Nucleic acids - Purines
NUCLEOTIDES

- Complex nitrogen-containing molecules required for cell growth and development
  - 1. Building blocks of nucleic acids
  - 2. Important in energy transformation
  - 3. Important metabolic pathway regulators
NCLEOTIDES

Composition
- 1. Nitrogenous base
  - 1. Purines
    - Composed of a six-membered heterocyclic ring fused to a five-membered imidazole ring
      - Adenine, Guanine, Hypoxanthine
  - 2. Pyrimidines
    - Composed of a single six-membered heterocyclic ring
      - Thymine, Cytosine, Uracil
- 2. Sugar (Pentose)
  - 1. Ribose
  - 2. 2-Deoxyribose
    - Linked to the base by a β-N-glycosidic linkage
- Base + Sugar = Nucleoside
- 3. Phosphate
  - Base + Sugar + 1, 2, or 3 Phosphate = Nucleotide
Purine metabolism
Nitrogen Metabolism - Purine Metabolism

Unit 6 - Objectives

1. List the precursors that provide nitrogen and carbon atoms to the purine ring.
2. Describe the synthesis of phosphoribosyl pyrophosphate and understand its central role in nucleotide metabolism and its relationship to carbohydrate metabolism.
3. Compare the energy requirements and regulation of the AMP and GMP branches.
4. Outline the synthesis of purine nucleotides by salvage pathways; note the biochemical lesion and clinical features of Lesch-Nyhan disease.
5. Describe the degradation of dietary and cellular purine nucleotides.
6. Describe the oxidation of purines to urate and the role of xanthine oxidase.
7. List the clinical features of gout; explain the rationale for using allopurinol in its treatment.
Ribonucleotide Synthesis

ribose 5-phosphate \(\xrightarrow{\text{PRPP synthetase}}\) PRPP

5-phosphoribosyl-1-pyrophosphate

"one-carbon" pool
- glutamine
- glycine
- HCO\(_3^-\)
- aspartate

"one-carbon" pool

IMP \(\xrightarrow{\text{GMP}}\) GTP

AMP \(\xrightarrow{\text{ATP}}\)

aspartate + carbamoyl phosphate \(\xrightarrow{\text{orotate}}\) UMP

UMP \(\rightarrow\) dTMP

UTP \(\rightarrow\) CTP

urate
NUCLEOTIDE SYNTHESIS

- May occur by either *de novo* synthesis or in a “salvage” pathways

- **Purine Biosynthesis**
  - Begins with **Ribose-5-phosphate** from PPP
  - Converted into **Phosphoribosyl pyrophosphate (PRPP)**
  - **Phase I**
    - Conversion of PRPP into **Inosine monophosphate**
    - The first purine nucleotide formed
    - Conversion of PRPP into 5-phosphoribosylamine
      - Committed step in purine biosynthesis
      - Source of atoms to construct the purine nucleus
      - N - Gln, Gly, Asp
      - C - Gly, N$^{10}$-formyl THF, CO$_2$
NUCLEOTIDE SYNTHESIS

- **Phase II**
  - Conversion of IMP into AMP or GMP
    - AMP
      - Amino group from Asp replaces keto group of IMP
      - Energy source for reaction: GTP
    - GMP
      - Addition of amino group from Gln to IMP

- The complete synthesis of AMP requires energy equivalent to 7 ATP
- The complete synthesis of GMP requires energy equivalent to 8 ATP
NUCLEOSIDE TRIPHOSPHATE FORMATION

1. AMP + ATP $\rightarrow$ 2 ADP
   - Enzyme: Adenylate kinase

2. Other nucleoside diphosphates
   - NMP + ATP $\leftrightarrow$ NDP + ADP
     - Enzyme: Nucleoside monophosphate kinases

3. Nucleoside triphosphates
   - $N_1$DP + $N_2$TP $\leftrightarrow$ $N_1$TP + $N_2$DP
     - Enzyme: Nucleoside diphosphate kinase
Ribose 5-phosphate + ATP $\xrightarrow{\text{Ribose phosphate pyrophosphokinase}}$ 5-Phosphoribosyl-1-pyrophosphate (PRPP)

