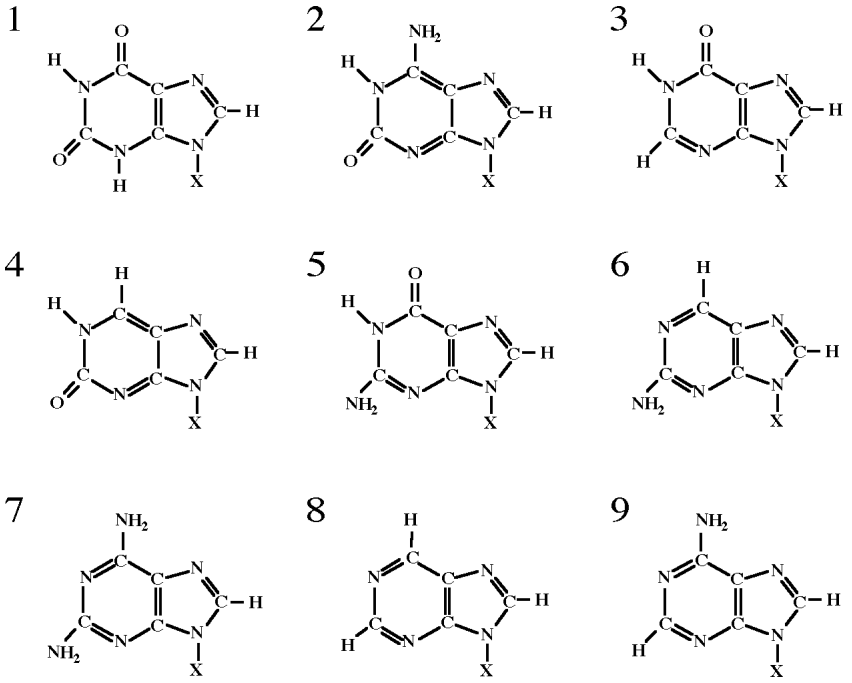
This copy contains questions from both the Midterm and Final Examinations

Decide which is the best answer and blacken the corresponding brackets.

1. Which of the following purines are imposters (not involved in nucleotide metabolism)?



A. Molecules 2, 4, 6, 7, 8 are fake (not involved in nucleotide metabolism).
B. Molecules 1, 3, 6, 8, 9 are fake.
C. Molecules 4, 5, 7, 8, 9 are fake.
D. Four or fewer are fake.
E. Six or more are fake.

2. Which of the following are substrates of thymidylate synthase?

A. dUMP, N^{10}-formyl THF, ATP
B. dUDP, N^5-N^{10}-methylene THF
C. dUTP, N^{10}-formyl THF, ATP
D. dUMP, N^5-N^{10}-methylene THF
E. UDP, N^5-N^{10}-methylene THF, ATP

3. Which of the following are precursors (contribute atoms) to <u>de novo</u> UMP biosynthesis?

A. N^{10}-formyl THF, glutamine, CO_2, PRPP
B. N^5-N^{10}-methylene THF, glutamine, CO_2, PRPP
C. aspartate, glutamine, CO_2, PRPP
D. aspartate, glutamine, CO_2, ATP
E. aspartate, glutamate, CO_2, ATP

Decide which is the best answer and blacken the corresponding brackets.

4. How many of the following statements are true?

i. Defects in adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) activities are causes of two different immunodefiency diseases.
ii. 5' Fluorodeoxyuridylate can be used to treat Lesch-Nyhan syndrome.
iii. Hydroxyurea is an allosteric inhibitor of ribonucleotide reductase.
iv. Defects in adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) activities lead to the elevated uric acid levels symptomatic of Lesch-Nyhan syndrome.
v. Diazidonorleucine (DON) is an analogue of aspartic acid and acts as an inhibitor of nitrogen transfer reactions.
vi. Orotic aciduria is a genetic disease associated with excess uridine or cytidine concentrations.

A. One statement is true.
B. Two statements are true.
C. Three statements are true.
D. Four statements are true.
E. Five or more statements are true.

5. Which list is made up of only bases?

A. inosine, orotic acid, thymine, guanosine, cytosine
B. xanthine, uracyl, thiamine, guanine, adenine
C. hypoxanthine, orotate, thymine, guanine, cytosine
D. xanthine, orotate, thiamine, guanine, hypoxanthine
E. inosine, orotate, uracyl, guanosine, xanthine

6. Consider the following statements on purine & pyrimidine biosynthesis.

i. Pyrimidine biosynthesis is subject to feedback inhibition by pyrimidine nucleoside triphosphates.
ii. PRPP activates biosynthesis of purines
iii. dATP inhibits ribonucleotide reductase
iv. AMP specifically inhibits the biosynthesis of GMP
v. GTP is required for the biosynthesis of AMP
vi. ATP stimulates ribonucleotide reductase

Which of the following best describes these statements.

