

Genetic Questions 1

September 2020

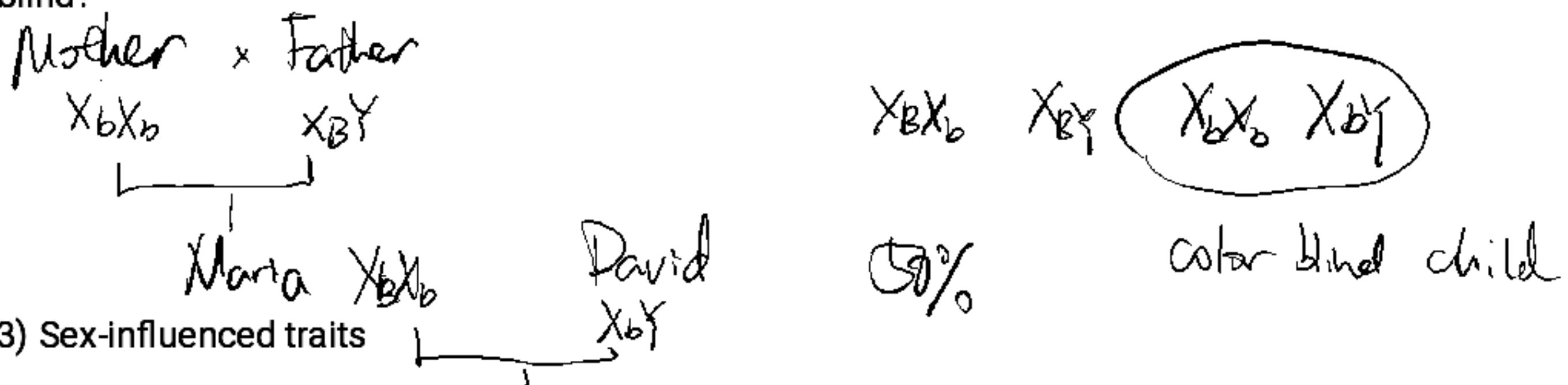
1) Monohybrid Cross

Short hair in rabbits (S) is dominant over long hair (s). The following crosses are carried out, producing the progeny shown. Give all possible genotypes of the parents in each cross.

- a) short haired female x short haired male => progeny:
4 with short hair and 2 with long hair
b) short haired female x long haired male => 12 short haired offsprings
- $Ss \times Ss \rightarrow Ss, SS, ss$
 - $SS \times ss \rightarrow Ss$ or $Ss \times ss$ at low chance

2) Sex-linked Inheritance

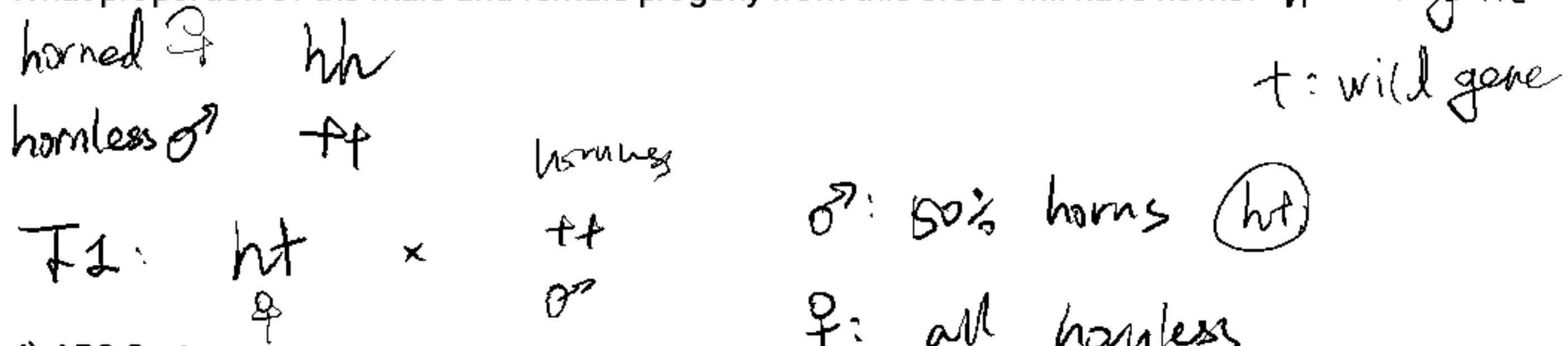
Color blindness in human is most commonly due to an X-linked recessive allele. Maria has normal vision, but her mother is color-blind. David is color-blind. If David and Maria have a child together, what is the probability the child will be color-blind?



3) Sex-influenced traits

In sheep, the presence of horns is produced by an autosomal allele that is dominant in males and recessive in females. A horned female is crossed with a hornless male. One of the resulting F1 females is crossed with a hornless male.

What proportion of the male and female progeny from this cross will have horns? h: horn gene



4) ABO System

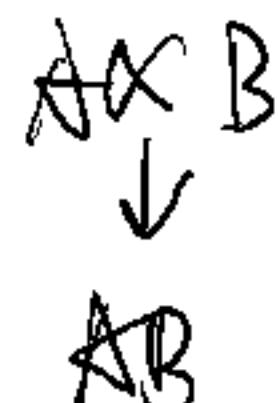
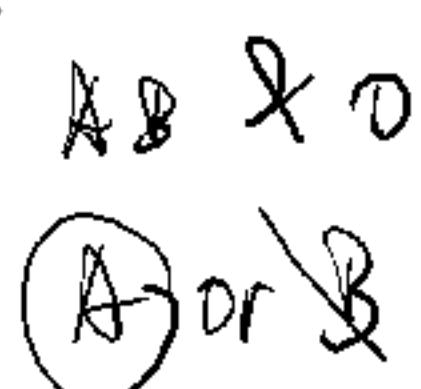
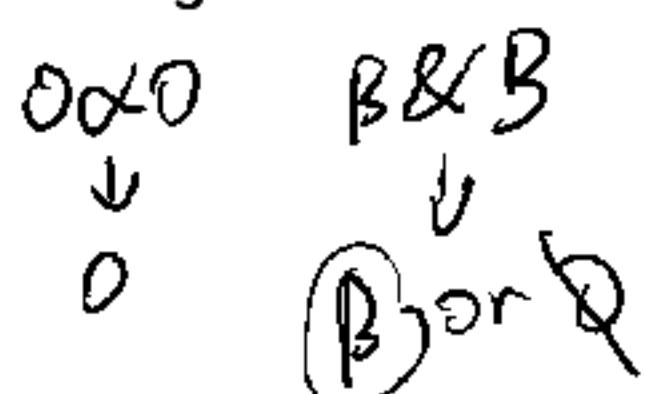
F2: ht, ff

At the neonate ward was big confusion. A new mother was convinced that she got the wrong infant back after a check up. Is it possible to correlate the infants lying in the ward with their parents unambiguously by simple blood typing?

The neonates have the following blood types: A, B, AB and O.

The parents on the ward: O & O, A & B, AB & O and B & B.

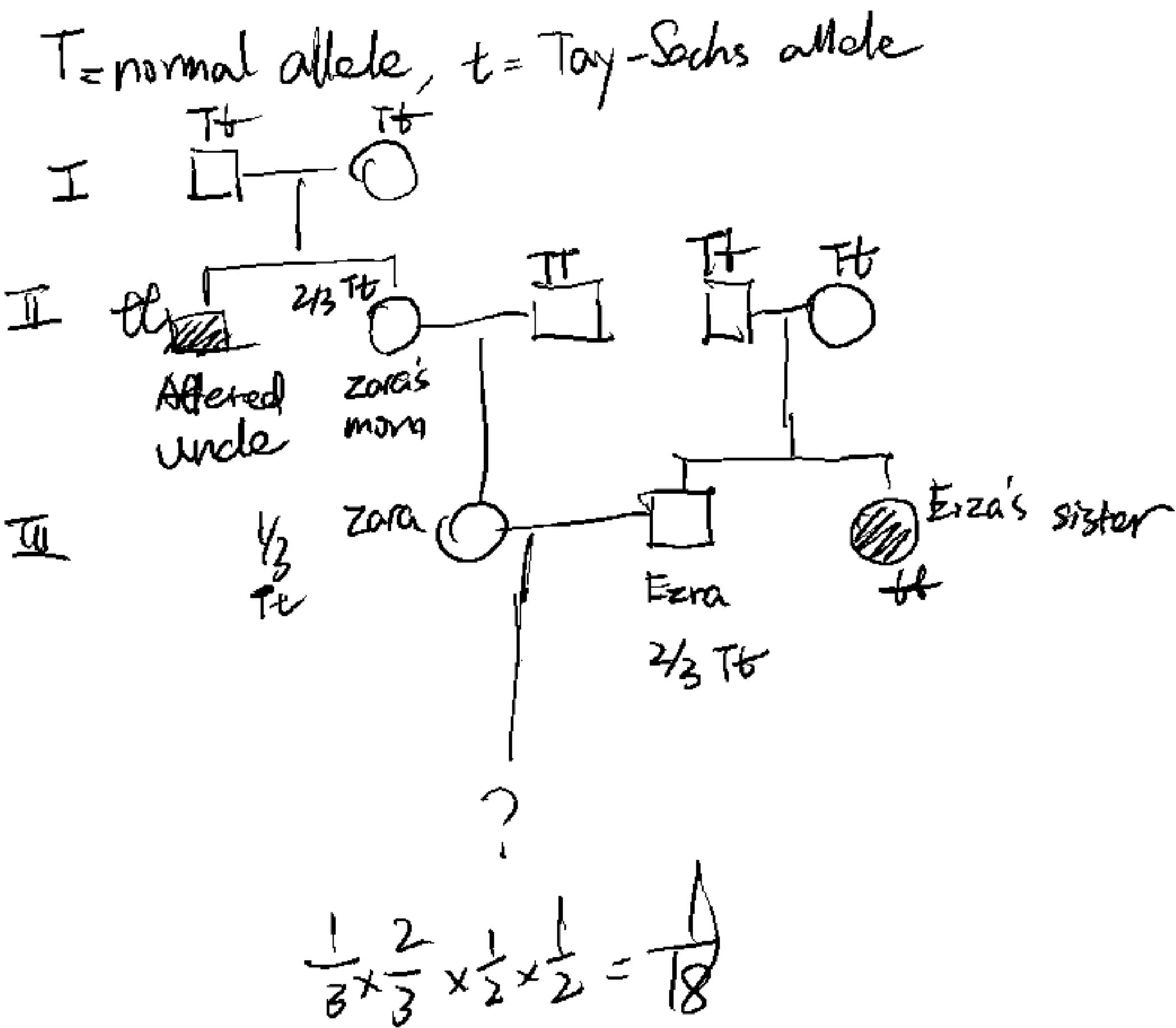
Which baby belongs to which set of parents?



5) Tay-Sachs is a recessive lethal disease in which there is neurological deterioration early in life. An affected person usually dies during early childhood or adolescence. The disease is rare in the population overall but is found at relatively high frequency in Ashkenazi Jews from Eastern Europe. Zara's maternal uncle had the disease. Zara is trying to determine the probability that she and her husband Ezra could have an affected child. Zara's father does not come from a high-risk population, while Ezra's sister died of the disease at an early age.

a) Draw the pedigree of the individuals described. Include the genotypes where possible.

b) Determine the probability that Zara's and Ezra's child will be affected.



6) A mouse sperm of genotype $aBcDE$ fertilizes an egg of genotype $a b c D e$. What are all the possibilities for the genotypes of

a) the zygote and

$aabbCcDDee$

$2 \times 2 \times 2 = 8 \text{ types}$

b) a sperm or egg produced by the mouse that develops from this fertilization?

$aBCDE$

$ab CDe$

$aBCD e$

$ab cDE$

$aBcDE$

$abc De$

$aBcDe$

$ab CDE$

Genetic Questions 2

September 2020

1) Palomino horses have a golden-yellow coat, chestnut horses have a brown coat, and cremello horses have a coat that is almost white. A series of crosses between the three different types of horses produced the following offspring:

Cross	Offspring
palomino x palomino	13 palomino, 6 chestnut, 5 cremello 2:1:1
chestnut x chestnut	16 chestnut
cremello x cremello	13 cremello
palomino x chestnut	8 palomino, 9 chestnut
palomino x cremello	11 palomino, 11 cremello
chestnut x cremello	23 palomino

The alleles are incompletely dominant

since palomino resulted from chestnut x cremello cross.

- a) Explain the inheritance of the palomino, chestnut, and cremello phenotype in horses knowing that these phenotypes are dependent on one gene only.
 b) Assign symbols to the alleles that determine these phenotypes, and list the genotypes of all parents and offspring given in the preceding table.

The given traits indicate that the cremello and chestnut are homozygous traits while palomino is heterozygous.
 a). The given results indicate that the cremello and chestnut are homozygous traits. Palomino is a heterozygous trait as it produces offspring in 2:1:1 ratio when palominos are self-crossed. The alleles are incompletely dominant because the Palomino phenotype resulted from chestnuts crossed with cremellos.
 b). Assume the genes coding for chestnut is CB, genes coding for cremello is CW and the gene coding for palomino is CBCW. Crossed

Given that,

palomino	X	palomino	=	13	palomino,	6	chestnut	5	cremello
CBCW	X	CBCW	=		CBCW		CBCB		CWCW
chestnut	X	chestnut	=			16			chestnut
CBCB	X	CBCB	=						CBCB
cremello	X	cremello	=			13			cremello
CWCW	X	CWCW	=						CWCW
palomino	X	chestnut	=	8	palomino,	9	chestnut		
CBCW	X	CBCB	=		CBCW		CBCB		
palomino	X	cremello	=	11	palomino,	11			cremello
CBCW	X	CWCW	=		CBCW		CWCW		
chestnut	X	cremello	=			23			palomino

$$CBCB \times CWCW = CBCW$$

- ~~2)~~ When a Chinese hamster with white spots is crossed with another hamster that has no spots, approximately 1/2 of the offspring have white spots and 1/2 have no spots. When two hamsters with white spots are crossed, 2/3 of the offspring possess white spots and 1/3 have no spots.

$$Tt \times Tt \rightarrow TT \quad Tt \quad tt$$

What is the genetic basis of white spotting in Chinese hamsters? \downarrow dead $\frac{2}{3}$ $\frac{1}{3}$

dominant phenotype and recessive lethality

- 3) A. C. Stevenson and E. A. Cheeseman studied deafness in a family in Northern Ireland and recorded the following pedigree (A.C. Stevenson and E.A. Cheeseman. 1956. *Annals of Human Genetics* 20: 177-231.)



