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Study questions for the lectures 1-4

1. What information(s) could you obtain from a genetic approach of studying mutants defective in a particular process?

Isolate genomic clone, sequence it, and determine amino acid sequence. This sequence can be compared with that of the healthy organisms to deduce protein function in the organism, which is not equivalent in the
mutant.

Firstly, genetic approach begins at the gene level, comparing a normal gene
to a mutant. It allows you to deduce amino acid sequence of the mutant protein and compare that with the sequence of the known protein to gain
insight into the functioning of the protein. You can also use expression
vectors to express this protein in other cells (i.e. in vitro tests on bacteria, or
other cell lines)

2. How would you define permissive conditions in respect to temperature
sensitive mutants?

Temperature-sensitive mutants exhibit the wildtype phenotype under low temperatures, but exhibit the mutant phenotype at higher temperatures. Thus, conditions are permissive when the mutants are allowed to grow normally, or under lower temperatures.

3. Define (or compare and contrast): a) gene expression; transcription;
replication; translation; b) gene; allele

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Gene expression is the process by which the information from a gene is used in the synthesis of functional protein or RNA product. Transcription is the process of synthesizing heteronuclear RNA transcripts from a gene, and is made by cell machinery using the template strand of DNA; RNA contains uracil instead of thymine in the nucleotide sequence, and is similar to the coding strand. Translation is the process of synthesizing peptide sequences from mRNA using cell machinery. Genes are sequences of nucleotides in a genome that contain information coding for the synthesis of a functional protein or RNA product. Alleles are alternative sequences of a particular gene; more specifically, two homologous chromosomes may contain different sequences of a specific gene at a particular locus, and thus contain different “alleles”.

4. Explain by using your own words the meaning/significance of gene expression.

Gene expression is the process of using the information stored in a genetic sequence to synthesize functional RNA or protein. When a gene is “expressed”, transcription occurs, and translation occurs to form protein product.

Gene expression is the use of the genetic information stored in a gene to create a functional protein or RNA product.

5. What are the roles of model organisms in molecular biology studies?

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Choose two model organisms and explain your reasoning.

Model organisms are used in molecular biology studies because they have
many benefits, including simplicity, relative ease to use, cheapness, and
often short reproductive times. They are also used as standards because so
much information has been collected on them and continue to be collected.
In this way, vast and specific amounts of information about these model
organisms are obtained, and can be extrapolated to other organisms,
including humans. For example, mice can be used to model mammalian
species such as humans due to highly conserved biological pathways and
processes, and they have relatively quick turnover rates compared to other
similar organisms. Escherichia coli are used to model metabolic processes
due to their simplicity, and the conservation of metabolic processes
throughout many species.

6. What are three main functions of DNA? Explain the importance of each
of them.

1) To store genetic information – allows for reproduction of cells into others,
and for production of protein and RNA products for cell function
2) To replicate faithfully – allows for production of nearly identical, viable
organisms to continue the species. life cycle.
3) To mutate – allows for variability in gene sequence and frequency,
allowing for selection and evolution to occur.

7. What is (are) the role(s) of phospho-diester bonds in DNA structure?

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What is (are) the role(s) of hydrogen bonds in DNA structure? What is (are) the role(s) of hydrophobic interactions in DNA structure?

Phosphodiester bonds provide the rigid backbone for DNA structure, and also help stabilize the molecule as they are negatively charged and repel
from each other, allowing H-bonding to occur between the bases instead.
Hydrogen bonds in DNA structure provide minimal stabilization, but also
allow for hydbridization between strands due to complementary hydrogen bonding between A and T, and between G and C. Hydrophobic interactions make it favourable for DNA strands to be hybridized in order to minimize the
water molecules that form the cage around the molecule – thus, provide an
entropically favorable way for DNA duplex formation.

8. What noncovalent interactions are involved in maintaining the double-
helical conformation of DNA?

Ionic forces, hydrogen bonds, van der Waals forces, and the hydrophobic
effect are involved in maintaining the double-helix formation.

9. Learn to recognize nitrogenous bases (A,T,G,C,U) and respective nucleotides.

OK

ANNH, GOHN, CNNO, UOHO, COHORT

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10. Describe Meselson-Stahl experiment and explain how it showed that DNA replication is semiconservative?

Bacteria were grown in 15N, and their DNA bases all contained this isotope
of nitrogen. The cells were then transferred to a 14N containing medium.
After cell replication, one distinct band was observed after centrifugation:
one for 15N14N DNA. This was because, as in the semiconservative model for DNA replication, each strand from the original 15N DNA was used to synthesize a new DNA using new 14N nucleotides. Thus, one strand for each of the new DNA molecules after replication had 15N and the other had
14N. After the second replication, only a 14N14N band and a thinner 15N14N band was observed. The 14N14N was due to new replication using the 14N strand on the 15N14N strand and new 14N nucleotides. The 1415N strand was due to new DNA synthesis using the 15N strands on some molecules. This experiment showed that DNA was being replicated by each
strand being used as a template for a new molecule, and the original DNA
duplex split and the strands were used to make new ones complementary to
them.

11. What is meant by saying that a DNA strand has polarity? That two strands of DNA are antiparallel? That the strands are complementary to one another?

A) Polarity . one strand is oriented such that it has 5. phosphates at one
end of the molecule and 3. OH groups at the other end of the molecule.
B) Antiparallel . in a duplex, one strand will go from 5. to 3. direction, while
the other strand will be opposite in orientation, or antiparallel, and

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go
from 3. to 5. direction.
C) The strands are complementary in that if one has a pyrimidine at one
location, the other strand will have a purine at that same location due to
proper H-bonding. A on one strand will bind T on the other DNA strand, and G will bond with C. Because of this, the sequence of the complementary strand of a known strand can be inferred.

12. If a C content of a preparation of double-stranded DNA is 20%, what is
the T content?

30% according to Chargaff.s rule.

13. What is the difference between nucleoside and nucleotide? What does
dNTP stand for?

A nucleoside contains a ribose sugar and nitrogenous base (A/T/C/U/G) while a nucleotide contains all that, and a phosphate. dNTP stands for deoxy-nucleotide triphosphate.

14. Describe the conformational characteristics of B DNA (or A DNA, Z DNA, triple-helical DNA). When does this (any of the above) form of DNA occur?

B-DNA . 10 bp per turn, 34 nm per turn. Right-handed helix, bases are perpendicular to the axis; this form is found in most cells (in vivo)

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A- DNA . found in low-water content and some RNA duplexes; 11 bp per turn over 28 nm, but bases are rotated to the axis, and so the molecule
appears to have bases more compact than B-DNA; also is left-handed, found in some spores

Z-DNA . found in high salt conditions; 12 bp per turn over 45 nm, small tilt
relative to axis; left-handed DNA, found at promoter regions, some binding
domains, possibly in chromosomal breakage/deletion/rearrangement

15. How does high salt concentration influence denaturation kinetics of
DNA? Explain your reasoning.

High salt stabilizes the negatively charged phosphates in the DNA backbone, reducing repulsion between the strands. This allows for a higher
melting temperature, due to the increased stability of the DNA duplex.

16. What are the classes of DNA sequences in genomic DNA (based on renaturation kinetics)?

Highly repetitive sequences . 100 000 or more sequences in the genome, renature very fast due to concentration/abundance.

Moderately repetitive sequences . 10 to 100 000 sequences/copies in

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the
genome, renature relatively quickly, but not as fast as copies cannot find
each other as fast

Unique . 1 to a few copies in the genome, very slow renaturation as it takes a long time for sequences to find each their complementary strand

17. What is Cot analysis?

Cot analysis is a measure of the rehybridization kinetics to determine relative complexities between genomes. Cot analysis involves shearing of
DNA and denaturation, and measurement of reannealing by absorption rates to determine % ssDNA (at 260 nm) relative to a polyA/polyT sequence. This allows for determination of unique portions of the genome,
and the Cot1/2 values can be compared to approximate the genome sizes.

18. Who received a Nobel Prize for 3D DNA structure?

James Watson, Francis Crick, and Maurice Wilkins. Rosalind Franklin did
not receive the Nobel Prize later on, even though she would of, as Nobel
prizes are not rewarded posthumously.

19. If you had two solutions of DNA, one single-stranded and one double-
stranded, with equivalent absorbance at 260 nm, how would the concentrations of DNA compare in these two solutions? (You can use a diagram if it makes it easier for you to explain.)

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The ssDNA sample would have a lower concentration than the dsDNA sample. This is because ssDNA exhibits high absorbance at 260 nm due to unstacking of bases (hyperchromic effect), while dsDNA exhibits low absorbance (hypochromic effect). To get equal absorbance, this would mean that the concentration of dsDNA is much higher than the ssDNA, in order to account for the difference in absorbance that would occur at equal concentrations.

cot
Study Question for lectures 5-7

1. How does complexity of bacterial genome differ from that of eukaryotic (for example calf) genome?

Bacterial genome has more unique sequences while eukaryotic genomes have a lower proportion of unique sequences, and a large amount of highly repetitive sequences. However, calf genome is more complex as it has a greater amount of unique DNA overall.

2. Explain C value paradox.

Organisms with higher DNA haploid genetic content (C value) do not correlate with apparent organism complexity. For example, ferns have a higher genetic content than humans, even though they would be considered less complex.

3. List and briefly explain factors that influence DNA renaturation kinetics.

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- Salt concentration . determines how the negatively charged phosphate backbone will repel strands
- DNA concentration . at higher concentration, strands will be able to find each other faster
- Genome complexity . a more complex genome will have more unique sequences that will renature very slowly as there are few similar copies, and so each strand must find its complement
- Time . over time, more strands will renature
- pH . affects H-bonds

4. You have found a new species of insects. To evaluate the complexity of the genome of this species, you isolate genomic DNA from, fragment the DNA to uniform 500 base pair pieces, denature the DNA and measure the rate of reassociation. Your data is represented in the curve below (sorry for the bad drawing):

(a) How many classes of DNA (in respect to sequence complexity) are found in this organism?

