



2025 Mitosisphere July Challenge

Part A: Multiple Choice

Anatomy & Physiology

1. Lipid nanoparticles (LNPs) are small, lipid-based particles utilized as drug delivery vehicles. Specifically, instead of heat killed viruses, they provide an efficient and effective way to deliver mRNA to patients. LNPs are typically spherical vesicles composed of a variety of lipids in order to maintain stability as well as facilitate interactions with target cells. Which of the following choices is NOT a reason why LNPs may be preferable to other forms of mRNA delivery?
 - A) LNPs are unlikely to elicit an unfavorable immune response.
 - B) LNPs allow for a higher degree of control to where drugs are released.
 - C) LNPs protect the mRNA from degradation by enzymes en route.
 - D) LNPs provide a long circulation period in the bloodstream.
 - E) LNPs may improve the solubility and stability of certain drugs.

Solution: *D, LNPs are cleared from the blood faster than other forms of mRNA delivery, not slower. All other options are true.*

2. John Billington has recently decided to go on a diet to prepare for his upcoming fight. However, he has limited what he is eating so much that he has begun suffering from various nutrient deficiencies. Specifically, he has become deficient in vitamin K (phyloquinone), Vitamin B3 (Niacin), and Vitamin B5 (Pantothenic acid). Which of the following symptoms is John LEAST LIKELY to be experiencing?
 - A) Fatigue
 - B) Excessive bleeding

- C) Muscle cramps
- D) Impaired coordination
- E) Impaired immune function

Solution: *E*, While impaired immune function may result, it is the least directly correlated with these deficiencies. Fatigue would result from *B3* deficiency, excessive bleeding from *K* deficiency, and muscle cramps as well as impaired coordination from *B5* deficiency.

Cell Biology

3. Histone H3 lysine 9 trimethylation (H3K9me3) is the most common form of constitutive methylation in eukaryotes. In which of the following regions would you least expect to find H3K9me3 modifications?

- A) Pericentric regions
- B) Telomeres
- C) Transposable elements
- D) X-inactivation regions
- E) Short tandem repeats

Solution: *D*, X-inactivation regions are facultatively methylated, not constitutively methylated. All other choices would likely be constitutively methylated.

4. Suzuko, who has taken a deep interest in Connor's peanut allergy, has decided to run an experiment to measure the relative concentration of antibodies present in Connor's blood before and after he gives him some Nutter Butters. In his experiment, he has a membrane with small bits of peanuts fixed to it. He runs both samples of Connor's blood (pre and post nutter butter exposure) across the membrane in the presence of fluorophore-conjugated antibodies which bind to the same antigen as Connor's peanut antibody. Which of the following choices best describes the data Suzuko would likely encounter and how he should interpret it?
- A) Increased fluorescence after exposure to peanuts indicating lower antibody concentration in Connor's blood.

- B) Decreased fluorescence after exposure to peanuts indicating lower antibody concentration in Connor's blood.
- C) Increased fluorescence after exposure to peanuts indicating higher antibody concentration in Connor's blood.
- D) Decreased fluorescence after exposure to peanuts indicating higher antibody concentration in Connor's blood.
- E) Equal levels of fluorescence since the amount of fluorescent labeled antibodies is the same in both tests.

Solution: *D, This is an example of a competitive assay. We know that higher antibody concentration would result from exposure to nutter butters since they would trigger an immune response. This would result in decreased fluorescence since Connor's antibodies are directly competing with the fluorophore-bound antibody for binding spots on the membrane. If we have more antibodies in Connor's blood, then that means less fluorophore bound antibodies will bind to the membrane.*

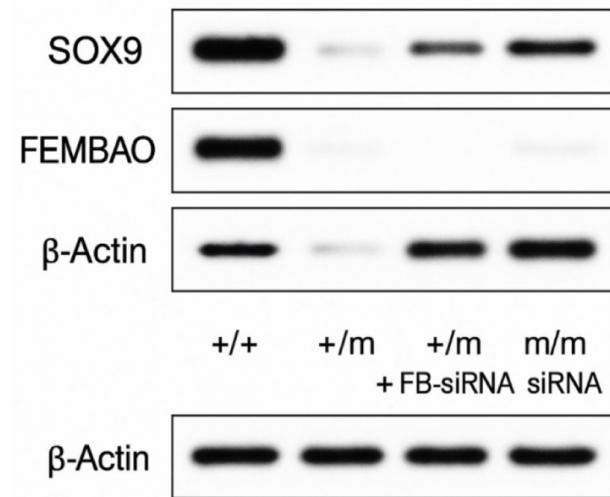
Genetics

5. Connor is studying the inheritance of elbow pointiness in gophers. He has concluded that 3 genes A, B, and C, all add quantitatively to increase elbow pointiness. Assuming each dominant allele for A, B, and C each provides 1 "point" of pointiness, and that pointiness ranges from 0-6. In a trihybrid cross, what fraction of offspring would you expect to have less than 5 points of "pointiness?"