- a) If you consider only generations I through III, what is the most likely mode of inheritance for this type of deafness?

Yes, every aspect of the pedigree can be explained by autosomal recessive inheritance, assuming that two different genes are involved

- b) Provide a possible explanation for the resulting phenotypes in generations IV and V.

III-7 and III-9 are homozygous for recessive alleles at two different loci that control hearing ability such that there is complementation in their offspring

- 4) For your work as a mouse geneticist you need a pure-breeding mouse strain that has a normal long tail and a black fur color. Luckily, you already have a pure-breeding line that has a short tail and black fur and another pure-breeding line that has a long tail and brown fur. By crossing the two lines you try to get your pure-breeding mouse line with long tails and black fur. The F₁ generation you get from this cross has short tails and black fur. You cross sisters and brothers from the F₁ generation. In the next generation (F₂) you will have some mice with long tails, some with short tails, some with black fur and some with brown fur. You need to eliminate the alleles for short tails and the one for brown fur. Which of these traits is easier to eliminate from your population by selective breeding? Show the crosses!



F₁: $\frac{T\text{short}}{\text{tlong}} \frac{F\text{black}}{f\text{brown}}$ x $\frac{T\text{short}}{\text{tlong}} \frac{F\text{black}}{f\text{brown}}$

F₂ progeny phenotype:

short tail, black fur 9

long tail, black fur 3

short tail, brown fur 3

long tail, brown fur 1

, easy to select against T
all with long tails are off

$\frac{\text{tlong}}{\text{tlong}} \frac{F\text{black}}{f\text{black}}$ x $\frac{\text{tlong}}{\text{tlong}} \frac{f\text{brown}}{f\text{brown}}$

$\frac{\text{tlong}}{\text{tlong}} \frac{F\text{black}}{f\text{black}}$ test cross
 $\frac{\text{tlong}}{\text{tlong}} \frac{f\text{brown}}{f\text{brown}}$

final cross

$\frac{\text{tlong}}{\text{tlong}} \frac{F\text{black}}{f\text{black}}$ x $\frac{\text{tlong}}{\text{tlong}} \frac{F\text{black}}{f\text{black}}$

October 2020

Genetic Questions 3

1) Distance between genes: A genetic map

In guinea pigs, white coat (c^w) is recessive to black coat (C^B) and wavy hair (h^w) is recessive to straight hair (H^S). A breeder crosses a guinea pig that is homozygous for white coat and wavy hair with a guinea pig that is homozygous for black coat and straight hair. The F₁ are then crossed with guinea pigs having white coats and wavy hair in a series of testcrosses.

$c^w c^w h^w h^w \times C^B C^B H^S H^S$ F₁: $C^w C^B h^w h^S$

a) Assuming independent assortment, what outcome would you expect?

$\times c^w c^w h^w h^w$

b) Knowing that the genes are linked, calculate the distance between the ~~two~~ genes:

progeny:	total	83	1:1:1:1
black, straight	30		
black, wavy	10		
white, straight	12		

$$\text{Dis. } \frac{22}{83} = 26.5\% = 26.5 \text{ cM}$$

2) Robin had problems with his genetics homework. At school they were working with fruit flies. They were looking at the following genes:

st = the gene for **scarlet eyes** (wild type: red eyes)

e = the gene for **ebony body color** (wild type: grey body)

ss = the gene for **spineless bristles** (wild type: long bristles)

For all genes: the wild type is dominant over the mutant allele.



On their first lab day they set up a cross with Drosophila males being homozygous for *st*, *e* and *ss* and wild type females. Two weeks later they crossed the resulting F1 females with their fathers.

Another two weeks later, they got progenies showing the following characteristics:

wild type	283
scarlet, ebony and spineless	278
ebony, spineless	50
scarlet	52
spineless	5
ebony	43
scarlet, spineless	41
scarlet, ebony	3

(5)

3

ss in center

200

st - ss - e

F1: *stR* *eb* *sl* ♀ × *stst ee ss* ♂

RR

GG

LL

The teacher asked the students to determine the proper order of the genes and to calculate the distance between the genes. Can you do it for Robin?

e-ss 92/755 ≈ 12.2 cM.

st-ss

110/755

≈ 14.6 cM.

3) Dog breeder John likes yellow and brown Labrador retrievers. In an attempt to produce yellow and brown puppies, he mated a yellow Labrador male and a brown Labrador female. Unfortunately, all the puppies produced in this cross were black.

a) Explain the result

e is recessive epistatic to *B*

b) How might John go about producing yellow and brown Labradors?

4) You're a passionate gardener and love your job. One day, you get a phone call from the city to plant some white flowering *Illegitimi noncarborundum* in front of the Government's main building. You have two white flowering pure-breeding lines. Unfortunately, both are a little bit sickly. So, you decide to breed them, in the hope to get rid of the factor that causes lethality. Your F1 plants are all white flowering as expected. You allow the plants to self-fertilize and spread the seeds abundantly in front of the Government's building. A few weeks later you get a phone call of the janitor asking you why you weren't seeding the flowers as ordered. You're puzzled and inspect the site. You find 126 white-flowering and 33 purple-flowering F2 plants.

a) What was the phenotype of the parents?

126:33 ≈ 3:1

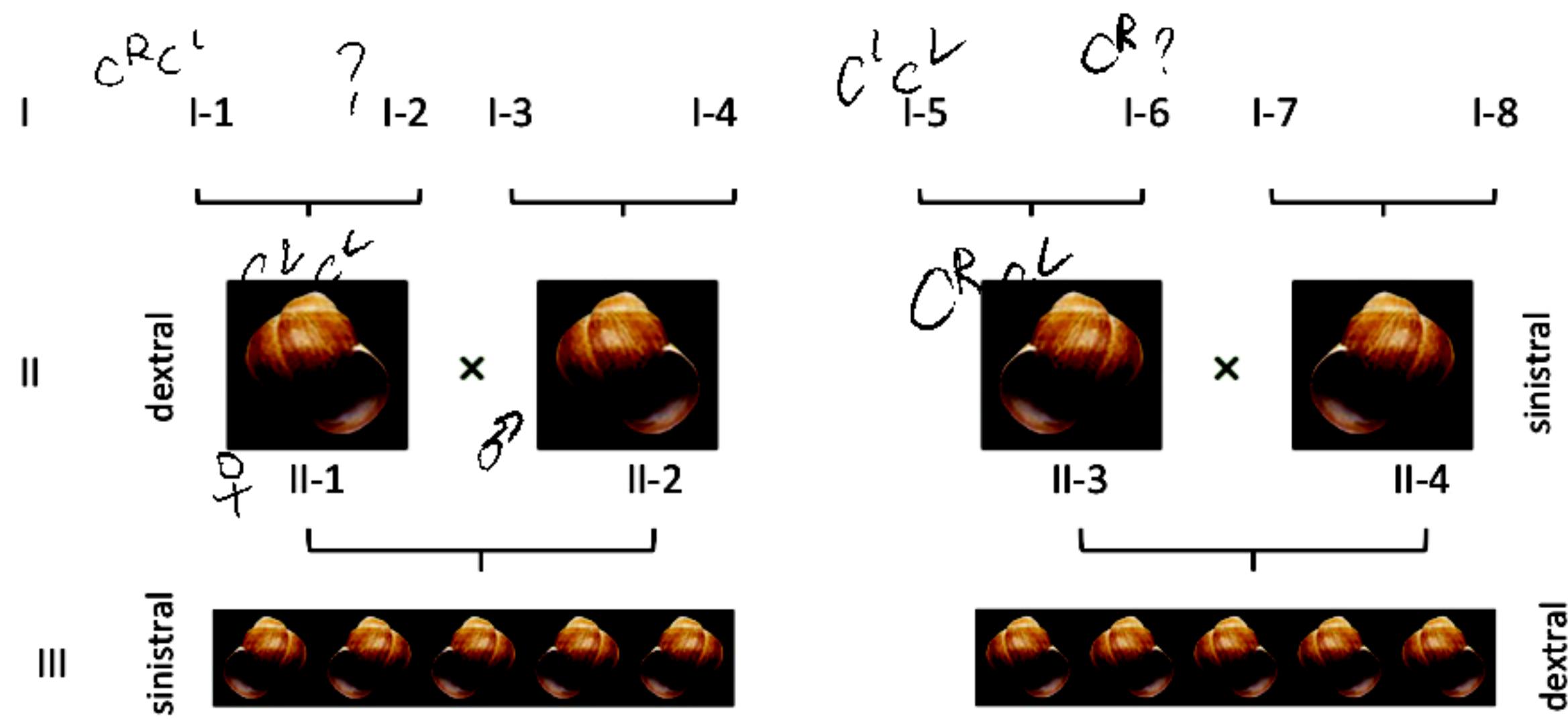
b) Explain the result.

AAbb × *aabb*

or

AA BB × *aabb*

5) Shell coiling in snails is maternally inherited but otherwise follows the Mendelian rules. Right coiling (dexter) is dominant over left coiling (sinistral). In the following snail-crossings the female is always indicated on the left side and the male on the right side.



- a) What can you say about the genotype of the animals II-1 and II-3?
 b) What about their parents? I-1, I-2, I-5 and I-6?

Question 3 continued

6)

a) I₁ $c^L c^L$

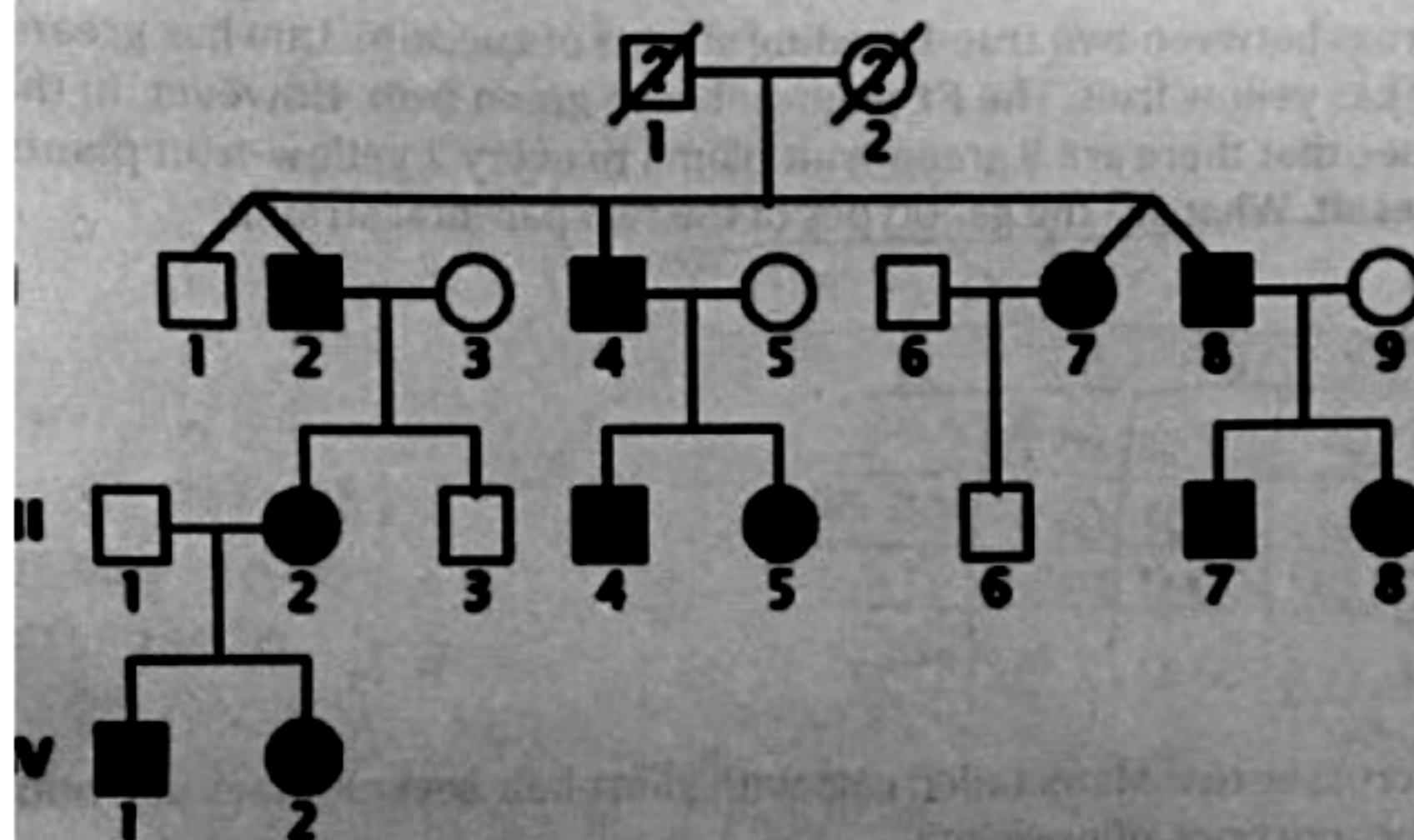
I₃ $c^R c^L$

b)

I-1 $c^R c^L$ I₂ not sure $c^L c^L$ or $c^R c^L$

I-5 $c^L c^L$ I-6 $c^R c^L$ or $c^R c^R$

The complete absence of one or more teeth (tooth agenesis) is a common trait in humans—indeed, more than 20% of humans lack one or more of their third molars. However, more severe absence of teeth, defined as missing six or more teeth, is less common and frequently an inherited condition. L. Lammi and colleagues examined tooth agenesis in the Finnish family shown in the pedigree below (L. Lammi. 2004. American Journal of Human Genetics 74:1043–1050). The affected status of I-1 and I-2 is unknown.



[Pedigree adapted from L. Lammi. 2004. American Journal of Human Genetics 74:1043–1050.]

- A. What is the most likely mode of inheritance for tooth agenesis in this family? Explain your reasoning.

Autosomal Dominant. Individuals of both genders are affected, and affected individuals usually produce affected children.

- B. Are the two sets of twins in this family monozygotic or dizygotic twins? What is the basis of your answer?