3; highly repetitive, moderately repetitive, and unique

(b) What can you say about the relative complexity of each class? What fraction of the genome falls into each class?

Highly repetitive, high copy number and high renaturation as copies can
find a complementary strand easily; 25% of genome here. Moderately repetitive: medium copy number, and quick renaturation as copies can find
complementary strand relatively fast; 25% of genome here. Unique: less than 10 copies of sequence, so it takes a long time for a strand to find its
complement; 50% of given genome.

6. List three (3) differences between prokaryotic Topoisomerase I and Gyrase.

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- Topoisomerase I makes cuts one strand, gyrase cuts two
- Topoisomerase I removes negative supercoils, gyrase introduces negative ones
- Topoisomerase I changes L by one, gyrase changes it by 2 as it is a topoisomerase type II

7. What are topological isomers of DNA?

Topological isomers are forms of DNA with the same sequence, but with differing levels of supercoiling.

8. Explain the importance of DNA supercoiling for the cell survival?

Supercoils can store energy which can be used to untwist double helixes and separate strands when needed (negative supercoil). They can also be over-wound to reduce interaction or to decrease the possibility of gene expression. Without supercoiling, there would be a lack of control of the regions of the chromosomal material available for transcription.

9. What are the differences between primary (or secondary, or tertiary) structures of RNA and DNA?

In RNA, primary structure contains ribonucleotides A, U, C, and G; DNA primary structure contains deoxyribonucleotides A, T, C, and G. Both have 5.-3. orientation and are linked by phosophodiester bonds. The secondary structure involves the possible interactions between nucleotides, such as the double helix formed by hydrogen-bonding in DNA between complementary strands, or hairpin and stem-loop formation in RNA. Tertiary structure in DNA is seen during supercoiling when complexed with proteins, while RNA tertiary structure can be seen during complex folding, i.e. into function tRNAs; RNA can do this because it is much less rigid as it does not have the double helix conformation.

10. You have a small 4800 bp long circular DNA. It has a linking

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number
of 450 (L=450). What are the twist (T) and the writhe (W) of this DNA? What assumptions about the structure of the DNA have you made in your answer? (Hint: what is the definition of the twist #?)

The twist is 480 assuming that the DNA is in B-DNA conformation and has
10 bp per turn, and thus 480 turns per 4800 bp. The writhe number then must be -30, which means that there are 30 negative supercoils.

11. Thinking question 1: Many different mutations have been observed in almost all genes, however, only a few have been isolated in histones. How would you explain this finding?

- Histones are required for silencing of genes, and thus small mutations
could result in drastic effects across the cell
- Histones are involved in DNA replication
- Histones are involved in DNA packing, and so mutations would result in inefficient packing and thus would affect cell viability and reproduction efficiency; also, failure for metaphase chromosomes to form and proper distribution into cells during mitosis/meiosis
- **mutations would be lethal or have drastic effects on cells, likely preventing proper folding and transmission to offspring due to the role
and nature of histones

12. Thinking question 2: would it be an easy task to produce polyclonal
antibodies to histones? Explain your reasoning.

It is unlikely since histones are highly conserved among organisms, and
so it would be difficult to get an animal to produce antibodies to them.

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13. What are some of the distinctive features of eukaryotic chromosomes? (note: I expect you to first define chromosomes and after that you have to briefly explain nucleosomes/histone proteins/octet +H1/wrapped DNA, different levels of chromosome condensation, centromere and telomere regions)

Eukaryotic chromosomes are the linear sequences of DNA found in eukaryotic organisms, and can exist in several states. Chromosomes are tightly packed into basic organization units known as nucleosomes – these
are composed of an octet core histone protein, linker DNA, and H1 histones. Histones are positively charged, and thus can associate with DNA. DNA will first wrap around the core 8-part histone protein (approx.
146 bp per histone, ~1.5 wraps) to give 4 x compression over the initial
amount. With the H1 histone, this compression is further increased by the
way the histone binds the DNA on the octet, linking and holding it tightly to
the core protein. This forms the 10 nm fibre. These nucleosome units then
associate through their N-terminal residues that stick out of the core protein and interact with each other like grooves on a screw to form a solenoid structure of 30 nm fibre. This generally forms at higher salt concentrations. Through association with nonhistone scaffolding proteins,
this 30 nm fibre is condensed much further to metaphase chromosomes of approximately 1400 nm diameter. This form is used in metaphase when sister chromatids are separated and distributed to each cell. Control loci,
telomeres, and centromeres also are found on the chromosomes. DNA can differ by being in either a euchromatin or heterochromatin state.

14. What is unusual about the amino acid composition of histones? How is the function of histones related to their amino acid composition?

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Histones are rich in lysine and arginine, which are positively charged. This
aids histones in associating with DNA, which is negatively charged.

15. Name a few non-histone proteins which are a part of chromatin structure and explain why you would expect them to be found there.

Scaffolding proteins are associated with 30 nm solenoid fibre, allowing for
tighter packing and eventually metaphase chromosome formation. Chaperone proteins assist with assembly of histones following DNA replication and formation of new strands, which require histones for condensation. Acetylases, deacetylases, and enzymes to methylate and phosphorylate residues on histones also affect dissociation of histones
from DNA and allowing replication and transcription of DNA. Transcriptional proteins and factors would also be present to enable transcription to take place.

16. What are heterochromatin and euchromatin? What is their importance in DNA replication and transcription?

Heterochromatin is the more condensed DNA that is less transcriptionally
active – it can either be facultative (transcribed in some tissues) or constitutive (highly repetitive, few genes, and inactive). Thus, depending
on its condensation, it may be transcribed but does not contain many genes. Euchromatin is transcriptionally active DNA and much less condensed, and is involved in transcription.

17. Does the degree of chromosomal condensation play a role in controlling replication and transcription? How (explain briefly; use your own words)?

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If a chromosome is highly condensed, proteins for replication and transcription will not be able to get at the genetic information stored in the
DNA nucleotide sequence. Thus, if histones are acetylated and DNA is uncondensed, machinery can reach the sequence and replicate or transcribe the required sequences.

18. Would you expect there to be more histones per kilobase in euchromatin or heterochromatin? Explain your reasoning.

One would expect there to be more histones per kilobase in heterochromatin. This is because heterochromatin is much more compact/condensed than euchromatin.

19. Thinking question (note: methodology is irrelevant for this question,
so do not think about it): the sequences of a particular set of genes are found by in situ hybridization to be heterochromatic in some cell types and euchromatic in different cell types. How would these sequences be categorized (what would be your conclusion about these sequences in respect to gene expression)?

These could be considered facultative heterochromatic. This is because in
some cells the genes are not expressed and are highly condensed, while in other tissues, there are regulation mechanisms for modulation of the
genes such that the chromatin is less condensed and available for expression.

20. Thinking question (note: methodology is irrelevant for this question,
so do not think about it): the sequences of a particular set of genes are found by in situ hybridization of muscle cells to be heterochromatic in early developmental stages and euchromatic in later developmental stages. How would these sequences be

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categorized (what would be your conclusion about these sequences in respect to gene expression)?

Study Questions for lectures 8-9

1. The human gametes have about 3 billion bp of DNA in their chromosomes.
a. Express the size of the diploid genome in kb (kilo bases; 1kb = 1000bp)

The diploid genome will have about 6 billion bp of DNA in chromosomes. Thus the diploid genome is about 6 million kb in size.

b. If the entire DNA was in relaxed B-DNA form, what would be the average length of a

chromosome expressed in cm?

Since there are 46 chromosomes in human diploid cells, each chromosome would have approximately 150 million bp. In B-DNA conformation, where 10 base pairs are 34 nm long in the helix, this would result in an unrelaxed chromosome length of approximately 51 mm.

c. On average, how many complete turns would be in each chromosome?

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There would be 15 million turns per chromosome on average, as there is one complete turn per 10 bp in B-DNA.

d. If there are around 30-40,000 genes in a human gamete, how many genes are there in an average chromosome?

On average, since there are 30-40000 genes per 23 chromosomes, there would be 1300-1750 genes per chromosome on average.

2. Define homologous chromosomes.

Homologous chromosomes are those that have the same genes located at the same loci, and synapse in meiosis, but may have different alleles of
these genes. For example, diploid cells will have 2 copies of chromosome
1. One will come from the father and one from the mother, with some differences due to recombination. Due to the different alleles, the chromatids will not be sister chromatids as they are not completely identical.

3. Define non-homologous chromosomes.

Non-homologous chromosomes are those that do not have the same genes located at the same loci. This is seen in humans between different
chromosomes, i.e. chromosome 1 and 22 which are totally different size and would contain different genes in different sequences.

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4. How many homologous chromosomes are there in a germ cell of a woman? Of a man?

A woman.s germ cell would contain 23 pairs of homologous chromosomes, as there are 22 autosomal chromosomes – which there will be 2 of each, one paternal and one maternal – and one sex chromosome; since females have two X chromosomes (which are the same size and contain the same genes at the same loci), these will also count as homologous chromosomes. Men will have only 22 pairs of homologous chromosomes, since they have X and Y sex chromosomes, which differ greatly and have different sizes and different genes at different locations.

5. Distinguish between homologous chromosomes and sister chromatids.

Sister chromatids are identical DNA molecules that make up one chromosome, and are the result of DNA replication in the S phase; these
are what are separated in equational division. Homologous chromosomes are separate chromosomes that have the same genes on the same loci, but may have different copies – i.e. they are not exactly the same as each
other. These are what are separated in reductional division, such as in
meiosis I.

6. What is the purpose of cell division in Prokaryotes? In Eukaryotes?

In prokaryotes, the purpose of cell division is solely for reproduction and
continuation of the species. In eukaryotes, cell division is for

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reproduction,
organism tissue growth (in higher organisms), and tissue repair (i.e. skin
and bone repair).

