

## Chapter 29. RNA Synthesis and Processing

### 29.1 RNA Polymerases Catalyze Transcription

The fundamental reaction of RNA synthesis is the formation of a *phosphodiester bond*. The 3'-hydroxyl group of the last nucleotide in the chain *nucleophilically* attacks the *alpha* phosphoryl group of the incoming nucleoside triphosphate with the concomitant release of a *pyrophosphate*.

The reaction is thermodynamically favorable. The catalytic sites of RNA polymerases include *two metal ions (magnesium)*. One ion remains tightly bound to the enzyme, whereas the other ion comes in with the nucleoside triphosphate and leaves with the pyrophosphate. Three conserved *aspartate* residues participate in binding these metal ions.

The polymerization reactions catalyzed by RNA polymerases takes place within a complex in DNA termed a *transcription bubble*. This complex consists of double-stranded DNA that has been locally unwound in a region of approx. 17 base *pairs*. The edges of the bases are exposed in the unwound region.

The first nucleotide (the start site) of a DNA sequence to be transcribed is denoted as +1 and the second as +2; the nucleotide preceding the start site is denoted -1. *These designations refer to the coding strand of DNA*. The *template strand* of DNA is the complement of that of the RNA transcript. The *coding strand* of DNA has the same sequence as that of the RNA transcript except for thymine in place of uracil. The coding strand is known as the *sense (+) strand*, and the template strand as the *antisense (-) strand*. *RNA synthesis can start without the requirement of a primer*. Most newly synthesized RNA chains carry a highly distinctive tag on the 5' end: the first base is either pppG or pppA.

After initiation takes place, RNA polymerase elongates the nucleic acid chain in the following manner. Ribonucleoside triphosphate binds in the active site of the RNA polymerase, directly adjacent to the growing RNA chain. *The incoming ribonucleoside triphosphate forms a base pair with the template strand. The 3'-hydroxyl group of the growing RNA chain, oriented and activated by the tightly bound metal ion, attacks the alpha-phosphoryl group to form a new phosphodiester bond, displacing pyrophosphate*. To proceed to the next step, the RNA-DNA hybrid must move relative to the polymerase to bring the 3' end of the newly added nucleotide into proper position for the next nucleotide to be added. This translocational step does not include breaking any bonds and the addition of the next nucleotide drives the polymerization reaction forward.

The DNA-RNA hybrid can also move in the opposite direction. This is very important for *proofreading*. When the wrong base is being incorporated the bonds between these bonds is less energetically costly. After the polymerase has backtracked, the phosphodiester bond one base pair before the one that has just formed is adjacent to the metal ion in the active site. *In this position, a hydrolysis reaction in which a water molecule attacks the phosphate can result in the cleavage of the phosphodiester bond and the release of a dinucleotide that includes the incorrect nucleotide*. Final rate of error: one mistake per 10<sup>5</sup> nucleotides. This is higher than that of DNA synthesis, as the consequences of mistakes are less extreme.

# Chapter 29 Rna Synthesis And Processing

**Frank Northen Magill**



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## **Table of Contents Chapter 29 Rna Synthesis And Processing**

1. Understanding the eBook Chapter 29 Rna Synthesis And Processing
  - The Rise of Digital Reading Chapter 29 Rna Synthesis And Processing
  - Advantages of eBooks Over Traditional Books
2. Identifying Chapter 29 Rna Synthesis And Processing
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Chapter 29 Rna Synthesis And Processing
  - User-Friendly Interface
4. Exploring eBook Recommendations from Chapter 29 Rna Synthesis And Processing

- Personalized Recommendations
  - Chapter 29 Rna Synthesis And Processing User Reviews and Ratings
  - Chapter 29 Rna Synthesis And Processing and Bestseller Lists
5. Accessing Chapter 29 Rna Synthesis And Processing Free and Paid eBooks
    - Chapter 29 Rna Synthesis And Processing Public Domain eBooks
    - Chapter 29 Rna Synthesis And Processing eBook Subscription Services
    - Chapter 29 Rna Synthesis And Processing Budget-Friendly Options
  6. Navigating Chapter 29 Rna Synthesis And Processing eBook Formats
    - ePub, PDF, MOBI, and More
    - Chapter 29 Rna Synthesis And Processing Compatibility with Devices
    - Chapter 29 Rna Synthesis And Processing Enhanced eBook Features
  7. Enhancing Your Reading Experience
    - Adjustable Fonts and Text Sizes of Chapter 29 Rna Synthesis And Processing
    - Highlighting and Note-Taking Chapter 29 Rna Synthesis And Processing
    - Interactive Elements Chapter 29 Rna Synthesis And Processing
  8. Staying Engaged with Chapter 29 Rna Synthesis And Processing
    - Joining Online Reading Communities
    - Participating in Virtual Book Clubs
    - Following Authors and Publishers Chapter 29 Rna Synthesis And Processing
  9. Balancing eBooks and Physical Books Chapter 29 Rna Synthesis And Processing
    - Benefits of a Digital Library
    - Creating a Diverse Reading Collection Chapter 29 Rna Synthesis And Processing
  10. Overcoming Reading Challenges
    - Dealing with Digital Eye Strain
    - Minimizing Distractions
    - Managing Screen Time
  11. Cultivating a Reading Routine Chapter 29 Rna Synthesis And Processing
    - Setting Reading Goals Chapter 29 Rna Synthesis And Processing
    - Carving Out Dedicated Reading Time
  12. Sourcing Reliable Information of Chapter 29 Rna Synthesis And Processing



- Fact-Checking eBook Content of Chapter 29 Rna Synthesis And Processing
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning
- Utilizing eBooks for Skill Development
  - Exploring Educational eBooks
14. Embracing eBook Trends
- Integration of Multimedia Elements
  - Interactive and Gamified eBooks

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