Both dizygotic

Both Zygotic

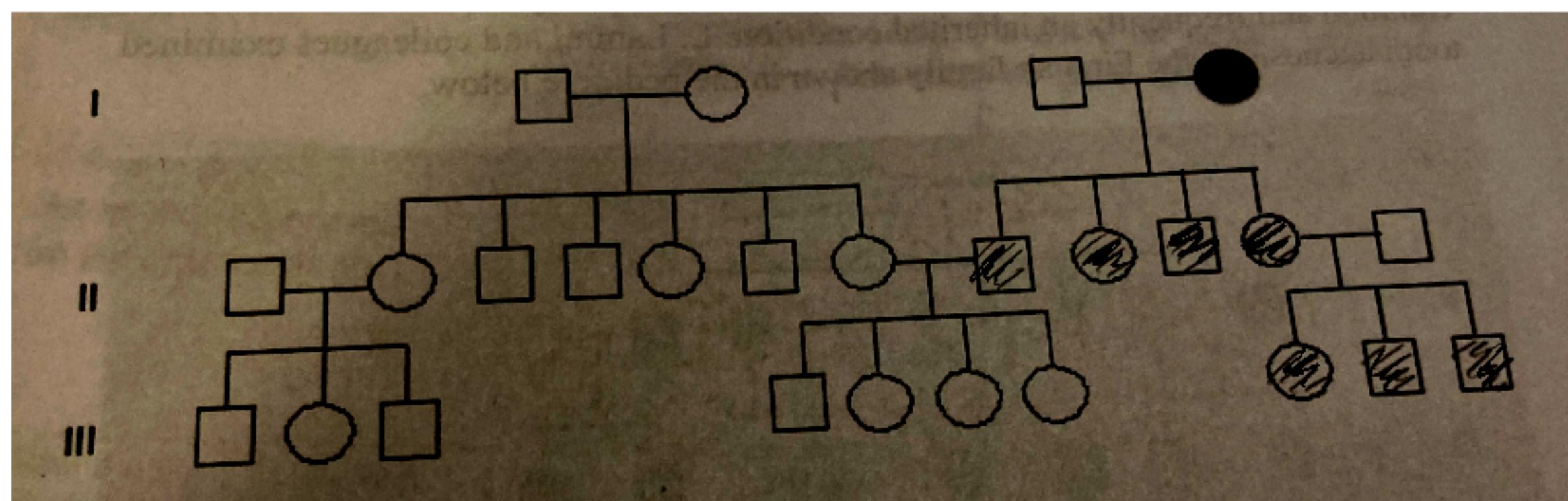
- C. If IV-2 married a man who has a full set of teeth, what is the probability that their child would have tooth agenesis?

50%

- 7) A female fruit fly with singed bristles was mated with a male from a true-breeding wild type stock with long bristles. All of the F1 females had wild-type bristles and all of the F1 males had singed bristles. If the F1 flies are intercrossed, the expected ratio of long to singled bristles in the F2 flies is:

- E) 1:1 in both sexes.
What is the mode of inheritance?

8) The lady marked with dark circle suffers from a mitochondrial disease. Fill in all individuals in generation II and III that will suffer from the same disease.



Questions Mitosis and Meiosis

October 2020

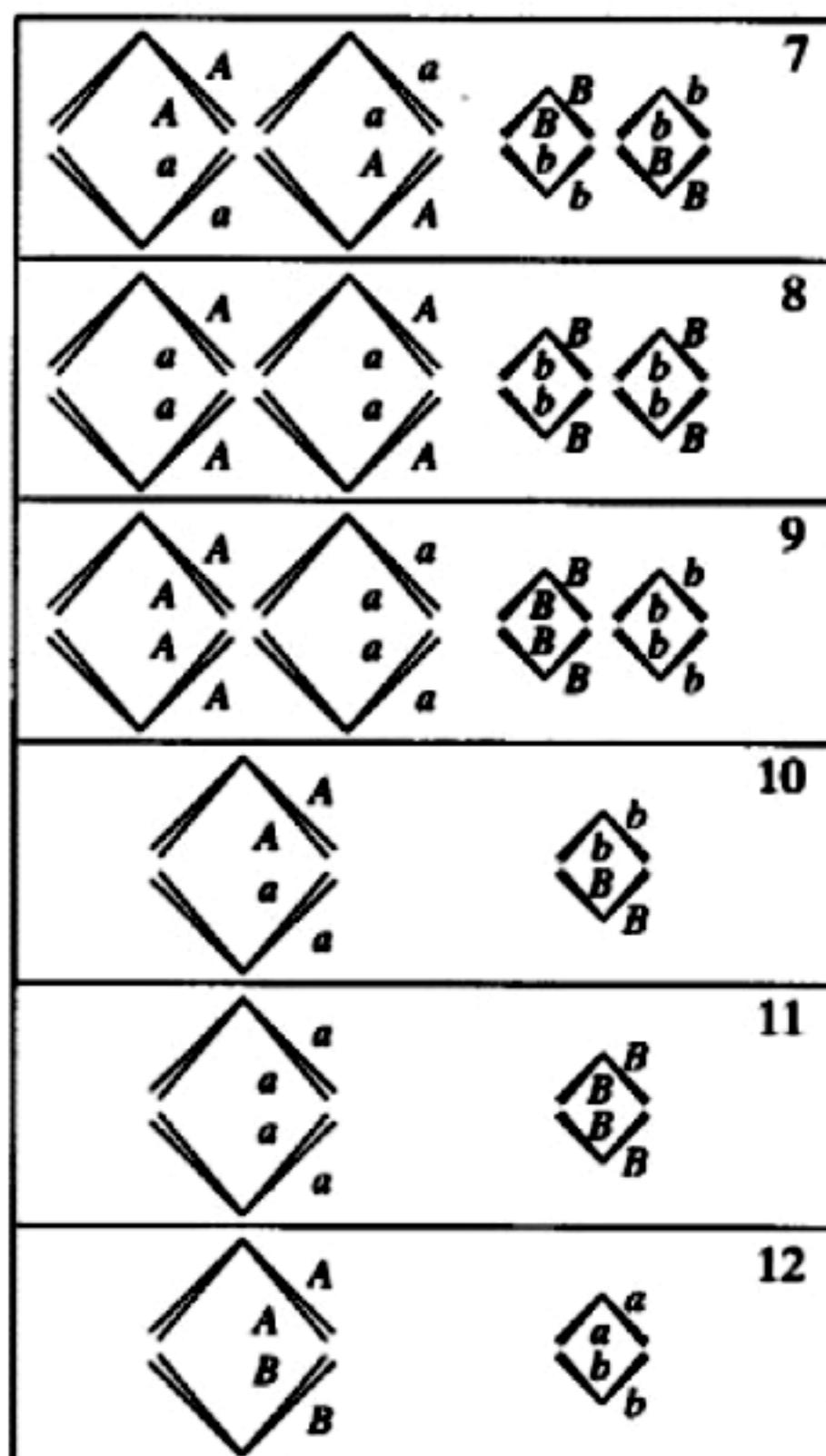
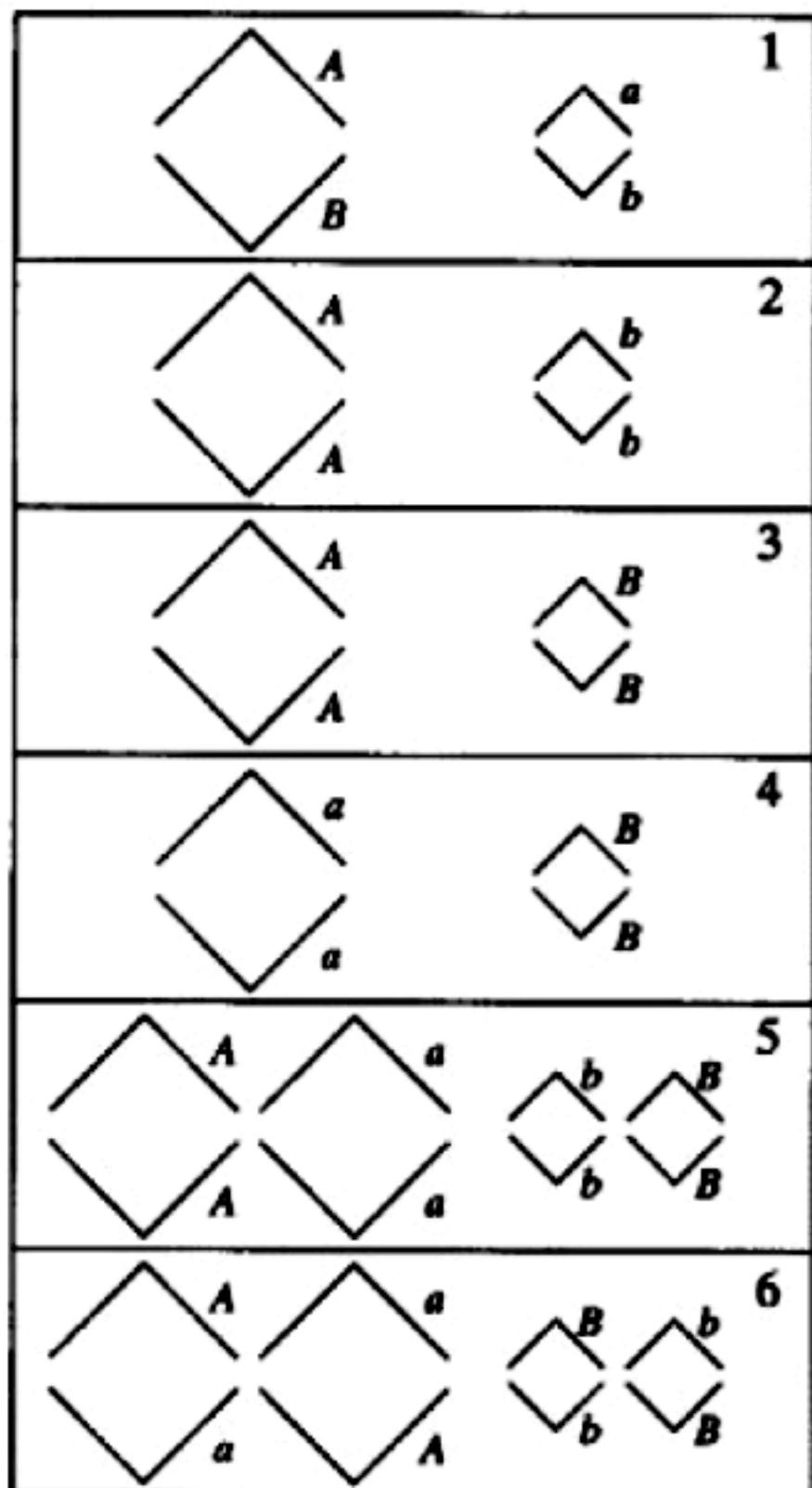
- 1) Mitosis Compared to Meiosis

	Mitosis	Meiosis

When does the process occur?	Somatic cell division after fertilization	germ maturation
Where in the body does it occur?	soma	germ line
How are the chromosomes organized on the equatorial plate? <small>赤道</small>	as single chromosomes	as paired chromosomes tetrades
How many cells are the result of the process?	2	4 human female: 1 oocyte and 3 polar bodies
How are the resulting cells compared to each other?	identical	different recombined
How many sets of chromosomes do the cells contain after the cell division?	2 → diploid	1 → haploid
How are the cells called?	somatic cells	germ cells: spermatocytes and oocytes
What is the significance of the process	<ul style="list-style-type: none"> - pass on identical information into the next generation of cells - growth body and for regeneration 	<ul style="list-style-type: none"> - to reduce the number of chromosomes (haploid) for functional germ cells - recombine, remix of DNA

2) Mitosis and Meiosis in *Haplopappus gracilis*

The plant *Haplopappus gracilis* is diploid and $2n=4$. It contains one long pair and one short pair of chromosomes. The diagrams below represent anaphases of individual cells in meiosis in a plant that is genetically a dihybrid (A/a ; B/b) for genes on different chromosomes. The lines represent chromosomes or chromatids, and the points of the V's represent the centromeres. In each case, say if the diagram represents a cell in meiosis I, meiosis II or mitosis. If a diagram shows an impossible situation, say so.



$AaBb$
 }

 } too many
 chromosomes

1. impossible : the alleles of the same genes are on non-homologous chromosomes
2. meiosis II
3. meiosis II
4. meiosis II
5. mitosis
6. impossible : appears to be mitotic anaphase but alleles if sister chromatids are not identical
7. impossible
8. impossible
9. impossible
10. meiosis I
11. meiosis I impossible : appears to be meiosis of homozygous the alleles of same genes on nonhomologous chromosomes
12. impossible

- 3) A horse has 64 chromosomes and a donkey has 62 chromosomes. A cross between a female horse and a male donkey produces a mule, which is usually sterile. How many chromosomes does a mule have? Explain why it usually is perfectly viable but sterile?

63

不育

Meiosis goes wrong. 1 chromosome fails to properly align in metaphase, perhaps due to mismatch between horse and donkey chromosomes.

- 4) The amount of DNA per cell of a particular species is measured in cells found at various stages of meiosis. The following amounts are obtained:

amount of DNA per cell: 3.7 pg
 7.3 pg
or 14.6 pg

Match the amounts of DNA above with the corresponding stage of meiosis.

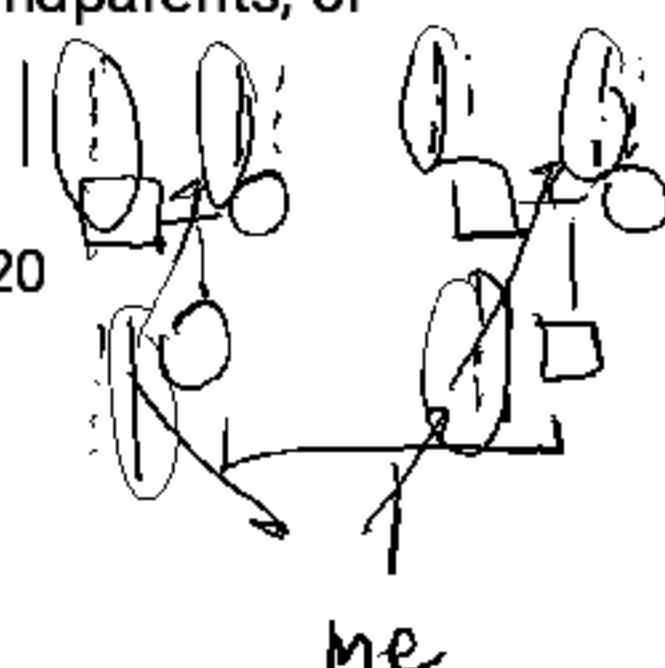
- a) G1 7.3 pg
b) prophase I 14.6 pg
c) G2 14.6 pg
d) telophase II and cytokinesis 3.7 pg
e) anaphase I 14.6 pg
f) metaphase II 7.3 pg

- 5) If a geneticist were to closely examine the make-up of a single autosomal chromosome from one of your cells, that chromosome would be found to be:

- A. entirely derived from just one of your grandparents
B. mosaic of genes from mother and father
C. mosaic from all 4 grandparents
D. mosaic of genes from 2 grandparents, either two grandfathers or two grandmothers
E. mosaic of genes from just two grandparents, either your maternal grandparents, or your paternal grandparents.