PRPP + Glutamine $\xrightarrow{\text{Amidophosphoribosyl transferase}}$ Glutamate + 5-Phosphoribosyl-1-amine + $\text{PP}_i$
5-Phosphoribosyl-1-amine \( \overset{\text{2}}{\rightarrow} \) Glycinamide ribonucleotide \( \overset{\text{3}}{\rightarrow} \) Formylglycinamide ribonucleotide

\[ \text{HC} = \text{O} \]
\[ \text{HN} = \text{C} \]
\[ \text{NH} \]
\[ \text{CH}_2 \]
\[ \text{Ribose -P} \]

5-Aminimidazole ribonucleotide \( \overset{\text{5}}{\rightarrow} \) Formylglycinimidine ribonucleotide
Schematic Representation of Purine Nucleotide Synthesis

NH₂, ATP, Glycine → Ribose 5-P → 5-Phosphoribosylamine

5,10-methenyl THF → R 5-P

Glutamine, ATP → R 5-P

CO₂ → R 5-P

Aspartate, ATP → R 5-P

IMP
Purine Biosynthesis:

5-Aminoimidazole ribonucleotide is carboxylated and this is followed by the addition of an aspartic acid to form 5-Aminoimidazole-4-N-succinocarboxamide ribonucleotide. Succinate is released and the resulting 5-Aminoimidazole-4-carboxamide ribonucleotide. The addition of a C-1 fragment and ring closure produces the first purine.
Inosinate (IMP) → Fumarate

Adenylosuccinate → Adenylate (AMP)

Inosinate (IMP) → NADH + H+

Xanthylate → Glutamine + ATP

Glutamine + ATP → Guanylate (GMP)
REGULATION OF PURINE NUCLEOTIDE BIOSYNTHESIS

- Overall pathway regulation occurs at:
  1. Ribose-5-phosphate pyrophosphokinase
  2. Glutamine-PRPP amidotransferase
  Classical End-product inhibition
     - The combined inhibitory effect of the end products is synergistic
     - The net inhibition is greater than the inhibition of each nucleotide acting alone

- Secondary regulation occurs at the IMP branch point
  - Each nucleotide regulates its own synthesis

- ATP and GTP hydrolysis drives the synthesis of the corresponding nucleotide
Control of purine biosynthesis

AMP AND GMP BIND TO SEPARATE SITES AND ARE SYNERGISTIC
PURINE SALVAGE PATHWAY

- Purine bases are retrieved from normal turnover of cellular nucleic acids or the diet
  - Provides an important source of purine nucleotides
    - Avoids the "Energetically Expensive" biosynthesis route

Enzymes

1. Hypoxanthine-guaninephosphoribosyl transferase
   - Reaction is irreversible
   - Hypoxanthine + PRPP $\rightarrow$ IMP + PP$_1$
   - Guanine + PRPP $\rightarrow$ GMP + PP$_1$

2. Adenine phosphoribosyltransferase
   - Reaction is irreversible
   - Adenine + PRPP $\rightarrow$ AMP + Pp$_1$
Purine Nucleotide Salvage Pathways

PRPP

HYPOXANTHINE-GUANINE PHOSPHORYL TRANSFERASE OR ADENINE PHOSPHORIBOSYL TRANSFERASE

PURINE RIBONUCLEOTIDE
Lesch-Nyhan Syndrome

The Lesch-Nyhan syndrome is characterized clinically by hyperuricemia, excess uric acid production and neurological problems, which may include spasticity, mental retardation and self-mutilization. This disorder is associated with a very severe or complete deficiency of HGPRTase activity. This is an "X" linked gene and thus the complete deficiency is almost always seen in males. It has been observed that if the HGPRTase activity is less than 2% of normal mental retardation is observed and if it is less than 0.2% of normal, the self mutilation is often seen. There seem to be several variants of this syndrome due to different mutations or enzyme defects. It is not clear why a deficiency in this salvage pathway should lead to neurological problems of this magnitude. The adenine phosphoribosyltransferase activity in these patients is normal or even elevated and thus an alternate reclamiation for purines in these individuals is available. A clue to this puzzle may lie in the observation that the concentration of HGPRTase is found to be 10 - 20 times as high in parts of the brain and CNS as in the liver or most other tissues. Thus the CNS may be particularly dependent on the HGPRT salvage pathway. While allopurinol can be used to lower the uric acid levels in these patients, it does not relieve the neurological symptoms. These patients often die from kidney failure resulting from sodium urate deposits.

