

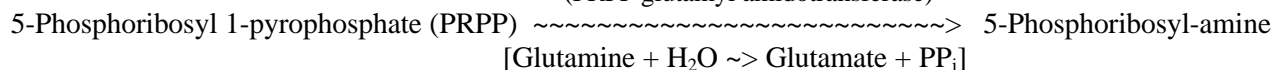
KEY to Cells TA Block 3 Questions (Part 1)

Any questions/comments on the answers, please direct to the individuals in brackets who contributed the answers!

1. What is the committed step in:

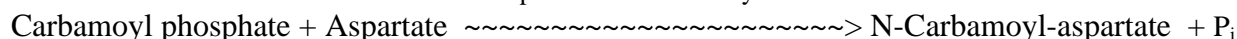
a. purine biosynthesis?

Amidophosphoryl-ribosyl transferase
(PRPP glutamyl amidotransferase)



b. pyrimidine biosynthesis?

Aspartate transcarbamoylase



To get full credit full credit for a question such as this on the test, you must name all the reactants, products, and enzymes.

[Marc Natter]

2. If C¹⁴-labelled glycine is used in the biosynthesis of AMP, which carbon atom(s) get labelled? Be specific.

Carbons C4 & C5 will be labelled.

[Marc Natter]

3. If C¹⁴-labelled aspartic acid is used in the biosynthesis of CTP, which carbon atom(s) get labelled? Be specific.

Carbons C4, C5, and C6 will be labelled.

You need to be able to draw out the full purine/pyrimidine nucleotide or nucleoside structure (including the ribose sugar and phosphate portions) with all bonds. Know which carbon and nitrogen atoms come from which reactants.

[Marc Natter]

4.a. What are the symptoms of gout?

A disease caused by the precipitation of urate crystals. Within joints ~~~~> **arthritis**, esp. of the big toe (“**podagra**”) & ankles. Long term untreated sufferers of gout get a deposition of urate under the skin which causes disfiguring, painful **tophi**. B/c of high blood uric acid levels, the body attempts to excrete in urine and urate crystals may deposit in the kidneys (renal parenchymal disease) and/or stones will form and lodge in the renal tubules, ducts, or ureters causing extreme pain.

b. What is the treatment for gout?

Administration of allopurinol.

c. Specify the enzymatic pathway involved and describe two ways that this treatment relieves gout.

1. Allopurinol blocks xanthine oxidase so you stop producing uric acid and excrete hypoxanthine instead (see diagram on p.115 of Marks)

2. Allopurinol reacts with PRPP to form a nucleotide. This lowers levels of PRPP and therefore reduces the de novo synthesis of purines.

You should be able to draw out the structure of allopurinol.

[Marc Natter]

5. Name the metabolic pathway(s) and branch(es) of which are used to:

a. make nucleotide precursors?

Purine and pyrimidine de novo biosynthesis pathways
purine salvage pathway
ribonucleotide reductase to make deoxyribonucleotides
thymidylate synthase to make dTMP
nonoxidative branch of pentose phosphate pathway

b. use nucleotides for energy?

This q is phrased incorrectly. It should ask - what metabolic pathway would be used if you ingested food high in DNA or RNA and the answer is the nonoxidative branch of the pentose phosphate pathway

c. synthesize NADPH for reducing power?

oxidative branch of pentose phosphate pathway (hexose monophosphate pathway)

[Svetlana Krasnokutsky]

6. Discuss Xeroderma Pigmentosum and Hereditary Non-Polyposis Colorectal Cancer (HNPPC):

a. What are the sign and symptoms of each disease?

X.P.: A genetic disease in which individuals are sensitive to UV radiation, leading to skin cancer and particularly melanoma. Afflicted individuals frequently die by age 30 from metastatic disease.

HNPPC: A genetic disease characterized by colorectal cancer which does **not** arise from intestinal polyps. These individuals are susceptible to colon, stomach, and uterine cancers (regions constantly making new cells), b/c their DNA accumulated mistakes following replication.

b. What causes each disease?

X.P.: Defect in DNA repair. UV light excites adjacent pyrimidine bases on DNA strands, causing them to form covalent dimers. Cultured skin cells from such individuals have a vast accumulation of thymine dimers.

