

# Study Guide- Exam 2

## (covers Classes 2.1 through 2.6)

### **Class 2.1---02/08/07: The Genetic Code**

#### Know the following terms:

Codon, anticodon, synonym, third-base degeneracy, wobble hypothesis, proofreading (relative to synthetase charging), suppressor tRNAs, free ribosomes, membrane-bound ribosomes, post-translational translocation, co-translational translocation, signal sequence, signal recognition particle (SRP), SRP receptor, 11S ribonucleoprotein complex, 7S RNA, translocon.

#### Understand the Following Concepts:

1. Be able to use the codon dictionary to translate a strand of mRNA.
2. Be able to pick out the wobble base in a codon and an anticodon.
3. Be able to recognize some of the more common modified bases found in tRNAs.
4. Know which bases Inosine, queuosine, and 2-thiouridine can base pair with.
5. Understand the relationship between the number of codons, tRNAs, and aminoacyl-tRNA synthetases.
6. Understand the mechanism of how aminoacyl-tRNA synthetases are charged.
7. Understand how a suppressor tRNA works to suppress a missense or nonsense mutation.
8. Know where and how protein synthesis takes place in different locations within cells.
9. Know the difference and understand the mechanisms for post-translational translocation and co-translational translocation of proteins.
10. Understand the structure and role of the signal sequence in synthesizing proteins that are membrane bound or secreted.
11. Know the structure of the SRP and the SRP receptor.
12. Understand the role of GTP in co-translational translocation.
13. Know the structure of the translocon.
14. Understand the general mechanisms by which proteins are imported into mitochondria and chloroplasts.

### **Class 2.2---02/15/07: Transcription**

#### Terms:

Promoter, startpoint, terminator, transcription unit, pre-mRNA, upstream sequence, downstream sequence, transcription bubble, closed promoter complex, open promoter complex, bridge, , holoenzyme, core enzyme, sigma factor, tight binding, abortive initiation, promoter clearance, up promoter mutations, down promoter mutations, gyrase, topoisomerase I, antitermination, intrinsic terminators, rho-dependent terminators, rut sites.

#### Concepts:

1. Be able to predict the sequence and orientation of an RNA transcribed from a labeled double-stranded DNA molecule.

2. Know what happens during each of the major stages of transcription.
3. Understand the difference an open and closed promoter complex.
4. Know the basic structure of the RNA polymerase complex and how the structure determines its different functions during transcription.
5. Be familiar with the holoenzyme structure of RNA polymerase and the concept of sigma factors.
6. Know how the binding of sigma factors affects the affinity of the RNA polymerase for promoter sequences.
7. Known the consensus sequences and conserved spacing found in prokaryotic promoters.
8. Know what makes the difference between a strong and weak promoter and an up and down promoter mutation.
9. Understand the role of supercoiling, gyrase, and topoisomerase I during transcription.
10. Understand how different sigma factors can control different sets of genes in prokaryotes.
11. Understand how terminators work to stop transcription, and how they are used to control the efficiency of gene expression.
12. Know the difference between intrinsic and rho-dependent terminators and how each works.

### **Class 2.3---02/20/07: Bacterial Operons**

#### Terms:

Trans-acting products, cis-acting sequences, diffuseable substance, structural genes, regulatory genes, negative regulatory circuit, positive regulatory circuit, lac repressor (lacI), lacZ, lacY, lacA, operator, inducer, gratuitous inducer, palindrome, inverted half site, high-affinity binding site, low-affinity binding site, de-repressed, activators, co-repressors, autogenous control.

#### Concepts:

1. Know the classical definitions of trans-acting products and cis-acting sequences. Be able to identify examples of each.
2. Know the difference between a genetic circuit that is regulated by negative control versus one that is regulated by positive-control, and be able to give an example of each.
3. Know all the components of the lac operon (promoter, operator, structural genes, repressor, inducer) and how they work.
4. Know the general structure for the lac repressor (DNA binding, inducer binding, oligomerization domains) and how it works to control gene expression of the lac operon.
5. Know the structure of the lac operator relative to the lac promoter.
6. Be able to predict what would happen to the expression of the lacZYA gene with different types of regulatory mutations (in operator, promoter, repressor).
7. Understand how the lac inducer acts to turn on expression of the lacZYA genes of the operon.
8. Know the complex structure of the lac operator (O1, O2, O3), and how repressor binding and higher-order repressor binding affect the DNA structure of the operator and the expression of operon genes.
9. Understand the concept of high and low affinity binding sites for the repressor.
10. Understand the relationship between activated, repressed, and derepressed states of an operon.