We obviously don't understand all that is going on here.
AMP → IMP → Hypoxanthine → Xanthine → Uric Acid

Xanthine Oxidase
Purine catabolism leads to hypoxanthine and then xanthine and uric acid. Uric acid is the primary nitrogenous excretion product derived from purines in humans. Abnormalities in biosynthesis, uric acid production or salvage can result in high levels of uric acid and the condition called Gout. Gout is due to elevated levels of uric acid and its limited solubility.
1. Elevated levels of phosphoribosyl pyrophosphate (PRPP)

2. Enhanced purine synthesis

3. Apparent loss of feedback inhibition

4. Partial deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRT) (salvage pathway)

5. Increased levels of uric acid exceed solubility of urate and leads to crystalization in joints and other areas

6. If HGPRT deficiency is total or near total a more serious consequence occurs in the form of Lesch-Nyhan Syndrome an X-linked genetic disorder with serious neurological consequences.
Allopurinol - a suicide Inhibitor used to treat Gout

Allopurinol → Xanthine Oxidase → Alloxanthine (The principal inhibitor)

Guanine → Xanthine → Urate

Hypoxanthine → Inhibited by allopurinol
Nucleic acids -2
Metabolism
Nitrogen Metabolism - Pyrimidine Metabolism

Unit 7 - Objectives

1. List the precursors that provide nitrogen and carbon atoms to the pyrimidine ring.

2. Explain the role of carbamoyl phosphate in pyrimidine synthesis; compare with its role in urea synthesis.

3. Compare the de novo purine and pyrimidine nucleotide synthetic pathways.

4. Note how nucleoside monophosphates acquire additional phosphoryl groups.

5. Describe the reaction catalyzed by ribonucleotide reductase and its regulation.

6. Relate the roles of thymidylate synthase and dihydrofolate reductase in the synthesis of TMP.

7. Account for the action of certain chemotherapeutic agents in altering purine
Pyrimidine Biosynthesis

Carbamoyl Phosphate Formation in the cytosol initiates the pathway, using a different enzyme from that used in the urea cycle and using glutamine as the nitrogen donor rather than ammonium ion. This enzyme is designated as carbamoyl phosphate synthetase II.

Glutamine + 2 ATP + HCO₃⁻ → Carbamoyl phosphate + 2 ADP + Pi + glutamate

\[
\begin{align*}
\text{Carbamoyl phosphate} &\quad \rightarrow \\
\text{Aspartate} &\quad \rightarrow \\
\text{N-Carbamoylaspartate}
\end{align*}
\]
- Pyrimidine biosynthesis begins with the formation of carbamoyl phosphate from glutamine and CO2. The enzyme which catalyzes this reaction occurs in the cytosol as opposed to the carbamoyl phosphate synthetase of the urea cycle which occurs in the mitochondrion. The second step in this pathway involves the formation of N-carbamoylaspartate.
<table>
<thead>
<tr>
<th></th>
<th>Carbamoyl Phosphate Synthetase I</th>
<th>Carbamoyl Phosphate Synthetase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Distribution</td>
<td>liver (primarily)</td>
<td>all</td>
</tr>
<tr>
<td>Cellular Location</td>
<td>mitochondrion</td>
<td>cytosol</td>
</tr>
<tr>
<td>Metabolic Pathway</td>
<td>urea synthesis</td>
<td>pyrimidine biosynthesis</td>
</tr>
<tr>
<td>Source of Nitrogen</td>
<td>ammonium ion</td>
<td>amide group of glutamine</td>
</tr>
</tbody>
</table>
This panel compares the two carbamoyl phosphate synthetases found in humans in terms of tissue location, cellular location and pathway identification. Note that the two enzymes derive their nitrogen from different sources (ammonium ion in the case of urea synthesis and glutamine in the case of pyrimidine biosynthesis.)
N-Carboxylaspartate

Orotate

Dihydroorotate

Dihydroorotate dehydrogenase

mitochondrial enzyme
• N-carbamoylaspartate can be cyclized by dihydroorotase to form dihydroorotate and subsequently orotate. Orotic acid contains the basic pyrimidine ring structure.
• Orotic acid can be converted to a nucleotide, orotidylate, with the transfer of a ribose and phosphate group from PRPP. Decarboxylation of orotidylic acid yields UMP or uridine monophosphate, a precursor of the other commonly found pyrimidine nucleotides.
Amidation at the Nucleoside Triphosphate Level