HNPPC: Caused by mutations in genes for proteins involved in the DNA mismatch repair system.

c. What reactions do or do not occur?

X.P.: Thymine (and equivalent) dimers are not repaired. Most of the time these individuals are deficient in the initial step of pyrimidine dimer removal – the endonuclease that detect defects are not sensitive enough or are missing (see p.175-76 of Marks).

HNPPC: Proteins involved in mismatch repair (hMSH1, hMSH2, hPMS1, hPMS2) are mutated. (The mechanisms in humans for distinguishing parental from newly synthesized DNA strands have not yet been clearly defined. In bacteria, parental DNA strands contain methyl groups attached to bases in specific sequences. Unmethylated mismatched regions can be recognized as damaged and are repaired.)

[Marc Natter]

7. What are the mechanisms of transcription termination in prokaryotes?

There are rho dependent and rho independent pathways. Rho independent relies on a hairpin turn in the DNA that stops the polymerase. In rho dependent transcription termination, a protein called, aptly enough, rho factor, binds to RNA polymerase and pulls it off the template.

[Adam Wos]

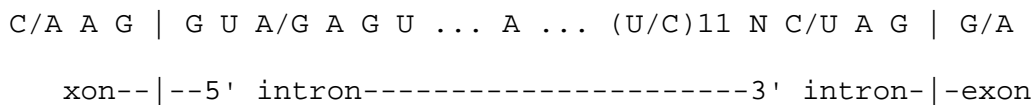
8. How are the differences between transcription in prokaryotes and eukaryotes used to advantage by antibiotics?

There are many differences between replication, transcription, and translation between prokaryotes and eukaryotes that are exploited by antibiotics. This question specifically asks about transcription - make sure that if this were an exam question, that you would answer properly by discussing only transcription. It is easy to proceed to hastily and answer everything and anything you know about antibiotics without answering specifically about the step that was asked about. in light of this, there are two major mechanisms. First, prokaryotes

use DNA Gyrase to unwind positive supercoils formed in the process of transcribing. This enzyme is ATP dependent. In humans, Topoisomerase II is used, and it is not ATP dependent. Novobiocin blocks the binding of ATP to the gyrase, and thus blocks gyrase's activity. Nalidixic acid also interferes with gyrase. A second mechanism involves the specific subunit design of prokaryote RNA polymerase. Rifampicin binds the beta subunit of RNA polymerase and interferes with the formation of the bond for the first nucleic acid to be added to the growing RNA transcript.

[Mike Buff]

9. How are introns spliced in pre-mRNA? Be specific as to how a splice region is recognized. The primary RNA transcript, hnRNA (heterogenous nuclear RNA), in addition to 5' capping and 3' polyadenylation, normally has introns removed before being passed out of the nucleus. It should be noted that not all eukaryotic primary transcripts contain introns. For those that do, introns are spliced in the following manner. Small nuclear RNAs (snRNAs) associate with proteins to form small nuclear RiboNucleoProteins (snRNPs). snRNPs then pair with conserved bases on the hnRNA, near the 5' and 3' ends of an intron, forming a complex called the spliceosome. The conserved sequence follows.



Following spliceosome formation, the intron is removed in the shape of a lariat (see Stryer, p.374 for diagram & further details).

[Ajit Janardhan]

10. a. What are the implications of pre-mRNA splicing for recombinant biotechnology? In your answer, specifically address the differences between a genomic and a cDNA library. To clarify, we will begin with basic definitions of two terms to help in answering the question.

Genomic libraries - contain a copy of every DNA nucleotide sequence in the genome. This is created by fragmenting the DNA from a cell and having it packaged within the lambda bacteriophage, a virus that infects bacteria. As the virus replicates, so will the DNA from the host cell.

cDNA libraries - contain only those DNA sequences that appear as mRNA molecules. Note, that these are cell specific. More importantly, they LACK introns and control regions of the DNA (i.e. junk DNA, promoters and enhancers.)

How does this impact pre-mRNA splicing for recombinant biotechnology?

