REPLICATION
INTRODUCTION TO MACROMOLECULAR SYNTHESIS

Macromolecular synthesis involves Replication (the polymerization of deoxyribonucleoside triphosphates into DNA (as the monophosphates)), Transcription (the polymerization of ribonucleoside triphosphates into RNA (as the monophosphates)), and Translation (the polymerization of amino acids into proteins).

A. Genetic Information. Genetic information stored in chromosomes (as nucleotide sequences in genes and sites) directs its own replication by:

1. Serving as template for DNA synthesis, and
2. Serving as template for messenger, transfer, and ribosomal RNA synthesis. The messenger RNA will be translated into proteins, the functions of which will be to catalyze all the reactions needed for growth, including DNA synthesis.

In replication, two complementary strands of DNA are unwound (separated) and the nucleotide sequence of each serves to direct the synthesis of complementary strands. The overall result is the production of two daughter chromosomes, each identical to the other and each identical to the parental chromosome.

In transcription, the nucleotide sequence of one strand of DNA is used to direct the polymerization of one complementary strand of RNA. Messenger RNA sequences, as codons, are translated into proteins, the functions of which are determined by their amino acid sequences. These proteins then function to catalyze all biochemical reactions of growth, including, for example, glycolysis and energy production, synthesis of low molecular weight precursors of macromolecules, and polymerization of macromolecules, including the chromosome itself. Thus, in cellular organisms, genetic information flows from DNA into RNA into Protein and the proteins then catalyze all the reactions necessary to duplicate the DNA which is to be passed on to the next generation.

B. Biochemistry.

11. Chromosomes are replicated once per cell cycle by activation of the replication origin, formation of two replication forks which move in opposite directions around the chromosome, and termination of replication. DNA polymerase III catalyzes the incorporation of deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP) onto growing 3' ends of leading and lagging strands in nucleoside transfer reactions. DNA chains are extended in the 5' to 3' direction. The anhydride bonds of the substrates yield the energy required to form the phosphodiester which link mononucleotides into DNA.

2. RNA polymerase recognizes promoters and synthesizes single strands of RNA, using the nucleotide sequence of one strand of DNA as the template from which to synthesize a complementary RNA sequence. RNA polymerase catalyzes the polymerization of ribonucleoside triphosphates, ATP, GTP, CTP, and UTP in the 5' to 3' direction. RNA polymerase has the ability to initiate new chains of RNA. That is, transcription does not require a primer, unlike replication.

3. Translation requires activated amino acids, messenger RNA, ribosomes, and energy in the form of GTP for polymerization of the amino acids. This polymerization is in the direction from the N terminus to the C terminus. The sequence of amino acids is dictated by the sequence of codons in the messenger, messengers being read 5' to 3'. Transfer RNAs serve two functions, (i) to carry activated amino acids and (ii) to serve as adaptors linking the amino acid to the codon (by codon-anticodon interaction). Peptidyl transfer reactions link the carboxyl of an amino acid or growing polypeptide to the amino group of the next amino acid to be polymerized, forming an amide bond. Amide bonds between amino acids are called peptide bonds. In prokaryotes, transcription and translation can be coupled; that is, messengers can begin to be translated while they are in the process of being transcribed. In other words, messengers which are in the process of being synthesized can be simultaneously translated.
CHROMOSOME REPLICATION

I. DNA PRECURSORS AND DNA STRUCTURE

Mononucleotides (Purines, Pyrimidines, Deoxyribose, Phosphates)
Complementary Nature of Double Stranded DNA
Antiparallel Structure; 5' > 3' and 3' > 5'
Hydrogen Bonds

II. DNA POLYMERIZATION

Nucleotidyl Transfer Reactions as General Reactions
5' > 3' Chain Elongation
Primer, Template

III. CHROMOSOME REPLICATION

Initiation; Origin
Replication Fork
Supercoils; Topoisomerase
Leading, Lagging Strands
Helicase
Primase
DNA Polymerase III
DNA Polymerase I
DNA Ligase

IV. DNA POLYMERASE III SUBUNITS

Dimeric Enzyme; Looping Model for Coordination of Leading/Lagging Strands
Processivity Clamp
Processivity Clamp Loading; Unloading
Proofreading Subunit