11. Understand how the trp operon is different from the lac operon.
12. Understand how cAMP/CRP can activate operons.

### **Class 2.4--02/22/07: Regulatory RNA/Phage Strategies**

#### Terms:

Regulatory RNA, secondary structure, antiterminator, attenuator, antisense RNA, sRNA, microRNA, RNA interference (RNAi),  $\lambda$  phage, lytic lifestyle, lysogenic lifestyle, immediate-early genes, delayed-early genes, late genes, Cro repressor, pN antiterminator, cI repressor, cII, CIII, Q factor, S and R genes, genes A-J,  $P_R$ ,  $P_L$ ,  $P_{R'}$ ,  $P_{RE}$ ,  $P_{RM}$ ,  $O_{R3}$ ,  $O_{R2}$ ,  $O_{R1}$ ,  $O_{L3}$ ,  $O_{L2}$ ,  $O_{L1}$ , cooperative binding, host proteases, immunity, release from lysogeny, host proteases.

#### Concepts:

1. Know 2 intramolecular mechanisms by which RNA structure controls gene expression.
2. Know the relationship between terminator, attenuator, and antiterminator.
3. Know at least 2 mechanisms by which small regulatory RNAs can control translation of target genes.
4. Know how sRNAs and microRNAs function to control gene expression.
5. Know the mechanism by which RNA interference (RNAi) works to control gene expression. Why is this discovery worthy of a Nobel Prize?
6. Know the difference between lytic and lysogenic strategies used by some phage.
7. Know the 3 different temporal sets of  $\lambda$  genes and how they are expressed during  $\lambda$  phage progression.
8. Know all the major regulatory genes in the  $\lambda$  genome (N, Cro, CI, CII, CIII, Q) and their role in gene expression and lifestyle progression.
9. Know what kinds of genes are produced from  $P_R$ ,  $P_L$ ,  $P_{R'}$ ,  $P_{RM}$ ,  $P_{RE}$  promoters.
10. Know how the lytic cycle progresses in  $\lambda$ .
11. Know that  $\lambda$  forms a circle upon entry in the bacterial cell, and know how each gene set is produced from that circular genome.
12. Know how pN works to shift expression of genes from immediate-early to delayed-early.
13. Know the domain structure of the cI repressor, know how cI can form higher-order oligomers (dimers, tetramers, octomers, 12 mers) to control gene expression from  $P_L$  and  $P_R$ .
14. Understand the complex structure of the  $O_L$  and  $O_R$  operators, how there are 3 separate repressor binding sites in each.
15. Know the events that lead to the establishment of lysogeny in  $\lambda$ . Understand the role of cII and cIII proteins, and why the  $P_{RE}$  promoter is only used later in the lifecycle.
16. Understand the autogenous circuit of how cI controls its own expression.
17. Understand how the Cro repressor works and how it is needed for the lytic cycle. Know the difference between Cro and cI binding at the  $O_L$  and  $O_R$  operators.
18. Know what influences the decision between lytic and lysogenic cycles (cII is critical switch). Understand the role that host factors play.
19. Understand the mechanism for  $\lambda$  immunity and release from lysogeny.

### **Class 2.5---02/27/07: Replicon Structures**

Terms:

Origin, replicon, single-copy replicons, multicopy replicons, bidirectional replication, oriC, ter sites, theta structure, Dam methylase, autonomous replicating sequence (ARS), origin replication complex (ORC), telomere, terminal replicating protein, rolling circle replication, conjugation, F episome, F<sup>+</sup> cell, F<sup>-</sup> cell, Hfr cell, pilis, pillin, *tra* and *trb* genes, crown gall, *Agrobacterium tumefaciens*, Ti-plasmid, T-DNA, *vir* genes,

Concepts:

