

Study Guide- Exam 3

(covers Classes 3.1 through 3.7)

Class 3.1---03/08/07: DNA Recombination

Know the following terms:

Homologous recombination, bivalents, cross over, chiasmata, recombination joint, branch migration, patch recombinant, splice recombinant, Holiday structure, D loop, synaptonemal complex, synapsis, axial elements, lateral elements, central element, cohesions, zip proteins, Hop proteins, Spo11 protein, RecA, RecBCD complex, chi sequence, Topoisomerase I, Topoisomerase II, gyrase, integrase, Cre/LOX, intasome, attP sites, attB sites, attL sites, attR sites, IHF host factors, intasome.

Understand the Following Concepts:

1. Know the structure of the homologous chromosomes as they pair during meiosis. Know that each chromosome contains 2 sister-chromatids (replicated DNA).
2. Be able to describe the major points of the single-stranded break model of homologous recombination.
3. Be able to distinguish between a patch recombinant and a splice recombinant.
4. Understand how a Holiday junction explains alternative resolutions during recombination.
5. Be able to reproduce the double-stranded break model of homologous recombination. Know that it is a more realistic model of recombination.
6. Know the structure of the synaptonemal complex, and how the major proteins (cohesions, zip proteins, hop proteins) are used to organize the structure.
7. Know how double-stranded breaks are generated during homologous recombination.
8. Know the role of RecBCD and RecA proteins during recombination in prokaryotes.
9. Know the role of the Ruv proteins in resolving Holiday junctions during recombination.
10. Know how Topoisomerases work, and know the difference between Type I and Type II topoisomerases in terms of moving strands of DNA.
11. Understand the role of gyrases and topoisomerases in producing or relaxing supercoils in DNA.
12. Know the major differences between homologous recombination and site-specific recombination.
13. Know the difference between recombinases and integrases.
14. Know how phage lambda integrates and excises into a bacterial host chromosome.
15. Understand the similarities and differences between integrase and topoisomerase I mechanisms of action.

Class 3.2---03/20/07: DNA Repair

Terms:

Base excision repair, nucleotide excision, mismatch repair, Thymidine (pyrimidine) dimer, bulky adducts, UvrAB, UvrA, UvrC, UvrBC, UvrD, base flipping, Alklyadenine DNA glycosylase

(AAG), error-prone repair, mismatch repair, DNA slippage, Umu proteins, 8-oxo-G, Mut enzymes, recombination repair, RecBC, RecF, RecA, RAD proteins, xeroderma pigmentosum, non-homologous end joining, Ku70/Ku80 heterodimers, Artemus.

Concepts:

1. Know the criteria for determining mutation rates.
2. Know the 5 types of DNA repairs (direct reversal, replacement, recombination repair, non-homologous end joining, resynthesis) and be able to identify each type.
3. Be able to classify the different types of DNA damages (single-base changes, structural distortions), and be able to give an example of each.
4. Understand the mechanism by which deamination of cytosine can lead to a mutation.
5. Understand the structure of a Thymine dimer and how they are usually repaired.
6. Know the 4 stages of excision repair in prokaryotic systems.
7. Understand how the Uvr complex works in excision repair.
8. Understand the mechanism of how base flipping is used to directly remove bases from DNA.
9. Know how the error-prone repair system works and why it contributes to lots of mutations
10. Understand the role of methylation in mismatch repair.
11. Know the stages of recombination repair.
12. Understand the mechanism by which non-homologous end joining can repair DNA. What is the role of Ku70 and Ku80 proteins?
13. Know the relationship between mutations in repair systems and some human genetic diseases.

Class 3.3---03/22/07: Transposable Elements

Terms:

Transposon, transposase, integrase, IS elements, inverted repeat, direct repeat, host target sequence, composite transposable element, drug resistance gene, armL, armR, Tn9, Tn10, TnA-family, replicative transposition, non-replicative transposition, resolvase, cointegrate, element excision, phage Mu, MuA, MuB, pseudoreplication fork, TnpA, TnpR, int sites, Ac/Ds maize elements, autonomous element, non-autonomous element, Drosophila P-elements, somatic tissues, germiline, hybrid dysgenesis, repressor protien, transgenic flies.

Concepts:

1. Understand how an IS element causes a duplication in host target sequences when it integrates.
2. Know the basic structures for IS elements, composite elements, and TnA family elements. Know the similarities and differences of each.
3. Know the similarities and differences between the replicative and nonreplicative mechanisms of transposition.
4. Know 3 criteria that are used by some transposases to choose a target site in host DNA.
5. Understand how recombination between transposons can lead to deletions and inversions of DNA.
6. Understand the phage Mu model for early events in transposition.
7. Understand how cointegrates are formed during replicative transposition.

8. Know the two pathways of non-replicative transposition.
9. Know the mechanism by which TnA transposons move.
10. Know the linkage between transposable elements and unstable genetic markers as first observed in corn.
11. Know the difference between autonomous and non-autonomous transposable elements.
12. Know the role of P-elements in hybrid dysgenesis and speciation.
13. Be able to predict the results of a cross between M and P cytotypic stocks that depend on the cytotypic of each parent.
14. Know how P-elements are used to make transgenic *Drosophila*.