A. Two or less of the six statements are true.
B. Exactly three of the six statements are true.
C. Exactly four of the six statements are true.
D. Exactly five of the six statements are true.
E. All are true.

Decide which is the best answer and blacken the corresponding brackets.

7. Sulfadrugs (sulfonamides) are antibacterial compounds used treat urinary tract infections. These compounds functions by:

A. preventing the binding of 5,10-methylenetetrahydrofolate to the enzyme thymidylate synthase
B. binding to the enzyme dihydrofolate reductase and preventing the reduction of dihydrofolate to tetrahydrofolate
C. interfering with the ribonucleotide reductase catalyzed conversion of ribonucleotides to deoxyribonucleotides, thereby preventing DNA synthesis (and cell proliferation)
D. reducing the intracellular pools of ribonucleoside triphosphates, thereby interfering with messenger RNA synthesis
E. blocking the synthesis of folate from *p*-aminobenzoic acid

8. Which of the following statements is correct?

A. N^{10}-formyl THF contributes carbon atoms 2 & 8 in purine biosynthesis.
B. N^{10}-formyl THF contributes nitrogen atoms 1 & 9 in purine biosynthesis.
C. Glycine contributes atoms 7, 8 & 9 in purine biosynthesis.
D. Nitrogen atoms occur in purine rings at positions 1, 5, 7 & 9 with the ribose at position 9.
E. N^5-N^{10}-methenyl THF contributes carbon atoms 4 & 8 in purine biosynthesis.

9. In animal cells, channeling occurs in pyrimidine biosynthesis...

A. at the level of the mitochondrial outer membrane enzyme dihydroortate dehydrogenase.
B. to increase the pool size of enzymatic intermediates.
C. at the level of two cytosolic multienzyme complexes.
D. between mitochondrial cabamoyl phosphate synthetase and aspartate transcarbamoylase.
E. between OMP decarboxylase and CTP synthetase.

10. The enzyme(s) responsible for generating nucleotide diphosphates is/are:

A. specific for each base type but non-specific for ribose/deoxyribose.
B. called diphosphonucleoside kinase (or nucleoside diphosphokinase).
C. called diphosphonucleosidases.
D. responsible for mantaining a low NTP/NDP ratio in the cell.
E. are specific for ribose/deoxyribose forms.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

1. Transcription in eukaryotes: indicate which of the following statements are correct.
   (A) The TATA-binding protein interacts with the DNA major groove of the TATA element.
   (B) The TATA-binding protein is involved in DNA binding and protein-protein interactions.
   (C) The phosphorylated form of RNA polymerase II is involved in promoter binding but not in transcription elongation.
   (D) On a DNA template that is not supercoiled, promoter clearance requires the participation of TFIIH.

2. Transcription in eukaryotes: indicate which of the following statements are correct.
   (A) Transcription initiation *in vitro* does not require the presence of TFIIB and TFIIH provided that the DNA template is negatively supercoiled.
   (B) The kinase that is responsible for the phosphorylation of RNA polymerase II is a cyclin-dependent kinase.
   (C) The kinase activity that is responsible for the phosphorylation of RNA polymerase II is provided by TFIIF.
   (D) TFIIH provides DNA-dependent ATPase and DNA helicase activities.

3. Transcription in prokaryotes: indicate which of the following statements are correct.

   (A) Binding sites for activators interacting with the σ (Greek letter sigma) factor must be located close to the -35 element.
   (B) The core RNA polymerase binds to the promoter to form the "closed complex".
   (C) The carboxy-terminal domain of the α (Greek letter alpha) factor makes specific protein-DNA contact with the UP element.
   (D) The change in α (Greek letter "alpha") factor can change the DNA binding specificity of RNA polymerase.

4. The regulation of a promoters in prokaryotes involves several elements. Indicate which of the following statements are correct.
   (A) A binding site situated in between the -35 or -10 promoter elements can function as an operator.
   (B) On one of the OR sites, bacteriophage lambda repressor functions as a repressor exclusively.
   (C) On one of the OR sites, bacteriophage lambda repressor functions both as a repressor and as an activator.
   (D) To activate transcription, an activator must be able to interact with either the sigma or the alpha subunit of RNA polymerase.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

5. Indicate which of the following statements are correct.
   (A) The catabolite activator protein requires an allosteric effector to bind to DNA.
   (B) DNA binding by the lactose repressor is stimulated in the presence of lactose.
   (C) A tetramer of bacteriophage lambda repressor has higher affinity for the operator region (OR3, OR2, OR1) than a dimer.
   (D) The tryptophan repressor binds specifically to DNA only in the presence of L-tryptophan.

6. Indicate which of the following statements are correct.
   (A) Cooperative binding to two sites on DNA is facilitated when the two sites are located on the same side of the double helix.
   (B) Cooperative binding to two sites on DNA involves both protein-protein and protein-DNA interactions.
   (C) DNA looping may occur when two interacting proteins bind to two sites at a distance.
   (D) DNA looping cannot be visualized by electron microscopy.

7. A major source of information about promoter function in prokaryotes is provided by mutations. Indicate which of the following statements are correct.
   (A) "Up mutations" often increase the homology with the -10 or the -35 consensus sequences.
   (B) "Down mutations" in the -10 sequence often involves the replacement of an A:T base pair with a G:C base pair.
   (C) "Down mutations" in the -35 sequence reduce the rate of closed complex formation.
   (D) Mutations that change the distance between the -10 and the -35 consensus sequences do not have an effect on promoter strength.

8. Various strategies are used by bacteriophages. Indicate which of the following statements are correct.
   (A) Promoters of bacteriophage early and late genes have different –35 sequences.
   (B) Some bacteriophages code for repressors and activators that control the action of the bacterial RNA polymerase.
   (C) In some bacteriophages, the transition from early to middle and then late genes is accomplished by synthesizing new alpha factors.
   (D) Some bacteriophages encode an RNA polymerase which is made of only one polypeptide.

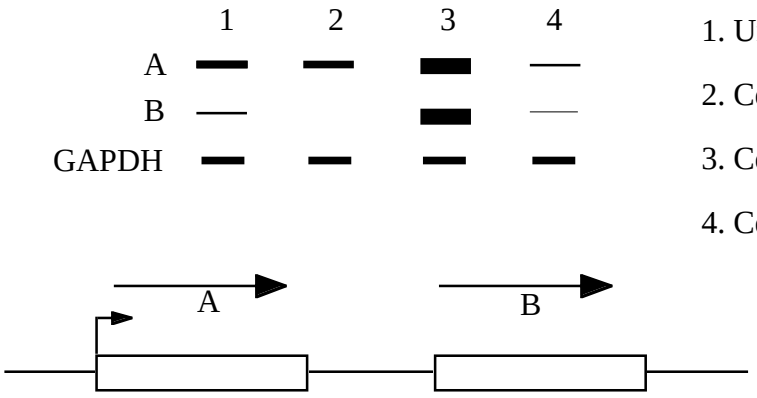
9. Transcription in prokaryotes: indicate which of the following statements are correct.
   (A) Activators in bacteria always contain a region capable of protein-protein interaction.
   (B) Depending on the position of its binding site on DNA, an activator may also function as a repressor.
   (C) Repressors in prokaryotes do not need to interact with other proteins in order to repress transcription.
   (D) To convert a weak promoter into a strong one, we could modify its -35 and -10 sequences or, alternatively, we could add a binding site for an activator.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

10. Transcription in eukaryotes: indicate which of the following statements are correct.
    (A) Transcriptional trans-activators are required for the effect of enhancers on gene expression.
    (B) Molecular targets for transcriptional trans-activators could be TFIIH, TAFs, TFIIB.
    (C) Transcriptional trans-activators may contain a specific DNA binding domain and a domain allowing interactions with one of the general transcription factors.
    (D) Some sub-units of TFIIF are also involved in DNA repair.

1. Interpretation of a nuclear run-on transcription assay. Indicate which conclusions can be drawn from the results of this experiment.



1. Untreated Cells
2. Cells treated with drug X
3. Cells treated with drug Y
4. Cells treated with drug Z

    (A) The GAPDH control serves to establish that there is equal level of radiolabeling in each sample.
    (B) Drug X causes a complete block to elongation of transcription.
    (C) Drug Z reduces transcription initiation.
    (D) Drug Y stimulates transcription initiation and transcription elongation.