Questions DNA Structure

October 2020



- 1) Multiple choice: more than one option can be correct
(from <http://www.biology-test.com/dna-online-test/index.html>)

- a) The backbone of each polynucleotide strand in a DNA double helix consists of alternating ...

- deoxyribose and phosphate groups
- base and phosphate groups
- ATP and phosphate groups
- histone and phosphate groups
- ribose and phosphate groups

b) A nucleotide consists of

- a nitrogen base and a five carbon sugar
- two nitrogen bases, a five carbon sugar, and a phosphate unit
- a nitrogen base
- a nitrogen base, a five carbon sugar, and a phosphate unit

c) In a DNA molecule, base pairing occurs normally between ...

- cytosine and guanine
- adenine and uracil
- thymine and cytosine
- guanine and uracil
 - adenine and thymine

d) A phosphate group in DNA consists of a central phosphorous surrounded by how many oxygens?

- three
- two
- four
- six

e) The two pyrimidine bases in DNA are ...

- adenine and guanine
- cytosine and guanine
- cytosine and thymine
- adenine and thymine
- adenine and uracil

f) In the DNA, complementary base pairs are held together by which kind of bonds?

- peptide bonds
- ionic bonds
- non-polar covalent bonds
- james bonds
- hydrogen bonds
- phosphodiester bonds

g) A DNA strand has the following bases: 5' -TACGATCATAT- 3'. What are the bases on its complementary strand?

- 5' -ATGCTAGTTA- 3'
- 3' -GCATATACGCG- 5'
- 3' -TACGATCATAT- 5'
- 3' -AUGCUGAUUA- 5'
- 3' -ATGCTAGTATA- 5'
- 5' -ATGCTAGTATA- 3'
- 3' -TATACTAGCAT- 5'
- 5' -ATATGATCGTA- 3'

3' -ATGCTAGTATA- 5'
or reverse

h) One strand in a DNA double helix runs 5' to 3' while the other runs 3' to 5'. The two DNA strands are ...

- identical
- antiparallel
- parallel
- heterocyclic

- i) How many hydrogen bonds link cytosine and guanine, and adenine and thymine, respectively?

- cytosine and guanine: 3, adenine and thymine: 2
- cytosine and guanine: 2, adenine and thymine: 3
- cytosine and guanine: 3, adenine and thymine: 3
- cytosine and guanine: 2, adenine and thymine: 2

- j) The DNA molecule of a certain organism is found to be composed of 28% guanine. What percentage of thymine would you expect?

$$\begin{array}{c} 44 \\ | \\ 56 \\ \hline (1 - 28 \times 2) / 2 = 22 \end{array}$$

$$\begin{array}{l} 28\% G \quad 28\% C \\ T \quad 22\% \end{array}$$

- BB
2) Farmer Henry has problems breeding his pigs. Three mother pigs seem to have problems to conceive and another 4 pigs had stillbirth. The veterinary realized that Henry's pigs are infected with a virus. Fortunately the virus could be isolated. An initial analysis revealed that its genome consisted of 10% adenine, 24% thymine, 30% guanine and 36% cytosine. What is the genetic material.

To be

single stranded DNA

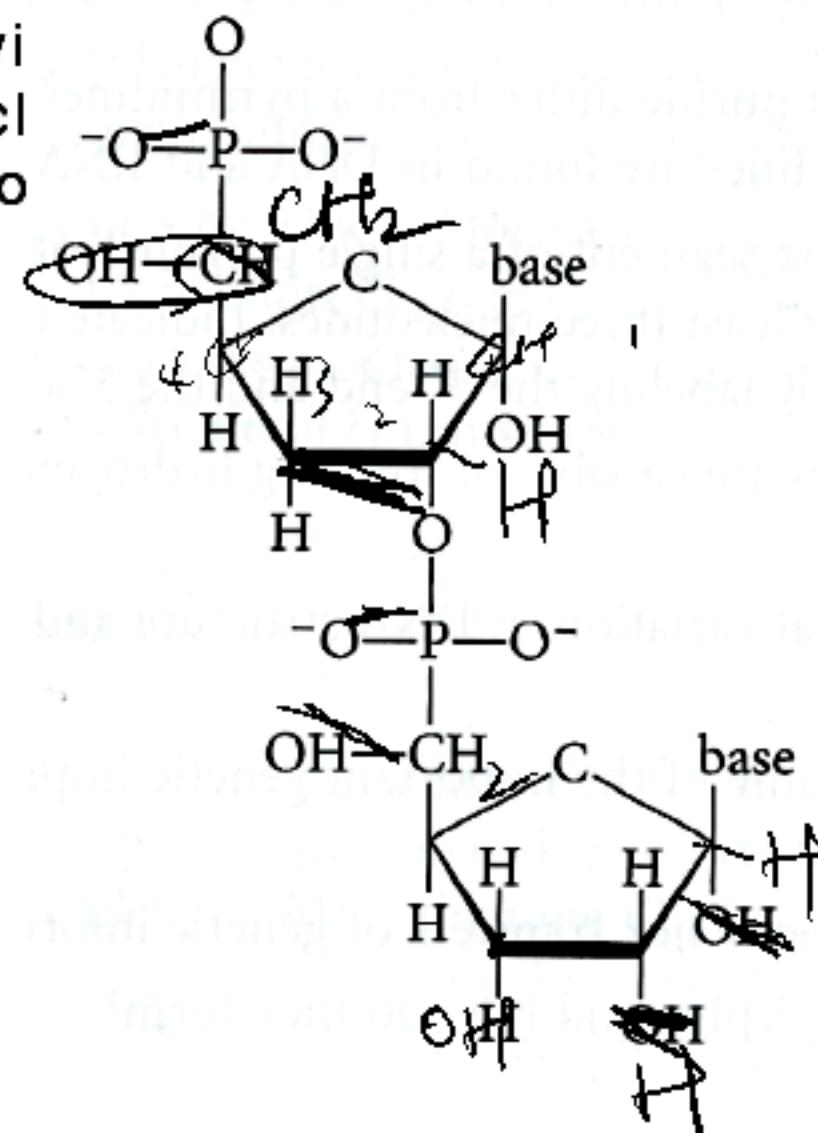
A ~~T~~
C ~~G~~

DNA &
protein
both
contain

- 3) Why did Hershey and Chase choose the radioisotopes ^{32}P and ^{35}S for use in their experiment? Could they have used radioactive isotopes of carbon (C) and oxygen (O) instead? Why or why not?

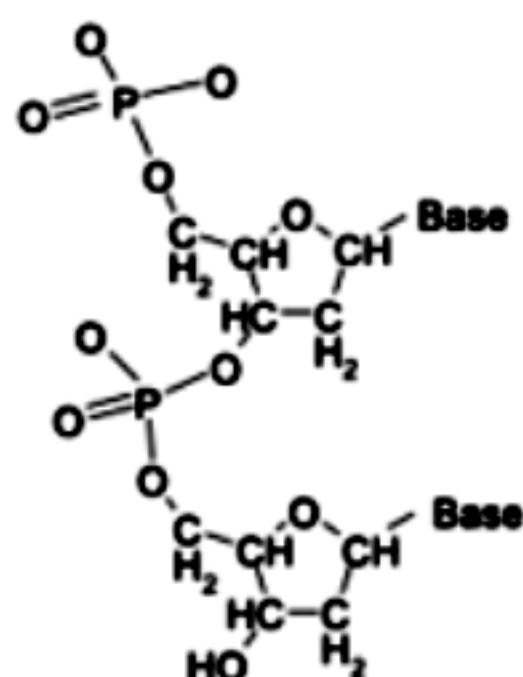
DNA contains phosphorus but not sulfur.
protein contains sulfur but not phosphorus.

- 4) For entertainment on a Friday night, genetics professor Charles Weiner proposed that his children diagram a polynucleotide strand of DNA. Having preschool, his 5-year-old daughter was able to draw a polynucleotide (here), but she made a few mistakes. Can you find them? Are you



- (a) Make a list of all the mistakes in the structure of this DNA polynucleotide strand.
- (1) Neither 5' carbon of the two sugars is directly linked to phosphorous.
 - (2) Neither 5' carbon of the two sugars has an OH group attached.
 - (3) Neither sugar molecule has oxygen in its ring structure between the 1' and 4' carbons.
 - (4) In both sugars, the 2' carbon has an -OH group attached, which does not occur in deoxyribonucleotides.
 - (5) At the 3' position in both sugars, only hydrogen is attached, as opposed to an -OH group.
 - (6) The 1' carbon of both sugars has an -OH group, as opposed to just a hydrogen attached.
- (b) Draw the correct structure for the polynucleotide strand.

136 Chapter Ten: DNA: The Chemical Nature of the Gene

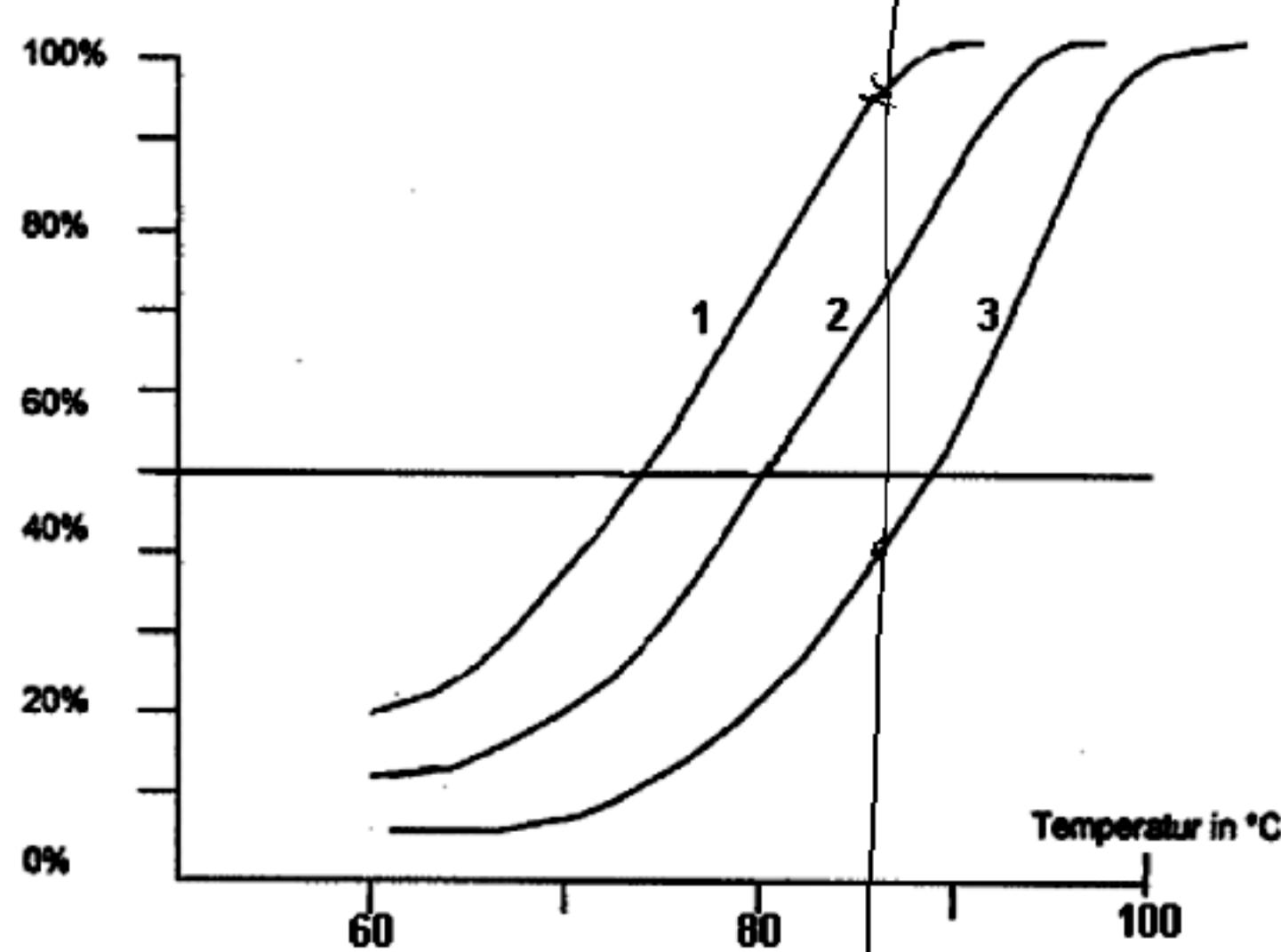


5) Melting temperature

Ornithologist Jessy Brown wanted to know whether the barn swallow is closer related to the common swift or to sunbirds. For this reason she measured the melting temperature of hybrid DNA. The curves are shown below:

- 1) Hybrid-DNA of barn swallow and common swift
- 2) Hybrid-DNA of barn swallow and sunbird
- 3) DNA of barn swallow

Percentage of single stranded DNA



closer related to sunbird
same temperature lower single stranded percentage

What is Jessy's conclusion?