V. STATIONARY REPLICATION FACTORIES

VI. AZT MECHANISM
Binary Fission - Replication

3-5 x 10^6 bp
2-3 x 10^9 MW
1 x 3 μm CELL

1100 μm CHROMOSOME
DOUBLE-STRANDED
REPLICATION FORKS

Origin Activation (Melted) ONCE/CYCLE

Complementary Daughter Strands

Terminus

Daughter Chromosomes

Semi-Conservative
NUCLEOTIDES

ESTER

HO−P−O−

HO−OH

NUCLEIC ACID BASE

N−GLYCO−

SIDIC BOND

DEOXYRIBOSE

DEOXY RIBO NUCLEOSIDE

DEOXY RIBO NUCLEOSIDE MONOPHOSPHATE

OR DEOXY RIBO NUCLEOTIDE
Anhydride Bonds

Deoxyribo Nucleoside Triphosphate

= Deoxyribo Nucleotide
NUCLEIC ACID BASES

PYRIMIDINES

N

Cytosine

Thymine

Uracil
PURINES

ADENINE

GUANINE
DNA CHARACTERISTICS

DOUBLE STRANDED DNA IS DS

COMPLEMENTARY STRANDS
A \rightleftharpoons T PAIRS
G \rightleftharpoons C PAIRS

ANTI-PARALLEL ORIENTATION
5' \rightarrow 3'
3' \leftarrow 5'

HYDROGEN BONDS
A \rightleftharpoons T 2
G \rightleftharpoons C 3

DENATURATION (MELT)
HEAT
MELT TEMP < (G-C CONTENT)
BASE PAIRS - HYDROGEN BONDS

C : G PAIR  CYTOSINE:GUANINE

T : A PAIR  THYMINE:ADENINE
NUCLEOTIDYL TRANSFER REACTION

SYNTHESIS - COENZYMES; FATTY ACIDS;

PROTEIN, RNA, DNA

\[
\begin{align*}
\text{HO-PO-P-O-P-O-P-O-CH}_2 & \\
\text{R-X-O=P-O-CH}_2 & \\
\text{HO-P-O-P-OH} & \quad \text{R-X-O-P-OH} \\
\text{PPi} & \quad \text{ATP}
\end{align*}
\]

\[ \text{NUCLEOPHILIC ATTACK} \]

[OR OTHER REACTIVE OXYGEN]

PYROPHOSPHATE
DNA POLYMERIZATION

3'          5'          DNA TEMPLATE
5'          3' OH       3' OH SERVES AS PRIMER

SINGLE STRAND REGION SERVES AS TEMPLATE

dATP, dGTP, dTTP, dCTP - LOW MOLECULAR WT BUILDING BLOCKS

ENZYME DNA POLYMERASE III

3'          5'          NOBEL DNA POLYMERASE II
5'          3' OH

+ PPi
NEW STRAND 5'→3' PRIMER
...-P-O-CH₂

NEW STRAND 5'→3' PRIMER
...-P-O-CH₂

DTTP deoxyTTP

3' PARENTAL STRAND 5'

DAUGHTER STRAND
...-P-O-CN₂

+PPI

5'→3'
CHROMOSOME REPLICATION STAGES

1. INITIATION - SPECIFIC SITE ORIGIN - ORIC - ESTABLISHES REPLICATION FORKS

2. POLYMERIZATION - MOVEMENT OF REPLICATION FORKS AROUND CIRCULAR CHROMOSOME - SYNTHESIS OF NEW DAUGHTER STRANDS
   - LEADING - CONTINUOUS
   - LAGGING - DISCONTINUOUS

3. TERMINATION - INCLUDES SEPARATION OF NEW DAUGHTER CHROMOSOMES
INITIATION
SUPERCOILED DNA - TOPOISOMERASES
ORIGIN SEQUENCE
ORIGIN BINDING PROTEIN: MELTS ORIGIN
GUIDE HELICASE TO ORIGIN
HELICASE - UNWINDS DUPLEx
PRIMASE - SYNTHESIZES PRIMERS

REPLICATION FORK MOVEMENT
LEADING STRAND - CONTINUOUS
LAGGING - DIS CONTINUOUS (MULTIPLE PRIMERS)
ORIGIN

INITIATION

POLYMERIZATION

REPLICATION FORKS

COUNTER CLOCKWISE

NEW SYNTHESIS

LEADING

LAGGING

TERMINATION
LEADING + LAGGING STRANDS

DNA POLYMERASE III
OKAZAKI FRAGMENT
PRIMER [RNA]
PRIMASE

HELICASE

DNA POLYMERASE III

TEMPLATE

LAGGING STRAND ONLY:

