※ 注意：請於試卷內之「非選擇題作答區」依序作答，並應註明作答之部份及題號。

Part A. (20 pts) Read the following story, and summarize precisely its center theme and provide at least 6 take-home messages to it.

[Topic of story and the source]

Drugs Of The Future Will Be Easier And Faster To Make, Thanks To mRNA – After Researchers Work Out A Few Remaining Kinks (https://www.discovermagazine.com/health/drugs-of-the-future-will-be-easier-and-faster-to-make-thanks-to-mrna-after, accessed 1/2024).

[Hint] You may include but not limited to

1. Basics of mRNA therapeutics

2. Why would non-self mRNAs activate our immune system?

3. Careful consideration on the utilization of mRNA beyond vaccine.

Messenger RNA, or mRNA, is made of four building blocks denoted by the letters A, C, G and U. The sequence of letters in an mRNA molecule conveys genetic information that directs how a protein is made.

An mRNA drug comprises two essential components: mRNA molecules, which code for desired proteins, and the lipid molecules – such as phospholipids and cholesterol – that encapsulate them. These mRNA-lipid nanoparticles, or LNPs, are tiny spheres about 100 nanometers in diameter that protect mRNA from degradation and facilitate its delivery into target cells. Once inside cells, mRNA molecules instruct the cell's machinery to produce the target protein required for a desired therapeutic effect. For example, the mRNA in the Pfizer-BioNTech and Moderna COVID-19 vaccines directs cells to produce a harmless version of the virus' spike protein that trains the immune system to recognize and better prepare for potential infection.

From a drug development perspective, mRNA drugs offer significant advantages over traditional drugs because they are easily programmable. Hundreds of pounds of mRNA can be made from readily available DNA templates, such that producing a different mRNA drug is as simple as changing the corresponding DNA templates.

More importantly, different mRNA drugs produced by the same set of methods will have similar properties. They will be delivered to the same tissues, trigger similar levels of immune responses and degrade in similar ways. This predictability significantly reduces the development risks and financial costs of developing mRNA drugs.

In addition to being easy to program, mRNA drugs have several other unique properties. For example, just like the mRNAs your body naturally produces, therapeutic mRNAs have a short half-life in cells: about one day. As a result, current mRNA technology is ideal for treatments that aren't meant to last long in the body.

This is why vaccines are popular candidates for mRNA technology: They provide long-term protection against disease after brief exposure to the drug with few side effects. There are currently more than 30 mRNA vaccine candidates, not including vaccines for COVID-19, in clinical trials.

Another critical feature of mRNA drugs is their intrinsic ability to stimulate the immune system. This may sound paradoxical – after all, your cells already contain large amounts of mRNAs. Why would other mRNAs activate your immune system? How does your immune system distinguish between self and nonself mRNAs?

One solution to this problem is to modify mRNA's building blocks – specifically, changing the U, or uridine, to its chemical cousins, pseudouridine and N1-methylpseudouridine. This subtle chemical change prevents the unwanted immune response while allowing the therapeutic mRNA to direct the cell to make the protein it encodes. The 2023 Nobel Prize in physiology or medicine was awarded to the scientists who made this breakthrough discovery. Both the Pfizer-BioNTech and Moderna COVID-19 mRNA vaccines use this technique.

The second source of unwanted immune response is impurities from mRNA production. To prepare mRNA from a DNA template, scientists use a protein called RNA polymerase that tends to make a small amount of side product called double-stranded RNA. Unlike mRNA, which is single-stranded, double-stranded RNA has two chains that form a double helix. RNA viruses also form double-stranded RNA when they replicate, and exposing cells to double-stranded RNA can lead to a strong

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immune response.

Removing double-stranded RNA is challenging, especially at the industrial scale. Fortuitously, for mRNA vaccines, the residual amount of double-stranded RNA can stimulate the immune system to enhance antibody responses. However, for applications other than vaccines, a cleaner RNA product is necessary to reduce side effects.

Although mRNA has the potential to transform drug development for various medical purposes, careful consideration is required to identify targets that align with the technology's strengths.

For example, because there is currently a limit to how long mRNA can last in the body, treatments that need a protein to be present for only a short period of time to achieve a lasting therapeutic effect are ideal. One promising example in development is using mRNA that encodes CRISPR-Cas9 gene-editing proteins to knock out genes that cause specific diseases.