Uridine triphosphate (UTP)

Glutamine + ATP + H₂O → Glutamate + ADP + P + 2H⁺

Cytidine triphosphate (CTP)
Following its conversion to the trinucleotide level (UTP), uridine monophosphate may be converted to CTP to provide the other common pyrimidine ribonucleotide. Note that glutamine is required for this conversion.
Ribonucleotide reductase catalyzes the conversion of ribonucleoside diphosphate to 2'-deoxyribonucleoside diphosphate. The reaction involves thioredoxin (reduced) and FAD, with NADPH and FADH₂ acting as electron donors and acceptors, respectively.
In order to generate the deoxyribonucleotide building blocks of DNA, ribonucleotides must be converted to 2-deoxyribonucleotides. This process takes place at the ribonucleoside diphosphate level and is carried out by ribonucleotide reductase. This enzyme complex contains thioredoxin and also requires FAD and NADPH.
dTMP Synthesis at the Nucleoside Monophosphate Level

deoxyuridine monophosphate (dUMP) → deoxythymidine monophosphate (dTMP)

N⁵,N¹⁰-Methylene tetrahydrofolate → Dihydrofolate
While deoxyCDP can be produced directly by ribonucleotide reductase, deoxythymidine is also required for DNA synthesis. The production of dTMP from dUMP is catalyzed by thymidylate synthetase. Note that the nucleotide phosphorylation is at the mono phosphate level and that the methyl group of thymidine is derived from a tetrahydrofolate derivative.
dTMP Synthesis at the Nucleoside Monophosphate Level

deoxyyuridine monophosphate (dUMP)

N$_5$,N$_{10}$- Methylene tetrahydrofolate

Dihydrofolate

deoxythymidine monophosphate (dTMP)
<table>
<thead>
<tr>
<th></th>
<th>Purine Pathway</th>
<th>Pyrimidine Pathway</th>
</tr>
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<tbody>
<tr>
<td>Synthetic sequence</td>
<td>1. N-glycosidic bond</td>
<td>1. ring assembly &amp; closure</td>
</tr>
<tr>
<td></td>
<td>2. Ring assembly &amp; closure</td>
<td>2. N-glycosidic bond</td>
</tr>
<tr>
<td></td>
<td>Formation of 5'-phosphoribosylamine (PRPP amidotransferase)</td>
<td>Formation of N-carbamoyl aspartate (aspartate transcarbamoylase)</td>
</tr>
<tr>
<td>Cellular location</td>
<td>Cytosol</td>
<td>Mitochondrion and cytosol</td>
</tr>
<tr>
<td>Enzyme organization</td>
<td>Individual and multifunctional (3)</td>
<td>Individual and multifunctional (2)</td>
</tr>
<tr>
<td>Regulation</td>
<td>Feedback inhibition by IMP, AMP and GMP at multiple sites</td>
<td>Feedback inhibition by UTP of carbamoyl-P synthetase</td>
</tr>
</tbody>
</table>
This panel shows a comparison of the purine and pyrimidine biosynthetic pathway. Both of these pathways must function normally in order to allow normal cell function and DNA replication during cell division.
THYMIDINE → DIHYDROTHYMIDINE → N-CARBAMOYLISOBUTYRATE → BETA-AMINOISOBUTYRATE → METHYLMALONYL CoA → Succinyl CoA
The general scheme of degradative metabolism for each of the thymidine ring structure is shown in this panel. Note that the end products are methylmalonyl CoA and subsequently succinyl CoA which can feed into central metabolism via the TCA cycle.
The general scheme of degradative metabolism for each of the pyrimidine ring structures is shown in this panel. Note that the end products are acetyl CoA or methylmalonyl CoA for uracin and thymine, respectively.
Nucleic acids - 3
DNA Structure & Chemistry
UNIT 1

THE FLOW OF GENETIC INFORMATION

LECTURES:
1. DNA Structure and Chemistry
2. Genomic DNA, Genes, Chromatin
3. DNA Replication, Mutation, Repair
4. RNA Structure and Transcription
5. Eukaryotic Transcriptional Regulation
6. RNA Processing
7. Protein Synthesis and the Genetic Code
8. Protein Synthesis and Protein Processing
THE FLOW OF GENETIC INFORMATION