POLYMERIZATION - DNA POL III
OKAZAKI PIECE COMPLETED, BUMPS INTO

PRIMER

PRIMER REMOVAL 1 NUCLEOTIDE AT A TIME [DNA POL I]

gap filling [DNA POL I]

dNTP
PRIMER REMOVAL; GAP FILLING PROCEED UNTIL:

THE GROWING END OF OKAZAKI PIECE 2 BUMPS INTO OKAZAKI PIECE 1 [LEAVES 5' PHOSPHATE ADJACENT TO 3' OH]

5' END OKAZAKI PIECE 1

3' END GAP FILLING-OKAZAKI 2
Phospho diester formed between two Okazaki pieces joined. Lagging strand is now intact [over this region].
DNA ligase joins adjacent 5' phosphates and 3' hydroxyls in series of steps (requires ATP)*

* OR NAD AS SOURCE OF AMP

ENZYME + ATP

OKAZAKI PIECE ONE

OKAZAKI PIECE TWO

3' PIECE ONE  5'

5'  3'

TEMPLATE
+ AMP + ENZYME
DNA POLYMERASE III - A COMPLEX ENZYME WHICH:

- POLYMERICIZES DNA STRANDS COMPLEMENTARY TO TEMPLATE

- REPLICATES LEADING AND LAGGING STRANDS TOGETHER - IT'S A DIMER

- POLYMERICIZES >10^5 NUCLEOTIDES WITHOUT DISSOCIATING FROM TEMPLATE

- CORRECTS ITS OWN MISTAKES

- CONTAINS A PROOFREADING SUBUNIT

- IT HAS A PROCESSIVITY CLAMP TO HOLD ON
A dimERIC Enzyme CoORDINATES LEADING + lagging StrandS

\[ \tau = \text{tau} \]

\( \tau \) binds itself to form dimer + binds polymerizing subunits
COORDINATING LEADING + LAGGING STRAND SYNTHESIS BY LOOPING THE LAGGING STRAND TEMPLATE

- LAGGING STRAND TEMPLATE PULLED BACKWARDS THRU Pol III UNTIL OKAZAKI PIECE IS COMPLETE
- Pol III THEN CYCLES TO PRIMER 3
Loop Model

- Okazaki Piece 2 Complete
- Pol III Cycles Toward Primer 3
- **POL III** extends primer 3
- Primer 4 has been synthesized
A clamp holds polymerizing subunit on template.

Clamp is processivity subunit.

Processivity = number of precursors polymerized without dissociating from template.

Clamps - hollow rings; encircle newly synthesized duplex; slide; bind to polymerizing subunit.
How do clamps get on duplex?
- A clamp loader loads & unloads

Clamp loader - loads clamp once for each Okazaki piece, unloads clamp when Okazaki piece is completed

Loads clamp once for leading strand
Opens + closes ring
Loading requires ATP
Proofreading - correcting last nucleotide incorporated if it is not complementary to template

ɛ - Epsilon - proofreading subunit

Epsilon - 3' to 5' exonuclease which clips out last 3' nucleotide if incorrect
Proof reading by DNA polymerase III

3' → 5' Exonuclease Activity

Growing new strand

ERROR!

3' → 5' Exonuclease

→ dCMP

Then, addition of dTTP
FACTORY

ORIGIN

STATIONARY REPLICATION
FACTORY - CHROMOSOME MOVES
AZT ACTION

REVERSE TRANSCRIPTASE

RNA:DNA

RNase H

RT

RNA:DNA

DNASE

SS RNA

RT +

AZT
NORMAL REVERSE TRANSCRIPTION

REVERSE TRANSCRIPTASE USES RNA TEMPLATE TO SYNTHESIZE DNA COPY

GROWING DNA STRAND EXTENDED BY ADDING ONE NUCLEOTIDE AT A TIME
AZT inhibits reverse transcription

1. Reverse transcriptase incorporates AZT into growing DNA strand

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\begin{align*}
\text{Parent} & \quad \text{RNA} \\
\text{G} & \quad \text{A} \\
\text{C} & \quad \text{A} \\
\text{G} & \quad \text{A} \\
\end{align*}
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Growing DNA strand cannot be extended

Growing strand cannot be extended

DNA synthesis stops