Questions: DNA Replication

October 2020

- 1) Discontinuous replication is a result of which property of DNA
 - a. Complementary bases
 - b. Five-carbon sugar
 - c. Charged phosphate group
 - d. Antiparallel nucleotide strands
- 2) Where on the lagging strand are primers synthesized?
 - a. Only at the 5' end of the newly synthesized strand
 - b. At the beginning of every Okazaki fragment
 - c. At multiple places within an Okazaki fragment
 - d. Only at the 3' end of the newly synthesized strand

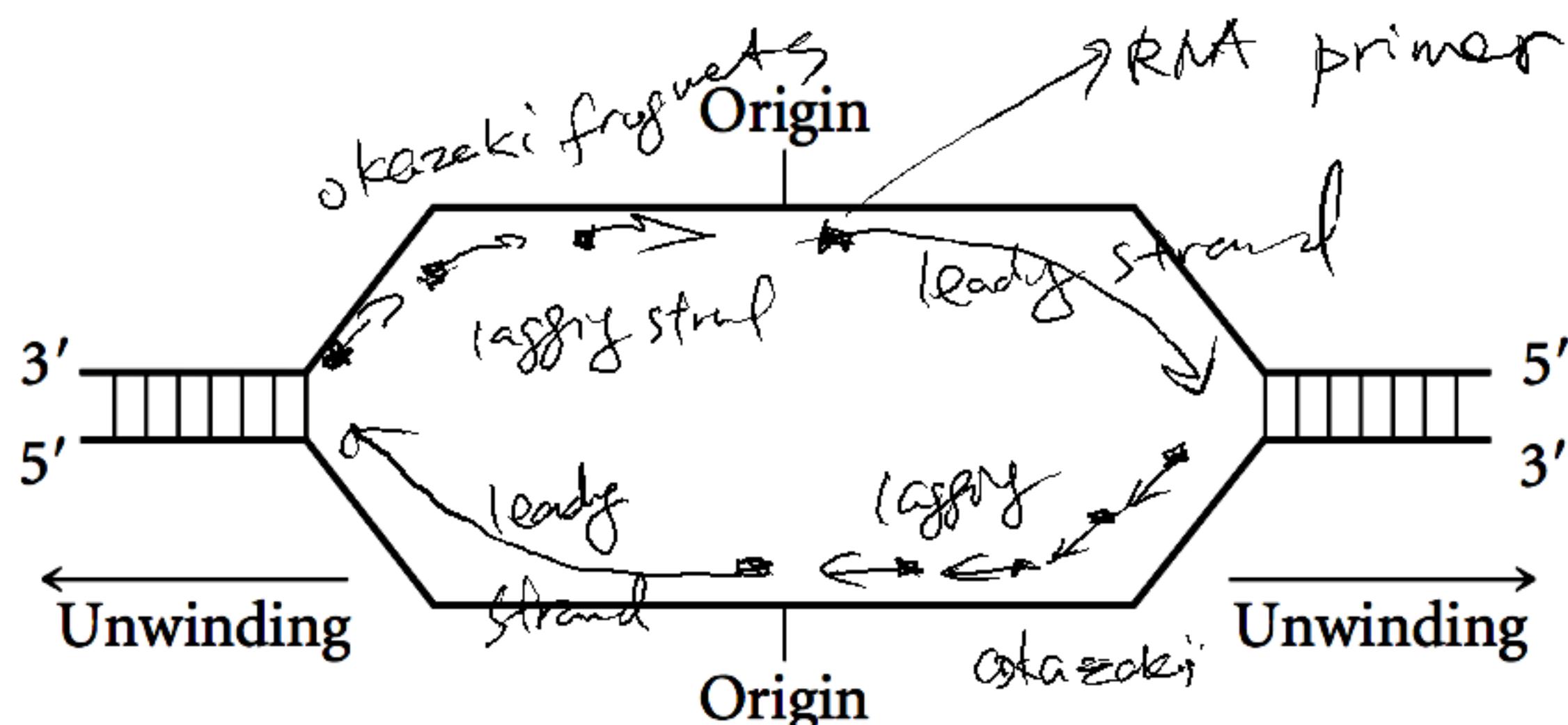
use nucleotides) What would be the effect on DNA replication if the following activity of DNA polymerase I were destroyed by mutation:

a. 3' → 5' exonuclease activity no proofreading, more errors in DNA (in primer region)

b. 5' → 3' exonuclease activity

c. 5' → 3' polymerase activity primers would not be replaced → big gaps, because it only eats!

- 4) The following diagram represents a DNA molecule that is undergoing replication. Draw in the strands of newly synthesized DNA and identify (a) the polarity of newly synthesized strands, (b) the leading and lagging strands, (c) Okazaki fragments, and (d) RNA primers.



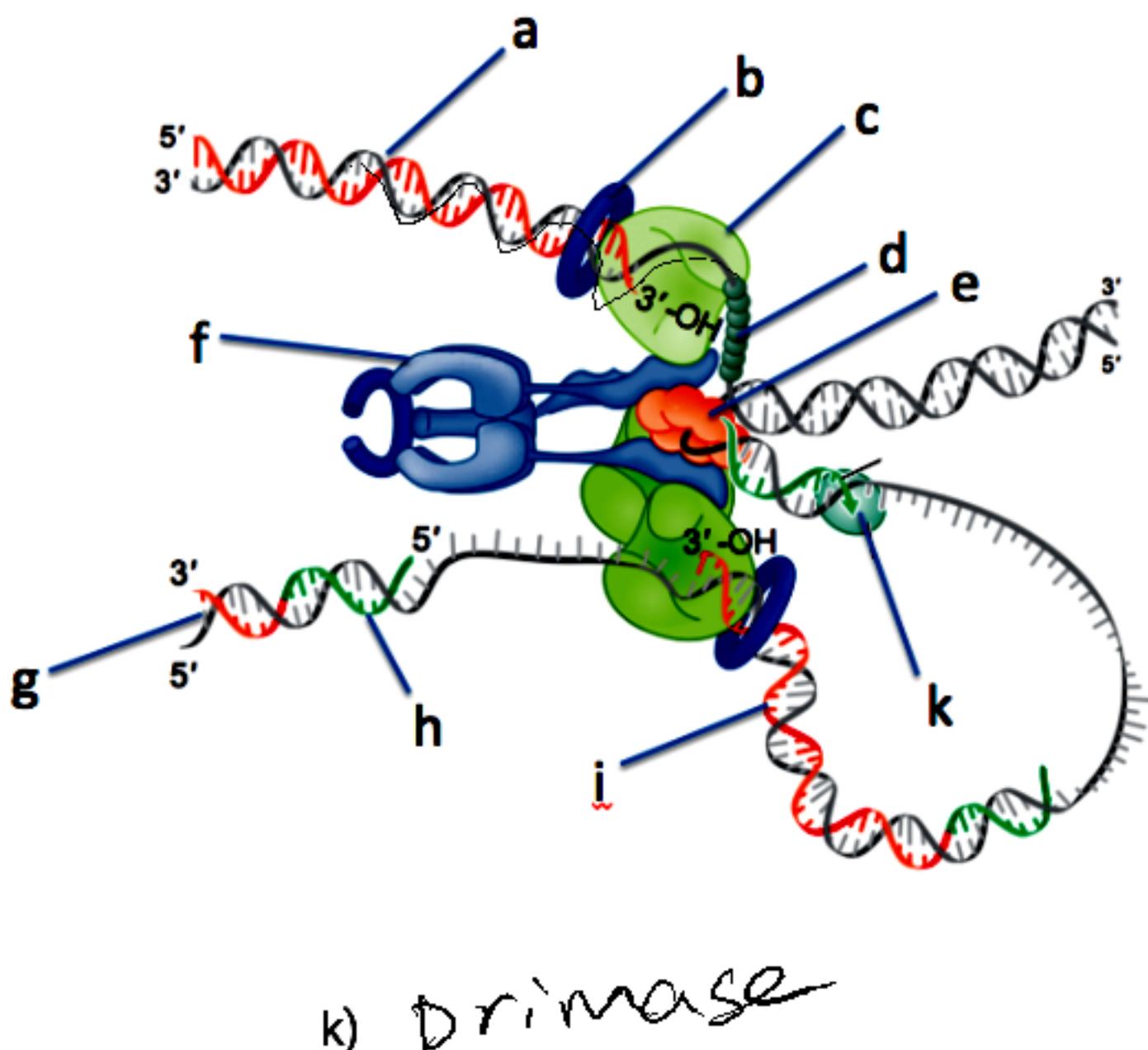
- 5) Phosphorus is required to synthesize the deoxyribonucleoside triphophates used in DNA replication. Peter Handrix grows some *E. coli* in a medium containing nonradioactive phosphorous for many generations. A sample of the bacteria is then transferred to a medium that contains a radioactive isotope of phosphorous (^{32}P). He removes one sample of the bacteria immediately after the transfer, another after one round and a third after two rounds of replication, respectively. What is the distribution of radioactivity? Will it be in none, one or both strands of the DNA?

immediately after	one round	two rounds
none	one strand	50% one strand
continuously only in		50% both strands
continuous direction		

- 6) Suppose a future scientist explores a distant planet and discovers a novel form of double-stranded nucleic acid. When this nucleic acid is exposed to DNA polymerases from *E. coli*, replication takes place continuously on both strands. What conclusion can you make about the structure of this novel nucleic acid?

Each stand of the novel double-stranded nucleic acid must be oriented parallel to the other, as opposed to the anti-parallel nature of earthly double-stranded DNA.

7) Label the following drawing



- leading strand
- - sliding clamp
 - DNA polymerase II
 - SSB protein
 - helicase
 - clamp loader
 - lagging strand
 - RNA primer
 - okazaki fragment

k) primase

Questions to Chapter 7 (from Hartwell, No. correlate more or less with 6th edition)

1) The following is a list of mutational changes. For each of the specific mutations described, indicate which of the terms at the end applies, either as a description of the mutation or as a possible cause. More than one term from the list can apply to each statement at the end.

1. an A-T base pair in the wild-type gene is changed to a G-C pair a, b

2. an A-T base pair is changed to a T-A pair c, b

3. the sequence AAGCTTATCG is changed to AAGCTATCG f, i

4. the sequence AAGCTTATCG is changed to AAGCTTTATCG g, i

5. the sequence AACGTTATCG is changed to AATGTTATCG a, b

6. the sequence AACGTCACACACACATCG is changed to AACGTCACATCG f, h, k

7. the gene map in a given chromosome arm is changed from bog-rad-fox1-fox2-try-duf (where fox1 and fox2 are highly homologous, recently diverged genes) to bog-rad-fox1-fox3-fox2-try-duf (where fox3 is a new gene with one end similar to fox1 and the other similar to fox2) g, k

8. the gene map in a chromosome is changed from bog-rad-fox1-fox2-try-duf to bog-rad-fox2-fox1-try-duf d, b

9. the gene map in a given chromosome is changed from bog-rad-fox1-fox2-try-duf to e, f, g, h

bog-rad-fox1-mel-qui-txu-sqm

pair with: a. transition, b. base substitution, c. transversion, d. inversion, e. translocation, f. deletion, g. insertion, h. X-ray irradiation, i. intercalator, k. unequal crossing-over

1.5	1,2,5	2	8
3,6,9	4,7,19	6,8,9	3,4
			6,7

3) The DNA sequence of a gene from three independently isolated mutants is given here. Using this information, what is the most likely sequence of the wild-type gene in this region? Reason your answer.

mutant 1 ACCGTAATCGACTGGTAAACCTTGCGCG
mutant 2 ACCGTAGTCGACCGGTAAACCTTGCGCG
mutant 3 ACCGTAGTCGACTGGTTAACCTTGCGCG

ACCGTAGTCGACTGGTAAACCTTGCGCG

5) Over a period of several years, a large hospital kept track of the number of births of babies displaying the trait achondroplasia. Achondroplasia is a very rare autosomal dominant condition resulting in dwarfism with abnormal body proportions. After 120,000 births, it was noted that there had been 27 babies born with achondroplasia. One physician was interested in determining how many of these dwarf babies result from new mutations and whether the apparent mutation rate in his area was higher than normal. He looked up the families of the 27 dwarf births and discovered that 4 of the dwarf babies had a dwarf parent. What is the apparent mutation rate of the achondroplasia gene in this population? Is it unusually high or low?

$$27 - 4 = 23 \text{ mutant gametes}$$

of 240,000 gametes

$$\frac{23}{240,000} = 9.6 \times 10^{-5}$$

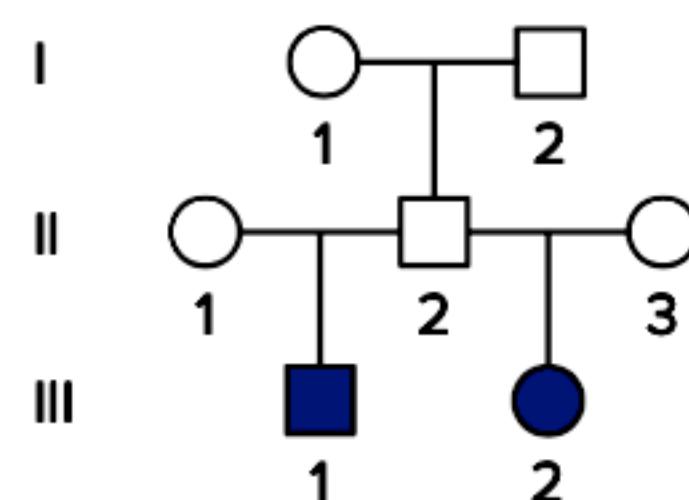
higher
normal

$2 \sim 12 \times 10^{-5}$

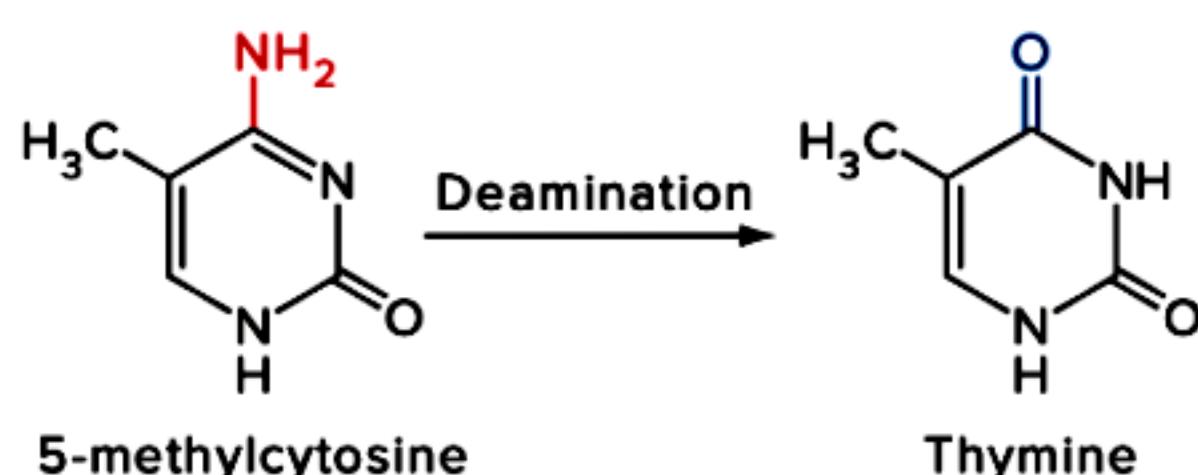
7) In a genetics lab, Kim and Maria infected a sample from an *E. coli* culture with a particular virulent bac- teriophage. They noticed that most of the cells were lysed, but a few survived. The survival rate in their sample was about 1×10^{-4} . Kim was sure the bacte- riophage induced the resistance in the cells, while Maria thought that resistant mutants probably already existed in the sample of cells they used. Earlier, for a different experiment, they had spread a dilute suspen- sion of *E. coli* onto solid

medium in a large petri dish, and, after seeing that about 10^5 colonies were growing up, they had replica-plated that plate onto three other plates. Kim and Maria decide to use these plates to test their theories. They pipette a suspension of the bacteriophage onto each of the three replica plates. What should they see if Kim is right? What should they see if Maria is right?