DNA → RNA → PROTEIN

1. REPLICATION (DNA SYNTHESIS)
2. TRANSCRIPTION (RNA SYNTHESIS)
3. TRANSLATION (PROTEIN SYNTHESIS)
1. DNA Structure and Chemistry

a). Evidence that DNA is the genetic information
   i). DNA transformation
   ii). Transgenic experiments
   iii). Mutation alters phenotype

b). Structure of DNA
   i). Structure of the bases, nucleosides, and nucleotides
   ii). Structure of the DNA double helix
      3’, 5’-phosphodiester bond
      polarity of the polynucleotide chains
      hydrogen bonding of the bases
      specificity of base pairing
      complementarity of the DNA strands

c). Chemistry of DNA
   i). Forces contributing to the stability of the double helix
   ii). Denaturation of DNA
      hyperchromicity
      melting curves and $T_m$
i). DNA transformation: in vivo experiment

Mice are injected either with Type R, non-virulent Streptococcus or with heat-killed, virulent Type S cells.

The mice are healthy.
• Mice are injected with both Type R, non-virulent and heat-killed, Type S Streptococcus DNA carrying genes from the virulent, heat-killed cells transforms the non-virulent bacterial cells, making them lethal to the mice
DNA transformation: in vitro experiment

Type R cells

Type R colonies

Type S cells

Type S colonies

Type R cells + DNA from Type S cells

Mixture of Type R and Type S colonies
Genotype: An organism’s genetic constitution.

Phenotype: The observed characteristics of an organism, as determined by the genetic makeup (and the environment).

• DNA from Type S cells (thus conferring the Type S genotype) transformed Type R cells into cells having the Type S phenotype
ii). Transgenic experiments

Plasmid DNA carrying the growth hormone gene injected into the nucleus of a fertilized mouse egg. The egg is then implanted into the uterus of a surrogate mother mouse, who gives birth to a transgenic mouse.
Mouse with growth hormone transgene

Normal mouse
iii). Mutation alters phenotype

- Phenotypic differences between individuals are due to differences between their genes
- These differences have arisen by mutation of DNA over many thousands of years
• Some inherited differences are less severe
  - rates of aging
  - drug responses

• Others are more severe
  - debilitating inherited diseases
b). Structure of DNA

i). Structure of the bases, nucleosides, and nucleotides

Purines

- Adenine (A)
- Guanine (G)

Pyrimidines

- Thymine (T)
- Cytosine (C)

5-Methylcytosine (5mC)
Nomenclature

<table>
<thead>
<tr>
<th></th>
<th>Nucleoside</th>
<th>Nucleotide</th>
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<tbody>
<tr>
<td><strong>Base</strong></td>
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<tr>
<td>Purines</td>
<td></td>
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</tr>
<tr>
<td>adenine</td>
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<td>guanine</td>
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<td>hypoxanthine</td>
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<td>Pyrimidines</td>
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<td>thymine</td>
<td>thymidine</td>
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</tr>
<tr>
<td>cytosine</td>
<td>cytidine</td>
<td></td>
</tr>
<tr>
<td>uracil</td>
<td>uridine</td>
<td></td>
</tr>
</tbody>
</table>

+ribose +phosphate
• polynucleotide chain
• 3',5'-phosphodiester bond
• hydrogen bonding of the bases

A-T base pair

Chargaff's rule: The content of A equals the content of T, and the content of G equals the content of C in double-stranded DNA from any species

G-C base pair
Double-stranded DNA

- Specificity of base pairing
- Complementarity of the DNA strands
- B-DNA has 10 base-pairs per turn
- DNA with fewer or more bp per turn is “supercoiled”
• base pairing during DNA synthesis

Parental DNA strands → Daughter DNA strands

• base pairing during RNA synthesis
DNA that is over- or underwound is “supercoiled”

• positive supercoiling results from overwinding DNA and normally occurs during DNA replication

• negative supercoiling results from underwinding DNA and normally occurs in the nucleosome

• negative supercoiling can give rise to Z-DNA
  • Z-DNA is a left handed helix with zigzagged (hence Z) phosphates
  • Z-DNA occurs where there are alternating pyrimidines and purines (on one strand)
  • the transition of B- to Z-DNA is facilitated by 5-methylcytosine

• negative supercoiling may affect RNA synthesis
  • by promoting Z-DNA formation
  • by making it easier to separate the DNA strands
• antiparallel polarity of the polynucleotide chains
• Nucleases hydrolyze phosphodiester bonds

