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DNA Replication and the Transmission of Genetic Information: A Molecular Mechanism Perspective

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Abstract. DNA replication, as the foundation of genetic information transmission and the continuity of life, relies on high fidelity and dynamic regulatory mechanisms that are central to maintaining genome stability. Through literature analysis, this review systematically summarizes the core molecular mechanisms of DNA replication initiation, elongation, proofreading, and repair, while comparing the commonalities and differences between prokaryotic and eukaryotic systems. Comparative analysis demonstrates that while prokaryotic and eukaryotic DNA replication share conserved high-fidelity principles, they exhibit fundamental mechanistic divergences. Advances in technologies such as cryo-electron microscopy have uncovered the structure and dynamics of the replication machinery and its coupling with epigenetic regulation. Future research should employ integrative multi-omics approaches to decipher the spatiotemporal regulation of replication, facilitating therapeutic interventions for replication-associated pathologies including cancer.

Keywords: DNA replication, origin recognition, replication and assembly, fidelity mechanisms, replication licensing control

1. Introduction

DNA replication serves as the foundation for the continuity of life and the high-fidelity transmission of genetic information. The discovery of semiconservative replication established a fundamental paradigm in molecular biology, revolutionizing our understanding of cell cycle regulation, genome maintenance, and genetic inheritance. Precise replication machinery safeguards genomic integrity during cellular proliferation and differentiation, whereas controlled replication errors serve as substrates for evolutionary adaptation, illustrating the dual requirements of genetic stability and variability. In recent years, advanced technologies such as cryo-electron microscopy, single-molecule imaging, and high-throughput sequencing have greatly advanced our understanding of the molecular mechanisms underlying replication. Research has progressively uncovered the highly precise and dynamically regulated process that spans from origin recognition and licensing, to replication fork formation and elongation, and ultimately to polymerase proofreading and error repair [1]. While replication mechanisms in prokaryotes and eukaryotes share a conserved framework of high fidelity, they differ significantly in complexity and regulation: prokaryotic systems are structurally simple with a single origin, whereas eukaryotic systems must coordinate multiple origins and are tightly coupled with chromatin architecture and epigenetic regulation. These

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conserved and divergent features illuminate evolutionary constraints and innovations, providing mechanistic insights into genome stability maintenance. This review aims to systematically examine the core processes of DNA replication initiation, elongation, proofreading, and repair, compare the similarities and differences between prokaryotic and eukaryotic systems, and discuss current frontiers and unresolved questions while envisioning future directions. This synthesis aims to establish conceptual frameworks for future investigations while highlighting translational implications for replication-associated diseases.

2. Replication elongation and replisome assembly

2.1. Replication fork structure and replisome composition

The replisome constitutes a sophisticated macromolecular machinery comprising helicase, single-stranded DNA-binding proteins (SSB in prokaryotes/RPA in eukaryotes), primase, DNA polymerases, the sliding clamp complex with its loader, topoisomerases, and DNA ligase. In the case of bacteriophage T7, a fully functional replisome is composed of only four proteins, which together form a replication loop to coordinate the synchronous synthesis of the leading and lagging strands [2]. In bacterial replisomes, helicase, the clamp loader, and DNA polymerase are tightly coupled, thereby constructing an efficient DNA replication machine [3].

2.2. Leading- and lagging-strand synthesis mechanisms

Leading-strand synthesis proceeds continuously, while lagging-strand synthesis occurs discontinuously via Okazaki fragment formation according to the trombone loop mechanism [4]. During this process, primase synthesizes RNA primers on the unwound template strand, which are subsequently extended by DNA polymerase in a coordinated, iterative manner. Single-stranded DNA-binding proteins (SSBs) bind to the ssDNA to protect its structural integrity, thereby ensuring the efficient operation of the replication complex [5].

3. Proofreading and repair mechanisms of DNA replication

The high fidelity of DNA replication is primarily maintained through coordinated proofreading (mediated by 3′→5′ exonuclease activity) and subsequent mismatch repair (MMR), which collectively establish a dual-fidelity checkpoint [6]. DNA polymerase proofreading (3′→5′ exonuclease activity) represents the primary fidelity mechanism. This process involves continuous surveillance of the nascent strand, with DNA polymerases excising misincorporated nucleotides through their intrinsic exonuclease activity. When a nucleotide mispairing occurs, the polymerase pauses synthesis and transfers the DNA strand from the polymerization site to the exonuclease site to excise the incorrect nucleotide. In prokaryotes, the ε subunit of DNA polymerase III carries out proofreading, with the θ subunit enhancing its activity, whereas in eukaryotes, the polymerases themselves contain intrinsic proofreading domains. Complementing proofreading, the mismatch repair system (MMR) specifically targets base mispairs and insertion/deletion loops that evade initial proofreading during replication or recombination [7]. The core components of MMR differ between prokaryotes and eukaryotes. In prokaryotes (such as Escherichia coli), MutS recognizes mismatched bases; the MutS–MutL complex then activates MutH, which introduces a nick in the hemimethylated DNA strand, thereby defining the starting point for excision of the mispaired base. In contrast, eukaryotic MMR is more complex. In humans, the major MMR components include hMutSα, hMutSβ, hMutLα, hMutLβ, and hMutLγ. Similar to the prokaryotic system, eukaryotic

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MMR exhibits substrate specificity, bidirectionality, and nick-directed strand specificity. The combined action of base-pair selectivity, polymerase proofreading, and MMR reduces the overall error rate of DNA replication to approximately 10^{-8}–10^{-10} [8].

4. Comparison between prokaryotic and eukaryotic mechanisms

4.1. Similarities and differences in the process of replication elongation

4.1.1. Dominant DNA polymerases

The primary DNA polymerases exhibit significant differences between prokaryotes and eukaryotes. In prokaryotic systems, DNA polymerase III functions as the core replicative enzyme. Notably, in Escherichia coli, the DNA polymerase III holoenzyme constitutes the principal enzymatic complex for DNA replication, consisting of two polymerase cores, a sliding clamp, a clamp loader, and a complete set of ten subunits. Eukaryotic replication complexes employ functionally analogous polymerases [9]. Eukaryotic DNA replication depends on three B-family polymerases: Pol α, a heterotetramer possessing primase activity that primarily generates RNA–DNA primers during the initiation phase; Pol δ and Pol ε, which mediate primer extension, with Pol δ predominantly synthesizing the lagging strand (Okazaki fragments) and Pol ε primarily extending the leading strand [10].

4.1.2. Primer removal

During DNA replication, the RNA primers at the 5′ ends of lagging-strand Okazaki fragments must be precisely removed and replaced with DNA. This process displays marked differences between prokaryotes and eukaryotes, establishing a distinct dual-mechanism framework. In prokaryotes, a single-enzyme dual-function mechanism predominates. The core of this process is DNA polymerase I (Pol I), which, owing to its unique 5′→3′ exonuclease activity, can directly recognize and remove RNA primers. Crucially, Pol I also exhibits 5′→3′ polymerase activity, enabling it to fill the resulting DNA gaps with deoxynucleotides while concurrently removing the primer, thereby accomplishing efficient “cut-and-fill” synthesis. Once this process is complete, the final phosphodiester bond is sealed by DNA ligase [11]. In contrast, eukaryotes employ a multi-enzyme cascade mechanism. Initially, RNase H (mainly RNase H1) recognizes and degrades the RNA portion of RNA–DNA hybrids, removing most of the RNA primer fragments. Notably, RNase H2 (typically existing as a heterotrimer) is specifically recruited to replication “foci” at the replication fork through interaction with proliferating cell nuclear antigen (PCNA), efficiently initiating primer removal [12]. This is followed by a precise trimming stage: any single-stranded flaps or residual RNA/DNA fragments remaining after RNase H action are removed by FEN1 (Flap Endonuclease 1), which has 5′→3′ endonuclease activity. FEN1 functions in close coordination with DNA polymerase δ (Pol δ), the main replicative polymerase responsible for extension [13]. Pol δ not only extends subsequent Okazaki fragments, generating “flap” structures for FEN1 cleavage, but also participates (directly or via associated factors) in filling the resulting gaps [14]. Finally, as in prokaryotic systems, all remaining nicks are sealed by DNA ligase I, completing continuous lagging-strand synthesis.

4.2. Core differences in cell cycle regulation

Regarding cell cycle regulation, prokaryotes lack licensing mechanisms to ensure each replication origin activates only once per cell cycle, whereas eukaryotes employ sophisticated regulatory hubs

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[15]. In yeast, Cdc6 and Cdt1 synthesized during the G1 phase bind to ORC and load MCM2-7; during the S phase, licensing is inhibited to prevent replication re-initiation [16]. Regarding replication re-initiation prevention, prokaryotic regulatory mechanisms differ from their eukaryotic counterparts. Prokaryotes primarily rely on the activity state of the replication origin-binding protein DnaA, the methylation level of DNA, and the saturation of binding sites at the replication origin (oriC) to ensure that DNA replication occurs only once per cell cycle. In contrast, eukaryotes exhibit more stringent spatiotemporal control and isolation mechanisms. For instance, the physical barrier function of the nuclear envelope isolates replication factors; in yeast, ORC6 can also prevent MCM loading by regulating the autoinhibitory state of the ORC·Cdc6 structure, thereby effectively preventing re-replication [17].

5. Frontiers and future directions

5.1. Chromatin assembly and epigenetic inheritance

The assembly of chromatin and faithful transmission of epigenetic information constitute indispensable events during DNA replication. Replication-coupled chromatin assembly, orchestrated by the interplay between histone chaperones and the replication machinery, ensures the precise inheritance of epigenetic states. Asf1 functions as the primary acceptor of H3/H4 dimers, transferring newly synthesized histones to CAF-1. Subsequently, CAF-1 directs the deposition of these H3/H4 dimers behind the replication fork and promotes their incorporation into tetrameric nucleosome cores. Acting as a central platform, PCNA recruits CAF-1 through its PIP-box domain, thereby establishing a tight spatiotemporal coupling between DNA replication and chromatin assembly. This mechanism operates not only throughout the cell cycle but also during DNA repair, ensuring the maintenance of epigenetic integrity [18]. When replication is challenged, for instance by fork stalling, the resulting defects extend beyond DNA breakage and genomic instability. Perturbations also disrupt the balance between histone recycling and de novo deposition, ultimately leading to epigenetic instability such as heterochromatin loss or misregulation of gene silencing. These findings highlight the fundamental role of epigenome stability in safeguarding genome function across the cell cycle [19].

5.2. Mechanistic insights enabled by emerging technologies

Recent technological breakthroughs, particularly in cryo-electron microscopy (cryo-EM), have propelled major advances in elucidating the mechanisms of DNA replication. Cryo-EM has revealed the stepwise conformational transitions of the CMG helicase complex (MCM2-7–Cdc45–GINS) from ATP binding to origin unwinding, identifying the pivotal role of the MCM2 gate in mediating DNA interactions that underlie the licensing-to-activation molecular switch. For the first time, the entire transition of the pre-RC into an active helicase has been reconstructed in vitro [20]. Complementary single-molecule approaches, including fluorescence imaging and force spectroscopy (magnetic and optical tweezers), have enabled real-time visualization of replication fork dynamics. Meanwhile, genome-wide profiling methods such as iPOND and TrAEL-seq have mapped replication landscapes at high resolution [21]. Together, these approaches have not only quantified essential replication parameters, such as fork progression rates and stalling frequencies, but also predicted hotspots of replication–transcription conflicts (TRCs), uncovering their links to replication stress and genomic instability. The integration of these multi-scale technologies—from atomic-level structural insights to cell-level dynamics and genome-wide mapping—has established a

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comprehensive framework that connects the molecular mechanisms of replication with their physiological consequences.

6. Conclusion

A clear framework for the core processes of DNA replication mechanisms—from the precise recognition of origins and the efficient assembly and progression of replication forks, to the stringent proofreading and repair systems—has been established in both prokaryotes and eukaryotes. Breakthroughs in technologies such as cryo-electron microscopy and single-molecule imaging have enabled visualization of the intricate structures and dynamic changes of replication machinery (e.g., the CMG helicase), as well as how replication is tightly coupled with the transmission of epigenetic information (e.g., through CAF-1/PCNA). However, a fundamental challenge remains. The replication machinery is a massive, rapidly operating, and highly dynamic "molecular factory," and existing technical approaches are still insufficient to fully understand how its numerous proteins coordinate with precision and respond instantaneously to environmental changes. Looking ahead, the key lies in integrating multiple powerful new technologies: using single-molecule tracking to observe replication in real time, leveraging artificial intelligence and supercomputing to simulate its complex dynamics, and adopting a holistic systems biology perspective for comprehensive analysis. These efforts aim to construct a more complete and more dynamic model of the replication process. Such advancements will enhance our understanding of the root causes of diseases, particularly how replication errors lead to the development and progression of severe conditions such as cancer and premature aging. Furthermore, they will enable the development of novel therapies, providing new strategies for designing targeted drugs—for instance, interfering with the runaway replication in cancer cells or protecting healthy cells from replication stress damage. In short, future research will strive to unravel the real-time operational landscape of the replication machinery and harness this understanding to address health challenges and drive technological innovation.

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