7. Distinguish between DNA replication and cell division.

DNA replication is a part of interphase during the S phase, when DNA chromatids are replicated to form identical sister chromatids which can
then be separated in cell division to provide nuclear material for two cells.
Cell division is different from this because it involves the other processes
of reproduction, including biomass production, division of the newly replicated DNA, and cytokinesis to form new cells.

8. Distinguish between reason/purpose for/of mitosis and
reason/purpose for/of meiosis?

The purpose of mitosis is solely to reproduce an exact copy of the original
cell – the DNA content is maintained, and allows for tissue growth. This is
why mitosis occurs throughout an organism.s lifespan. Meiosis is solely
required for gamete production for sexual reproduction. It allows for unique haploid gametes to be formed, which can combine to form a zygote. This allows for maintaining variability while still preserving the
genetic information of an organism. This is important because variability is
important for survival if life and environmental conditions change.

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9. How is variability of genetic information attained by meiosis and fertilization?

Through crossing over of tetrads and recombination during prophase of meiosis I, paternal and maternal chromosomes can produce unique chromatids containing different combinations of alleles. Through independent assortment of chromosomes during metaphase, haploid cells are formed with further unique chromosome combinations. This occurs again during meiosis II, when sister chromatids are separated, which can
contain different alleles due to previous recombination. This variability is
further magnified by the combination of male and female gametes, both which can contain highly variable allele combinations, to form the diploid
zygote.

10. What is a cell cycle? What are the stages of cell cycle?

Cell cycle is the orderly set of events that takes place for a cell to properly
duplicate its genetic information and biomass for formation of two new daughter cells. The two main stages of the cell cycle are interphase, where cell mass and DNA content doubles for the cell division. Mitosis and
cytokinesis are the second stages of the cell cycle: mitosis involves nuclear division to give each new cell the same DNA content, and cytokinesis involves separation of the cell cytoplasm, resulting in two
independently-functioning cells. Mitosis involves chromatin condensation,
attachment to spindle fibres, alignment at the metaphase plate, and separation of sister chromatids to the poles of the original cell.

11. List and briefly describe the checkpoints in cell cycle. What is their

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purpose?

The checkpoints in the cell cycle are for conserving cell viability and functionality – they do this by making sure that everything is in order in the cell for healthy growth and biological function to occur, and also by making sure that DNA is only replicated once. The M checkpoint occurs right after mitosis to ensure that proper division has occurred. The G1 checkpoint occurs to check for damaged DNA before the cell invests energy in replicating the DNA for mitosis. The G2 checkpoint occurs to check if DNA is damaged or has been replicated improperly, which would affect the products of mitosis. Lastly, the M checkpoint checks for proper chromosome attachment to kinetochores so that chromatids can be distributed to the daughter cells. If there are problems that are not fixed, the cell may undergo apoptosis to prevent production of unhealthy daughter cells.

12. What can trigger arrest during the cell cycle?

Arrest can be triggered by DNA damage at the G1 or G2 checkpoints, or by incomplete DNA replication at the G2 checkpoint. Improper chromosome attachment to kinetochores during M can arrest cell division. Also, improper distribution of chromatids and proper nuclear division after mitosis can trigger arrest. Lack of nutrients, improper size also.

13. How many chromosomes are there in a somatic cell of a person with Down syndrome (trisomy of chromosome 21)?
a. How many autosomes does this person have in a somatic cell?

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A person with Down syndrome will have 3 chromosomes of chromosomes 21. Thus, they will have 45 autosomes in a somatic cell.

b. How many sex chromosomes does this person have in a somatic cell? In a germ cell? In a gamete? In a spermatozoid? In an ovum? In a zygote?

This person will have 2 sex chromosomes in a somatic cell; 2 in a germ cell; 1 in a gamete; 1 in a spermatozoid and 1 in an ovum; 2 in a zygote.

c. How many DNA molecules does this person have in mitotic metaphase? In G1 phase?

94 DNA molecules in mitotic metaphase; 47 in G1 phase, before DNA replication.

d. How many telomeres are there in a person’s somatic cell during G2 phase?

After DNA replication, there are 94 DNA molecules, and so there are 188 telomeres.

e. There are 4 alleles for a certain gene carried by chromosome 21 in

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human population. How many allele copies does a person with Down syndrome have in a somatic cell in G1 phase? How many different alleles for this gene could the same person have?

A person with Down syndrome could have 3 allele copies in G1 phase. The same person could only have 2 different alleles, as one would come from one parent, and 2 from the other.

*Next 3 questions: there are 13 alleles for a certain gene carried by chromosome 21 in human population.

f. How many allele copies does a person with Down syndrome have in a somatic cell in G1 phase? How many different alleles for this gene could the same person have?

3; 2

g. How many allele copies does a person with Down syndrome have in a cell which is in meiosis I anaphase?

6

h. How many different alleles for this gene could the same person have in a cell which is in meiosis I anaphase?

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Suggestion: re-make the questions e-h but for a gene carried by chromosome 5.

14. When does chromosome segregation happen in mitosis? In meiosis? When does chromatid segregation happen in mitosis? In meiosis?

Mitosis . does not occur as homologous chromosomes do not pair

Meiosis . occurs in anaphase I because homologous chromosomes pair in prophase I

Mitosis . chromatid segregation occurs in anaphase

Meiosis . chromatid segregation during anaphase II

Study Questions for lectures 10-11

1. List proteins (enzymes and other factors) involved in the process of
DNA replication in E. coli. Explain briefly the role of each of these proteins in replication.

- oriC site, or origin of replication contains DnaA boxes and 3 x 13 bp
sites that are AT-rich
- DnaA protein recognizes DnaA boxes, binds and opens 13 bp sites

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(AT-rich) if negative supercoiled, requires ATP
- DnaB/helicase carried to DnaA by DnaC chaperone
- Helicase unwinds DNA at replication fork, forming positive supercoils
ahead of it
- Toposiomerase II/gyrase/DnaG converts positive supercoils to negative ones that form in front of the primeosome
- Single-stranded binding proteins bind single stranded DNA, keeping it
from reforming the double helix
- DNA primase adds a ~10 nucleotide RNA primer at the primosome (associated with helicase)
- DNA polymerase III adds dNTPs downstream of the primer from 5. to 3. end by adding them to the 3. free OH group
- Multiple subunits of the DNA polymerase III holoenzyme
- Lagging and leading strand synthesized simultaneously
- DNA polymerase I removes RNA primer nucleotides and fills it in with new nucleotides, also proofreads added nucleotides
- DNA ligase ligates newly added nucleotides with ones from nearby strand

2. What is meant by replication being bidirectional? Semi-conservative? Continuous and discontinuous?

Bidirectional in that it starts from a site of origin on the chromosomal
material and replicates in both directions from that site – replication bubble
formed, which expands. Semi-conservative in that the newly formed DNA will contain one strand of the previous DNA, and a new synthesized strand. Continuous in that on one strand, DNA is continually added off of
one primer in the 5. to 3. direction. On the other strand, synthesis is
discontinuous in that primers are continually added and replication occurs
in the 5. to 3. direction until the previous strand is reached, when b-subunit
releases core polymerase.

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3. Contrast the role of DNA polymerase I and III in E. coli DNA replication.

DNAPI removes rNTPs from primer and fills them in with dNTPs. Has 5. to
3. exonuclease activity, 5. to 3. polymerase activity, and 3. to 5. exonuclease activity to remove mismatches that were added by it. Low processivity also. Not “required” for cell viability and DNA replication, as in
mutants are still viable.

DNAPIII is highly processive and is absolutely required for DNA replication. It is a complex enzyme that adds dNTPs to both leading and
lagging strands. It also has 3. to 5. exonuclease activity, but does not
remove primers. It requires a primer to begin synthesizing DNA.

4. Which subunit of DNA polymerase III provides processivity? Which protein complex loads this subunit onto the DNA?

The B-subunit provides processivity. The gamma-complex loads this subunit onto the RNA primer of DNA.

5. How can discontinuous synthesis of the lagging strand keep up with continuous synthesis of the leading strand?

Primers are added and one of the core enzymes synthesizes DNA from it. A loop is formed because the core enzymes are attached together by the tau-subunits. Once the 135 bp or so fragment is synthesized, the core polymerase on the lagging strand is released, and binds upstream to another RNA primer. In this way, the lagging strand can keep up with the
leading strand, which only runs off of one primer.

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6. Why is decatenation required after replication of circular DNAs?

Because the two strands of circular DNA are interlinked.

7. Why do eukaryotes need telomeres but prokaryotes do not?

Prokaryotes have circular DNA, and thus there is always a 5. end available for primers to be added to for DNA polymerase to bind to and synthesize the lagging strand. Eukaryotes do not have fixed ends as they
are linear, and so there comes a point where a 3. overhang will occur at
the end of the chromosome, as RNA primase cannot add a primer right onto the exact end.

8. Thinking question: What would be the components necessary to make DNA in vitro by using DNA polymerase I?

You would need a template DNA strand, lots of RNA primers (or RNA primase to synthesize them), and DNA ligase.

9. Thinking question: What properties would you expect an E. coli cell to have if it had a temperature – sensitive mutation in the gene for DNA ligase?

At high temperatures, DNA ligase would not be able to ligate strands together. Thus, at a certain temperature, the strands would dissociate.

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This would result in massive repercussions throughout the cell as DNA would not be able to be properly duplicated, or transcribed and translated
into products.

10. Thinking question: What properties would you expect an E. coli cell
to have if it had a temperature – sensitive mutation in the gene for DNA polymerase I?

It would not be able to remove the RNA primer. This would result in errors
in transcription and later replication. Also, the primer could fall off –
however, the gap could be filled in by other DNA polymerases. Also, RNase H can remove RNA primers. A mutation for DNA polymerase I would still result in viable cells.