Remember, pre - mRNA is modified to RNA by excising introns and combining exons. The exons from the mRNA transcript that will be translated into protein. A cDNA library is composed of exons, hence, will be directly translated into protein without any splicing. This is of great value when the cDNA is fully known. A genomic library, however, is of value when only a portion of the protein or DNA that encodes the mRNA is known. In these cases, a probe can be made that will hybridize to the portion of the gene in interest in the library. This allows you to possibly identify the gene and subsequently create a specific cDNA library from it.

b. Under what circumstances would you use a cDNA rather than a genomic library?

A cDNA library is advantageous when you know the exact protein and the cell type you want to amplify. This allows selective replication of a protein such as insulin.

A genomic library is useful when you only have a portion of the protein you are interested in. You create a probe that will hybridize with your genomic DNA, thereby, allowing you to identify the gene of interest.

c. What is a YAC?

Yeast Artificial Chromosome (YACs) are useful when analyzing larger pieces of DNA. They contain a centromere, an Autonomous Replication Sequence (ARS), a pair of telomeres, marker genes and a cloning site. These can be used to selectively characterize eukaryotic genes such as the Dystrophin gene.

[Natalie Digoia]

11. a. List four features of human ribosomal RNA genes. What differences between prokaryotic and eukaryotic ribosomal RNA are used to advantage by antibiotics?

(1) rRNA is made in the nucleus (nucleolus)

(2) the ribosomes 70S (50S made of 23S,5S rRNA and 30S subunit made of 16S rRNA)

(3) has hnrRNA and snrRNA

(4) tandem repeats

The main difference between prokaryotes and eukaryotes that are used by antibiotics is (2). This is illustrated by several antibiotics, ie erythromycin which binds to the 50S subunit of bacteria and stops translocation, chloramphenicol binds to 50S subunit and inhibits the peptidyl transferase and streptomycin which prevents the binding of f-Met-tRNA thereby blocking the initiation step.

b. What is the advantage for E. coli to have the three ribosomal RNAs (16s, 23s, and 5s) as part of the same RNA precursor?

Regulation. Since they are one the same precursor they can be coordinately regulated (need only one promoter to get all three at same time) and hence only the amount needed will be made (no excess or scarcity)

c. What is the mode of action of diptheria toxin?

Diphtheria toxin is a powerful inhibitor of human protein synthesis. The toxin contains an enzyme that transfers ADP-ribose from NAD to elongation factor 2 (EF2), blocking translocation.

[Joyce Rubin]

12. List four differences between introns in tRNA genes and protein coding genes.

tRNA genes may or may not contain an intron (present about 15 % of time). If present, only one intron exists and it is always in the same site - 1 nt 3' of the anti-codon. Unlike introns in protein coding genes, these introns are less than 60 nt long and always are spliced according to shape.

[Ben Greller]

13. What is the function of modified bases in tRNA?

Examples of modifications:

methylation

hydroxymethylation

glycosylation

acetylation

deamination

Methylation prevents formation of certain base pairs rendering some bases accessible for other interactions; also imparts hydrophobicity, which may be important for interaction with synthetases and ribosomal proteins. Other modifications alter codon recognition, aid in recognition by specific enzymes, or protect it from being degraded. (From Lippincott, Stryer 3rd ed. (old), Lehninger 2nd ed.)

[Ingrid Marquardt]

14. There are six codons for the amino acid serine in the genetic code. Using the rules of codon-anticodon pairing (and the wobble hypothesis):

a. what is the minimum number of tRNAs needed to read these six codons?

There are several rules to keep in mind when answering this type of question. Consider the following chart of codons, the base pair rules for the Wobble hypothesis, and the convention that all codes are written in the 5' to 3' direction.

serine codons	1st base of anticodon	3rd base of codon
5'- UCU -3'		
UCC	C	G
UCA	A	U
UCG	U	A or G
AGU	G	U or C
AGC	I	U, C or A

The first two positions of the codon are non-negotiable. However, in this case, that represents only two types: UC and AG. When you apply the Wobble rules for the third position, there are a minimum of three (3) tRNAs needed.

b. what are the anticodons for each of these tRNAs?

These three are: ICU, IGA, and CGA [or you could choose UGA for the third]. Compare these to the codons for serine above. Remember that these are written 5' to 3', and therefore would be "backward" when lined up with the codon in the ribosome. (Other possibilities exist – consult the chart).

[Jack Leavens]