Class 3.4--03/27/07: Retroviruses and Retroposons

Terms:

Provirus, Gag, Pol, Env, Protease, Gag-Pol protein, Reverse transcriptase, plus strand RNA, minus strand RNA, Long-terminal repeat (LTR), RNase H activity, tRNA primer, strong-stop minus DNA, strong-stop plus DNA, transduction, oncogenes, v-onc, c-onc, retrotransposons, Ty elements, delta element, viral-like particles, copia elements, LINES, SINES, Alu elements, processed pseudogenes.

Concepts:

1. Know the general life cycle of a retrovirus.
2. Know the genome organization of a retrovirus, and the mechanisms used by the virus to produce Gag, Pol, and Env proteins.
3. Know two mechanisms by which the Gag-Pol fusion protein can be generated from a retrovirus genome.
4. Know the detailed mechanism by which reverse transcriptase can make a double-stranded DNA from a single-stranded RNA virus molecule.
5. Know how a tRNA is used as a primer for reverse transcriptase.
6. Understand how double-stranded retroviral DNA is integrated into a host genome.
7. Understand how retroviruses can become oncogenic retroviruses.]
8. Know the difference between transposable elements and retroposons.
9. Know the 3 classes of retroposons.
10. Know the structure of the Ty element in yeast.
11. Know the relationship between viral-like particles and transposition in Ty elements.
12. Know the similarities and differences between LINES and viral-superfamily retroposons.
13. Know the steps of LINE transposition.
14. Know the differences between LINES and SINES.
15. Be able to describe Alu elements and a possible explanation for their origin.
16. Know how processed pseudogenes are different from standard pseudogenes.
17. Be able to recognize the structure of a processed pseudogene.
18. Be able to describe the consequences of transposons in terms of genome organization.
19. Know some of the differences between the mouse and human genomes in terms of active retroviruses and retroposons.

Class 3.5--03/29/07: Eukaryotic Transcription I: Promoters and Enhancers

Terms:

Transcription factor, RNA pol I, RNA pol II, RNA pol III, promoter, enhancer, nucleolus, nucleoplasm, carboxyl terminal domain (CTD), core promoter for RNA pol I, upstream promoter element (UPE) for RNA pol I, upstream binding factor (UBF), SL1, TBP, positioning factor, BoxA, BoxB, BoxC, TF_{III}B, TF_{III}C, TATA box, initiator sequence, downstream promoter element (DPE), TFIID, basal apparatus binding factors, TF_{II}H, CAAT box, GC box, enhanceosome, insulator, CpG islands.

Concepts:

1. Know the 4 different types of transcription factors in terms of what molecules they recognize.
2. Know the 3 types of eukaryotic RNA polymerases, where they are located within cells, and which genes they transcribe.
3. Know the basic differences between a eukaryotic promoter and a eukaryotic enhancer.
4. Know the structure and importance of the carboxy-terminal domain of RNA pol II.
5. Know the structure of the RNA pol I promoter (core promoter and upstream promoter element).
6. Understand the structure and role of SL1 and its relationship to the TBP positioning factor.
7. Be able to describe the 3 different types of RNA pol III promoters and which genes they transcribe.
8. Know the role of TF_{III}B and TF_{III}C in RNA pol III transcription.
9. Know the relationship between startpoint (initiator sequence), TATA box, and downstream promoter elements (DPE) in RNA pol II promoters.
10. Understand the TF_{II}D complex in terms of binding sequences and associated proteins (TBP, TAFs)
11. Know what comprises the two types of core promoters in RNA pol II genes.
12. Know how the TBP positioning factor is localized in the 3 different types of polymerase promoters.
13. Know the structure and functions of the TF_{II}H transcription factor. Know the role of TF_{II}H in initiation and repair.
14. Know what functions are regulated by a phosphorylated CTD tail.
15. Know the similarities and differences between promoters, activator sequences, and enhancers. Know that they all are DNA sequences.
16. Know the relationship between enhancers, transcription factors, basal apparatus, and promoters.
17. Be able to describe the concept of the enhanceosome.
18. Know how enhancers function and how they are similar and different than promoters.
19. Know how CpG islands are regulatory targets for methylation and repression.

Class 3.6---04/03/07: Eukaryotic Transcription II: Transcription Factors

Terms:

Basal factors, activator proteins, coactivator proteins, chromatin remodeling regulators, DNA-binding domains, protein-protein interacting domains, connector domains, holoenzyme, mediator, response elements, HSE, GRE, MRE, zinc-finger motifs, steroid receptor motifs, helix-turn-helix motif, helix-loop-helix motif, leucine zipper, Cys₂/His₂ finger, lipid hormones, steroids, sex steroids, corticoids, vitamin D₃, thyroid hormone, retinoid acid, Cys₂/Cys₂ finger, SMRT corepressor, CBP/p300 activator, amphipathic alpha helix, HLH and bHLH transcription factors, bZIP proteins.