11. Compare and contrast major eukaryotic and prokaryotic DNA polymerases.

Prokaryotic . one main polymerase synthesizing DNA on leading and lagging strand, no primase activity

Eukaryotic . multiple polymerases present; primase function, involved in
telomere region, initiation of replication; much slower due to complex chromatin structure

12. What is telomerase and why is it important?

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Telomerase is a nucleoprotein that adds repetitive sequences to the ends
of chromosomes (telomeres), extending the length and reducing the chance of important genetic information being removed in DNA replication
(end-replication problem). It carries its own RNA primer. It is important
because it keeps the ends of the chromosomes long, preventing loss of important information in every cycle of duplication and mitosis.

13. What is the major difference between bacterial and eukaryotic replication that allows a eukaryotic cell to replicate its DNA in a reasonable amount of time?

Eukaryotic cells have a large number of replicons/origins of replication,
whereas bacteria only have one. This allows for multiple replisomes to be
activated at once, thus greatly speeding up the replication of the chromosome(s).

14. Describe the events that occur at an origin of replication during initiation of replication in E. coli?

DNaA will bind A boxes near the ori region, and will melt the 13-bp AT-rich
regions. SSB will bind ssDNA, and helicase (DnaB, brought by DnaC) will
unwind, forming a replication fork. Gyrase/DnaH will release positive supercoils that form in front of the replication fork. DnaG/primase will lay
down RNA primers, and is associated with the helicase at the primeosome. DNAP will bind at the replication fork.

15. What are cis elements? Trans-elements?

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Cis-elements are DNA sequences on a nearby genome sequence from the desired sequence. These include DnaA boxes or 13 bp regions such as in OriC in E. coli. Trans-factors are proteins made somewhere else in
the cell that can diffuse and recognize/bind/act on the cis-elements or on
the target genome sequence.

16. Is the following statement true or false: Regardless of whether a gene is expressed in a given cell type, it will replicate at the same, characteristic time during S phase. Explain your reasoning.

False. All of the genome is replicated in the S phase, regardless of whether genes are expressed. However, genes are not all replicated simultaneously, but sequentially depending on their location from the replicons and the degree of condensation – genes being expressed will be
in euchromatin, which is less condensed and will be replicated quicker/earlier. Genes in heterochromatin will be replicated slower/ later
due to greater condensation.

17. Thinking question: Is making of RNA primers (by primase), which have to be subsequently removed and replaced with dNTPs (by DNA polymerase I) actually wasteful and energetically inefficient process? (Hint: think about fidelity of primer-making vs. proofreading capability of DNA pol I, in other words what is the only purpose of RNA primers – is the accuracy of this process very important at this point?)

RNA primers do not have to be accurate as it is not what matters. They are only there for DNA polymerase to bind and form the complementary DNA strand. Thus this is most likely not a very wasteful process as it does
not use up rNTPs and it is not carried out with high fidelity.

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18. You preformed Cot analysis using genomic DNA samples obtained from a 2 year-old child and a 76 year-old individual. Results of this analysis show that one of the most rapidly reassociating classes of DNA is substantially reduced in the older individual with respect to the 2 year-old. How can you explain this finding? (hint: think about termination of linear DNA replication.)

The older individual has shorter telomeres, if any, which are highly repetitive 6-7 nucleotide sections of DNA. These would rapidly reassociate
due to repetitiveness and high abundance. In a 2 year old child, more telomeres would be observed.

Study Questions for lectures 12-14

1. Distinguish between the terms “DNA repair” and “recombination”.

DNA repair is when damaged DNA is fixed so to maintain cell viability –
DNA may not necessarily be repaired to its correct/original form. Recombination is the exchange of genetic material between two DNA molecules, causing rearrangement. Some forms of DNA repair incorporate recombination, such as when a nick is encountered in DNA during replication.

2. List and briefly explain three major causes for mutation in DNA.
- Errors from replication: small chance for wrong base being put in , such
as A instead of C
- Spontaneous error: depurination (loss of AT pair) or deamination (C converted to U)
- External factors: other outside forces acting on the DNA, including ionizing/nonionizing radiation, intercalating agents, or alkylating agents

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3. Explain how errors in DNA replication can lead to mutations.

If there is an error in DNA replication, such as an A being inserted instead
of a C to form an A–G pair, the next replication of DNA will result in formation of an A–T pair on one strand and a C–G pair on the other; at this
point, the mutation could not be fixed and could result in visible mutations
to the cell products.

4. Distinguish between the effects of mutations on the somatic and germ cells of multicellular organism.

Mutations in somatic cells can result in tissue loss/damage, or even cancer if cells are allowed to uncontrollably reproduce. Germ cell mutations can be carried on to the offspring through the gametes, and thus make mutations hereditary.

5. List the various types of DNA repair mechanism (we have mentioned seven).
- Exonuclease activity of polymerase
- Base excision repair
- Nucleotide excision repair
- Non-homologous end joint repair
- Direct reversal repair
- Recombination repair
- Error-prone repair (translesion repair, NHEJ)

6. Give the detailed description of the base excision (or nucleotide excision) repair process in bacteria.
a. Lesion-specific glycosylases hydrolyze glycosidic bonds between sugar and base; recognizes specific mismatches
b. AP (Pu/Pyr) endonuclease removes abasic sugar, nicking upstream DNA to make 3.OH end
c. DNA pol. I extends DNA strand from here

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d. DNA ligase fills in hole

In nucleotide excision repair, UvrA scans for damage/lesion. UvrB is then
bound, and UvrA released. UvrC then joins, and two incisions are made surrounding the lesion (10 bp chunk). UvrD unwinds, releases ssDNA

segment. DNA polymerase I excises segment, replaces with dNTP. DNA ligase connects strands together.

7. What is the direction of excision by DNA polymerase I during nucleotide excision?

3. to 5.

8. Describe the mismatch repair process of bacteria (pay attention at the ways in which the daughter and parent strand are recognized by repair system).

In the mismatch repair process, errors are corrected during replication
when a DNA backbone distortion is detected. mutS will bind to the mismatch, and mutL will find a GATC sequence and bind at 2 locations to
create a loop, using ATP. mutH will then bind and nick the unmethylated
strand (newly synthesized, as it is “assumed” that is where the mutation
will be found. The strand will be excised from GATC up to the mismatch by
exonuclease I or III. DNA polymerase III and DNA ligase will fill in the new
strand.

9. Describe briefly the mechanism of direct reversal of damage in bacteria.

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Some bacteria contain photolyase enzyme to directly reverse the effects of nonionizing radition, such as UV, on DNA forming thymine dimmers, which can cause addition/deletion in DNA during replication. This enzyme uses light.

10. Define and briefly explain recombination repair.

Recombination repair involves using an intact strand to serve as a template for another damaged strand. It involves DNA polymerase III stalling once reaching the replication fork due to a nick. An exonuclease (RecBCD) degrades the end where the nick is to create a 3. overhang, which is bound by RecA. Strand invasion occurs, and the intact strand is used as a template. The strand is then cut and the remaining nucleotides are added by DNA polymerase and ligated together, fixing the break.

11. What is SOS repair mechanism, when is it used (induced) and why is it important?

The SOS repair mechanism involves highly erroneous, low-processive DNA polymerases which add nucleotides in a haphazard way during heat shock conditions. This is important because the cell has a very low chance of survival, and so it is just trying to maintain its DNA and get through replication and cell division.

12. Describe the term “non-homologous end joining” and explain how

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this process results in the repair of double strand breaks in DNA molecule.

In non-homologous end joining, there is a double strand break, and the two strands resulting are cut away or filled by a DNA polymerase to form
blunt ends which are ligated together. It is non-homologous because there
is no need for a homologous template, unlike in homologous recombination.

13. Which repair mechanisms involve both strands of DNA? How are they activated?

Homologous recombination involves both strands, and is activated by nicks in the DNA encountered during DNA replication. Non-homologous end joining also involves both strands of DNA, and is a result of double
strand breaks during which there is no alternative strand to provide a template. Translesion synthesis also involves a double-strand break, but is
even more inaccurate, and is encountered under cell stress conditions.

14. Distinguish between the two DSB repair mechanisms we talked about in class in respect to their accuracy. What is the role of each of
them in a survival of a cell?

NHEJ involves formation of blunt ends from a double strand break and reattachment to attempt to reach the previous conformation with no knowledge of the sequence. Translesion synthesis is induced under the heat shock stress response (SOS) and the main goal is just to reach chromosomal replication, even if highly erroneous. Recombination repair
is much more efficient and accurate, and is used under normal conditions.

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15. What are biological roles of DNA recombination?

Recombination allows for novel gene/allele combinations in offspring, and
may allow for duplications or deletions, or formations of new genes depending on the sites of recombination and the genetic content being exchanged.

16. List and briefly describe the ways genomic DNA can be rearranged (there are three of them).
- Insertion: DNA sequence inserted into genome
- Deletion: DNA sequence removed from genome
- Inversion: DNA sequence flipped in orientation from within genome
- General recombination
- Site-specific recombination (deletions, insertions, inversions)
- Transposition

17. What are the key steps in single stranded model of homologous DNA recombination?
a. DNA duplexes align
b. Introduction of break into one of each strands
c. Strand invasion
d. Branch migration
e. Resolution

18. Explain the relationship between hybrid duplex and heteroduplex. (You
can use diagram.)

A hybrid duplex is any duplex between DNA and DNA or RNA without

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perfect complementarily. A heteroduplex is a double-stranded molecule of nucleic acid originated through the genetic recombination of single complementary strands derived from different sources.

19. Describe the function of proteins involved in homologous recombination of E. coli.

RecBCD binds DNA, enters at DSB and unwinds and degrades DNA until a chi sequence is reached, where it continues to make a 3. overhang. Then, RecA associates with the 3. overhang and carries out strand invasion. Ruv proteins then catalyze branch migration and resolution.