Endonucleases cleave internally and can cut on either side of a phosphate leaving 5’ phosphate or 3’ phosphate ends depending on the particular endonuclease.

e.g., proofreading exonucleases

e.g., restriction endonucleases
c). Chemistry of DNA

i). Forces affecting the stability of the DNA double helix

- hydrophobic interactions - stabilize
  - hydrophobic inside and hydrophilic outside
- stacking interactions - stabilize
  - relatively weak but additive van der Waals forces
- hydrogen bonding - stabilize
  - relatively weak but additive and facilitates stacking
- electrostatic interactions - destabilize
  - contributed primarily by the (negative) phosphates
  - affect intrastrand and interstrand interactions
  - repulsion can be neutralized with positive charges (e.g., positively charged Na$^+$ ions or proteins)
Hydrophobic core region

Hydrophilic phosphates

Hydrophilic phosphates
Stacking interactions

Charge repulsion
Model of double-stranded DNA showing three base pairs
ii). Denaturation of DNA

Double-stranded DNA

Extremes in pH or high temperature → A-T rich regions denature first

Cooperative unwinding of the DNA strands

Strand separation and formation of single-stranded random coils

Extremes in pH or high temperature

A-T rich regions denature first

Cooperative unwinding of the DNA strands

Strand separation and formation of single-stranded random coils
Electron micrograph of partially melted DNA

- A-T rich regions melt first, followed by G-C rich regions

A-T rich region of DNA has melted into a single-stranded bubble

Double-stranded, G-C rich DNA has not yet melted
The absorbance at 260 nm of a DNA solution increases when the double helix is melted into single strands.
- DNA melting curve

- $T_m$ is the temperature at the midpoint of the transition
• $T_m$ is dependent on the G-C content of the DNA

E. coli DNA, which is 50% G-C, has a $T_m$ of 69 °C.

• average base composition (G-C content) can be determined from the melting temperature of DNA
Nucleic acids - 4
DNA Replication, Mutation, Repair
3. DNA Replication, Mutation, Repair

a). DNA replication
   i). Cell cycle/ semi-conservative replication
   ii). Initiation of DNA replication
   iii). Discontinuous DNA synthesis
   iv). Components of the replication apparatus

b). Mutation
   i). Types and rates of mutation
   ii). Spontaneous mutations in DNA replication
   iii). Lesions caused by mutagens

c). DNA repair
   i). Types of lesions that require repair
   ii). Mechanisms of repair
      - Proofreading by DNA polymerase
      - Mismatched repair
      - Excision repair
   iii). Defects in DNA repair or replication
The mammalian cell cycle

- **G0**: Quiescent cells
- **G1 phase**: Rapid growth and preparation for DNA synthesis
- **S phase**: DNA synthesis and histone synthesis
- **G2 phase**: Growth and preparation for cell division
- **M phase**: Mitosis
DNA replication is semi-conservative

Parental DNA strands → Daughter DNA strands

Each of the parental strands serves as a template for a daughter strand
Origins of DNA replication on mammalian chromosomes

- Origins of DNA replication (every ~150 kb)
- Bidirectional replication
- Fusion of bubbles
- Daughter chromosomes
Primasome

dna B (helicase)
dna C
dna G (primase)

5’

3’

OH

RNA primer
(~5 nucleotides)
Reaction catalyzed by DNA polymerase

- all DNA polymerases require a primer with a free 3’ OH group
- all DNA polymerases catalyze chain growth in a 5’ to 3’ direction
- some DNA polymerases have a 3’ to 5’ proofreading activity
Discontinuous synthesis of DNA

Because DNA is always synthesized in a 5’ to 3’ direction, synthesis of one of the strands...

...has to be discontinuous.

