

BIO240 Midterm Essay Study Guide

Essay Question: You will be asked to write a detailed essay describing the process one of the following processes: DNA replication; Transcription; Translation. This essay will count for between 1/3 and 1/2 of your possible midterm exam points.

DNA Replication: Below is a list of elements to include in a solid essay on DNA replication. You should probably write the essay from the 'prokaryotic perspective' and then distinguish how eukaryotes differ in the process.

Semi-conservative replication mechanism

Bidirectional replication with DNA polymerase reading the template strand from 3' to 5' and building the new strand from 5' to 3' direction

Stages:

1. Unwind DNA: DnaA binding; DnaB and DnaC; DnaB is Helicase; Single-Stranded Binding Proteins and function; Gyrase to release tension caused by replication bubble.
2. Primer: Primase to lay down primer of RNA nucleotides (DNA polymerase α in eukaryotes); DNA polymerase must reference a 3' – OH group to begin polymerization.
3. DNA Polymerization: DNA Polymerase III (DNA polymerases δ and ϵ in eukaryotes) references primer and begins polymerization in both directions along the replication bubble using dNTPs as material and energy source for reaction. Leading vs lagging strands; continuous vs. discontinuous replication; Okazaki fragments. Self-correction properties of DNA polymerase (3' to 5' exonuclease activity).
4. Clean up: excision of RNA nucleotides of primer sequences by DNA Polymerase I and replaced. RNA flap or tag generated by DNA polymerase activity carving out primer on lagging strands. Ligase must make final – OH to Phosphate bond.

Eukaryotes: DNA polymerase γ for mtDNA replication. Telomerase to deal with telomeres. Thousands of origin sites for replication to begin vs. one in prokaryotes.

Transcription: Below is a list of elements to include in a solid essay on the transcription. Here it may be more difficult to focus on prokaryotes and then site changes in eukaryotes. It can be done, however, and you need to explain both systems.

Stages:

1. Initiation: RNA polymerase reads template DNA from 3' to 5' direction and builds mRNA (or tRNA or rRNA) from 5' to 3' direction. RNA polymerase bonds to promoter regions upstream of the transcription initiation site. Strong or weak promoter sequences – and why? Basal promoter sequence, e.g. the TATA box. Proximal promoter sequences, e.g. CAAT box or GC box. Assembly of a preinitiation complex:
 - a. TFIID binds to the TATA box.
 - b. TFIIB binds to the TFIID-TATA box complex.
 - c. RNA Polymerase II and TFIIF bind to the TFIID-TFIIB complex forming a **minimal transcription initiation complex**.
 - d. TFIIE and TFIIH bind to produce the **complete transcription initiation complex or preinitiation complex (PIC)**.

Enhancers, activators; silencers and repressors.

2. Elongation: RNA Polymerase reads DNA sequence and uses triphosphate RNA nucleotides to build a complementary sequence using the DNA as the template. RNA pairing rules versus DNA pairing rules. Three regions of an mRNA strand. Function of three eukaryotic RNA polymerases.

3. Termination: rho dependent and rho independent factors in prokaryotes. Cleavage of 3' end after AAUAAA sequence and addition of Poly-A tail in eukaryotes.

Modification of mRNA in eukaryotes: Poly-A tail; 7-methylguanosine (7mG) cap at 5' end. Excision of introns and splicing together of exons. Spliceosomes. Ribozymes. RNA lariat structure and function of snRNPs. Substitution editing. Insertion and deletion editing.

Translation: Below is a list of elements to include in a solid essay on translation. Here it is easier to simply write about the general process and site how prokaryotes and eukaryotes differ while you write. mRNA is read from 5' to 3' end in translation (there is a mistake in my course notes stating the opposite).

Three RNAs necessary: mRNA, rRNA, and tRNA. How tRNA is charged and specific for a single amino acid and an appropriate anticodon. How the initial sequence of mRNA is set in correct frame in small ribosomal subunit using Shine-Delgarno sequence in prokaryotes and Kozak sequence in eukaryotes. Use of formylmethionine tRNA (f-Met) as first amino acid in prokaryotes. Use of a special initiator form of Met-tRNA for eukaryotes.

Formation of initiation complex for translation – mRNA transcript, initiation factors, small subunit of ribosome: attachment of large subunit with GTP and removal of some initiation factors, setting initial AUG in P site of ribosome. Use of eIFs in eukaryotes – stands for eukaryotic initiation factors, and a modified AUG sequence.

Elongation: GTP mediated movement of ribosome/mRNA complex moving new charged tRNAs from A site, to P site to E site (uncharged). Ribozyme cleavage of amino acid from P site and bond to amino acid in A site. Peptidyl transferase. GTP and elongation factors (EFs and eEFs) interaction for translocation.

Function of Genetic Code in determining sequence of amino acids in gene product.

Termination: stop codon signaling GTP dependent release factor that cleaves final amino acid and causes disassociation of small and large subunits.

Distribution of gene products after translation – signal sequences and distribution through Golgi complex (not details, just knowledge that it happens).