- A) $7/64$
- B) $1/8$
- C) $21/32$
- D) $57/64$
- E) $63/64$

Solution: *D, For there to be less than 5 points of pointiness, we may have anywhere from 0-4 dominant alleles. Rather than counting this, we'll count the number of ways we can have 5 or 6 dominant alleles and subtract this from the total number of possibilities. This is just $[2^6 - ((\binom{6}{6}) + (\binom{6}{5}))]/2^6 = 57/64$.*

6. Joseph has recently discovered the protein *FEMBAO*, which he is studying in mice. Specifically, he is interested in its effects on the expression of *SOX9*, a gene located on chromosome 17 responsible for the proper formation of testis. In order to better understand the mechanisms behind *FEMBAO*, Joseph has decided to run a western blot in the presence of siRNAs for *FEMBAO* (FB-siRNA). The data may be found below.



Which of the following statements is NOT true?

- A) If FEMBAO acted genetically downstream of FOX9, Joseph would have observed different results on his western blot.
- B) In a cross between a heterozygous female and a WT male mouse, 25 percent of offspring would show mutant phenotypes.
- C) FB-siRNA is insufficient to rescue the heterozygous condition of FEMBAO for proper development of testis.
- D) Female mice homozygous for the mutant FEMBAO gene would be indistinguishable from heterozygote females.
- E) The mutant form of FEMBAO may disrupt wild type FEMBAO by directly interacting with it.

Solution: C, Looking at the western blot, we can see that the lane with the heterozygous condition +/m and FB-siRNA shows little to no expression of FEMBAO, as well as reduced expression of SOX9.

Evolution

7. Which of the following is an example of negative frequency-dependent selection?

- A) The ratio of left to right mouthed fish in a lake periodically oscillates.
- B) Antibiotic resistant bacteria becoming more prevalent over time.
- C) Black mice become more prevalent after lava has cooled into igneous rocks.
- D) Multiple toxic species of butterflies resemble one another.
- E) A certain population of finches evolves to have either extremely short or extremely long beaks.

Solution: A, *The periodic oscillation indicates that as left and right mouthed fish become more prevalent respectively, they are selected against. Definitionally, that is negative frequency dependent selection.*

Plant Biology

8. In order to determine the function of 2 unnamed plant hormones, you decide to inject 5 different groups of *Aridopsis Thaliana* samples with radioactively labeled linoleic acid and beta-carotene. You scan the plants after injection and proceed to subject group 1 to hungry grasshoppers, group 2 to flooding, group 3 to mechanical stress, group 4 to ethylene, and group 5 is your control, which receives just water. Then, you rescan all the plants and observe the following data:

	Group 1	Group 2	Group 3	Group 4	Group 5
¹³ C	+	-	+	-	-
³ H	-	-	-	+	-
¹⁴ N	+	-	+	+	-

“+” indicates relocation of isotope following treatment, “-” indicates no relocation.

Which of the following statements is false?

- A) The two hormones being studied work antagonistically in regulating seed germination.
- B) Linoleic acid was radioactively labeled with ^{13}C .
- C) Both hormones being studied influence protein relocalization.
- D) The hormone radioactively labeled with ^3H is involved in the plant's response to xerophytic conditions.
- E) Neither hormone being studied incorporates nitrogen.

Solution: *A, The two hormones being studied are jasmonic acid and abscisic acid. Jasmonic acid plays no role in seed germination. We know B is true since linoleic acid is the precursor to jasmonic acid, and jasmonic acid relocalization occurs in groups 1 and 3, which we should expect since jasmonic acid is involved in the response to both herbivory and mechanical stress. We know C is true since both groups 1 and 4 lead to nitrogen relocalization, however neither jasmonic acid nor abscisic acid incorporate nitrogen into their structures. We know D is true since abscisic acid is the hormone labeled with hydrogen, and ABA does indeed regulate the response to xerophytic conditions. Finally, E is true for the same reasoning as C, neither hormone incorporates nitrogen yet it gets relocalized.*

Ecology

9. Johnny the jackrabbit has recently turned green (Oh no!) following the dropping of large amounts of garbage onto his grassland. Feeling horrible since this garbage was released from your helicopter, you decide to go on a mission to clean up all the trash you dropped. Miraculously, after cleaning up all the garbage, Johnny begins to gain his normal color back. Which of the following types of species best describes Johnny?