1. Know the types of replicons that are found in prokaryotic and eukaryotic systems.
2. Understand the relationship between the origin and bidirectional replication.
3. Know the 4 stages of replication in *E. coli*.
4. Know how *terE,D,A* and *terC,B* work to terminate replication in *E. coli*.
5. Know the general models for how replication affects transcription in *E. coli*.
6. Know what Dam methylase does, and understand its role in DNA replication.
7. Understand how Eukaryotic chromosomal replicons are different for prokaryotic chromosomal replicons.
8. Understand the structure of the ARS (yeast ori) and the relationship between ARS, ORC, licensing factor, and nuclear membrane breakdown to Cdc6 and MCM complex.
9. Know the 4 strategies employed by various systems to overcome the problem of replicating linear DNA ends. Be able to give an example of each.
10. Know how rolling circle replication works. Be familiar with two diverse examples of rolling circle replication.
11. Know the role of protein A in  $\phi$ X174 phage replication.
12. Know the overall organization of the F genome in terms of size and genes that it carries.
13. Know the mechanism by which F factor is transferred to an F<sup>-</sup> cell during conjugation. Know the difference modes of transfer if F is an episome or integrated into the host genome.
14. Understand the role of the pilis in conjugation and DNA transfer.
15. Know the mechanism by which the *Agrobacteria* can transfer DNA to dicot plant cells.
16. Know how T-DNA is transferred from the Ti plasmid using bacterial and host signals (from wounding).
17. Understand the role of the 6 *vir* genes in the transfer process.

**Class 2.6---03/01/07: DNA Replication**

Terms:

Replicase, translesion DNA polymerase, frameshifts, substitutions, proofreading function, DNA-binding domain, palm, fingers, thumb, 3'-5' exonuclease activity, catalytic core ( $\alpha$ ,  $\epsilon$ ,  $\theta$ ), dimerization subunit ( $\tau$ ), clamp ( $\beta, \beta$ ), clamp loader, processivity, discontinuous replication, leading strand, lagging strand, Okazaki fragment, helicase (DnaB), single-stranded binding (SSB) protein, priming, primer, primase (DnaG), dipolymerase model, Ligase, 5'-3' exonuclease.

Concepts:

1. Know the general characteristics of DNA polymerases and know that not all are used for semiconservative replication.

2. Understand the limitations of DNA polymerase in terms of its synthesizing activity (5' to 3'). Be sure to know why the 3' end can be extended while the 5' end cannot.
3. Know the 5 types of DNA pols and their general roles in DNA synthesis in *E. coli*.
4. Understand the 2 general types of errors that DNA polymerase can make during replication, and mechanisms that are used to prevent or correct them.
5. Understand the general proofreading function of DNA pol III, how it works, and where it is produced in the enzyme complex.
6. Know the general structure for the DNA binding domain, exonuclease activity, and N-terminal domains of DNA pol III.
7. Understand how positioning of DNA occurs within DNA pol III.
8. Know how changes in conformation lead to DNA polymerization or DNA editing due to competition between the catalytic site of the polymerizing and exonuclease domains.
9. Know the role of each subunit within each subcomplex:  $\alpha$ ,  $\epsilon$ ,  $\theta$ ,  $\beta$ ,  $\tau$ .
10. Know the structure of the  $\beta$  clamp and how it is loaded onto DNA by the clamp loader. Understand how the  $\beta$ -clamp is related to processivity of the enzyme.
11. Understand how eukaryotic DNA polymerases compare to prokaryotic DNA polymerases.
12. Know that each replication bubble contains 2 replication forks, and each replication fork contains a leading and a lagging strand of replicating DNA.
14. Know the role of helicase (DnaB) and single-stranded binding (SSB) protein, in the replication process.
15. Understand the limitation of DNA polymerases to replicate without a primer. Know the 4 ways that DNA can be primed. Be able to give an example of each
16. Know the relationship between primed DNA, primer, primase (DnaG), and the primasome.
17. Understand the Dipolymerase model to explain how both leading and lagging strands are synthesized at the same time. Be able to explain the role of: core polymerase,  $\beta$ -clamp, clamp loader, primase, SSB protein, helicase, template loop. Know how new Okazaki fragments are produced relative to the leading strand.
18. Know how Okazaki fragments are jointed by DNA pol I and ligase.