BS1940  Course Topics  
Fall 2001  
Drs. Hatfull and Arndt

Introduction to molecular biology  
Combining genetics, biochemistry, structural chemistry

Information flow in biological systems:  
The Central Dogma  
The Sequence Hypothesis

Macromolecular structures and properties  
DNA  
History: Identification of DNA as the genetic material  
Chemical composition  
Structural features: Basepairing, Antiparallel structure  
Nomenclature  
Denaturation and annealing  
G+C content

RNA  
Chemical composition  
Structure

Protein  
Primary, secondary, tertiary and quaternary structural features

Some useful facts:  
Average MW of a base pair=660  
Size of average bacterial gene=1000bp  
Average protein size=30kDa  
Average MW amino acid=110Da

The Genetic Code:  
Transcriptional units  
Gene-protein relationships  
Role of tRNA, tRNA synthetases  
Ribosome structure  
Degenerate genetic code  
Signals for translation initiation and termination

Molecular cloning methods  
Restriction enzymes  
Site recognition, cleavage  
Frequency of occurrence of sites  
Where they come from; modification

Joining DNA fragments with DNA ligase  
Transformation  
Chemical treatment  
Electroporation
Vectors
  Plasmids
  Features
  Examples
  Phages
  Advantages
Selecting recombinants
  Use of alkaline phosphatase to reduce vector background
  Blue/white color screen
  Hybridization
  Immunological methods
Other vectors:
  Cosmids
  ssDNA phage vectors
  Phagemids
Polymerase Chain Reaction (PCR)
  Basics
  Applications
Techniques in molecular biology
  Molecular separations
    Gel Electrophoresis
    Chromatography
  Importance of radioactive labels
  Methods for labeling nucleic acids
  Southern blotting
  Northern blotting
  Site-directed mutagenesis
  Restriction Mapping
  Mapping ends of RNA
    S1 nuclease mapping
    Primer extension
  Reporter gene expression
  Methods for analyzing protein-DNA interactions
    Gel mobility shifts
    DNaseI footprinting
Genomics
  DNA sequencing methods
  Sanger
  Maxam Gilbert
  Automation
  Shotgun approaches
  Sequencing Large genomes
In silico workshop
   Constructing recombinant plasmids in silico
   Restriction digestion in silico
   Translation into amino acid sequences in silico
   Identifying open reading frames in silico
   Analyzing G+C content in silico
   Start and stops of genes in silico

Prokaryotic transcription machinery
   Initiation, elongation, termination
   RNA polymerase structure
      \( \alpha_2\beta\beta' \) (core) + \( \sigma \) (specificity)
   Promoters
      -10, -35, consensus
      UP elements and \( \alpha \)-CTD
   Melting DNA (closed complex --> open complex), abortive initiation
   Termination
      \( \rho \)-dependent
      \( \rho \)-independent (stem-loop structure)

Regulation of transcription in prokaryotes
   Lac operon
      Operon concept
      Negative control by repressor
      Mechanism of repression
      Inducer
      Positive control by cAMP and CAP
      Interaction of CAP with \( \alpha \)-CTD
      Role of DNA bending
   Trp operon
      Regulation by repressor
      Sensing trp as co-repressor
      Regulation by attenuation (in E. coli)
      Alternative secondary structures of mRNA leader
      Sensing trp as charged tRNA\(^{Trp}\)
      Role of termination
      Different (related) mechanism in B. subtilus
      TRAP protein

Protein-DNA interactions in prokaryotes
   Structural features
   Origins of DNA binding specificity
      Contributions of the protein
      Contributions of the DNA

Transcription in eukaryotes
   Machinery
      Three RNA polymerases
      Identification
      Functions
Common features
Unique features
Structural properties
Post-translational modification

Promoters
Roles of promoter elements for each polymerase
Locations of the promoter elements
Regulatory vs. core promoter elements

General transcription factors for each polymerase
Models for assembly
Functions of the individual factors
Common themes

Regulation
Properties of eukaryotic activators
Structural features
DNA binding domains
Dimerization domains
Activation domains
Modular structure of activators

Coactivators
Major classes
Properties
Interactions with the general transcription machinery

Mechanisms of activation
Targets of activators
Steps in initiation that are affected
Importance of coactivators