9) The following pedigree shows the inheritance of a completely penetrant, dominant trait called *amelogenesis imperfecta* that affects the structure and integrity of the teeth. DNA analysis of blood obtained from affected individuals III-1 and III-2 shows the presence of the same disease-causing mutation in one of the two copies of an autosomal gene called *ENAM* that is not seen in DNA from the blood of any of the parents in generation II. Explain this result, citing Fig. 4.19 and Fig. 7.5. Do you think this type of inheritance pattern is rare or common?



- 17) When a particular mutagen identified by the Ames test is injected into mice, it causes the appearance of many tumors, showing that this substance is carcinogenic. When cells from these tumors are injected into other mice not exposed to the mutagen, almost all of the new mice develop tumors. However, when mice carrying mutagen-induced tumors are mated to unexposed mice, virtually all of the progeny are tumor free. Why can the tumor be transferred horizontally (by injecting cells) but not vertically (from one generation to the next)? *The mutagen induces mutations in somatic cells and not in gametic cells*
- 23) In human DNA, 70% of cytosine residues that are followed by guanine (so-called CpG dinucleotides, where p indicates the phosphate in the phosphodiester bond between these two nucleotides) are methylated to form 5-methylcytosine. As shown in the following figure, if 5-methylcytosine should undergo spontaneous deamination, it becomes thymine.



- Methylated CpG dinucleotides are hotspots for point mutations in human DNA. Can you propose a hypothesis that explains why?
- Making the simplifying assumptions that human DNA has an equal number of C-G and A-T base pairs, and that the human DNA sequence is random, how frequently in the human genome would you expect to find the base sequence CpG?
- It turns out that, even after taking into account the actual GC content of human DNA (~42%), the frequency of CpG in human DNA is much lower than predicted by the calculation in part (b). Explain why this might be the case.

43) The following non-complementing *E. coli* mutants were tested for growth on four known precursors of thymine, A–D.

Mutant	Precursor/product				
	A	B	C	D	Thymine
9	+	-	+	-	+
10	-	-	+	-	+
14	+	+	+	-	+
18	+	+	+	+	+
21	-	-	-	-	+

a. Show a simple linear biosynthetic pathway of the four precursors and the end product, thymine. Indicate which step is blocked by each of the five mutations.

b. What precursor would accumulate in the following double mutants: 9 and 10? 10 and 14?

2) when his-salmonella strain used in ames test is exposed to substance X, no his+ revertants are seen. however, if rat liver supernatant is added to the cells along with sub X, revertants occur. is sub x a potential carcinogen for human cells?

ANS: yes

the rat liver supernatant contains enzymes that convert substance X to a mutagen and his+ revertants occur

our liver contain similar enzymes that process various substances, converting them into other forms that cause mutation and can lead to cancer

-10: is consensus sequence for binding of sigma; if sigma still binds it could have an effect on opening -10 complex (mainly if it is one of the nucleotides that flip out). A T \rightarrow A or A \rightarrow T mutation has probably a smaller effect.

-30: a point mutation has little effect, deletions or insertions change the spacing between -10 and -30 element and this is detrimental.

Questions Transcription

November 2020

1) What would be the most likely effect of a mutation at the following locations in an E. coli gene?

- a) -10
- b) -20
- c) -35
- d) start site of transcription

\rightarrow -35: sigma does not bind, polymerase is not loaded in \rightarrow reduced transcription

the sequence at the start site is not critical

2) The following sequence of nucleotides is found in a single-stranded DNA template.

Assume the RNA polymerase proceeds along this template from the left to the right.

a) Which end of the DNA template is the 5' end?

b) Give the sequence of the RNA copied from this template and label its 5' end 3' ends.

5' to 3'
Synthesis

3' GACAATGTCCATGCCA 5'

read from

5' CUGUUAACAGGUACGGU 3'

3' to 5'

3) The following DNA nucleotides are found near the end of a bacterial transcription unit. Find the terminator in this sequence.

3' -AGCATAACAGCAGACCGATCTGGTCTGAAAAAAAGCATACA-5'
stem loop stem

- a) Mark the point at which transcription will terminate.

- b) Is this terminator rho independent or rho dependent?
- c) Draw a diagram of the RNA that will be transcribed from this DNA, including its nucleotide sequence and any secondary structures that form.

b) no; rho-independent
→ use hairpin (UUUWW)

c)

5' AGCAUACAG CUGACC GAUCUU GGUCC

- 4) What protein associated with a transcription factor is common to all eukaryotic promoters? What is its function in transcription?

TBP (TATA binding protein) in TFIID. It binds the TATA Box and positions the polymerase over the transcriptional start site.

- 5) Compare and contrast transcription and replication. How are these processes similar and how are they different?

	replication	transcription
template	DNA usually both strands	DNA, template strand
polymerase	DNA polymerase	RNA polymerase
building blocks	dNTP, dATP, dCTP, dGTP, dTTP	NTP, ATP, GTP, UTP
product	usually dsDNA (chromosome)	mRNA, tRNA etc. usually single stranded affected by regulation of protein synthesis
time	synchronised during cell cycle	regulated

- 6) The gene for muscular dystrophy is dystrophin. With 2'400'000 nucleotides it is the longest human gene known. Assuming a rate of 40 bases per second, how long does it take the polymerase to transcribe this gene?

The full length protein contains 3685 amino acids. If you construct a cDNA clone with 200 bp for leader and trailer sequences, and you transferred it into cultured cells, how long would it take to be transcribed?

$$2'400'000 \div 40 \text{ s} = 60000 \text{ min}$$

$$(3685 \times 3 + 200) \div 40 \text{ s} = 4.69 \text{ min}$$

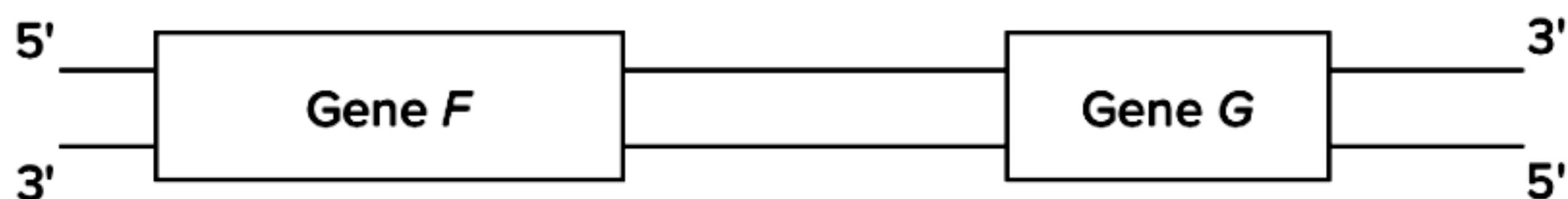
7) solved problem from Hartwell, question 1:

The doublestranded circular DNA molecule that forms the genome of the SV40 tumor virus can be denatured into singlestranded DNA molecules. Because the base composition of the two strands differs, the strands can be separated on the basis of their density into two strands designated W(atson) and C(rick). When each of the purified preparations of the single strands was mixed with mRNA from cells infected with the virus, hybrids were formed between the RNA and DNA. Closer analysis of these hybridizations showed that RNAs that hybridized with the W preparation were different from RNAs that hybridized with the C preparation. What does this tell you about the transcription templates for the different classes of RNAs?

i.e. large Tag

Tag protein is required for initiation of replication and Initiation and maintenance

question 21) The coding sequence for gene F is read from left to right on the accompanying figure. The coding sequence for gene G is read from right to left. Which strand of DNA (top or bottom) serves as the template for transcription of each gene?



9) questions to section 8.2 from Hartwell, ie

question 23) In studying normal and mutant forms of a particular human enzyme, a geneticist came across a particularly interesting mutant form of the enzyme. The normal enzyme is 227 amino acids long, but the mutant form was 312 amino acids long. The extra 85 amino acids occurred as a block in the middle of the normal sequence. The inserted amino acids do not correspond in any way to the normal protein sequence. What are possible explanations for this phenomenon? How would you distinguish among them?

from unspliced Intron due to mutation in a splice site sequence or from a mutation caused by insertion of DNA sequence.

An intron sequence would be present in the genomic DNA of the wild type, but an insertion sequence would not.

Answers:

- 1a) -10: is consensus sequence for binding of sigma; if sigma still binds it could have an effect on opening the complex (mainly if it is one of the nucleotides that flip out a T → A or an A → T mutation has probably a smaller effect)
1b) 20 a point mutation has little effect, deletions or insertions change the spacing between the -10 and -35 element and that is not flexible
1c) -35 sigma binds. Polymerase is not stalled in → reduced transcription?
1d) the sequence at the start site is not that critical

2) 3' G A C A A T G T C C A T G C C A 5'

RNA 5' C U G U U A C A G G U A C G G U 3'

3a) 3' -AGCATAACAGCAGACCGATCTTGGTCTGAAAAAAA / GCATACA-5'

3b) no; rho-independent

3c) AGCATAACAG **CAGACC** GATCTT **GGTCTG** AAAAAAA
 stem loop stem

4) TBP in TFIID. It binds the TATA box (even if it is not present) and positions the polymerase over the transcriptional start site

5) both

- use DNA as a template
- molecules are synthesized from 5' to 3'
- newly synthesized molecules are antiparallel and complementary
- use nucleotide triphosphate as substrate
- complex of proteins and enzymes is necessary for catalysis

transcription:

- unidirectional synthesis of only a single strand of nucleic acid
- initiation doesn't need primer
- subject to numerous regulatory mechanisms
- individual genes or small groups of genes are transcribed at a time

replication:

- bidirectional synthesis
- needs primer
- initiation at replication origin
- at specific time during cell cycle (S-phase)

6) it takes

$$(2'400'000 : 40) \text{ seconds} = 60'000 \text{ seconds} \rightarrow 1000 \text{ minutes} \rightarrow \mathbf{16 - 17 \text{ hours}}$$

transcription of cDNA clone: $3x 3685 + 200 = 11055 + 200 = 11'255 \text{ bp}$
time: $(11255 : 40) \text{ seconds} = 281 \text{ seconds} = \mathbf{4 \text{ min } 41 \text{ sec}}$

7-9) see Hartwell

Questions Translation

November 2020

1) For each of the following sequences, place a check mark in the appropriate space to indicate the process most immediately affected by deleting the sequence. Choose only one process for each sequence (i.e., one check mark per sequence)

	Process most immediately affected by the deletion			
Sequence deleted	Replication	Transcription	RNA processing	Translation
ori site	✓			
3' splice-site consensus			✓	
sequence for poly(A)tail			✓	
start codon				✓
-10 consensus sequence		✓		
Shine Dalgarno				✓

2) Several experiments were conducted to obtain information about how the eukaryotic ribosome recognizes the AUG start codon. In one experiment, the gene that encodes methionine initiator tRNA ($tRNA_i^{Met}$) was changed. The nucleotides that specify the anticodon on $tRNA_i^{Met}$ were mutated so that the anticodon in the tRNA was 5'-CCA-3' instead of 5'-CAU-3'. When this mutated gene was placed in a eukaryotic cell, protein synthesis took place, but the proteins produced were abnormal. Some of the proteins produced contained extra amino acids, and others contained fewer amino acids than normal.

a) What do these results indicate about how the ribosome recognizes the starting point for translation in eukaryotic cells? Explain your reasoning.

obviously the start site is

b) If the same experiment had been conducted on bacterial cells, what results would you expect?

3) Mutations that introduce stop codons cause a number of genetic diseases. For example, from 2% to 5% of the people who have cystic fibrosis possess a mutation that causes a premature stop codon in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). This premature stop codon produces a truncated form of CFTR that is nonfunctional and results in the symptoms of cystic fibrosis.

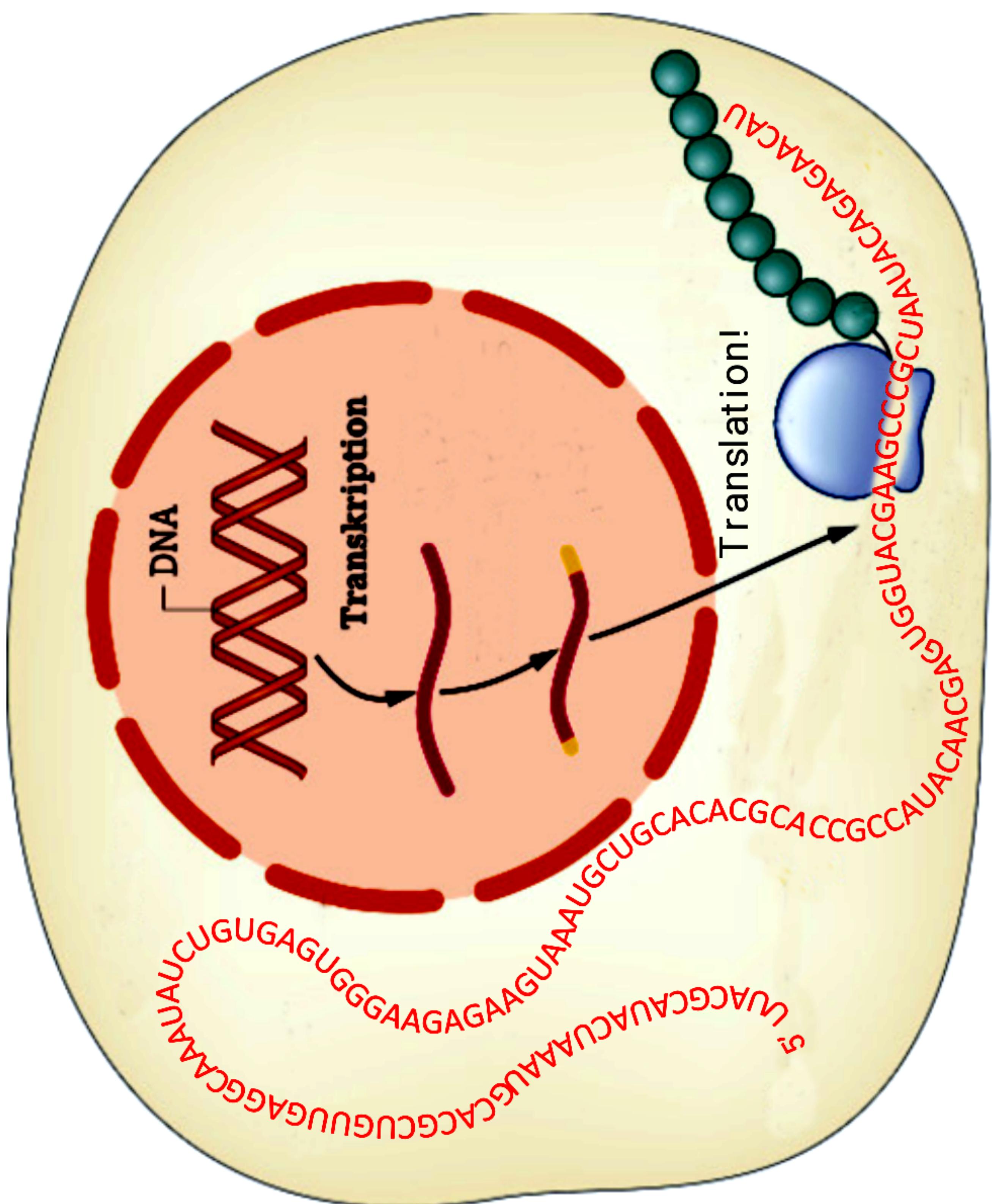


In *Drosophila*, such nonsense mutations can be cured by introducing a suppressor tRNA into the genome of the fly. Using a tRNA^{Ty} gene, how would you change the anticodon to