- A) Keystone species
- B) Pioneer species
- C) Indicator species
- D) Invasive species

E) Flagship species

Solution: *C, An indicator species is a species whose abundance or health can provide insights into the health of an ecosystem. Since Johnny's color seems to be directly correlated with the level of trash in the grassland, he can best be described as an indicator species.*

Ethology

10. You've recently decided to start training your cat Sparky to use and flush the toilet himself. You need to get this done within the next month or so, since your family is coming for Christmas and you want to impress them. You only see them once a year though, so you do not care if he continues to do so after they've left. In order to train Sparky, which of the following reinforcement schedules should you use?

A) Fixed ratio

B) Fixed interval

C) Variable ratio

D) Variable interval

E) C or D

Solution: *B, The fixed interval schedule is reliable and consistent, and since you have adequate time to prepare, Sparky will likely adapt. Fixed interval schedules are also highly susceptible to extinction after ceasing them, and since you do not care whether Sparky continues after your family leaves, this makes fixed interval the best option.*

Biosystematics

11. While on a hike through the Costa Rican jungle, you notice a colony of ants all traveling together towards their ant hill. Fascinated by their behavior, your tour guide tells you these ants can carry tens of times their body weight and that these ants are all bringing these leaves to their queen within the ant hill.



Which of the following orders of insects are these ants a part of?

- A) Archeagnatha
- B) Zygenotema
- C) Orthoptera
- D) Hymenoptera
- E) Himenptera

Solution: *D, Hymenoptera includes colony animals such as ants, bees, and wasps.*

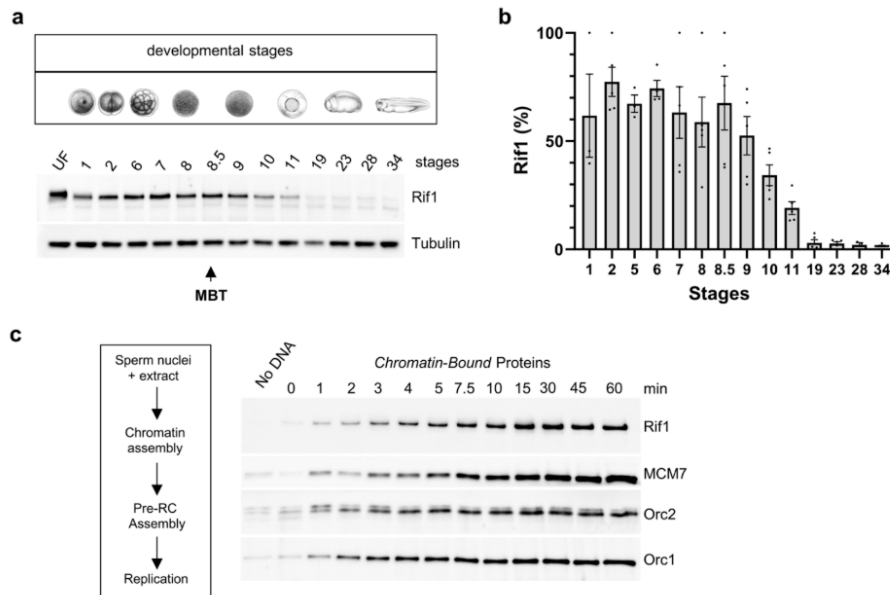
Part B: Multiple True/False (MTF)

Instructions: For each of the following prompts, mark each statement as True or False.

Rif1 is a key replication timing factor that has been studied in developing frogs of the species *Xenopus laevis*. *Rif1* recruits protein phosphatase 1 (PP1) to chromatin, counteracting S phase kinases. *Rif1* depletion increases the chromatin-binding of the S phase kinase *Cdc7/Drf1*, the firing factors *Treslin* and *MTBP*, *Cdc45*, *RecQL4*, and the phosphorylation of both *Treslin* and *MTBP*.

