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Genetic Regulatory Network of DNA Replication Initiation in E. coli and Its Biological Significance

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Abstract. This review outlines the mechanisms and regulation of DNA replication in Escherichia coli within the oriC region, focusing on DnaA nucleotide state and stepwise occupation of high- and low-affinity boxes, origin DNA topology, and architectural proteins (IHF, Fis, SeqA). We outline helicase loading by the DnaB–DnaC complex, the role of the β-clamp and Hda in controlling reinitiation, and how small RNAs such as DsrA remodel transcriptional programs that impinge on initiation fidelity through the regulation of proteins associated with the DNA replication process and inhibition of. We discuss coupling between replication and transcription, the contribution of DNA gyrase to the need for unwound DNA to replicate, and strategies cells use to avoid replication–transcription conflicts. Finally, we highlight synthetic biology applications, including orthogonal replication systems in E. coli that decouple engineered DNA propagation from the host chromosome. These insights provide an integrated view of initiation control and suggest opportunities to engineer replication for biotechnology and genome stability research.

Keywords: DNA replication initiation, DnaA, oriC, IHF, DsrA, E. coli, orthogonal replication

1. Introduction

Arguably one of the most impactful and researched bacteria within the field of molecular biology [1], Escherichia coli's (E. coli) notable division period, in optimal conditions, of less than 20 minutes and a chromosomal replication period of approximately 40 minutes [2], has been the subject of interest due to its multiforking replication abilities.

In a bacterial chromosome, replication begins at a singular origin of replication, oriC. The oriC sequence and factors of replication initiation vary among species. In the case of E. coli, E. coli's oriC contains unique sequences for DnaA binding called DnaA boxes, which, once bound, are the main contributors to replication initiation. Alongside that, the oriC region contains an AT-rich sequence known as the DNA unwinding element (DUE), and a variety of other protein-binding sites for negative and positive regulators, all of which are actors within the replication process, as well [3,4]. In E. coli, chromosomal replication occurs bidirectionally and semiconservatively, with simultaneous rounds of replication allowing for the multiforked nature of E.coli chromosomal replication [5,6].

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2. Significance of DNA replication initiation regulation

Genomic stability relies on the accuracy of replication, with instabilities in this process posing a significant threat to chromosomal replication [7]. To prevent replication errors in the initiation process of replication, multiple regulatory safeguards are placed on the oriC. The tight regulation of the DNA replication process is primarily conducted by the periodic concentration of DnaA-ATP, which initiates replication once the quantity bound to DnaA boxes satisfies a certain threshold and falls shortly after. The fall in concentration after initiation is caused by the hydrolysis of DnaA-ATP to DnaA-ADP, while the rise in concentration is linked to the activity of the DnaA-ATP rejuvenating sequences 1 and 2, DARS1 and DARS2, respectively. While the deletion of these measures does not affect the viability of replication [4], genomic instabilities within the oriC region may jeopardize the daughter cell's DNA replication function.

Expanding on the molecular mechanism behind the binding of DnaA-ATP and its boxes to the unwinding of DNA, it is important to note that while DnaA may have similar affinities to ATP and ADP, only DnaA-ATP can bind to the numerous low-affinity and single-stranded DnaA boxes (R3, R5/M, I1, I2, I3, C1, C2, C3, τ1, and τ2), while the three high to medium-affinity DnaA boxes (R1, R4, and R2) can bind to both [4]. The drop in DnaA-ATP concentration is associated with two different conversion processes that change DnaA-ATP to DnaA-ADP: the Hda protein, which is associated with DNA-loaded β-clamp (DnaN), activates DnaA's intrinsic ATPase activity, and the IHF protein and its binding site within the datA locus, which hydrolyzes DnaA-ATP [6].

Once transcription factors activate transcription in the oriC, multiple factors alter the local DNA conformation to allow for complexes tasked with transcription to form. For the binding of DnaA to the oriC, three regulators, SeqA, factor for inversion stimulation (Fis), and IHF, affect the binding of low-affinity DnaA boxes. [8] While the protein SeqA does not play a significant role in altering DNA conformation, it negatively regulates DNA replication initiation, preventing the premature reinitiation of DNA replication at the origin, integral to upholding a tightly regulated replication system. [9] In the context of altering the DNA conformation, Fis and IHF become the main focus. DnaA and Fis binding prevent IHF from binding to its recognition site by constraining the origin with DnaA interactions. However, the incremental binding of DnaAs to boxes eventually knocks Fis off, allowing for the binding of IHF. IHF induces a ~180-degree bend within the DNA conformation that allows for interactions between DnaA-ATP and the DUE and the transfer of extra DnaA-ATP from one site to another, ensuring enough DnaA-ATP has accumulated [8].

Within bacteria, small non-coding RNA (sRNA) are responsible for multiple regulatory roles within a cell in response to changes in cellular conditions. Within E. coli, a sequence downstream of the positive regulator of capsular polysaccharide synthesis, rcsA, lies the DsrA sRNA, an 87-base sequence for an Hfq-dependent RNA that is noted to regulate multiple RNAs, including the Histone-like nucleoid structuring protein (H-NS) and the alternative sigma factor RpoS, both of which are significant contributors to regulating transcription. The Hfq chaperone protein is crucial for facilitating binding between DsrA and target mRNAs [10-12].

H-NS contains a C-terminal DNA-binding region that, once bound, reduces DNA-binding activity, therefore preventing DNA replication, after the region is oligomerized [13]. Though the mechanism behind how DsrA represses H-NS' activity remains elusive, researchers have suggested that DsrA's base pairs bind with the start codon of H-NS' mRNA to repress activity, alongside stimulating its degradation through the use of the RNA-degrading protein RNase E [14].

RpoS is crucial to transcription due to its role as a secondary sigma factor, a group of RNA polymerases tasked with determining the specificity of promoter DNA binding and initiation of transcription [15]. DsrA positively regulates RpoS by altering the RpoS mRNA to allow ribosomal

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transcription of the sequence and by preventing RpoS mRNA degradation; alongside that, it has been noted that Hfq is needed to increase the DsrA to RpoS mRNA annealing process [11].

Once the ssDNA is in an open bubble, the bubble is expanded by the presence of ATP-bound DnaC, the replicative helicase loader ATPase DnaB, from the DnaB-DnaC complex, which transfers to both ssDNA. The DnaB-DnaC complex is composed of 6 DnaC proteins tightly bound to the DnaB hexamer in the presence of ATP and Mg2+, inhibiting DnaB's DNA unwinding properties at the moment. DnaC is a dual ATP/ADP switch protein whose bound ATP is hydrolyzed to ADP in the presence of ssDNA and DnaB, thus no longer inhibiting its bound DnaB once the DnaB binds with the oriC, allowing for the assembly of two replisome complexes on opposing sides with replication forks [16-19].

Found on both replication forks and responsible for DNA synthesis, DNA Polymerase III is a complex multisubunit enzyme that is first found bound, in twos, to a DNA clamp loader. The DNA clamp loader also carries a sliding clamp. When the DNA clamp loader is in proximity of the replication fork, a sliding clamp is placed on the strands with a DNA Polymerase III, creating the complex necessary for DNA synthesis on that strand. Within the process of DNA replication, it is important to note that DnaCs are found on the strands that form lagging strands, strands of DNA with inconsistent backbones, with DNA Polymerase III, and the replication complexes on each strand are antiparallel to each other. [20-22]

Once DnaC begins cleaving the hydrogen bonds between base pairs, DNA gyrase is seen ahead of the replication fork, catalyzing the negative supercoiling in the DNA, in other words, unwinding it [19]. DNA gyrase carries out its function through a process called "sign inversion," where the A-subunit of DNA gyrase catenates and decatenates DNA's phosphodiester bonds. This process is powered and induced by ATP, whose facilitation is covered by the B-subunit of the protein [23,24].

3. Applications of understanding

With the understanding of E. coli's DNA replication system, applications of such information, such as developing orthogonal replication systems, provide many opportunities for fields of research in the sphere of microbiology. Orthogonal replication systems are engineered genetic components that achieve a systematic purpose with minimal interactions with their host cell. These systems have been a focus within the field of molecular biology in response to the challenges faced while attempting to modify a host, such as the difficulty in translating from model to reality and the finicky nature of editing a host's genome without affecting its core abilities to express and read genes. [25] In the context of E. coli, there have been recent developments in creating an orthogonal replicon for the species. In one 2024 study, a team established a stable system for orthogonal replication in E. coli, also known as EcORep. [26]

With developments like these, there arises the idea of utilizing our knowledge of DNA replication initiation onto a multitude of concepts. Take, for example, a targeted antimicrobial target that is designed to eradicate microbes by inhibiting DNA replication. Or, the possible application of DNA damage repair, noting the potential in the bridge between these two fields.

4. Conclusion

In sum, the DNA replication initiation of E. coli is a tightly regulated system with multiple regulators inhibiting and promoting different aspects of the process, ranging from the binding of DNA-ATP to boxes to the transcription of the DNA itself, to the unwinding of the DNA strand. As displayed by this research, while there is a vast amount of studies conducted on this particular

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subject, some aspects of how E. coli replication proteins interact are yet to be explained. But, as further knowledge on the mechanisms of E. coli replication comes to light, innovative applications are bound to provide leaps in the field.

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