Researchers are exploring this strategy to develop a single-dose treatment for hereditary transthyretin amyloidosis, a rare genetic disease caused by the accumulation of misfolded proteins in the heart and nerves. This disease is an ideal target for mRNA-based CRISPR gene therapy because the target protein is produced by the liver. Because most drugs pass through the liver, this makes it easier to deliver CRISPR-Cas9 mRNA to its target. In the next few years, a new generation of more precise mRNA-based genome editing therapies will enter clinical trials.

The first reason involves location. Therapeutic mRNAs enter cells using endosomes – sacs made of the cell's membrane that take in materials from the cell's environment. Your immune system can detect mRNA in endosomes because this is usually a sign of an RNA virus infection – cellular mRNAs normally don't enter endosomes. When your immune system labels therapeutic mRNAs as viral material, it triggers a strong inflammatory response that can lead to severe side effects.

For treatments that need a specific protein to be present in the body for long periods of time or need to prompt little to no immune reaction, further advancements in mRNA technology are necessary to extend mRNA's half-life and eliminate immune-triggering contaminants. Notable new developments in these areas include using computational algorithms to optimize mRNA sequences in ways that enhance their stability and engineering RNA polymerases that introduce fewer side products that may cause an immune response.

Further advancements have the potential to enable a new generation of safe, durable and effective mRNA therapeutics for applications beyond vaccines.

Part B. (30 pts, 2 pt for each) Match the vocabulary word with the definition. Write the letter for the definition that matches the word in the blank.

1. Hypothesis ____
2. Dependent variable ____
3. Independent variable ____
4. Materials ____
5. Procedure ____
6. Observation ____
7. Data____
8. Replicate____
9. Investigation ____
10. Scientific Method ____
11. Scientific inquiry ____
12. Control group ____
13. Experimental group ____
14. Scientific theory ____
15. Conclusion____

A. The steps you take to complete the experiments.
B. The process scientists follow to complete an investigation (question, hypothesis, materials, procedure…).
C. Things you need to complete your experiment.
D. The results of experiment.
E. The information you collect from the experiment.
F. To repeat the experiment.
G. Watching and noticing events that happen during an experiment
H. A prediction about what will happen with the experiment.
I. An experiment design to answer a question.
J. This group shows the effect of the variable being tested.
K. This is the one variable that is changed.
L. A well-tested explanation for experimental results.
M. The many ways in which scientists study the natural world.
N. This describes an observed pattern in nature.
O. This group is left alone and not experimented on.
P. This is the variable that gets measured.

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Part C. (30 points, 2.5 pt for each) Reading comprehension. Please read each paragraph and answer the following questions.

The human skin microbiome contributes to skin protection from external factors such as pathogens; however, it is also involved in other processes such as wound healing or pH regulation, and it has an active role in the interaction with immune cells. Interestingly, sebaceous sites are predominated by *Cutibacterium acnes*, a facultative anaerobic and Gram-positive bacterium that is found especially within pilosebaceous units of the skin. In this acidic niche, deep inside the skin, *C. acnes* feeds from sebum, especially triglycerides, produced by sebocytes. The low environmental pH induced by the production of propionic acid impedes the growth of pathogens and contributes to skin homeostasis. Additionally, it has been shown that *C. acnes* compositions can be applied successfully on human skin to modulate the original microbiome composition. Furthermore, genomic studies observed a low turnover of microbial species within the skin and found that the *C. acnes* population in individual follicles is mainly clonal. Therefore, this species seems to be an attractive synthetic biology chassis for treating skin diseases due to its engraftment potential, specific niche, importance to skin homeostasis and close contact with relevant therapeutic targets.

Acne is a common skin condition caused by the blockage or inflammation of pilosebaceous follicles, with severe cases commonly treated with isotretinoin, a vitamin A derivative, which produces serious side-effects such as teratogenicity or extreme scaling of the skin. Isotretinoin treatment is associated with an upregulation of the LCN2 gene encoding NGAL (neutrophil gelatinase-associated lipocalin), a protein that contributes to an abatement of acne symptoms by inducing apoptosis in sebocytes. In this work, the researchers have successfully edited the genome of *C. acnes* to secrete and produce NGAL protein to induce the death of sebocytes and further mitigate the symptom of acne. [modified from *Nat Biotechnol* (2024). https://doi.org/10.1038/s41587-023-02072-4]