2. Attenuation within the tryptophan operon *in vivo* could be affected one way or another by the following manipulations. Assuming that all amino acids are present in high amounts, indicate which of the following manipulations will cause an increase in attenuation.

    (A) deletion of the entire leader region between the transcription start site and the trpE gene
    (B) replacement of all the tryptophan codons with alanine codons in the leader coding region.
    (C) deletion of the sequence "1" in the leader region.
    (D) introducing mutations that increases the stability of the 3:4 hairpin.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

3. Regarding rho-dependent termination:
   (A) rho is capable of unwinding the RNA.
   (B) the polarity of some nonsense mutations may be linked to their effect on rho-dependent termination.
   (C) functions as a hexamer.
   (D) rho binds to RNA that contain a rho consensus binding site.rho.

4. Regarding the process of antitermination in bacteria,
   (A) antitermination during delayed early transcription of bacteriophage lambda requires the action of the bacterial proteins NusA, NusG, NusB and S10.
   (B) in the absence of N, the NusA protein functions as a termination factor.
   (C) antitermination during late transcription of bacteriophage lambda requires the action of the viral protein, Q.
   (D) antitermination functions better on rho-independent than rho-dependent termination sites.

5. In the case of imprinted genes,
   (A) the pattern of methylation may change in the germ cells.
   (B) The origin of a given allele (whether it is maternally or paternally inherited) determines whether it is expressed or not.
   (C) maintenance of the imprint pattern in somatic cells requires the action of the cytosine methyl-transferase (CMT).
   (D) deletion of the expressed allele would result in a phenotype identical to the homozygous null phenotype.

6. Regarding DNA methylation,
   (A) 5-aza-2'-deoxycytidine (5azaC) can make a covalent interaction with the enzyme DNA methyltransferase.
   (B) The incorporation of 5-aza-2'-deoxycytidine (5azaC) in the DNA does not affect gene expression.
   (C) The promoter region of house-keeping genes is not methylated.
   (D) DNA methylation plays a role in the control of gene expression in yeast.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

7. Regarding DNA methylation,
   (A) The observed rate of point mutations in mammalian cells is higher at CG dinucleotides than at any other dinucleotides
   (B) In tumor cells, certain CpG-rich islands can be hypermethylated even when the overall rate of methylation is decreased.
   (C) DNA methylation can be used as a marker to determine whether the BRCA1 gene is expressed in breast tumors.
   (D) In the "min" mice system, inhibition of DNA methylation led to an increase in tumor formation.

8. Regarding DNA methylation,
   (A) Repetitive DNA sequences are generally methylated in the adult mouse.
   (B) The level of methylation in the genome of mammals changes drastically before and after the time of implantation.
   (C) Cells deficient for the DNA methyltransferase 1 exhibit a higher rate of mutations.
   (D) The rate of recombination is not affected by the level of DNA methylation.

9. Regarding silencing at the telomeres and the silent mating type loci:
   (A) Rap1 is one of the proteins that recruit the silencing complex at the telomere and at the silent mating type loci.
   (B) The same complex of proteins recruit the silencing complex at the telomere and at the silent mating type loci
   (C) In the reaction catalyzed by the silencing complex, NAD acts as a co-factor and O-acetyl-ADP-ribose is a product of the reaction.
   (D) The Sir4 protein carries an enzymatic reaction that is required for the repression of transcription by the silencing complex.

10. In the technique to identify DNase hypersensitive sites:
   (A) The DNA fragments generated in the procedure are detected by Southern blot hybridization
   (B) The DNA is first digested with a restriction enzyme, and then with DNase 1.
   (C) As a control in the reaction, some DNA is digested with a restriction enzyme only.
   (D) The treatment with DNase 1 is performed on the "naked" DNA.

11. Regarding nuclear receptors:
   (A) The ligand-binding domain can bind alternatively to a co-repressor or a co-activator complex.
   (B) Co-activator complexes prefer to interact with a ligand binding domain that is bound to its ligand.
   (C) Nuclear receptors bind to DNA as homo- or hetero-dimers.
   (D) The ligand is usually a hydrophilic molecule.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

12. Silencing
    (A) Position effect variegation results from the fact that spreading of a particular chromatin structure along the chromosome varies from one cell to another. .
    (B) Specifically modified histones H3 and H4 are found at silenced loci.
    (C) Genes that are located closed to a silent mating type locus may be subject to position effect variegation.
    (D) Telomeric silencing involves the association between the Rap1 protein and Sir3 or Sir4 proteins.

