC* blocking | SeqA | Spo0A | CtrA |
| DNA methylation | Dam | - | Ccrm |
| Phosphorylation cascade | - | KinA, Spo0F, Spo0B | DivK, ChpT, CckA |
| Proteolysis | - | - | ClpXP |

* Orthologous to the *E. coli* components. - unidentified.

Table 2. Comparison of the controls that regulate DNA replication in *E. coli*, *B. subtilis* and *C. crescentus.*

the deprivation of amino acids, carbon, and limitations of nitrogen and phosphate. Under these conditions the cells suffer a reduction in size and restrict the content of their genetic information to only one nucleoid per cell (Schreiber et al., 1995).

The signal which triggers the stringent response is mediated by the accumulation of small-molecule nucleotides. These are guanosine tetra- and penta-phosphates; ppGpp and pppGpp (Ferullo & Lovett, 2008). These alarmones are synthesized as a response to the nutritional limitations by the proteins ReIA (synthetase I) and SpoT (synthetase II), (Bernardo et al., 2006). During the stringent conditions the elongation phase of DNA replication is inhibited because ppGpp and pppGppp specifically block the activity of the primase enzyme (DnaG). This is caused by the binding of a phosphate group of ppGpp to the primase resulting in an allosteric inhibition of the replication complex, the primase cannot therefore bind to the helicase. High cellular levels (up to millimolar concentrations) of these small nucleotides completely arrest DNA replication whereas lower levels only diminish the rate of replication (Wang et al., 2007).

Another path of regulation of DNA replication under a stringent condition is produced by the fact that the promoter of *dnaA* is also subject to the stringent response and the transcription of *dnaA* is also repressed under these conditions (Chiaramello & Zyskind, 1990; Levine et al., 1995).

## 5. DNA replication and asymmetrical bacterial cell-division

In *B. subtilis* the arrest of DNA replication takes place around the *oriC*, from the *gnt* gene on the left arm over an equal distance to the *gerD* gene of right arm, covering at least 190 kpb on both sides of the *ori*C (Levine et al., 1991). During this process the stages of chromosomal segregation in cell division differ between prespores and vegetative cells. First, the newly replicated chromosomes are attached at each of the cell poles (one pole will become the spore and the other pole the mother cell). Upon the asymmetric septation, under stress, about 30% of one of the replicated chromosomes is trapped in the prespore (Wu & Errington, 1994). The protein SpoIIIE forms a pore in the invaginating septum around the trapped DNA and permits the transfer of the remaining chromosome through the septum into the prespore (Lewis, 2001). All this produces an imbalance among regulators in the forespore and vegetative cell that results in an asymmetrical cell division in *B. subtilis*.

Another example of asymmetrical cell-division happens in *C. crescentus*, this bacterium differentiates into two different progeny: a flagellated swarmer cell and a stalked cell. The swarmer cells are incapable of replicating their DNA (prevented by the mechanisms previously mentioned in this chapter), until they differentiate into a stalked cell, this cell-type immediately enters into a new period of chromosome replication and cell division, and generates again the two cell types (Ryan & Shapiro, 2003). When *C. crescentus* is starved of carbon sources, its DnaA protein is degraded in a manner that depends on the stringent response mediated by the protein Spo, a ppGpp synthetase (Lesley & Shapiro, 2008). Additionally starvation increases the degradation of DnaA leading to the stabilization of CtrA resulting in the inhibition of DNA replication (Gorbatyuk & Marczynski, 2005).

## 6. DNA replication in bacteria with two chromosomes

Until now, in this chapter we have discussed replication focusing on bacteria with one chromosome, but some bacteria have more than one chromosome, one example of this is *Vibrio cholerae*, a human pathogen, which possesses two chromosomes, chrI and chrII (Heidelberg et al., 2000). The components and regulation of DNA replication for chrI in *V. cholera* are similar to the *oriC* of *E. coli* whereas the *oriC* of chrII shares some characteristics with plasmid replicons. Both cases (chrI and chrII) also require a specific repeated sequence for the replicative machinery (Zakrzewska-Czerwińska, et al., 2007). One of the specific requirements is that chrI initiates replication assisted only by DnaA whereas chrII requires the activity of the RctB protein that binds specifically to its *oriC* (Duigou et al., 2006), and an untranslated trans-acting RNA (*rctA*) (Egan et al., 2005). However the two chromosomes replicate synchronously although each has requirements for specific components which reduces the competition between both origins of replication for the replicative machineries (Duigou et al., 2006).

The proper regulation of DNA replication in bacteria with multiple chromosomes must involve interesting strategies to control the replication of both chromosomes. Unfortunately our knowledge about the regulation of DNA replication in these cases is poorly understood. It has been suggested that organisms with two chromosomes have an advantage for regulation of replication in some environmental conditions such as in free-living aquatic conditions or in association with a host, since faster replication of all DNA content is facilitated (Egan & Waldor, 2003).

## 7. Bacteria with multiple nucleoids

Another interesting phenomenon associated with DNA replication is endoreduplication (duplication of DNA in the absence of cell-division) as happens in the differentiation of *Rhizobium etli*, when these bacteria form a nodule and enter on it, in an endosymbiotic association with roots of leguminous plants. Irreversible cell differentiation occurs in these bacteria, which generates a nitrogen fixing bacteroid that is metabolically and morphologically different from the original pre-nodule cell. The differences between these types of cells result from cellular elongation and endoreduplication, without cell division. These bacteroid cells result from normal cells suffering repeated rounds of DNA replication and since the cell division is blocked they have multiple nucleoids (Mergaert et al., 2006). Interestingly this endoreplicative process is controlled by factors that are nodule-specific cysteine-rich (NCR) peptides generated from the host plant and targeted to the bacterial periplasm, with the ability to penetrate the bacteria membrane and function in its cytoplasm (Van de Velde et al., 2009).

## 8. Future perspectives

There are many details pending even in the best studied bacterial models. Some of the advantages of knowing in detail the replication process and its regulation are the possibilities for controlling the replication rates in bacteria, for example, to block the DNA replication of a pathogen or achieve cell-synchronization in bacterial cultures. Using this last premise, Ferullo et al. (2009), developed a method for synchronizing *E. coli* cultures, by treating the bacteria with DL-serine hydroxamate, a structural analogue of the amino acid serine, this treatment induces a natural stringent response, causing the arrest of the initiation of DNA replication, once the stringent signal is released, cells initiate a synchronized round of DNA replication.

Another advantage of knowing the details of the replication process and its regulation is to allow us to control and use it as a clock in some bio-engineered systems, an example of this is the ON and OF switch, generated by the methylated or hemimethylated state of DNA in *E. coli* (Low & Casadesús, 2008), specially at the GATC sites of the regulatory regions of many genes and the possibility of timing the replication rate in this organism.

Some organisms with reduced genomes such as the obligate endosymbionts *Baumannia cicadellinicola* and *Carsonella ruddii*, have lost most of the relevant components of the replicative machinery, such as DnaA. It is suggested that, the lack of DnaA allows the host to control DNA replication of the symbiont avoiding over-reproduction of the bacteria in its cytosol (Akman et al., 2002). Another possibility is that DNA replication happens at a low basal- rate in these stable conditions, in an unrepressed manner. It is postulated that the association between different organisms leads to adaptation in the rate of DNA replication of the bacteria in balance with the developmental status of their hosts (Gil et al., 2003).

## 9. Conclusions

The replication of DNA is a complex process in which a great number of regulators and mechanisms are involved, one of the most important is the DnaA protein. Replication normally begins by the formation of a complex of DnaA at the *oriC* region, with the assistance of DiaA, and the incorporation of some proteins that form the replisome, subsequently the formation of the open complex takes place, followed by a complex

interaction of the proteins needed to execute and complete the DNA replication. The process finalizes with the recognition of the *ter* site and disassembly of the replisome. Many of the proteins are broadly conserved within the bacteria but some special factors are required in bacteria which undergo particular processes such as asymmetrical cell division.

In general these processes are controlled by a series of circuits, which usually center on the *oriC* and affect the activity of DnaA. The result is regulation of the initiation step of DNA replication. Some of the regulatory mechanisms are time-dependent allowing only one DNA replication event per cell cycle. The methylation state of the DNA-strands is another important condition that not only controls the possibility of starting DNA replication but also regulates the transcription of many genes important for the execution of this function. All or certain of these mechanisms are adjusted under some special conditions, such as when the stringent response is triggered by amino acid starvation. In some bacteria with extremely reduced genomes it is still a mystery as to how DNA replication takes place and how it is controlled. Many of these latter organisms lack several important proteins implicated in the control and execution of DNA replication, and these bacteria can be useful as models for generating a system with the minimal components necessary for DNA replication.

## 10. Acknowledgments

Authors thank June Simpson for critical comments to the ms and David Velázquez Ramírez for assistance with Figures 1-5. This work was supported by CONACYT grant (102854) for young researchers given to AM-A. CQ-V thanks CINVESTAV-IPN and UAA for a PhD scholarship and LE-S thanks CONACYT for a PhD scholarship (208153).

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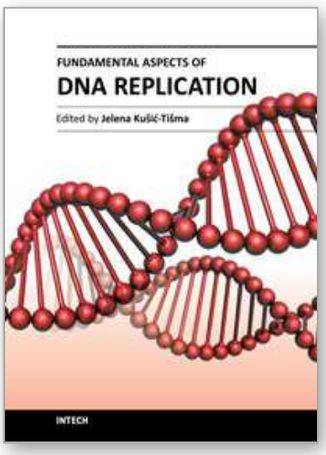


**Fundamental Aspects of DNA Replication**

Edited by Dr. Jelena Kusic-Tisma

ISBN 978-953-307-259-3

Hard cover, 306 pages

**Publisher** InTech

**Published online** 26, September, 2011

**Published in print edition** September, 2011

DNA replication, the process of copying one double stranded DNA molecule to produce two identical copies, is at the heart of cell proliferation. This book highlights new insights into the replication process in eukaryotes, from the assembly of pre-replication complex and features of DNA replication origins, through polymerization mechanisms, to propagation of epigenetic states. It also covers cell cycle control of replication initiation and includes the latest on mechanisms of replication in prokaryotes. The association between genome replication and transcription is also addressed. We hope that readers will find this book interesting, helpful and inspiring.

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