16. What is the primary role of *C. acnes* in the skin microbiome?
    (a) Inducing inflammation in pilosebaceous follicles
    (b) Feeding on sebum and triglycerides in sebaceous sites
    (c) Producing vitamin A derivatives for skin health
    (d) Triggering apoptosis in immune cells

17. What contributes to the acidic environment in sebaceous sites and its impact on skin homeostasis?
    (a) Production of propionic acid by C. acnes
    (b) Increased sebum production by sebocytes
    (c) Presence of pathogens in the skin microbiome
    (d) Inflammation in the pilosebaceous units

18. How does isotretinoin treatment affect the LCN2 gene in the context of acne?
    (a) Downregulation of LCN2, leading to increased acne symptoms
    (b) Upregulation of LCN2, contributing to the abatement of acne symptoms
    (c) Activation of immune cells in response to LCN2
    (d) Inhibition of NGAL protein production

19. Why is *C. acnes* considered an attractive synthetic biology chassis for treating skin diseases?
    (a) It induces inflammation, promoting skin healing
    (b) It has a high turnover of microbial species in the skin
    (c) It is associated with severe side effects of isotretinoin
    (d) It has engraftment potential, a specific niche in the skin, and a low turnover of microbial species

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The glioblastoma multiforme (GBM) is the most common and lethal primary brain tumor in adults with a median survival of less than 15 months, despite continuous efforts in treatment innovations. A major roadblock that contributes to such a dismal prognosis is tumor relapse, which is observed in over 90% of patients. While the standard of care for primary treatment has been largely established, recurrent GBM remains therapeutically unresolved due to the complexity of tumor heterogeneity and limited understanding of the evolutionary dynamics after therapy.

To address these challenges, several studies generated genomic data from two or more longitudinal GBM samples and characterized the evolution of the molecular profiles in response to treatment. These studies showed that recurrent GBMs undergo a complex clonal evolution under therapeutic pressure driving genetic drifts that follow both neutral and selective evolutionary models. Whereas emerging knowledge of GBM evolution is covered through the profiling of their genomes and transcriptomes, these platforms have failed to identify consistent trajectories of evolution that could explain the therapeutic resistance invariably associated with recurrent disease.

When combined with genomics and transcriptomics, proteomics provides another paradigm for investigating a hidden dimension of cancer biology that has gone largely undetected. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) has implemented a significant effort to profile a wide spectrum of malignant tumor types using multiple proteogenomic platforms. This approach has generated tumor classifications and subgroups that are associated with distinct patient prognoses and potential therapeutic vulnerabilities. Here, a research team performed comprehensive proteogenomic analyses of 123 matched primary and recurrent GBMs and adopted the integration of genomics and deep proteomics characterization to extract the significant changes that drive GBM evolution under therapy. The proteogenomic inspection of the longitudinal GBM cohort identified the activation of neuronal programs as the primary mechanism of evolution driving tumor recurrence after therapy. Integrated multi-omics analyses uncovered the activation of the WNT/PCP pathway and the BRAF kinase as molecular determinants of neuronal transition in recurrent GBM, thus offering diagnostic and therapeutic tools to manage tumor recurrence in patients with GBM. [modified from *Cancer Cell* (2024) https://doi.org/10.1016/j.ccell.2023.12.015]

20. What is a major challenge contributing to the dismal prognosis in GBM?
    (a) Serious side effects after treatments
    (b) Tumor relapse observed in over 90% of patients
    (c) Limited genomic data availability
    (d) Neutral evolutionary models

21. Why does recurrent GBM remain therapeutically unresolved according to the paragraph?
    (a) Lack of molecular profiling in response to treatment
    (b) Complexity of tumor heterogeneity and limited understanding of evolutionary dynamics
    (c) Standard of care for primary treatment
    (d) Emerging knowledge of GBM evolution

22. What role does proteomics play in investigating cancer biology, as mentioned in the paragraph?
    (a) To establish tumor classifications
    (b) To identify transcriptional profiles of tumors
    (c) To profile a wide spectrum of malignant tumor types
    (d) To generate genomic data

23. What did the research team's comprehensive proteogenomic analyses of primary and recurrent GBMs reveal about the evolution of recurrent GBM under therapy?
    (a) Activation of the WNT/PCP pathway and the BRAF kinase
    (b) