

14 DNA METABOLISM

Executive Summary

The integrity of an organism's genome is under constant threat from both internal and external forces, yet its faithful transmission from one generation to the next is a prerequisite for life. This chapter explores the suite of sophisticated molecular processes that cells have evolved to meet this fundamental challenge. The core of this system involves three highly integrated and essential pathways: DNA Replication, DNA Repair, and DNA Recombination.

DNA Replication is the process of accurately duplicating the entire genome before cell division. It operates under a strict set of rules, ensuring each new DNA molecule is a perfect copy of the original. This process is not simply about forming chemical bonds; it is about forming the *correct* bonds with extraordinary precision, a feat carried out by a complex machinery of specialized enzymes.

DNA Repair acts as the genome's quality control system. Despite the high fidelity of replication, errors occur, and DNA is constantly damaged by spontaneous chemical reactions and environmental factors. Cells employ a diverse array of repair pathways, each tailored to recognize and correct specific types of lesions. These systems are crucial for preventing the accumulation of mutations, which are strongly linked to diseases such as cancer. However, the rare errors that escape correction are not merely failures; they provide the raw genetic variation that serves as the fuel for evolution.

DNA Recombination involves the physical exchange of DNA segments between molecules. This process serves a dual purpose: it is a powerful mechanism for repairing severe DNA damage, such as double-strand breaks, and it is a primary driver of genetic diversity during meiosis in sexually reproducing organisms.

These three processes are not isolated events but are deeply interconnected aspects of DNA metabolism. This chapter focuses on the fundamental principles governing these pathways, the key enzymatic players that execute them, and their profound relevance to both evolution and medicine.

1. The Fundamental Principles of DNA Replication

DNA replication is the foundational process of heredity, enabling a cell to pass its genetic blueprint to its daughter cells. The paramount strategic importance of this process lies in its accuracy; a failure to copy the genome faithfully can have catastrophic consequences for the organism. Early research, primarily in bacterial systems, established several universal rules that govern how this duplication is achieved across all forms of life.

Core Concepts

- **Semiconservative Replication:** As established by the Meselson-Stahl experiment, each new DNA molecule consists of one parental strand and one newly synthesized strand, ensuring that the original genetic information is conserved in each copy.
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- **Origin and Bidirectional Synthesis:** Replication does not begin randomly but initiates at a specific sequence known as an **origin**. As visualized by John Cairns in his work with *E. coli*, synthesis then proceeds in both directions away from the origin, creating two **replication forks** that move simultaneously around the chromosome. In circular chromosomes, this creates an intermediate that resembles the Greek letter theta (θ).
- **5' to 3' Synthesis and the Lagging Strand:** All known DNA Polymerases can only add new nucleotides to the 3' end of a growing DNA strand, meaning synthesis always occurs in the **5' to 3' direction**. This biochemical constraint requires an elegant solution for the antiparallel nature of the DNA double helix. One strand, the leading strand, is synthesized continuously. The other, the lagging strand, must be synthesized discontinuously in short segments known as **Okazaki fragments** (typically 150 to 200 nucleotides long in eukaryotes), which are later joined together by the enzyme DNA ligase to create a continuous strand.

These fundamental rules dictate the "what" and "how" of DNA replication. Executing these rules requires a coordinated team of sophisticated molecular machines—the enzymes of DNA metabolism.

2. The Key Enzymes of DNA Metabolism

The complex choreography of DNA metabolism is performed by a diverse cast of specialized enzymes. These molecular machines work in concert to synthesize, degrade, unwind, and ligate DNA molecules with remarkable speed and precision. This section highlights the major classes of enzymes that carry out these critical functions.

DNA Degradation and Synthesis

Enzyme Class	Primary Function & Key Details
Nucleases	These enzymes are responsible for degrading DNA by breaking phosphodiester bonds. They are broadly classified into exonucleases , which remove nucleotides from the ends of a DNA strand, and endonucleases , which cut at specific internal sites within the molecule.
DNA Polymerases	The first of these enzymes, DNA Polymerase I , was discovered by Arthur Kornberg. They catalyze the synthesis of new DNA strands. Their defining characteristic is that they require both a template strand to read and a primer to provide a free 3'-hydroxyl group onto which they can add new nucleotides.

The Replication Machinery in *E. coli*

The replication fork in *E. coli* serves as a model system for understanding the key players involved in DNA synthesis.