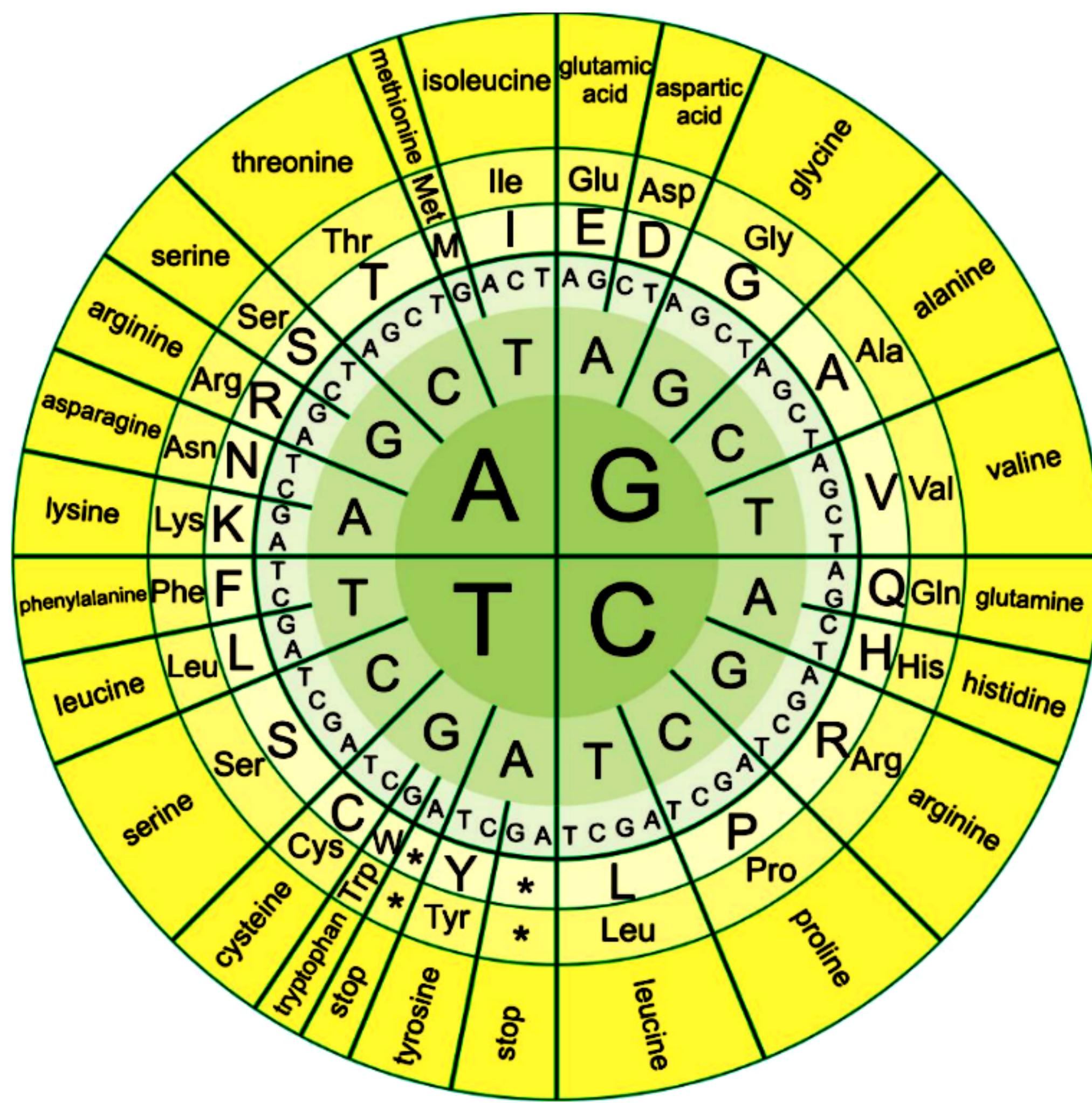
rescue an amber (UAG) nonsense mutation? *Drosophila* has only one type of tRNA^{Tyr}.

4) Genetic Code

Using the one letter code for amino acids, translate the mRNA on the following page into the corresponding protein sequence.



Have a nice week



Some additional example questions from the book (you should be able to answer at the end of the book)

Solved problem III: Geneticists interested in human hemoglobins have found a very large number of mutant forms. Some of these mutant proteins are of normal size (but have amino acid substitutions) while others are short, due to deletions or nonsense mutations. The first extra long example was named Hb Constant Spring, in which the β -globin has all of its normal amino acids plus several extra amino acids attached after the normal C-terminal end of the protein.

1. What is the most plausible explanation for its origin?
2. Is it possible that Hb Constant Spring arose from failure to splice out an intron?
3. Estimate how many extra amino acids might be added to the C terminal end of the mutant protein.

Question 7: The following diagram describes the mRNA sequence of part of the A gene and the beginning of the B gene of phage ϕ X174. In this phage, some genes are read in overlapping reading frames. For example, the code for the A gene is used for part of the B gene, but the reading frame is displaced by one base. Shown here is the single mRNA with the codons for proteins A and B indicated.

aa#	5	6	7	8	9	10	11	12	13	14	15	16
A												mRNA
	Ala	Lys	Glu	Trp	Asn	Asn	Ser	Leu	Lys	Thr	Lys	Leu
	GCUAAAGAAUGGAACAAACUCACUAAAAACCAAGCUG											
B												Met
	Met	Glu	Gln	Leu	Thr	Lys	Asn	Gln	Ala			
aa#	1	2	3	4	5	6	7	8	9			

Given the following amino acid (aa) changes, indicate the base change that occurred in the mRNA and the consequences for the other protein sequence.

- a. Asn at position 10 in protein A is changed to Tyr.
- b. Leu at position 12 in protein A is changed to Pro.
- c. Gln at position 8 in protein B is changed to Leu.
- d. The occurrence of overlapping reading frames is very rare in nature. When it does occur, the extent of the overlap is not very long. Why do you think this is the case?

Question 31: The human genome contains about 500 genes for tRNAs.

- a. Do you think that each one of these tRNA genes has a different function?
- b. Can you explain why the human genome might have evolved so as to house so many tRNA genes?

Question 43:

43. The following is a list of mutations that have been discovered in a gene that has more than 60 exons and encodes a very large protein of 2532 amino acids. Indicate whether or not each mutation could cause a detectable change in the size or the amount of mRNA and/or a detectable change in the size or the amount of the protein product. (Detectable changes in size or amount must be greater than 1% of normal values.) What kind of change would you predict?

- a. Lys576Val (changes amino acid 576 from lysine into valine)
- b. Lys576Arg
- c. AAG576AAA (changes codon 576 from AAG to AAA)
- d. AAG576UAG
- e. Met1Arg (at least two possible scenarios exist for this mutation)
- f. promoter mutation
- g. one base pair insertion into codon 1841
- h. deletion of codon 779
- i. IVS18DS, G-A, + 1 (this mutation changes the first nucleotide in the eighteenth intron
of the gene, causing exon 18 to be spliced to exon 20, thus skipping exon 19)
- j. deletion of the polyA addition site
- k. GtoA substitution in the 5' UTR
- l. insertion of 1000 base pairs into the sixth intron (this particular insertion does not alter splicing)

Solutions

1)

	Process most immediately affected by the deletion			
Sequence deleted	Replication	Transcription	RNA processing	Translation
ori site	✓			
3' splice-site consensus			✓	
sequence for poly(A)tail			✓	
start codon				✓
-10 consensus sequence		✓		
Shine Dalgarno				✓

2a) Obviously the start site is not clear anymore. The ribosome migrates along the mRNA to find the first AUG, but the tRNA can't bind properly. The results suggest that, to initiate translation, the mRNA is scanned to find the appropriate start sequence.

2b) The initiation of translation in bacteria requires the 16S RNA of the small ribosomal subunit to interact with the Shine-Dalgarno sequence on the mRNA. This interaction serves to line up the ribosome over the start codon. If the anticodon has been changed such that the start codon cannot be recognized, then protein synthesis is not likely to take place.

3) Tyr = UAU or UAC (use the table for the genetic code)

anticodon in tRNA^{Tyr} = NUA where N is the wobbling base that recognizes C or U in the wobbling position (you could look it up in the book...)

Stop Codons are UAA, UAG and UGA

by changing the anticodon in the tRNA^{Tyr} to CUA the stop codon UAG will be read

4) Have a nice week

isolates several constitutive mutations affecting this operon, meaning the operon is always transcribed. Where might these constitutive mutations occur? How would the mutations cause the operon to be constitutive?

2) Can you imaging what role RNA stability plays in gene regulation? What controls RNA stability in eukaryotic cells?

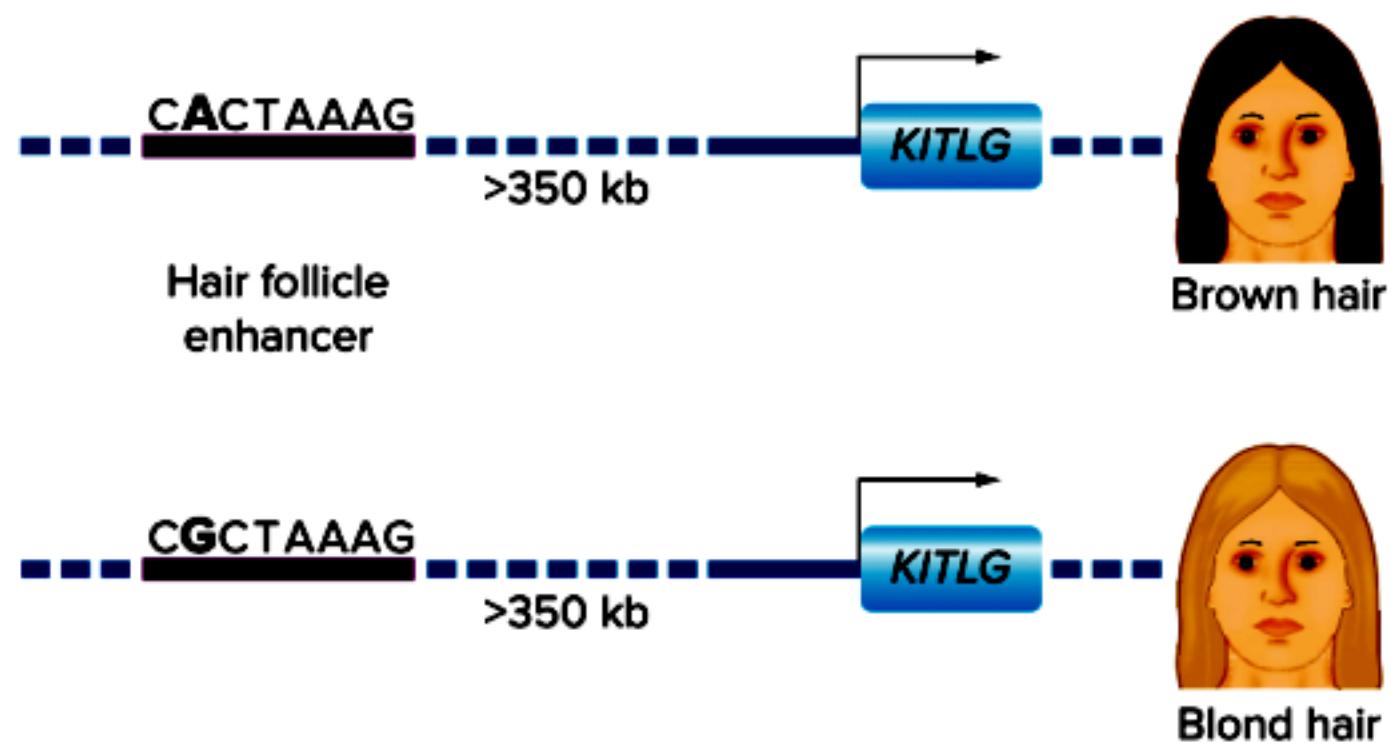
3) What will be the effect on sexual development in newly fertilized *Drosophila* embryos if the following genes are deleted?

10. ***sex lethal (Sxl)***

11. ***transformer (tra)***

12. ***male specific lethal 2 (msl-2)***

13. 4) As shown in the following diagram, a single nucleo- tide difference in a hair follicle enhancer of a human gene called *KITLG* contributes to the trait of hair color. People with an A-T base pair in the enhancer tend to have dark hair, while people with a G-C base pair at the same position tend to have blond hair. The base pair difference affects the level of *KITLG* transcription: The blond-associated allele is transcribed only 80% as frequently as the dark hair-associated allele. Explain how a single base pair difference in an enhancer sequence can have this effect.



Answer

1) An inducible operon is normally not being transcribed, meaning that the repressor is active and binds to the operator, inhibiting transcription. Transcription takes place when the inducer binds to the repressor, making it unable to bind to the operator. Constitutive mutations cause transcription to take place at all times, whether the inducer is present or not. **Constitutive mutations** might occur in the **regulator gene**, altering the repressor so that it is never able to bind to the operator. Alternatively, **constitutive mutations** might occur in the **operator**, altering the binding site for the repressor so that the repressor is unable to bind under any conditions.

2) The total amount of protein synthesized depends on the amount of mRNA available for translation. The amount of available mRNA depends on the rates of mRNA synthesis and degradation. Less stable mRNAs degrade faster than stable mRNAs, and so fewer copies of the mRNA are available as templates for translation.