20. Describe the role of chi sequences in homologous recombination of E. coli.

Chi sequences serve as hotspots for recombination, as RecBCD will cleave DNA out up to these sequences and form 3. overhangs there so that recombination will occur around these sequences. Hence they are known as crossover hotspot instigators.

21. What is the major role of homologous recombination in prokaryotes?

To repair DNA breaks. prokaryotes do not carry out meiosis, and do not have recombination for the purpose of unique gamete production.

22. What are the roles of homologous recombination in eukaryotes?

In eukaryotes, homologous recombination can serve to repair DNA, as

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well as to allow for gene/chromosomal exchange for production of novel gametes and maintenance of variability.

23. When does the programmed creation of DSBs occur in eukaryotes? In which type of cells? Briefly describe the process.

Eukaryotes . in germ cells during meiosis for cross-over. Spo11 generates DSB, and Mre11 creates 3. overhangs much like in recombination repair. Dmc1/Rad1 (RecA type) allow for strand invasion. This allows for exchange of DNA segments, and will vary depending on the number of crossing over and the resolution.

24. Define gene conversion (use your own words). What is the significance of gene conversion?

It is a process by which DNA sequence information is transferred from one
DNA helix (which remains unchanged) to another DNA helix, whose sequence is altered. It is one of the ways a gene may be mutated. Gene conversion may lead to non-Mendelian inheritance and has often been recorded in fungal crosses.

If sequence in homologous recombination repair is a different allele and
used as a template for repair, there will be mispaired bases in the heteroduplex. Mismatch repair will excise and replace one of the strands
to restore complementarity, and the result could be that a gene is converted to a different allele. This is significant because this allows for
genetic recombination and different combinations of alleles outside of normal Mendelian inheritance.

25. What is the role of mismatch repair mechanism in gene conversion?

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Mismatch repair mechanism recognizes the mispaired bases in the heteroduplex – it then excises one of the strands to restore complementarity, and may convert one allele to another. This can result in
a higher abundance of a certain allele over another in gametes.

26. Explain the role of site-specific recombination in infection of E. coli
genome by lambda phage.

Lambda phage will target host DNA with its transposase and splice its own
RNA into the host DNA via lambda integrase. This allows the phage to enter the lysogenic cycle, where it can remain in the host genome and be
replicated many times. Under certain stresses, the phage DNA can be induced to be deleted out of the genome, and enter the lytic cycle where it
can form new phage and lyse the cell.

27. What are potential effects of transposons on the genome?

Can disrupt regulation sequences of genes, or disrupt genes themselves.

28. List and briefly describe three mechanisms used by genetic elements
to randomly move from one site in the genome to another.
a. Simple cleavage by transposase and insertion into another sequence
b. Transcription of transposon sequence to make enzymes and cDNA for reverse transcriptase which is integrated into a target via transposase
c. Transcription of RNA containing LINE from downstream promoter, and poly-A tails for priming reverse transcription, integration directly
into host by nicking (non-discriminatory)

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29. Draw a fully annotated diagram illustrating the transposition mechanism of a simple IS transposon (or retroviral-like retrotransposon, or non-retroviral retrotransposon).

30. Who was Barbara McClintock and what was her major scientific contribution (no more then three sentences).

Through studies on corn, Barbara McClintock discovered and published information on transposable elements and their role in mutating genes through insertion of genetic information.

16. You have discovered base changes in the promoter region of the operon in a bacterial chromosome. Would you expect these changes to act in trans on another copy of the operon? Explain your reasoning.

You would expect these changes to act within the operon. This is because
the promoter is involved in transcription of the downstream coding region,
and cannot “diffuse” through the cell to act on other regions on other chromosomes.

17. What are cis- elements? What are trans- factors? Give an example from the Trp operon (or form the Ara-operon).

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Cis-elements are regions of DNA that generally affect processes happening on the same strand of DNA. For example, in the Ara-operon, the araO2 is an operator that when bound to AraC dimer, blocks RNAP from binding and thus inhibits transcription. Thus, it acts in cis by affecting
the events taking place in the region of the DNA. In the Trp operon, the
operator is also like a cis-element since the repressor dimer (bound with
tryptophan) can bind it and transcription of the Trp genes is inhibited.

Trans-factors are proteins that can be produced somewhere else in the cell and diffuse through the cell, binding to regions of the DNA and influencing transcription. In the ara-operon, AraC is a trans-factor since it
is produced by one set of genes and can diffuse and act on the ara genes.
Also, CAP is a trans-factor since it is produced somewhere else in the cell
and can bind promoters in the presence of cAMP and enhance transcription of catabolic genes.

18. Draw the diagram of the lac operon that illustrates negative control
(be careful here and think about the complete picture!!!!!).

lacR is produced and binds the lac operator when lactose is absent, preventing transcription. Lactose acts as a co-repressor or inducer by binding lacR, keeping it from binding the operator and thus allowing transcription. This lactose-lacR combination also works to inhibit lacR
synthesis, through „autoregulation..

19. You have isolated a protein that binds to DNA in the region upstream
of the promoter sequence of the gene of interest. If this is a positive

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regulator (activator) which would be true:

A) Loss of function mutation in the gene encoding this DNA binding protein would cause constitutive expression

B) Loss of function mutation in the gene encoding this DNA binding protein would result in lower or no expression.

Explain your reasoning.

Lower or no expression (B) would be expected because normally, an activator is produced that binds the enhancer, increasing the ability of RNAP to bind and for transcription to be initiated.

20. Discuss why are lac Oc mutants cis-acting.

These mutants are cis-acting because the operator within that region of DNA is mutated, preventing lacR from inhibiting transcription. If a merodiploid situation is introduced, this mutated operon will not affect the expression of the plasmid lac operon, which will be repressed by lacR as normally.

21. Discuss why are lac I- mutants trans-acting.

These mutants are trans-acting because the repressor is a protein that is produced and can diffuse through the cell. Thus, if non-inducible lacR is produced, it will bind lac operons throughout the cell, even if they are on a plasmid.

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22. Discuss positive and negative regulation of L-ara operon.

Positive: an abundance of arabinose binds the AraC regulator, allowing it
to bind as a dimer to araI1 and araI2 . in this form, it does not bind the
operators and thus does not inhibit binding of RNAP. Also, with low glucose concentrations, cAMP levels rise, binding CAP which works through the promoter to increase transcription of the ara genes.

Negative: a lack of arabinose allows the AraC regulator to bind the araI1
and araO2 regions, thus preventing RNAP binding by hiding the ara promoter.

23. Regarding the regulation of Trp operon, what do we call the amino acid tryptophan? Why?

The amino acid tryptophan is a co-repressor. This is because it binds the
aporepressor TrpR, which is normally not able to repress the trp genes,
and allows it to then inhibit transcription of the trp genes. Because trp itself
is not repressing transcription directly, and because the aporepressor requires it for repression, the amino acid tryptophan acts as a „co-repressor..

24. What is meant by polycistronic mRNA? Give an example.

Polycistronic mRNA means that there are several genes under the influence of one promoter. An example is in the lac operon, where

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beta-
galactosidase, permease, and the lacA product are all under control of one promoter. Initiation of transcription at the promoter will allow all genes
to be present on the mRNA product. Translation of this cluster of genes
can then take place, allowing the important components of the processes
to be present.

25. What is catabolite repression? What is the role of Catabolite Activator Protein? Explain its action (remember the # of operons it activates!).

Catabolite repression is the effect observed when glucose is present in a
cell . because glucose is favoured for metabolism, transcription of other
catabolic genes will be repressed as they do not need to be on. CAP is activated by cAMP, which is produced during low levels of glucose – it works by activating over 100 catabolic genes including the arabinose and
lactose genes, thus allowing alternative forms of energy to be utilized.

26. Define: repressor, co-repressor, aporepressor and inducer.

A repressor is a molecule that can inhibit transcription. A co-repressor is a
molecule that can activate an apo-repressor, forming a repressor that can
inhibit transcription. The co-repressor cannot have inhibitory effects on its
own, and the apo-repressor is unable to be inhibitory without the co-repressor bound. An inducer is a molecule that induces gene expression by binding to a repressor, preventing it from binding DNA and having inhibitory effects.

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Repressor . trans-acting factor that will block transcription initiation when
bound to operator.

Effector . inducer or co-repressor – when it binds, it changes the repressor's conformation, thus changing its activity.

Auxiliary operons . additional repressor binding sites to further fine tune
inhibition.

27. Define effector and inducer. Give examples.

An effector is a molecule that can bind a repressor and affect its ability to
bind an operator. Arabinose acts as an inducer, binding AraC and preventing it from inhibiting transcription. Tryptophan acts as a co-repressor, binding the TrpR aporepressor and allowing it to inhibit transcription of the trp genes by binding the operator.

28. What are activators? What are enhancers?

Activators are trans-factors that can bind enhancer elements and positively influence RNAP binding and transcription initiation at the promoter through interactions. Again, the affinity of activators for enhancer
elements may depend on the presence of effectors.

29. What is the role of auxiliary operators?

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Auxiliary operators can work to provide finer tuning of gene expression by
allowing complex repression and DNA looping , preventing RNAP from accessing the promoter. i.e. tetramer in lac repressor.

30. Discuss the type of regulation of gene expression by two-component regulatory systems in bacteria?

Various stimuli can activate a sensor-transmitter protein, which has kinase
activity to activate a response regulator. This regulator then can stimulate
or repress gene regulation. Stimuli can be pH, osmotic levels, nitrogen
levels, etc., and these systems allow the bacteria to modulate its responses based on the environment.

31. Glutamine and arginine in DNA-binding proteins tend to make what kind of bonds with DNA?

Hydrogen bonds, van der Waals, and hydrophobic interactions. (recall Gln
and Arg have amine groups and Gln has a carboxyl group).

32. List different ways of control of prokaryotic transcription initiation
and give one example for each of them (this question could be separated in few smaller questions; make sure you understand

review slides and that you have at least one example for each of the “control ways”).