Concepts:

1. Know the basic steps that must occur for a gene to be expressed (chromatin remodeling and promoter access, transcription initiation, RNA processing, RNA export, translation).
2. Know why transcriptional initiation is the rate-limiting step in eukaryotic gene expression.
3. Know the 4 different types of general transcription factors. Be able to briefly describe each type.
4. Know how prokaryotic promoters are different from eukaryotic promoters. Also be aware of how prokaryotic transcription factors act differently than eukaryotic transcription factors.
5. Be familiar with the modular domain structure of transcription factors (DNA-binding, protein-protein, connector domains).
6. Know how activator proteins interact with the basal apparatus of the promoter and how coactivators may be employed.
7. Understand how the recruitment model and cofactor model explains how activators stimulate transcription in eukaryotes.
8. Be familiar with at least two types of repression mechanisms (general and specific models).
9. Know how the prokaryotic RNA pol holoenzyme is different from the eukaryotic RNA pol II holoenzyme.
10. Be clear about the relationship between response elements (DNA sequences) and activator proteins (transcription factors that bind to them).
11. Know how the heat-shock response differs in prokaryotes versus eukaryotes. Know how the heat-shock genes are turned on in each system.
12. Be familiar with the regulation of the Metallothionein gene. What does a large collection of response elements in the promoter/enhancer region say about how this gene is expressed?
13. Know the 5 types of DNA-binding domains found in some activator proteins.
14. Know how a classic zinc-finger domain differs from the zinc finger domain found in steroid (lipid hormone) receptors.
15. Be able to recognize common lipid hormones (steroids, vitamin D₃, thyroid hormone, retinoic acid).
16. Be able to define the concept of a ligand-activated transcription factor.
17. Know the basic modular structure of a lipid-hormone receptor.
18. Know the difference between lipid-hormone receptors that form homodimers and those that form heterodimers. Also know the general difference in the response elements that they recognize (inverted versus direct repeats).
19. Be able to describe how lipid binding can activate the receptor to stimulate transcription. Know the role of the SMRT co-repressor and the CBP/p300 activator in this process.
20. Know how helix-loop-helix proteins with and without a basic region can regulate transcription. Understand the role of dimer formation in this process.
21. Know how leucine zippers interact to form active transcription factors.

Class 3.7---04/05/07: RNA Splicing and Processing

Terms:

pre-mRNA, hnRNA, ribonucleoprotein particle (RNP), donor junction, acceptor junction, branch point, lariat, snRNA, snRNP, snurp, scRNA, snoRNA, spliceosome, nucleophilic attack, U1, U2, U4, U5, U6, E complex, A complex, B1 complex, B2 complex, C1 complex, C2 complex, splicing factor, ASF/SF2, U2A, SF1, transesterification reaction, Exon Junction Complex (EJC), TAP/Mex transport proteins, nuclear pore, alternative splicing, translicing, splice leader (SL-RNA), PolyA addition, RNA pol II termination, AAUAAA sequence.

Concepts:

1. Recall the steps needed to convert pre-mRNA into mRNA (capping, splicing, polyA tail addition).
2. Know that splicing involves removing introns from pre-mRNA (not DNA).
3. Know the conserved elements of an intron: donor (5' or left) site, branch site, acceptor (3' or right) site.
4. Know the 5 general stages of splicing.
5. Know the structure of the lariat and the unusual 5'-2' phosphodiester bond.
6. Know the relationship between U1 RNA and U1 snurp. Know how the U1 RNA recognizes the splice donor sequence of pre-mRNA.
7. Know the general components of the spliceosome and how splicing factors are related to the snurps.
8. Know the basic events involved in spliceosome assembly. Know the major events in the formation of each complex (what factors bind and dissociate) from E complex to C2 complex.
9. Know the role of each snurp (U1, U2, U4, U6, U5) and the major splicing factors discussed in class (ASF/SF2, U2A, SF1).
10. Know the dynamic interactions between the U6 snRNA, U4 snRNA, and U2 snRNA (clamp), and how these control the nucleophilic attack and transesterification of the A base (in the branch site) to the donor site of the intron to form the lariat.
11. Know how splicing can control RNA transport from the cell. Know the role of the Exon Junction Complex and the transport proteins.
12. Know that alternative splicing can generate more than one type of RNA and sometimes protein from the same pre-mRNA. Be able to give at least one example of alternative splicing. Know at least one mechanism by which this can be controlled (i. e. the role of ASF/SF2 or SF5).
13. Know how transplicing occurs in *C. elegans*. Understand the role of the splice leader and how different RNAs (in trans) contains half intron sequences.
14. Understand the general mechanism for how RNA pol II transcripts are terminated and how the 3' end is processed. Know the role of the AAUAAA sequence and how it relates to cleavage.
15. Know that 3' end processing: cleavage and polyA addition is accomplished by a large multi-protein complex with different activities.