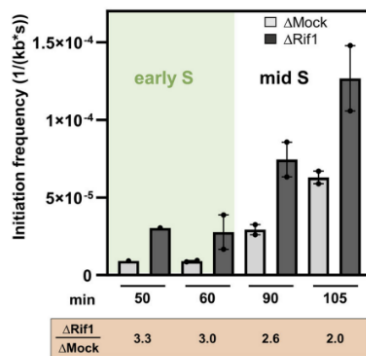
Treslin is a protein crucial for DNA replication and cell cycle control. It plays a role in initiating DNA replication, working in conjunction with *TopBP1* and *Cdc45*. *Cdc45* plays a key role in the formation of the CMG complex. The

CMG complex acts as a DNA helicase, unwinding the DNA double helix to allow replication to begin. *Cdc45* also interacts with other proteins and is involved in double-strand break repair.



a) Time course analysis of Rif1 expression throughout development; whole embryo protein extracts were analyzed by western blotting against indicated proteins, tubulin was used as loading control; MBT (midblastula transition), UF (unfertilized egg). b) Quantification of Rif1 abundance in three biological replicates and two technical replicates of western blot series of embryonic whole cell extract, plotted as mean $OD_{normalized}$ to Tubulin, scaled using min and max: $x_{scaled} = (x - x_{min}) / (x_{max} - x_{min})$, Mean with SED, $n = 5$ (data points). c) Time course analysis during the S phase; sperm nuclei were incubated in *Xenopus* egg extracts and chromatin was isolated for immunoblotting at indicated time points before and during DNA replication.

Haccard, O., et al. (2023). *Comm Bio*, 6(1). <https://doi.org/10.1038/s42003-023-05172-8>



Mean initiation frequencies ($I(t)$) with standard error of mean with SEM, $n = 2$ (data points) and ratio $\Delta Rif1 / \Delta Mock$ ($I(t)$) were calculated

Haccard, O., et al. (2023). *Comm Bio*, 6(1). <https://doi.org/10.1038/s42003-023-05172-8>

12. Indicate whether the following are True or False.

i) *Rif1* expression accelerates the rate of replication firing in *Xenopus laevis*.

ii) If a *Xenopus laevis* zygote in the 4-cell stage was treated with siRNAs specific to *Rif1*, its cells' S-phase would likely be accelerated.

- iii) *Rif1* disrupts cell cycle progression via perturbation of the cytoskeleton.
- iv) A possible mechanism of *Rif1* may be recruitment of initiation factors to chromatin.
- v) Inhibition of *Rif1* in cancerous cells may be effective in increasing the rate of double-stranded break repair.

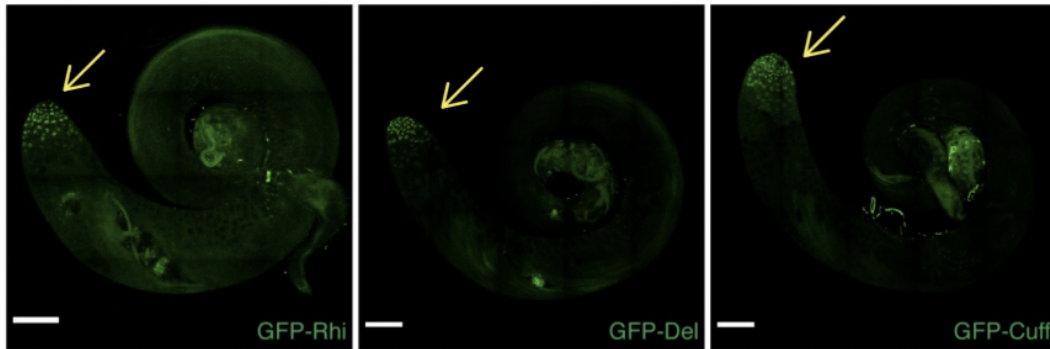
Solution:

- i) *False. Rif1 represses the rate of origin firing. We know that Rif1 depletion “increases the chromatin-binding of the S phase kinase Cdc7/Drf1, the firing factors Treslin. . .” — It is later stated that “Treslin is a protein crucial for DNA replication and cell cycle control. It plays a role in initiating DNA replication”. Since Rif1 depletion inhibits DNA replication through Treslin activation, Rif1 must inhibit replication, not accelerate it.*
- ii) *True. Rif1 inhibits origin firing, so by inhibiting Rif1 with siRNAs, we would increase the rate of origin firing and therefore accelerate the S-phase.*
- iii) *False. There’s no evidence to support this based on the data. The tubulin in the western blot was utilized as a loading control and its expression can be seen to be essentially constant throughout.*
- iv) *False. Rif1 inhibits origin firing and thus DNA replication. Therefore a possible mechanism may be repression of initiation factors and not recruitment.*
- v) *True. Inhibiting Rif1 leads to the chromatin binding of Cdc45, which is involved in double stranded break repair.*

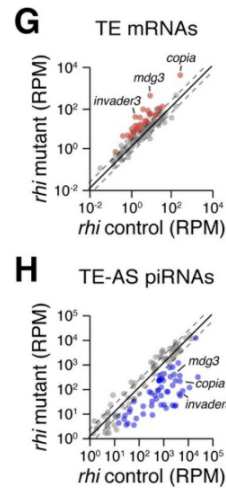
Piwi-interacting RNAs, or piRNAs are small non-coding RNAs which guard the germ line from transposons by silencing them. The Rhino-Deadlock-Cutoff (RDC) complex in the *Drosophila* line has been seen to be essential for expression of dual-strand piRNA clusters and transposon silencing in testis. RDC is critical to transposon repression in males, and males without RDC exhibit reduced fertility and loss of germ cells.