Autoregulation: presence of inducer and repressor, or co-repressor and

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aporepressor works through autoregulation to inhibit repressor synthesis
in ara and trp operons respectively.

Also:

- Catabolite repression
- Positive and negative regulation through regulators (ara)
- Negative regulation and co-repressor-aporepressor (trp)
- Negative regulation and inducer-repressor (lac)
- Auxiliary operons for fine tuning

33. Define constitutive and regulated proteins. What is the difference between expression of constitutive and expression of regulated proteins?

Constitutive proteins are required for cell survival, while regulated proteins
may not be. Constitutive protein synthesis is ongoing, while regulated proteins are produced by inducible genes that may be controlled by environmental effects, etc.

34. What is the most important characteristic of binding sites for prokaryotic regulatory proteins (for example lac operon operator)? How are those binding sites different from the RNAP binding site (promoter)?

Binding protein regulatory sites are symmetrical, while promoter RNAP binding sites are assymetrical so that RNAP can only proceed in one direction.

35. Describe the most common structural motif found in a DNA binding domain of prokaryotic regulatory proteins.

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The helix-turn-helix is the most common structural motif, containing a helix
that fits into the major groove of DNA, a short turn of a few amino acids,
and a second helix that stabilizes the first helix.

36. Apart from the DNA binding domain, we have mentioned other two domains found in prokaryotic binding proteins. What are they?

The oligomerization domain allows multiple TFs to bind each other, and possibly form a homodimer. The effector binding domain is the region where effector molecules may bind and influence TF conformation and activity.

37. Thinking question: three adjacent genes are involved in arginine biosynthesis. (They are structural genes of arginine operon.) You have three DNA fragments, each containing coding sequence for one of these three structural genes, and you have used these fragments to make three different probes for northern blotting (one probe for each of the three genes).

You have isolated mRNA from bacteria and you have performed the agarose gel electrophoresis followed by northern blotting.

What result do you expect to get if you use each of these three probes as a probe in northern analysis? What would be the result if three genes do not make up an operon? You could use diagrams to explain your reasoning.

You would expect a single band to appear, since all genes are found on
the same mRNA and are thus in the same vicinity – probing will thus

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light
up the same region.

If they were not on the same operon, you would expect multiple bands to
be observed as the probes would bind different mRNAs with most-likely different lengths and positions on the gel.

1. What are the major differences between prokaryotic and eukaryotic transcription?

Eukaryotic transcription has 3 different RNAPs, there are no operons, DNA can be in euchromatin or heterochromatin, GTFs are required for RNAP to bind promoters, and pre-mRNA is subject to processing.

2. Which hypothesis regarding eukaryotic RNAPs was proven with .
-
amanitin and actinomycin D (be specific)?

It was proven that there were 3 distinct RNAPs that acted under different
ionic conditions, in different locations in the cell, and using Mn and/or Mg
to transcribe three different sets of genes with completely different promoters.

3. Which genes are transcribed by RNAP I? RNAP II? RNAP III?

RNAPI makes 28S, 18S, and 5.8S rRNA (45S, then cleaved). RNAPII makes hnRNA and snRNA. RNAPIII makes U6 sNRA, tRNA precursors, and 5S rRNA.

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4. What does CTD stand for? Explain the role of CTD tail in eukaryotic gene expression?

CTD stands for carboxy terminal domain. The dephosphorylated (II a) form is required for RNAPII binding to the PIC. The phosphorylated form
(II o) is required for elongation. Also roles in splicing, methylation, and
polyadenylation.

5. How would you define enhancers? What are their characteristics? What is the difference(s) between enhancer and upstream control element?

Enhancers are genetic elements to which activators can bind and increase
the ability of RNAP to bind the promoter – they can be found anywhere. Upstream control elements are only found upstream of the promoter and are sites for regulatory proteins to bind and influence transcription.

6. Explain the use of reporter genes for estimation of promoter strength.

Reporter genes are used because they can produce some kind of product that can be measured when the genes are expressed. When placed under control of a promoter, the strength of the promoter can be measured by measuring the level of product production (GFP, beta-galactosidase, luciferase are examples).

7. Explain briefly 5’ deletion series. What kind of information do they

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reveal?

This involves deletion of regions of the DNA starting from the 5. end. Expression of a reporter gene is measured, and thus the necessary parts
of the measured DNA region can be determined based on when expression is inhibited.

8. Explain the modular nature of RNAP II promoters.

RNAP II promoters are modular in that there are many elements and factors that can interact with promoters and influence transcription. More
specifically, different combinations can achieve different levels of expression, and no single element is essential for all promoters.

9. Draw a diagram of RNAP II promoter (show all types of elements that we have mentioned in class).

- Enhancer elements throughout
- Promoter

- GC boxes upstream of TATA box
- TATA box at ~−30
- Initiator elements with consensus sequence may be present
- (core promoter/promoter and regulatory elements)
- Silencers throughout
- Boundary elements
- insulators

10. Explain the tissue (cell type) specificity of eukaryotic cis elements.

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The same cis elements are found throughout all cells of an organism, but
what differs is the transcription factors that are produced and that can
interact with the cis elements and influence gene expression.

11. Knowing that different genes may have the same promoter and enhancer elements and that different transcription factors contain the same structural features, how would you explain transcriptional specificity?

Genes with the same cis elements and under the influence of the same TFs will be expressed.

12. If you know the binding site for certain transcription factor (TF),
outline experiments you would use to purify this TF and to assay its activity.

Fix an oligonucleotide sequence (containing the binding site sequence) to
a binding column, and run isolated proteins through the column. At high
salt, the solution that elutes will contain the proteins that had the highest
affinity for the sequence.

13. Distinguish between the function of promoters and enhancers in transcriptional regulation.

??

14. Distinguish between the function of general transcription factors

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and
transcription activators in transcriptional regulation.

General transcription factors are involved in binding the promoter and recruiting RNAPII, stabilizing it, and preparing for transcription. Transcription activators work to increase the affinity of RNAPII for the
promoter by working with a mediator complex and bending DNA.

15. List and briefly explain four major domains in eukaryotic transcription factors.

DNA-binding domain . generally helixes to fit into DNA major grooves and serve as recognition sites for the proper sequences

Dimerization domain . alpha helix in C6 zinc finger, leucine zipper, and
bHLH with alpha helix), serves to form dimmers

Transcription activation domain . portion that interacts with other factors/elements to allow transcription initiation.

Ligand-binding domain . region that binds effector, such as steroids binding C4/nuclear receptors

16. List most frequent structural motifs in eukaryotic DNA binding domains.

Zinc fingers are the most frequent structural motifs in eukaryotic DBDs,
particularly the C2H2 and C4 motifs. There are also homeodomains,

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basic
Leucine zippers, and basic helix-loop-helix.

17. List three classes of transcription activation domains in eukaryotic
transcription factors.

Acidic, proline-rich, and glutamine-rich.

18. What is achieved by the ability of some transcription factors to form
heterodimers (basically two things/players in regulation)?

Allows fine tuning by different repressor TFs and activator TFs binding,
and/or allows different combinations of enhancers to be recognized.
(heterodimerization = combinatorial control).

19. What is meant by the independence of the DNA-binding and transcription-activating domain of a transcription factor?

The DBD is only responsible for binding the enhancer/silencer element, while the transcription-activating domain has specific roles. The level of
transcriptional control is primarily affected by the TAD, not the DBD.

20. What is the role of the TATA box? What happens when TATA box is removed from the RNAP II promoter?

The TATA box is bound by TBP, allowing the RNAP binding cascade to begin. It basically serves to locate the start site. Without TATA boxes,
TAFs must bind initiator elements of CG boxes somewhere in the

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promoter region and facilitate TFIIB binding.

21. What is combinatorial control of transcription?

Combinations of different TFs can allow for fine tuning. This is done through heterodimerization or combination (i.e. repressor-activator TFs).

22. What are the roles of TFIIH in transcription initiation by RNAP II?

TFIIH works to phosphorylate the CTD tail when the initiation complex is
properly bound, and also to work as a helicase to unwind DNA in the region about to be translated.

23. What are the roles of TFIID in transcription initiation by RNAP II (be
as specific as possible; have to talk about TBP and TAFs)?

TFIID works to locate the promoter. TBP binds the TATA box, and provides the foundation for the transcriptional complex while preventing
nucleosome stabilization. TAFs work in TATA-less promoters, tethering TBP to initiator elements or CG boxes. TAFs also interact with TFs and have roles in looping to determine the level to which TFIID stays on the
promoter.

24. What are the roles of TFIIB in transcription initiation by RNAP II?

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TFIIB orients the PIC by binding BRE upstream of the TATA box. RNAPII binds TFIIB, and so it aligns the RNAP on the DNA and aligns other GTFs.

25. What are TATA-less promoters? How could transcription be initiated at TATA-less promoters?

See above

1. Describe the role of histone acetylation/deacetylation in regulation of
transcription.

Histone acetylation allows for DNA to be remodelled so that transcription
factors and activators can reach genetic elements and initiate transcription. This occurs because acetylation provides a negative charge
to the histones, allowing them to dissociate from DNA more freely, which
also has a negative charge. The nucleosomes are still intact but can be
repositioned to expose promoter elements.

Deacetylation allows for transcription to be inhibited, as transcriptional
machinery is not able to access the promoter which is held up in tightly
wound nucleosomes.

2. Describe the role of chromatin remodelling complexes in regulation of transcription.

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Chromatin remodelling complexes reposition the nucleosomes and can expose promoter elements for binding of GTFs. (ATP is used in this process)

3. Describe an influence of activators and repressors on assembly of initiation complexes.

Activators and repressors can help in recruiting coactivator or corepressor
complexes that have histone acetylase or deacetylase activity. They also
can act with each other and provide a form of fine tuning through their
interactions; they can recruit GTFs by interacting with TAFs, etc.