- **DNA Polymerase III:** Discovered after DNA Polymerase I was found to be too slow and insufficiently processive for the job, DNA Polymerase III is the principal replication enzyme in *E. coli*. It possesses the high speed and processivity (the ability to add many nucleotides without dissociating) required to duplicate the entire bacterial chromosome in a timely manner.

- **Helicase (DnaB):** This ring-shaped enzyme is responsible for unwinding the DNA double helix at the replication fork, separating the two parental strands to make them available as templates for synthesis.
- **DNA Ligase:** The "glue" of the replication machinery, DNA ligase seals the nicks between adjacent Okazaki fragments on the lagging strand, creating a continuous, unbroken phosphodiester backbone.
- **Topoisomerases (DNA Gyrase):** As helicase unwinds the DNA, it introduces torsional strain and overwinding (supercoiling) ahead of the replication fork. DNA topoisomerases, such as DNA gyrase (a **type II topoisomerase**) in *E. coli*, work to relieve this strain by transiently cutting and resealing the DNA backbone.

While these core enzymatic functions are conserved, the scale and complexity of eukaryotic genomes require additional layers of regulation and machinery.

3. Comparing Replication in Prokaryotes and Eukaryotes

While the fundamental principles of DNA replication are universal, the strategies employed by prokaryotes and eukaryotes differ significantly to accommodate the vast disparity in genome size, structure, and cellular organization. The increased complexity of the eukaryotic genome necessitates more sophisticated mechanisms for initiating, regulating, and terminating replication.

Key Distinctions

- **Origins of Replication:** A typical prokaryotic chromosome, like that of *E. coli*, has a **single origin of replication** (*oriC*). In contrast, the much larger eukaryotic genomes contain thousands of origins. Yeast has about 400 defined origins, while human chromosomes have 30,000 to 50,000. This multiplicity of origins is essential; given the slower speed of eukaryotic replication forks, copying an average human chromosome from a single origin would take over 500 hours.
- **Regulation:** Eukaryotic replication is tightly regulated to ensure that the entire genome is copied exactly once per cell cycle. This control is orchestrated by **cyclins** and **cyclin-dependent kinases (CDKs)**. These proteins trigger the formation of a **prereplicative complex (pre-RC)** at each origin, an event called **licensing**, which ensures that each origin "fires" only once. In prokaryotes, regulation is simpler, involving mechanisms such as the methylation of the origin sequence by **Dam methylase** to control the timing of initiation.
- **Termination:** In the circular *E. coli* chromosome, replication terminates when the two forks meet in a specific region containing **Ter sequences**. The Tus protein binds to these sites, and the **Tus-Ter complex** creates a trap that a replication fork can enter but cannot leave, acting as a one-way gate to ensure the forks meet in the proper region. The two newly synthesized daughter chromosomes are often interlinked (catenated) and must be separated by **DNA topoisomerase IV** before cell division can complete.

The process of copying DNA is remarkably accurate, but errors are inevitable. The focus thus shifts from synthesis to the critical mechanisms that protect the genome from damage and mistakes.

4. DNA Repair: Safeguarding the Genome

The chemical stability of DNA is finite, and the molecule is susceptible to damage from both cellular metabolism and environmental agents. A permanent change in the nucleotide sequence is called a **mutation**. While most mutations are neutral or harmful, they are also the ultimate source of genetic variation that fuels evolution. However, the accumulation of mutations in somatic cells is strongly correlated with diseases, most notably cancer. Inherited defects in DNA repair genes, such as **BRCA1 and BRCA2** which are involved in repairing double-strand breaks, are linked to a dramatically increased risk of breast and ovarian cancer. To counteract this constant threat, cells have evolved a sophisticated network of DNA repair pathways.