Invader3, *mdg3*, and *copia* are three of the most highly active transposons in the *rhi*-mutants. *Copia* sits shortly upstream of the *lacZ*-gene, in order to monitor its expression, X-gal staining was utilized in control as well as mutants for all 3 genes *rhi*, *del*, and *cuff*.

Del has been seen to recruit the transcription initiation factor *TFIIA-L* and the protein Moonshiner (moon) to initiate transcription in hostile heterochromatin environments. Moon has been observed to be necessary only in ovaries, while not in testis. Moon expression in testis using a GFP-tagged Moon transgene was observed to be able to rescue the female sterility caused by moon mutation. In these moon mutants, Rhi localization was perturbed, however in two other moon mutants, moon1 and moon28, Rhi localization was found to be undisturbed.

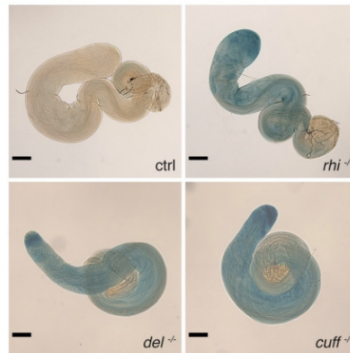


Expression of GFP-tagged Rhi (left), Del (middle) and Cuff (right) transgenes driven by their respective regulatory regions. Scale bar: 100 μ m.



(G) De-repression of TEs in testes of *rhi* mutants measured by polyA+ RNA-seq. Scatter plot showing expression of TE mRNAs in *rhi2/KG* mutant versus heterozygous control testes. TEs that show 2-fold de-repression (FDR < 0.05) and 1 RPM averaged levels are marked red. The mean of two biological replicates is shown. (H) Loss of TE-targeting piRNAs in testes of *rhi* mutants. Scatter plot showing expression of TE-antisense piRNAs in *rhi2/KG* mutant versus heterozygous control testes. TE-antisense piRNAs that show 2-fold reduction (FDR < 0.05) and 10 RPM averaged levels are marked blue. Shown are averages of two biological replicates.

X-gal staining of *copia-lacZ* reporter



De-repression of *copia* reporter in testes of *rhi* mutants. Brightfield images showing heterozygous control and *rhi2/KG*, *delHN/WK* and *cuffWM25/QQ37* mutant testes expressing *copia-lacZ*, after X-gal staining. *copia* LTR containing its promoter is fused upstream to *lacZ* gene. Note that part of *copia* LTR is transcribed as well. Scale bar: 100 μm.

All 3 images:

Chen, P., Luo, Y., & Aravin, A. A. (2021). RDC complex executes a dynamic piRNA program during *Drosophila* spermatogenesis to safeguard male fertility. *PLoS Genetics*, 17(9), e1009591–e1009591. <https://doi.org/10.1371/journal.pgen.1009591>

13. Indicate whether the following are True or False.

- Rhi* co-localizes with both *del* and *cuff* at the apical tip of the testes.
- Moon* acts genetically upstream of the RDC complex.
- Rhi* mutants show constitutively repressed transposition of *copia*.
- RDC mutant germline stem cells likely upregulate nucleotide excision repair.
- Copia* is a more active transposable element than *Invader3*.

Solution:

- i) *True, As seen in the first image with GFP labeled rhi, del, and cuff, all three proteins colocalize towards the apical tip of the testis.*
- ii) *False, We can conclude this based on the three different moon mutants. Since the moon mutation was seen to disrupt rhi localization, however moon1 and moon28 mutations did not affect rhi localization, we can conclude that moon must act downstream of rhi, not upstream.*
- iii) *False, Rhi is a key protein in the RDC complex which inhibits transposon movement. Rhi mutants show increased levels of transposon activity, not decreased. We may also see this in scatterplot G, which shows increased copia production in rhi mutants.*
- iv) *False, There is no evidence in the data to support this. Furthermore, if RDC mutants showed change in any DNA repair mechanism, it would be homologous recombination, not NER. .*
- v) *True, This can simply be seen in scatterplots G and H, which show copia to be more active than Invader3.*