4. Explain the role of enchancesomes and architectural proteins in regulation of transcription initiation?

The combinations of transcription factors binding enhancers and the GTFs
form different initiation complexes, or enhanceosomes, that provide combinatorial control of gene expression through the different combinations and concentrations of activators. This allows for specific
gene expression in various tissues based on which TFs are present and in
what concentration.

Architectural proteins bind elements of the DNA and can change DNA shape to allow other TFs and GTFs to interact and affect transcription.
Through this, architectural TFs can cause bending or looping of the DNA
to stimulate transcription initiation.

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5. Explain the role of mediators (or insulators – different question) in
regulation of transcription initiation.

Mediators link TFs binding other elements to the basal complex/pre-initiation complex and allow for control of transcription initiation from a
different element.

Insulators prevent certain elements from reacting with certain promoters
by isolating them. They also may work to define the boundary between heterochromatin and euchromatin.

6. What is the role of DNA methylation in regulation of transcription initiation?

DNA methylation keeps TFs from binding certain regions and GTFs from binding promoters, thus keeping them from stimulating transcription. Also,
other proteins may recognize the methylated DNA and compete against TFs for these sites.

7. Describe how TFs from nuclear receptors superfamily regulate transcription (one example is enough; remember: nuclear receptor could be in cytoplasm, bound to chaperone protein, or in nucleus, already bound to DNA).

Steroid hormones bind transcription factors which are in the cytoplasm,
bound to heat-shock proteins. The hsp is released, allowing the TF to

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enter the nucleus and bind a response element on DNA, influencing the transcription of genes.

8. What is (are) the role(s) of transcription factors during development?

During development, TFs affect the localization of mRNAs in the cell, which contributes to further differentiation through differences in concentrations and activities of TFs and other protein products. Different
poles of the embryo will have different sets of proteins synthesized, resulting in differentiation.

9. Describe the role of TBP during transcription (think about promoters
for all three eukaryotic RNAPs and TATA-less RNAPII promoters – how do RNAPs bind to them; also, think about coordination of activities of all three polymerases).

TBP binds DNA upstream of the transcription initiation site and acts as the
positioning factor to which TFIID or TFI/III B can bind to and the rest of the
GTFs can bind, allowing RNAP to bind and begin transcription. In TATA-less promoters, TAFs bind initiator elements or CG boxes through Sp1, and anchors TBP to the region (as if it was a TATA box). TBP can then initiate TFIID binding to the promoter region.

10. What is the role of Sp1 protein? What is the role of SL1 protein? What do they have in common?

Sp1 protein has a role in TATA-less promoters involving GC boxes – it binds GC boxes and interacts with TAFs which anchor TBP and allow TFIID binding to the promoter. SL1 protein is involved in RNAP I promoters – it is bound by UPE-bound UBF, and allows for RNAPI

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positioning at the core promoter. They are similar in that they do not bind
TATA boxes, but they still provide the basis for RNAP binding.

11. What is unusual about type 1 and 2 promoters for RNAPIII polymerase?

These promoters are unusual because they contain promoter elements that are in the transcribed region (internal promoters).

12. Describe the mechanism of attenuation of the Trp operon. Explain the importance of this mechanism for a bacterium?

13. Could you imagine a mechanism similar to the mechanism of attenuation of the Trp operon in Eukaryotes? Explain your reasoning.

It is unlikely that an attenuation mechanism would exist because transcription
is taking place in the nucleus and translation is occurring in the cytoplasm.
Even if translation can possibly occur in the nucleus (which is still debated), it
is unlikely that amino acid concentrations would be significant enough for
something like a leader peptide to be produced. Basically, conditions in the
nucleus would likely not mirror the conditions in the cytoplasm, which is where
the transcript would be needed for translation, and so an attenuation mechanism would be pointless since most biochemical reactions are taking
place outside of the nucleus.

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14. Describe two distinctly different ways in which the trp operon is controlled by the overall availability of tryptophan (one way is through the formation of repressor protein and the other through attenuation of transcription. Think about fine tuning of Trp production; the questions below might be helpful).

The abundance of tryptophan determines the formation of repressor protein through autoregulation, and also the production of a leader peptide
and transcription inhibition.

15. Describe the mechanism responsible for shutdown of the trp operon when a plentiful supply of free tryptophan is available.

Free tryptophan acts as a co-repressor to bind the trpR aporepressor, which binds the trp operon, thus inhibiting transcription.

16. Describe the mechanism by which the leader-attenuator region fine tunes the extent of transcription of the structural genes in the trp operon when Trp is available (but not to the point to completely saturate the apo-repressor).

The leader sequence contains two codons for tryptophan. The level of tryptophan presents works to fine tune the level of transcription that occurs, since if the leader peptide is produced, regions 3 and 4 of the
sequence will form a stem loop and cause termination of transcription by
the U-rich region melting and RNAP dissociating. With low tryptophan levels, the ribosome cannot pass synthesize the leader peptide, and so regions 2 and 3 form a stem loop, but termination does not cease due to

the lack of a U-rich region. Thus, the level of tryptophan fine tunes the

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transcription of trp genes.

17. Describe rho-dependant transcription termination

Rho-factor binds at a rut site on the primary transcript and moves up to
RNAP which is stuck at some termination site (possibly a secondary RNA hairpin forms). Rho is a helicase and works to unwind the DNA-RNA hybrid, allowing the transcription components to dissociate. ATP is used
by rho factor.

18. Describe rho-independent transcription termination

As in the trp attenuation method, GC-rich regions form a stem loop structure which is followed by U residues. Formation of this structure leads
to termination of transcription by DNA-RNA melting. RNAP then dissociates.

38. Describe the process of mRNA cleavage and polyadenylation. (Don’t forget the role of CTD tail)

CTD tail, when phosphorylated, recruits enzymes that recognize the polyA
signal and upstream elements, cleave out the GU-rich region, and provide
PAP to add the 3. polyA tail.

mRNA cleavage in eukaryotes primarily occurs due to spliceosome formation. The spliceosome is assisted by the CTD tail in recognizing exons and branch sites, interacting with components, and for proper mRNA folding during and after cleavage.

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39. How is 5’ cap added to the nascent RNA?

Enzymes are recruited by the CTD tail for phosphate cleavage, GMP addition, and 7-methyl transfer. Note: this occurs almost immediately pending transcription initiation.

40. What is the relationship between hnRNA and mRNA?

hnRNA is the incompletely processed product that is formed off of RNAPII,
whether it end up as cytoplasmic mRNA or not. mRNA is the final product
after exons are spliced together and the 5. cap and polyA tail are added.

41. What are the general steps in processing of a pre-mRNA into a mRNA?

The 5. cap is added right at the start of transcription (20-30 nt in). The
poly-A tail is added to the 3. end following cleavage of the GU-rich downstream element. Introns are cleaved out by spliceosome or are self-
cleaved out.

42. What is the role of snRNAs in the spliceosome?

snRNAs serve to recognize complementary sequences and form the branch sites in the introns for cleavage. They act as ribozymes and make
up the catalytic part of the spliceosome for the esterification reactions.

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43. What is the role of Sm proteins in the spliceosome? What other proteins (apart from Sm) are found to be associated with splicing?

Sm proteins bind and associate with snRNAs as a circle around the snRNA. Other associated proteins associate with snRNAs and Sm proteins (i.e. U1-associated proteins A, C, and 70K). Protein complexes
on the CTD tail also have roles in recognizing splice sites and branch sites, and help in interactions and spliceosome assembly and catalysis,
and prevention of secondary and tertiary structures from forming (helicase
activity).

44. What is the difference between splicing of group I and group II introns? Between splicing of group II introns and spliceosomal splicing?

Group I use G as branch site, while Group II uses A as branch site. Spliceosome splicing is different in that it uses snRNAs, Sm proteins, and
uses the CTD tail of RNAPII.

45. Describe the current model of spliceosomal splicing.

In the current model of spliceosomal splicing:

- U1 attaches to the 5.splice site via complementary sequences
- U2 binds to the branch point and forms the “A complex” where A is sticking out and available to react
- U4 and U6 and U5 bind the 3. introns splice site – U4 inhibits U6 activity
- A conformational change results in the “B1 complex” forming
- U4 dissociates, and U6 invades the 5. splice site causing U1 to be released forming the activated “B2 complex”
- U5 positions and holds, while U2 and U6 act catalytically to

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splice at both sites
- Lariat produced and degraded
- snRNPs released and likely recycled

46. List the roles of 5’ methyl cap. List the roles of polyA tail. List the
roles of CTD tail (yes, again).

5. methyl cap protects the 5. end from degradation, and methylation of hydroxyl groups keeps them from being reactive. The poly-A tail provides
a site for PAB proteins to bind and stabilize the transcript, preventing it
from 3. exonuclease activity. The CTD tails has roles in recruiting enzymes for polyadenylation, it is associated with enzymes for methylation, and has roles in stabilizing the spliceosome complex and recognizing exon splice sites, etc.

47. What are the two types of transcriptional units in Eukaryotes? Use diagrams.

Simple . all exons are cleaved together and used

Complex . exons can be optional or mutually exclusive, allowing for alternative splicing for different products.

48. List and explain two means of control of gene expression that could
happen during pre-mRNA processing.

Alternative splicing

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Control of polyadenylation

49. Describe one case of control of gene expression at the level of mRNA processing by means of splicing.

Alternative splicing of the fibronectin gene results in constitutively different
mRNA for fibroblasts and hepatocytes.

50. List and briefly explain different mechanisms of post-transcriptional
control of gene expression (think about examples)?

Alternative splicing where different splicing factors present in different
types of cells lead to differentiation of the same transcript.

This could include using different polyadenylation signals on the same transcript, resulting in different mRNA such as in thyroid and neuronal
cells.

Alternative spliceosomes could also splice alternatively off of the same
transcript.

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mRNA silencing or degradation can prevent translation from occurring.

Regulation of poly-adenylation will determine how fast the transcript will be
degraded.