Major DNA Repair Pathways

- **Mismatch Repair** This system acts as a proofreader, correcting errors made during DNA replication that escape the polymerase's own error-checking mechanisms. In bacteria, the system distinguishes the newly synthesized strand from the parental template. The parental strand is methylated, while the new strand is transiently *unmethylated*. The repair machinery specifically targets the unmethylated strand, ensuring the error is corrected, not the original template. Inherited defects in this pathway are the cause of **Hereditary Nonpolyposis Colon Cancer (HNPCC)**.
- **Base-Excision Repair** This pathway targets damage to a single base, such as the deamination of cytosine to form uracil. A specific **DNA glycosylase** recognizes and removes the damaged base, creating a baseless site (an AP site). This site is then recognized by other enzymes that cut the DNA backbone, remove the abasic sugar, and fill in the gap with the correct nucleotide.
- **Nucleotide-Excision Repair** This mechanism repairs "bulky" lesions that distort the DNA double helix, such as the pyrimidine dimers formed by exposure to ultraviolet (UV) light. Instead of removing a single base, this pathway excises a short segment of the damaged strand, which is then re-synthesized using the intact complementary strand as a template. Defects in this pathway in humans lead to the genetic disorder **xeroderma pigmentosum (XP)**, characterized by extreme sensitivity to sunlight and a high incidence of skin cancer.
- **Direct Repair** In some cases, damage can be reversed directly without removing any part of the DNA. A prime example is the action of **DNA photolyases**, enzymes that use energy from visible light to directly break the covalent bonds forming pyrimidine dimers. These enzymes are common in bacteria, fungi, and plants but are notably absent in placental mammals, including humans.

Handling Extensive Damage: The SOS Response

When bacterial DNA sustains overwhelming damage that stalls replication forks, a last-resort system known as the **SOS response** is activated. This involves **error-prone translesion synthesis (TLS)**, where specialized, low-fidelity DNA polymerases replicate past the damaged sites. This process often introduces mutations, but it represents a crucial trade-off: the cell accepts a higher mutation rate for the chance to complete replication and survive.

Beyond simply repairing damage, cells can also fundamentally rearrange their DNA through the process of recombination.

5. DNA Recombination: Reshuffling and Repairing DNA

DNA recombination is a process that results in the exchange of genetic information between DNA molecules. It encompasses a family of pathways that rearrange genetic material for various purposes, from high-fidelity repair to the generation of new gene combinations. The three main classes are homologous recombination, site-specific recombination, and transposition.

Homologous Recombination: A Dual-Purpose Tool

This pathway involves the exchange of DNA segments between two molecules that share an extended region of nearly identical sequence. Its two primary roles are mechanistically related: the cellular machinery that evolved to repair broken chromosomes is co-opted during meiosis to intentionally create and then resolve breaks to generate genetic diversity.

1. **High-Fidelity DNA Repair:** Homologous recombination is the most accurate way to repair severe DNA lesions, particularly double-strand breaks. It uses an undamaged homologous chromosome or a newly replicated sister chromatid as a template to flawlessly restore the original DNA sequence at the break site. Key proteins, such as **RecA** in bacteria, are essential for mediating this strand exchange.
2. **Meiosis and Genetic Diversity:** In eukaryotes, this process is essential for **meiosis**, the specialized cell division that produces gametes. Recombination creates physical links, or **chiasmata**, between homologous chromosomes. These links are required to ensure the chromosomes align and segregate correctly into daughter cells. This shuffling of genetic material between parental chromosomes also creates new combinations of genes, dramatically increasing the genetic diversity of the offspring.

When Homologous Repair Fails

When a homologous template is not available, cells can use an alternative pathway called **Nonhomologous End Joining (NHEJ)**. This process simply ligates the two broken ends of a double-strand break back together. While effective at preventing chromosome loss, NHEJ is an error-prone process that often results in the loss or addition of nucleotides at the junction.

Programmed Recombination: Generating Antibody Diversity

The vertebrate immune system harnesses site-specific recombination to solve a complex problem: how to generate millions of different antibodies from a limited number of genes. The genes encoding immunoglobulins are stored as segments (V, J, and C segments). During the development of each B lymphocyte, a site-specific recombination event joins one V segment with one J segment, creating a unique, functional gene. For the kappa light chain, combining one of roughly 40 V segments with one of 5 J segments generates about 200 different chains. When combined with the more than 5,000 possibilities for the heavy chain, this combinatorial strategy allows the immune system to produce over **1 million different antibodies** from a surprisingly small number of gene parts.

Transposition: "Jumping Genes"

Transposons, also known as "jumping genes," are segments of DNA that can move from one location in a chromosome to another. This movement, or transposition, is mediated by an enzyme called a **transposase** and does not require sequence homology between the transposon and its new insertion site. While sometimes viewed as a form of molecular parasitism, transposition is also a major driver of genome evolution.

Ultimately, the constant interplay between high-fidelity replication, vigilant repair, and dynamic recombination is not just a maintenance program; it is the engine that drives both the stability of life and the genetic variation that fuels its evolution.