Stability affected by	5'	3'	poly(A)	cap
	3'			tail
	5'			UTR
	3'			UTR
coding	region	in	an	mRNA
(= secondary and tertiary structure of RNA)				

. Poly(A)-binding proteins bind at the 3' poly(A) tail. These proteins contribute to the stability of the tail and protect the 5' cap through direct interaction. When a critical number of adenine nucleotides have been removed from the tail, the protection is lost and the 5' cap is removed. The removal of the 5' cap enables 5'-to-3' nucleases to degrade the mRNA.

3) What will be the effect on sexual development in newly fertilized *Drosophila* embryos if the following genes are deleted?

14. **sex lethal:** If *Sxl* is off, then the embryo develops towards the male phenotype. If it is an XY zygote it will become a normal healthy male. If it is an XX zygote it will die because its two X chromosomes will be hypertranscribed and too much of X chromosomal product result
15. **transformer:** All animals will develop into males. But XX males will be sterile.
16. **double-sex:** both XX and XY animals survive and will show a development somewhere between female and male (intersex).

4) The site of the point mutation is critical for high affinity of the enhancer binding protein (activator protein) to the DNA. If this affinity is reduced then the transcription rate is usually reduced

Questions where answer was hard to find:

Questions to Chapter 7:

- 9) Becomes 8) in my book -> no correction
- 17) Becomes 16) in my book -> no correction
- 23) not in my book

Questions Transcription	to	Chapter	8:
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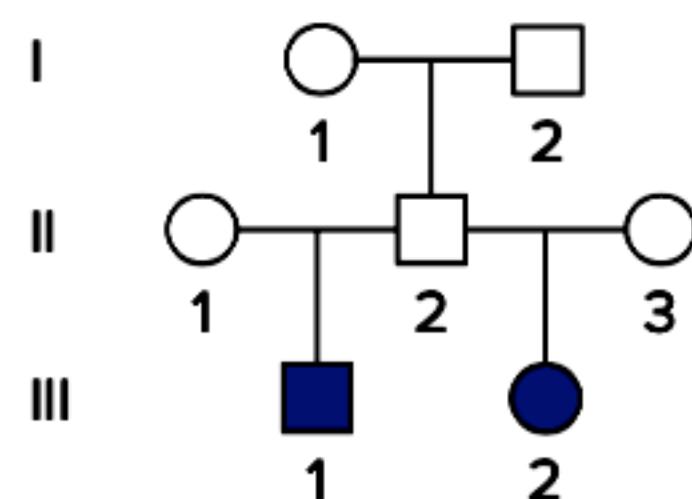
- 9) question 23) in your book but not present in mine

Translation

- 7) Becomes 6) in my book -> no correction
- 31) not in my book
- 43) not in my book

Chapter 7, Question 9:

The following pedigree shows the inheritance of a completely penetrant, dominant trait called *amelogen esis imperfecta* that affects the structure and integrity of the teeth. DNA analysis of blood obtained from affected individuals III-1 and III-2 shows the presence of the same disease-causing mutation in one of the two copies of an autosomal gene called *ENAM* that is not seen in DNA from the blood of any of the parents in generation II. Explain this result, citing Fig. 4.19 and Fig. 7.5. Do you think this type of inheritance pattern is rare or common?



Answer: A large proportion of the sperm of individual II-2 contain the mutant *ENAM* allele. A new mutation occurred in an early spermatogonial cell; this is rare because fewer germline cells present early in development mean they went through few cell divisions and therefore had less opportunity for random mutations to occur.

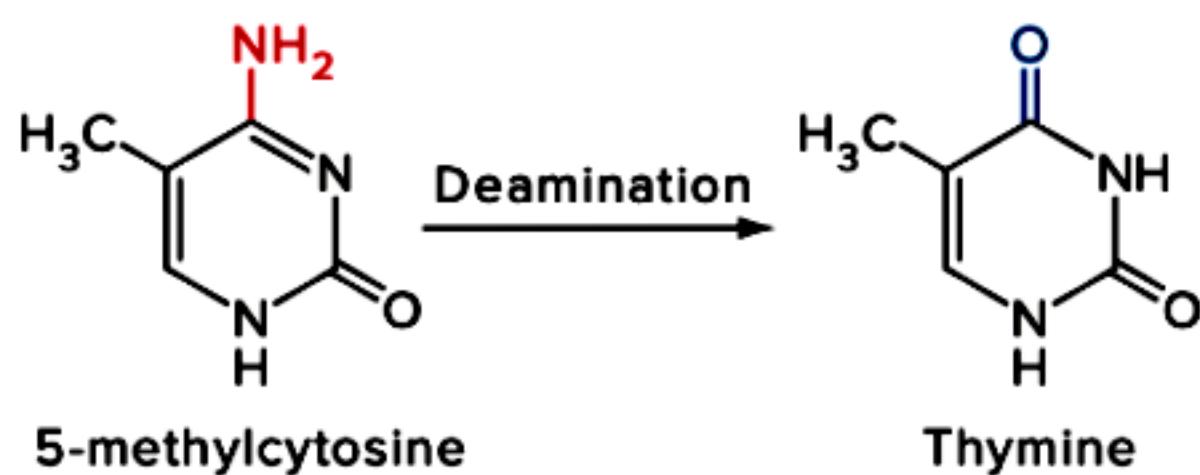
Chapter 7, Question 17:

When a particular mutagen identified by the Ames test is injected into mice, it causes the appearance of many tumors, showing that this substance is carcinogenic. When cells from these tumors are injected into other mice not exposed to the mutagen, almost all of the new mice develop tumors. However, when mice carrying mutagen-induced tumors are mated to unexposed mice, virtually all of the progeny are tumor free. Why can the tumor be transferred horizontally (by injecting cells) but not vertically (from one generation to the next)?

Answer: The mutations are in the somatic cell, not the germ line.

Chapter 7, Question 23:

In human DNA, 70% of cytosine residues that are followed by guanine (so-called CpG dinucleotides, where p indicates the phosphate in the phosphodiester bond between these two nucleotides) are methylated to form 5-methylcytosine. As shown in the following figure, if 5-methylcytosine should undergo spontaneous deamination, it becomes thymine.



- Methylated CpG dinucleotides are hotspots for point mutations in human DNA. Can you propose a hypothesis that explains why?
- Making the simplifying assumptions that human DNA has an equal number of C–G and A–T base pairs, and that the human DNA sequence is random, how frequently in the human genome would you expect to find the base sequence CpG?
- It turns out that, even after taking into account the actual GC content of human DNA (~42%), the frequency of CpG in human DNA is much lower than predicted by the calculation in part (b). Explain why this might be the case.

Answers:

a. Since the C in CpG is often methylated, the dinucleotide changes sometimes spontaneously to TpG

CpG	changes to	TpG	after replication -->	TpG
GpC		GpC		ApC

this event happens more often than random mutagenesis

b. 1/4 of all nucleotides are Cs and 1/4 of them is followed by a G => CpG in 1/16 of all dinucleotides

genome size 3×10^9 bp => CpG should show up roughly 2×10^8 times (on each strand)

c. There is a spontaneous disappearance of CpG because it rarely easily mutates to TpG (and probably almost never back to CpG) ==> much lower than calculated. On the other hand, TpG should be more frequent than calculated.

Chapter 8, Transcription, Question 9) questions to section 8.2 from Hartwell , question 23

In studying normal and mutant forms of a particular human enzyme, a geneticist came across a particularly interesting mutant form of the enzyme. The normal enzyme is 227 amino acids long, but the mutant form was 312 amino acids long. The extra 85 amino acids occurred as a block in the middle of the normal sequence. The inserted amino acids do not correspond in any way to the normal protein sequence. What are possible explanations for this phenomenon? How would you distinguish among them?

Answer:

The 85 amino acids could have come from an unspliced intron due to mutation in a splice site sequence, or from a mutation caused by insertion of DNA sequence. An intron sequence would be present in the genomic DNA of the wildtype, but an inserted sequence would not.

Chapter 8, Translation, Question 7:

The following diagram describes the mRNA sequence of part of the A gene and the beginning of the B gene of phage φX174. In this phage, some genes are read in overlapping reading frames. For example, the code for the A gene is used for part of the B gene, but the reading frame is displaced by one base. Shown here is the single mRNA with the codons for proteins A and B indicated.

aa#	5	6	7	8	9	10	11	12	13	14	15	16
A	AlaLysGluTrpAsnAsnSerLeuLysThrLysLeu mRNA											
	GCUAAAGAAUGGAACACUCACUAAAAACCAAGCUG											
B	MetGluGlnLeuThrLysAsnGlnAla											
aa#	1	2	3	4	5	6	7	8	9			

Given the following amino acid (aa) changes, indicate the base change that occurred in the mRNA and the consequences for the other protein sequence.

- a. Asn at position 10 in protein A is changed to Tyr.
- b. Leu at position 12 in protein A is changed to Pro.
- c. Gln at position 8 in protein B is changed to Leu.
- d. The occurrence of overlapping reading frames is very rare in nature. When it does occur, the extent of the overlap is not very long. Why do you think this is the case?

Answers:

- a. protein A: AAC (Asn10) -> UAC (Tyr); protein B: CAA (Gln3) -> CUA (Leu) = substitution
 - b. protein A: CUA (Leu12) -> CCA (Pro); protein B: ACU (Thr5) -> ACC (Thr) = silent mutation
 - c. protein B: CAA (Gln8) -> CUA (Leu); protein A: AAG (Lys15) -> UAG (stop) = nonsense
 - d. It doesn't happen often because random point mutation would affect two proteins instead of one. This means the evolutionary pressure exists to avoid overlapping ORFs. Also, it is difficult for ORF's to evolve under constraint to encode functional proteins on both DNA strands.
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Chapter 8, Translation, Question 31:

The human genome contains about 500 genes for tRNAs.

- a. Do you think that each one of these tRNA genes has a different function?
- b. Can you explain why the human genome might have evolved so as to house so many tRNA genes?

Answer:

- a. no. definitely not. there are still only maximal 61 codons that are used for incorporation of amino acids and not 500!! A high redundancy for the same codon/amino acid pair exists.
 - b. Human cells have a considerably larger genome than for example *E. coli* cells. Due to these larger and complexer genome, more genes have to be expressed. Therefore, a large number of tRNAs is essential for the process to occur reasonably fast. More tRNAs are either produced by a higher transcription rate of single genes (more efficient promoter), by a higher stability of tRNAs or more of the same tRNA encoding genes.
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Chapter 8, Translation, Question 43:

The following is a list of mutations that have been discovered in a gene that has

more than 60 exons and encodes a very large protein of 2532 amino acids. Indicate whether or not each mutation could cause a detectable change in the size or the amount of mRNA and/or a detectable change in the size or the amount of the protein product. (Detectable changes in size or amount must be greater than 1% of normal values.) What kind of change would you predict?

- a. Lys576Val (changes amino acid 576 from lysine into valine)
- b. Lys576Arg
- c. AAG576AAA (changes codon 576 from AAG to AAA)
- d. AAG576UAG
- e. Met1Arg (at least two possible scenarios exist for this mutation)
- f. promoter mutation
- g. one base pair insertion into codon 1841
- h. deletion of codon 779
- i. IVS18DS, G-A, + 1 (this mutation changes the first nucleotide in the eighteenth intron
of the gene, causing exon 18 to be spliced to exon 20, thus skipping exon 19)
- j. deletion of the polyA addition site
- k. GtoA substitution in the 5' UTR
- l. insertion of 1000 base pairs into the sixth intron (this particular insertion does not alter splicing)

Answers:

- a. no detectable difference in size
- b. no "
- c. no
- d. no detectable change in size of RNA, (but maybe in amount, since stability might be reduced if it is not completely translated),
protein will be considerably shorter: stop after 575 aa instead of 2532 aa
- e. no detectable change in size of RNA (but probably in amount, since stability will be reduced if mRNA is not translated)
either no protein is produced or ribosome finds another Met to start translation (in this case protein is shorter and the difference in size could be more than 1 %, ie 25 aa)
- f. amount of transcript is most likely affected, but not size of mRNA
if no mRNA is present, than also no protein will be present
but if protein is translated it will have the normal length
- g. no detectable change in size of RNA (but probably in amount)
an insertion of one base pair into the protein coding region results in a frame shift. statistically the proteinsynthesis will stop 20 aa later. => a shorter protein results and since there is a smaller amount of mRNA the amount of protein is also reduced
- h. as g) the deletion of one base pair in the protein coding region has the similar effect as the insertion of one base pair
- i. there is a mistake in this question: the mutation of the last nucleotide in intron 18 would result in a skipped exon 19
skipping an exon results in a shorter mRNA
the protein is also shorter, either through a frame shift or through the aas encoded by exon 19

- j. the length of the mRNA is most likely similar. the amount of the mRNA would be considerably decreased, since an mRNA without poly(A) is a lot less stable
 - k. a point mutation in the 5' UTR has most likely no effect on the expression of mRNA or protein
 - l. an insertion into an intron that does not effect splicing has most likely no effect on the expression of mRNA or protein
-

60

1) Organisation of nucleosomes: How do histones influence folding in eukaryotic DNA?

2) What is the difference between heterochromatin and euchromatin? How does acetylation of histones affect the chromosome structure?

3) The bacterial chromosome is usually a circular DNA molecule with some associated proteins. Although the proteins that cause the *E.coli* chromosome to coil are not histones, what property would you expect them to share with histones?

The major difference between Euchromatin and heterochromatin is that euchromatin is uncoiled packed and genetically active form of chromatin. While heterochromatin is firmly packed form and is genetically inactive part of chromosomes. Acetylation of histones alters accessibility of chromatin and allows DNA binding proteins to interact with exposed sites to activate gene transcription and downstream cellular functions.

Answers

1) Histones are the basic proteins of the nucleosome. DNA is wrapped around the octameric H2A, H2B, H3, H3, H4, H4 complex. H1 serves as a clamp and tightens the binding.

Histones and DNA together form the 10 nm and the 30 nm fiber, respectively.

2) Euchromatin is chromatin that becomes less compacted during interphase and is accessible to the cellular machinery responsible for gene activity.

Heterochromatin, on the other hand, remains quite condensed during interphase and contains genes that are largely inaccessible to this machinery.

Histones in transcriptionally active DNA regions are often acetylated. The acetyl group binds to the positively charged NH₃-group of lys on histone tails -> histones loose positive charge and, therefore, do not bind DNA as tightly.

3) Since DNA is acidic and proteins bind to it, we expect the proteins to be basic with positively charged amino acids (which are mostly lys and arg)