51. Explain the role of mRNA stability (or editing, or iRNA, or translational control switch or mRNA localization – five different questions possible) in control of gene expression.

mRNA stability is important because translation will only occur if the mRNA is not degraded and if the ribosome can reach the mRNA and begin translation. With the translational control switch, the presence of a
certain nutrient can affect whether the mRNA is degraded or not (transferrin) or translation is inhibited (ferritin). iRNA can be made to inhibit
mRNA translation by binding regions through a miRNP or siRNP complex.

52. Explain the connection between pre-mRNA splicing and transport of mRNA from the nucleus.

There is some kind of mechanism that normally prevents pre-mRNA from exiting the nucleus before it is spliced. This was illustrated in an experiment where hsp30 mRNA was not observed unless cells were treated with cyclohexamide. This caused protein synthesis inhibition, resulting in hsp30 mRNA being visible due to the mechanism for preventing pre-mRNA exiting the nucleus being inhibited.

53. What is trans-splicing? Give an example.

Trans-splicing is the creation of multiple monocistronic mRNA from a single polycistronic pre-mRNA. An example of this is in the Trypanosoma,
where the leader sequences are transcribed, capped, and spliced to

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form
mini exons; these are then trans-spliced to polycistronic
transcription units
which are spliced, after which a poly-A tail is added to the form the
final
product.

54. What does S in 16S stand for? What is the numerical value of this constant?

S stands for svedberg units, and one S is equivalent to 10E-13 s. It
is a
measure of the velocity of sedimentation rate of suspended particles
centrifuged under constant conditions.

55. What are the roles of three major RNAs in protein synthesis?

mRNA provides the genetic information in the form of codons. rRNA adds amino acids to a peptide chain from tRNAs which recognize the codons
on
the mRNA and carry amino acids to the ribosomes.

56. What is the name of the region of tRNA molecule which attaches to an amino acid?

CCA sequence of acceptor stem.

57. How many different tRNAs are there in an eukaryotic cell? How many different aminoacyl tRNA synthetasis are there in an eukaryotic cell?

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Around 60 tRNAs and 20 aminoacyl tRNA synthetases – one for each amino acid.

58. What are the roles of tRNA in translation?

Bind appropriate amino acids and bring them to appropriate sites matching mRNA codon during translation.

59. What is the wobble position for an anticodon? For a codon?

Position 3 on the codon and position 1 on the anticodon. Some tRNAs can
have a nucleotide in this sequence that does not have to properly base pair for the amino acid to be transferred, as there are 61 possible codons
for amino acids and only 50 tRNAs.

60. Explain what does it mean when we say that the code is degenerate? Codons are synonymous?

The code is degenerate as in there are possibly several codons coding for
a single amino acid, and so there may be a large number of nucleotide sequence possibilities that code for the same amino acid sequence. Codons are synonymous in that there is more than one codon coding for the same amino acid in many cases.

61. What is the role of Shine-Dalgarno sequence?

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It is the site for prokaryotic ribosomal binding, as recognized by the 3. of
16S rRNA of the 30 S subunit.

62. What is the role of Kozak sequence?

It is the proper sequence for the eukaryotic 43S ribosome with initiator
tRNA to scan to, thus properly positioning the ribosome at the start site.

63. What are the major differences in initiation of translation between
eukaryotes and prokaryotes (remember: first AA and the way mRNA
and tRNA bind to ribosomes)?

Prokaryotes have formyl-methionine initiator tRNA, while eukaryotes have
just methionine. Also, in eukaryotes, a GTPase + GTP + tRNAi will bind
the 40S subunit as a pre-initiation complex, and there are proteins to keep
60S subunit from binding until this complex is formed. the 60S subunit
cannot bind until the 43S initiation complex reaches the Kozak AUG, after
which GTP is used, factors are released, and the 40S initiation complex
forms.

In prokaryotes, the 16S subunit binds at the Shine Dalgarno sequence
directly, and there are factors that bind to prevent tRNA entry into the A
site and 50S addition until the initiator tRNA binds the P site (IF1, 2, and
3). The GTPase complex does not bind as a ternary complex, as in

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eukaryotes, although GTP is used for the initiation complex to be formed.
binding of tRNAi initiates factor release, allowing 50S to bind.

In eukaryotes, the tRNA binds BEFORE the preinitiation complex binds the mRNA. In prokaryotes, the preinitiation complex binds mRNA, and then initiator tRNA enters the P site.

64. Describe in detail events during initiation of the Eukaryotic (or Prokaryotic) translation.

Prokaryotes: initiation factors bind to prevent tRNA binding the A site,
prevent 50 S subunit from binding the 30S subunit, and GTPase complexed with GTP. This preinitiation complex (with 30S) will bind mRNA
at the Shine-Dalgarno sequence (via the 16S subunit complementarity). fMet-tRNAi will then enter at the P site. The initiation factor that prevents
50S subunit binding (IF3) will leave, allowing the 50S subunit to bind and
displace various other factors, hydrolyzing GTP in the process.

Eukaryotes: various initiation factors bind the 40S subunit to keep it from
binding the 60S subunit. The ternary complex of tRNAi, GTPase, and GTP will bind this complex to form the 43S preinitiation complex, which can
then interact with the m7G 5. cap and scan along for the Kozak sequence.
Here, factors will be released to form the 40S initiation complex, after
which the 60S subunit can bind with GTP hydrolysis (by eIF2), and translation can begin.

1. Briefly explain two cases of RNA having enzymatic capability (how many ribozymes did we mention in class)?

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Some RNA molecules can self-splice introns out, or act in snRNPs as the
catalytic units of spliceosomes to remove introns. RNase P is another example of a ribozymes that removes the 5. tail of a tRNA following transcription. Also prokaryotic 23S ribosome as peptidyl transferase.

2. What is a peptidyl transferase? What is catalyzed by peptidyl transferase?

A peptidyl transferase is an enzyme that catalyzes the transfer of an amino acid from a tRNA to a growing peptide chain, and catalyzes the peptide bond between two amino acids. In prokaryotes, this has been found to be from ribozymatic activity of the 23S rRNA subunit.

3. How is gene expression controlled at the level of translation (mRNA is now in contact with ribosomes or their subunits/translation factors; three things mentioned in the class)?

Multiple AUG codons allows for potentially different proteins to be produced from the same transcript.

Internal ribosomal entry sites, which can be controlled under different
circumstances, can allow ribosomes to bind downstream of the 5. cap and
transcribe from different coding sequences.

Translational factors such as eIF2 can be phosphorylated, affecting their
activity. For example, eIF2 phosphorylation will inhibit its GTPase activity
and thus inhibit translation initiation.

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4. Which two factors greatly influence efficiency of protein synthesis?

Polyribosome formation and circularization of the mRNA, as done by PABI
and other initiation factors that form a complex to which the 40S and 60S
machinery can rapidly bind.

5. What are the roles of PABI? What is the role of PABII?

PABI has a cytoplasmic role to stabilize mRNA by binding the poly-A tail –
it also has a role in circularizing mRNA, forming a complex with other initiation factors and allowing ribosomes to rapidly bind the mRNA and translate proteins. PABII is the nucleus version of PABI, and has a role to
stabilize the mRNA while in the nucleus, preventing it from degradation,
and speeding up the process of polyadenylation.

6. Explain what happens with polypeptides after translation.

After translation, polypeptides are released from the ribosome and fold up
spontaneously, but can be modified in various ways.

7. How are protein modifications related to the control of gene expression? List possible protein modifications.

Protein modifications are also related to the control of gene expression,
because the modification mechanisms can themselves be under control,
and modified proteins will allow for further possibilities of activity in the

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organism. Modifications can affect protein activity as well as how proteins
will bind other subunits, and whether the proteins will be degraded (determined by specific amino acid sequences).

Transient modifications such as phosphorylation may modify protein activity, and can be under the control of various mechanisms. Permanent
modifications such as sulfation will have a much greater effect of proteins
since the change cannot be reversed. Acylation, glycosylation, methylation, prenylation, and cleavage can all contribute to protein activity.

8. Define cytoplasmic male sterility in plants.

Due to mutations in mtDNA, plants are unable to make male gametes (pollen) and thus cannot self-pollinate. These plants are useful in making
hybrids.

9. Explain endosymbiotic theory.

Mitochondria and chloroplasts were likely prokaryotes that were endocytosed by eukaryotes for a symbiotic relationship. Proofs include:
mitochondria have circular DNA, they use prokaryotic-like rRNA and ribosomes, they are affected differently by antibiotics and translation
inhibitors than the rest of the cell, and fMet is used as in prokaryotes.

10. Which genome encodes for proteins found in mitochondria. Explain.

The mtDNA codes for tRNA and rRNA, while ribosomal proteins are coded for by the nucleus. Other proteins are coded for by either or both genomes, although the nucleus codes for most proteins.

11. What are the characteristics of mitochondrial transcripts?

Mitochondrial transcripts are produced from either the H or L strand, and
are polycistronic. They are then cleaved following transcription. There are
no introns, and there are only a few nucleotides between coding sequences, if any.

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12. How is normal tRNA : rRNA ratio maintained during the transcription
of mitochondrial DNA?

rRNA genes are transcribed more often since they are closer to the promoter, and the transcription mechanism wil often stop before synthesizing the full H-strand.

13. Define and explain the importance of heteroplasmy and homeoplasmy.

Heteroplasmy is when there is a mix between mutated and normal mtDNA in mitochondria, while homoplasmy is when there is either all mutated or
all normal mtDNA present. Imperfect transmission of mitochondria during
mitosis allows for somatic mtDNA mutations to possibly accumulate in cells with homoplasmy, leading to late onset diseases.

14. Is mutation rate in mitochondrial DNA high or low? Explain why.

The mutation rate is high, especially in the D-loop due to: exposure to free
radicals from respiration, lack of protective histones, high replication rate,
and less efficient repair systems.

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