This is the lagging strand.
Each replication fork has a leading and a lagging strand

- The leading and lagging strand arrows show the direction of DNA chain elongation in a 5’ to 3’ direction
- The small DNA pieces on the lagging strand are called Okazaki fragments (100-1000 bases in length)
RNA primer

replication fork

direction of leading strand synthesis

3’ 5’

3’ 5’

direction of lagging strand synthesis
Movement of the replication fork
Movement of the replication fork

5’ RNA primer Okazaki fragment RNA primer
Defects in DNA repair or replication

All are associated with a high frequency of chromosome and gene (base pair) mutations; most are also associated with a predisposition to cancer, particularly leukemia

• Xeroderma pigmentosum
  • caused by mutations in genes involved in nucleotide excision repair
  • associated with a 2000-fold increase of sunlight-induced skin cancer and with other types of cancer such as melanoma

• Ataxia telangiectasia
  • caused by gene that detects DNA damage
  • increased risk of X-ray
  • associated with increased breast cancer in carriers

• Fanconi anemia
  • increased risk of X-ray
  • sensitivity to sunlight

• Bloom syndrome
  • caused by mutations in a DNA helicase gene
  • increased risk of X-ray
  • sensitivity to sunlight

• Cockayne syndrome
  • caused by a defect in transcription-linked DNA repair
  • sensitivity to sunlight

• Werner’s syndrome
  • caused by mutations in a DNA helicase gene
  • premature aging
Nucleic acids -5
RNA Structure and Transcription
4. RNA Structure and Transcription

a). Chemistry of RNA
   i). Bases found in RNA
   ii). Ribose sugar
   iii). RNA polynucleotide chain
   iv). Secondary and tertiary structure

b). Characteristics of prokaryotic RNA
   i). Classes of prokaryotic RNA
   ii). Structure of prokaryotic messenger RNA

c). Transcription initiation in prokaryotes
   i). Transcription
   ii). Promoter structure
   iii). Prokaryotic RNA polymerase structure
   iv). Initiation of transcription and the sigma cycle

d). Regulation of the lactose operon
   i). Function of the lactose operon
   ii). Negative control: Lac repressor and inducer
   iii). Postive control: CAP and cAMP
The major bases found in DNA and RNA

DNA
- Adenine
- Cytosine
- Guanine
- Thymine

RNA
- Adenine
- Cytosine
- Guanine
- Uracil (U)

Thymine-adenine base pair
Uracil-adenine base pair
Examples of modified bases found in RNA

Dihydrouridine  Pseudouridine  1-methylguanosine  7-methylguanosine
1-methyladenosine  2-thiocytidine  5-methylcytidine  Ribothymine
RNA polynucleotide chain

• 2' -OH makes 3', 5' phosphodiester bond unstable
Secondary structure

Tertiary structure
Classes of prokaryotic RNA

- ribosomal RNA (rRNA)
  - 16S (small ribosomal subunit)
  - 23S (large ribosomal subunit)
  - 5S (large ribosomal subunit)
- transfer RNA (tRNA)
- messenger RNA (mRNA)

Structure of prokaryotic messenger RNA

The Shine-Dalgarno (SD) sequence base-pairs with a pyrimidine-rich sequence in 16S rRNA to facilitate the initiation of protein synthesis.
Transcription

- Closed promoter complex
- RNA polymerase
- Open promoter complex

initiation

elongation

termination

RNA product
Promoter structure in prokaryotes

**-30 region**
TTGACA
AACTGT

**-10 region**
TATAAT
ATATTA

**transcription start site**

**Pribnow box**

<table>
<thead>
<tr>
<th>Letter</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>T</td>
<td>82</td>
<td>84 79 64 53 45%</td>
</tr>
<tr>
<td>G</td>
<td>64</td>
<td>82 41 95 96%</td>
</tr>
<tr>
<td>A</td>
<td>79</td>
<td>79 95 44 59 51 96%</td>
</tr>
<tr>
<td>C</td>
<td>53</td>
<td>53 79 82 31 45%</td>
</tr>
<tr>
<td>A</td>
<td>45</td>
<td>45 53 79 82 31 45%</td>
</tr>
</tbody>
</table>
Prokaryotic RNA polymerase structure

RNA polymerase of E. coli is a multisubunit protein

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Number</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>2</td>
<td>uncertain</td>
</tr>
<tr>
<td>β</td>
<td>1</td>
<td>forms phosphodiester bonds</td>
</tr>
<tr>
<td>β’</td>
<td>1</td>
<td>binds DNA template</td>
</tr>
<tr>
<td>σ</td>
<td>1</td>
<td>recognizes promoter and facilitates initiation</td>
</tr>
</tbody>
</table>

\[
\alpha_2\beta\beta’\sigma \rightleftharpoons \alpha_2\beta\beta’ + \sigma
\]

holoenzyme \quad \text{core polymerase} \quad \sigma \quad \text{holoenzyme} \quad \text{core polymerase} \quad \sigma

\text{sigma factor}
The function of sigma factor

- the sigma subunit of RNA polymerase is an “initiation factor”
- there are several different sigma factors in E. coli that are specific for different sets of genes
- sigma factor functions to ensure that RNA polymerase binds stably to DNA only at promoters
  - sigma destabilizes nonspecific binding to non-promoter DNA
  - sigma stabilizes specific binding to promoter DNA
  - this accelerates the search for promoter DNA

<table>
<thead>
<tr>
<th>DNA Type</th>
<th>$K_a \ (M^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any DNA (nonspecific)</td>
<td>$2 \times 10^{11}$</td>
</tr>
<tr>
<td>Promoter DNA (specific)</td>
<td>$10^{13}$ to $10^{15}$</td>
</tr>
</tbody>
</table>

- promoters vary in “strength” by ~two orders of magnitude
RNA polymerase holoenzyme (+ $\sigma$ factor)

- closed promoter complex (moderately stable)
- the sigma subunit binds to the -10 region

- open promoter complex (highly stable)
- the holoenzyme has very high affinity for promoter regions because of sigma factor

- once initiation takes place, RNA polymerase does not need very high affinity for the promoter
- sigma factor dissociates from the core polymerase after a few elongation reactions

- elongation takes place with the core RNA polymerase

The sigma cycle

- sigma can re-bind other core enzymes
Mechanism of RNA synthesis

- RNA synthesis usually initiated with ATP or GTP (the first nucleotide)
- RNA chains are synthesized in a 5’ to 3’ direction
- Termination of some transcripts makes use of the Rho protein, which is a termination factor that catalyzes the dissociation of the RNA and polymerase.
The lactose operon in E. coli

- promoter binds CAP and RNA polymerase
- operator binds the lac repressor

- the function of the lactose (lac) operon is to produce the enzymes required to metabolize lactose for energy when it is required by the cell.
Regulation of the lactose operon - negative control

**promoter - operator**

| lac I | P | O | lac Z | lac Y | lac A |

- lac repressor

- **the repressor tetramer** binds to the operator and prevents RNA polymerase from binding to the promoter

| lac I | P | lac Z | lac Y | lac A |

- RNA polymerase is blocked from the promoter
Alleviation of negative control - action of the inducer of the lac operon

- when lactose becomes available, it is taken up by the cell
- allolactose (an intermediate in the hydrolysis of lactose) is produced
- one molecule of allolactose binds to each of the repressor subunits
- binding of allolactose results in a conformational change in the repressor
- the conformational change results in decreased affinity of the repressor for the operator and dissociation of the repressor from the DNA

IPTG (isopropyl thiogalactoside) is also used as a (non-physiological) inducer
- repressor (with bound allolactose) dissociates from the operator
- negative control (repression) is alleviated, however...

- RNA polymerase cannot form a stable complex with the promoter
Affinity of lac repressor for DNA (M⁻¹)

<table>
<thead>
<tr>
<th>DNA</th>
<th>Repressor</th>
<th>Repressor + Inducer</th>
</tr>
</thead>
<tbody>
<tr>
<td>lac operator</td>
<td>2 X 10¹³</td>
<td>2 X 10¹⁰</td>
</tr>
<tr>
<td>All other DNA</td>
<td>2 X 10⁶</td>
<td>2 X 10⁶</td>
</tr>
</tbody>
</table>

Specificity¹  10⁷  10⁴

¹ Specificity is the ratio of 
(Kₐ for binding to operator DNA) / (Kₐ for binding to random DNA)
Regulation of the lactose operon - positive control

- in the presence of both lactose and glucose it is not necessary for the cell to metabolize lactose for energy
- in the absence of glucose and in the presence of lactose it becomes advantageous to make use of the available lactose for energy
- in the absence of glucose cells synthesize cyclic AMP (cAMP)
- cAMP\(^1\) serves as a positive regulator of catabolite operons (lac operon)
- cAMP binds the dimeric cAMP binding protein (CAP)\(^2\)
- binding of cAMP increases the affinity of CAP for the promoter
- binding of CAP to the promoter facilitates the binding of RNA polymerase

\(^1\) cAMP = 3’, 5’ cyclic adenosine monophosphate
\(^2\) also termed catabolite activator protein
Activation of lac operon transcription

- the function of the lactose (lac) operon is to produce the enzymes required to metabolize lactose for energy when it is required by the cell.