13. Chromatin structure and gene regulation.
    (A) The extent to which transcription can be activated by a transcriptional activator *in vitro* can approach the level of activation observed *in vivo* provided that chromatin DNA is used as a template for transcription.
    (B) In the context of chromatin, antirepression refers to the process by which the chromatin DNA template is made accessible to transcription factors.
    (C) The method that measures the sensitivity of a given locus to DNase 1 has revealed a link between gene expression and the state of the chromatin.
    (D) The DNase sensitivity of the globin locus is constant among various cell types.

14. Acetyltransferases and chromatin remodeling complexes.
    (A) Acetylation of histones results in the loss of a positive charge.
    (B) The process of transcription elongation results in the progressive acetylation of histones within a gene.
    (C) The RNA pol II holoenzyme is associated with a chromatin remodeling complex.
    (D) Some but not all chromatin remodeling complexes contain an ATPase activity.

15. Chromatin structure and gene regulation.
    (A) A functional globin locus control region induces DNA replication of the globin gene cluster early during S phase in erythroid cells.
    (B) At the molecular level, the consequence of the Hispanic deletion is to remove most of the globin locus control region.
    (C) Inclusion of a locus control region within a transgene ensures that the transgene will be expressed independently of its site of integration.
    (D) In transgenic mice, the locus control region confers tissue-specific gene expression and copy number dependent gene expression.

For each of the statements below ONE or MORE are correct. Decide which combination of statements is correct and blacken the brackets with:

1) If A, B and C are correct
2) If A and C are correct
3) If B and D are correct
4) If D is correct
5) If all are correct

16. Phosphorylation of eIF2 in mammalian cells leads to
    (A) Prevention of ternary complex formation
    (B) Trapping of eIF2B in a complex with eIF2
    (C) Inhibition of cap-dependent and cap-independent translation
    (D) Degradation of viral and cellular mRNAs

17. Phosphorylation of eIF2 by GCN2 in yeast leads to
    A) Degradation of viral and cellular mRNAs
    B) Decreased amounts of eIF2-GTP-$tRNA^{met}$
    C) Induction of oligoisoadenylate synthase
    D) Induction of GCN4 mRNA translation

18. Eukaryotic mRNA can be polycistronic because of

    (A) Leaky "scanning"
    (B) Shine-Dalgarno sequence
    (C) Termination and reinitation by ribosomes
    (D) Lack of cap structure

19. The iron responsive element (IRE) in the transferrin receptor mRNA is:

    (A) Required for efficient ribosome binding
    (B) Recognized by a translational activator
    (C) Recognized by hemin
    (D) Present in the 3’ non-coding region of the mRNA

20. Insulin stimulation of translation involves

    A) phosphorylation of 4E-BP1 (PHAS-I)
    B) PI3 kinase activation
    C) translation initiation
    D) Dissocaition of 4E-BP1 from eIF4E

Decide which is the best answer and blacken the corresponding brackets.

21. The Shine-Dalgarno sequence is

    A) Pyrimidine rich
    B) Complementary to 18S rRNA
    C) Functions in eukaryotes and prokaryotes
    D) Located 3' to the initiator AUG
    E) Approximately 3-10 nucleotides long

22. Poliovirus infection causes inhibition of translation of cellular mRNAs by:

    A) Inhibiting cap-dependent translation
    B) Phosphorylation of eIF2_
    C) Dephosphorylation of eIF4G
    D) Degradation of cellular mRNAs
    E) None of the above

23. The poly A binding protein in eukaryotes functions in translation and interacts with:

    A) eIF4E
    B) One of the eIF3 subunits
    C) eIF4G
    D) eIF2
    E) Directly with the cap structure

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

24. You label your protein in vivo with radioactive phosphate [^{32} P] and you believe that your protein is phosphorylated on **only** tyrosine residues. How would you prove this and identify which tyrosine is phosphorylated (you know the sequence of your protein and you have more than one tyrosine in your protein).

    A) Cleave your protein with a protease, separate peptides and sequence radioactive peptides.
    B) Subject your protein to partial alkali hydrolysis and separate amino acids by chromatography.
    C) Subject your protein to high temperatures and separate amino acids by chromatography
    D) Subject your protein to acid hydrolysis and separate amino acids by chromatography.

    1. A and B
    2. A, C and D
    3. A and C
    4. A and D
    5. C and D

25. Which of the following are true for CRE binding protein (CREB).
    A) Phosphorylation of CREB on its transactivation domain is required for recruitment of a coactivator
    B) Phosphorylation of CREB causes it to translocate from the cytoplasm to the nucleus.
    C) Phosphorylation of CREB on its DNA binding domain stimulates transcription
    D) Phosphorylation of CREB stabilizes its association with DNA.

    1. A and D
    2. A and C
    3. C
    4. A
    5. B and D

26. You know that a complex between transcription factor X and co-activator Y is essential to activate transcription from promoters containing a cyclic AMP response (CRE) element. Phosphorylation of your transcription factor (X) by A kinase is required for activation of transcription from CRE elements <u>but you do not know if this is required for DNA binding of X or association of X with Y</u>. What experimental approaches would allow you to establish if DNA binding or association of X and Y is regulated by phosphorylation.

    A) Synthesise protein X and protein Y in bacteria and determine if X associates with Y in the absence of phosphorylation.
    B) Gel shift assays using radiolabelled double stranded CRE oligonucleotides plus bacterially synthesised X. Where X is +/- phosphorylated by A kinase in vitro.
    C) Take a cellular extract, +/- stimulation by activators of protein kinase A. and bind proteins to an affinity column containing a CRE oligo. Determine if X is bound.
    D) Transfect cells that do not express X or Y, with a CRE CAT reporter gene plus plasmids expressing nothing, factor Y, each with and without a plasmid expressing A kinase. Measure CAT activity.

    1. A, B, and D
    2. A, B and C
    3. B, C and D
    4. A, C and D
    5. A and D

27. The following compounds act as sugar donors in glycoprotein or proteoglycan biosynthesis:
    A) GMP-sialic acid
    B) Dolichol-P-galactose
    C) GDP-mannose
    D) UDP-iduronic acid

    1. A
    2. B
    3. C
    4. D
    5. B and C

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

28. Targeting of most lysosomal enzymes to the lysosomes:

A) requires the activity of a specific N-acetylglucosaminidase
B) requires formation of N-linked complex oligosaccharides
C) requires ERGIC 53
D) requires mannose-6-P

1. A
2. B
3. B and C
4. D
5. A and D

29. For normal glycosylation in mammalian cells, the oligosaccharide precursor to N-linked oligosaccharides

A) contains 3 glucose, 9 mannose and 2 N-acetylglucosamine residues
B) contains 3 glucose, 9 mannose and 1 N-acetylglucosamine residues
C) is synthesized in a step-wise manner directly from nucleotide sugars
D) is synthesized exclusively in the lumen of the endoplasmic reticulum

1. A
2. B
3. B and C
4. D
5. B and D

30. In the glycosylation of proteins, which of the following statements are true regarding glycopeptide linkages:

A) GlcNAc residues are only found attached to asparagine residues
B) Asn-X-Ser is essential for O-linked glycosylation
C) Asn-X-Ser in proteins is always glycosylated
D) Xylose is involved in the glycopeptide linkage of glycosaminoglycans

1. A
2. B
3. B and C
4. D
5. B and D

For each of the statements below, ONE or MORE are correct. Decide which combination of statements is correct and blacken the corresponding brackets.

31. The carbohydrate structure responsible for the B blood group antigenic determinant:
    A) occurs only on type 1 oligosaccharide chains
    B) contains α1,2-linked fucose residues
    C) contains α1,3-linked fucose residues
    D) contains terminal α1,3-linked galactose

    1. A
    2. B
    3. B and C
    4. D
    5. B and D

32. The sulfate groups in glycosaminoglycans:
    A) are added after formation of the oligosaccharides
    B) are added from sulfated nucleotide sugars during formation of the oligosaccharides
    C) are added in the Golgi
    D) are distributed uniformly along the length of glycosaminoglycans

    1. A
    2. C
    3. A and C
    4. D
    5. B and D

SECTION IV - G. Shore

Give a clear and concise answer to each question.

33. As head of cancer research at a major pharmaceutical company, you must assess a proposal to develop a drug that activates BAK. What is your recommendation? **(7 pts)**

34. pPY is a newly discovered protease. Unexpectedly, in cells expressing pPY, the protein was found both in the cytosol and in the mitochondrial matrix. Extensive analysis revealed that the protein in both locations had the identical structure. Suggest a model to account for this finding and describe experiments to test your hypothesis. **(5 pts)**

35. In a genetic screen in yeast, a mutation in a single gene was identified that caused many lysosomal proteins to localize to the outside of the cell. Suggest an explanation. **(5 pts)**

36. What is the difference between “initiator” caspases and “effector” caspases. **(7 pts)**