Chromatin
Structural components
Histones
Structural features
Modification
DNA
Packaging states
Effects on transcription
Strategies used by factors to contend with chromatin
Chromatin remodeling and modification factors

RNA Processing
Types of modification and evidence for each
Splicing
Alternative splicing
Capping
Polyadenylation
Importance of each to gene expression
RNA signals involved
Protein factors involved
Involvement of snRNAs
Mechanisms of RNA processing

Translation
Initiation
Prokaryotes
  tRNA charging: formation of fMet.tRNA
  Ribosome subunits and dissociation
  Formation of 30S initiation complex
    Roles of IF1, IF2, IF3
    Choice of initiation site
  Formation of 70S complex
Eukaryotes
  Scanning model for initiation
    Evidence; role of mRNA secondary structures
  Initiation factors
    Roles of eIF1, eIF1A, eIF4, eIF5, eIF3 etc.
    Roles of exchange factors eg. eIF2B
    Adapter role of eIF4G
Regulation of initiation
Prokaryotes
  e.g. L11 operon
Eukaryotes
  Regulation of eIF2α
  Regulation of eIF4E

Elongation
The Genetic Code: Use of termination codons
  Codons and anticodons; pairing
Ribosomes: A, P, E sites
  The elongation cycle
  Roles of elongation factors; recycling
  Uses of antibiotics; puromycin

Termination
Use of termination factors
Roles of release factors
Nonsense mutations
Nonsense suppression

DNA Replication
General features of the process and evidence for each
  Semiconservative
  Semidiscontinuous
  Primers
  Modes of replication
    Bidirectional
    Rolling circle
Fidelity

Proteins involved
- DNA polymerases and their roles in replication
  - Activities of *E. coli* DNA Pol I and III
  - Subunits of *E. coli* DNA Pol III
  - Eukaryotic DNA polymerases

Helicase
- SSB
- DNA gyrase
- Primase

Initiation of replication
- Identification of bacterial and yeast origins
  - *E. coli* priming reaction
    - OriC
    - DnaA
    - HU
    - Helicase (DnaB)
    - Primase (DnaG)
- Eukaryotic origins
  - SV40
  - Yeast

Elongation of replication
- Beta-clamp
- Processivity
- Structure
- Clamp loading
  - Structure of DNA Pol III at the replication fork
    - Role of the subunits
- Coordination of leading and lagging strand synthesis

Termination of replication in *E. coli*
- Proteins and sites
- Structural ramifications

Replication of linear chromosomes
- Telemores
  - Properties
  - Synthesis
  - Telomerase

DNA Repair
- DNA damage
  - Types
  - Causes
    - Replication errors
    - External agents
- Mechanisms of repair
  - Direct reversal mechanisms
  - Base excision repair
Nucleotide excision repair
   Uvr ABC system
   XP proteins and their activities
Mismatch repair
   Mut HLS system
   Role of DNA methylation
Recombination repair
Error prone repair
   RecA, UmuD, UmuC

Recombination
   Topological effects of recombination
      Between inverted repeats
      Between direct repeats
Homologous recombination
   Holliday model
   Central role of RecA
   RecBCD mediated formation of Holliday junction
   Branch migration (RuvA, RuvB) and resolution (RuvC)
      Alternate products
Site-specific recombination
   General features;
      Control of directionality
      Absence of DNA synthesis
Tyrosine recombinases:
   \( \lambda \) integration and excision
      Organization of attP and attB
      Structure of Int
      Role of IHF and Xis
      Protein bridges
      Mechanism of strand transfer (phosphotransfer Rx)
      Sequential strand exchange
      Directional control through DNA-protein complexes
Serine recombinases:
   Cointegrate resolution
      Organization res site
      Structure of resolvase
      Mechanism of strand transfer
      Concerted strand exchange
      Directional control through DNA topology

Transposition
   Products:
      Simple insertions
      Replication fusions (cointegrates)
Common features:
   Ubiquity
Frequency
Target duplication
Random target choice
Transposon Features
Inverted repeats
Transposase gene
Mechanism of transposition
Target duplication
Cut and paste transposition
Replicative transposition
Formation of cointegrate
Resolution