Central Dogma

DNA → RNA → Protein

Discussing DNA replication
(Nucleus of eukaryote, cytoplasm of prokaryote)

Recall
Replication is semi-conservative and bidirectional

Lecture 10

DNA Replication
*Leading and lagging strand synthesis
Biochemistry of replication
DNA mutation and repair
Transcription

ECB 6-10

DNA polymerase -
adds nucleotides
at 3' end of
existing strand
Synthesis occurs
in 5' → 3' direction

Template
Specificity of which base adds depends on base pairing
with template strand (strands are complementary)
Problem: Strands are antiparallel; DNA made only 5' to 3'

Fork moves at rate of ~1000 nucleotides/sec in prokaryotes (~100 NTs/sec in humans)

Leading and lagging strands

Replication fork is asymmetrical

Lagging strand synthesis occurs from RNA primer
Other Proteins at Replication Fork

Helicase unwinds DNA duplex; breaks H bonds; requires ATP; Single stranded binding proteins coat strand and prevent renaturation

Sliding clamp keeps DNA polymerase bound

Lecture 10
DNA Replication
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Mutation: a permanent change in DNA sequence
Mutations accumulate with age and cause cancer.

Cancer = loss of control of the cell cycle.

Mistakes during replication cause mutations.

Mutations and Repair

During Replication:
- Incorrect nucleotide incorporated
  - Cell mechanism to reduce: proofreading

Post Replication:
- Many sources of DNA damage
  - Cell mechanism to reduce: Many mechanisms of repair
Proofreading

DNA Polymerase
- Catalyzes phosphodiester bond formation
- Has exonuclease activity
- Performs proofreading

Proofreading mechanism

Exonuclease activity removes the incorrect nucleotide
Polymerase activity then adds correct nucleotide
After proofreading, mistakes about 1/10^7 nucleotides

Replication
5' Triphosphate
- Energy from PP
- At 5' end
- Error
  - Incorrect dNTP removed
  - No PPP left
  - At 5' end
  - Correct dNTP cannot be added

Replication
3' OH
- Energy from pyrophosphate
- Correct dNTP
- Energy from pyrophosphate
- Corrected!
Mutations and Repair

During Replication:
Incorrect nucleotide incorporated

Post Replication:
Many sources of DNA damage

Post-replication repair also removes 99% of errors made in replication; Final error rate 1/10^9 NT

Causes of Errors:
Deamination
Depurination
Pyrimidine dimers

Cell mechanisms to reduce:
proofreading

Cell mechanisms to reduce:
Many, involve removing damaged DNA

Post-replication repair requires excision, resynthesis, ligation

How damaged strand is recognized is not understood

Lecture 10

DNA Replication
Semi-conservative replication
Biochemistry of replication
End replication problem

DNA mutation and repair

*Transcription
Central Dogma

DNA → RNA → Protein

Replication

Nucleus of eukaryote
Cytoplasm of prokaryote

What is a gene?

Double stranded DNA

Transcription control region

Coding region

Protein A
Protein B
Protein C

"each gene contains the information required to make a protein"

How much of genome is composed of genes?

Genome Projects:

Bacteria - about 500 genes, most of genome
Eukaryotes - about 20,000-40,000 genes, represents much less of genome
Humans - about 30,000 genes, only a few percent of the total genome!!!
Rest is repetitive DNA sequences - junk DNA
Much of repetitive DNA is transposable elements that have mutated and can no longer move
15% of human genome is the L1 element
11% is Alu sequence, about 300 nucleotide pairs
DNA Structure:
Double stranded
Double helix
Deoxyribonucleotides
ATCG
Organized as chromatin

RNA Structure:
Single stranded
Variety of 3D structures
Ribonucleotides
AUGG
Organized as RNA-protein complexes

RNA Structure

DNA codes for RNA
Single-stranded product
(5' to 3')
Not H bonded to DNA

RNA Polymerase

Coded by DNA template strand (also called antisense strand)
DNA template

Base pairing

Rate ~ 30 NT/sec

4 major RNA classes

<table>
<thead>
<tr>
<th>TYPE OF RNA</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNAs</td>
<td>code for proteins</td>
</tr>
<tr>
<td>tRNAs</td>
<td>form part of the structure of the ribosome and participate in protein synthesis</td>
</tr>
<tr>
<td>tRFAs</td>
<td>used in protein synthesis as adapters between mRNA and amino acids</td>
</tr>
<tr>
<td>Small RNAs</td>
<td>used in pre-mRNA splicing, transport of proteins to the ER, and other cellular processes</td>
</tr>
</tbody>
</table>

RNA Polymerases

Prokaryotes: One RNA Polymerase, composed of four subunits, plus additional factors that can confer promoter specificity

Eukaryotes: Three RNA Polymerases (RNA Pol I, II, III), each composed of >10 different proteins, transcribe different types of genes.

RNA Pol I: synthesizes ribosomal RNA (rRNA)

RNA Pol II: synthesizes mRNA (protein coding) and some small RNAs

RNA Pol III: synthesizes a variety of small RNAs, including tRNAs
Lecture 10

DNA Replication
- Semi-conservative replication
- Biochemistry of replication
- End replication problem

DNA mutation and repair

Transcription-general
- *Prokaryotes
- Eukaryotes

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Prokaryotic Gene Organization

Most genes in Operons:
- genes organized together, with one shared transcription start site

One mRNA codes for:
- Several proteins

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Transcription Initiation in Bacteria - overview

RNA Polymerase contacts DNA, slides along strand, "looking" for promoter sequences.

Promoter: "Nucleotide sequence in DNA to which RNA polymerase binds and begins transcription." ECB definition

At the promoter, the RNA Polymerase binds tightly, opening up a small single stranded region.

Transcription of one strand

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Sigma subunit - binds RNA Pol., recognizes DNA sequences in the promoter approximately 35 and 10 bases ‘upstream’ of transcription start site.

**Transcription Initiation in Bacteria (cont’d)**

Initiation and termination of prokaryotic transcription

Terminator (stop sequence)

Transcription termination in bacteria
How does the cell know where to transcribe? ... and when to transcribe?

Proteins bind to specific DNA sequences, some activate transcription, some repress. Terminated transcription factors.

Genome of Mycoplasma genitalium

One of smallest genomes of any cell: codes for 470 proteins

Gene regulation in prokaryotes

ECB 8-6

Operator sequence associated with promoter

Repressible operon

Default state is on, can turned off
Inducible operon; lac operon

Default state is off, turned on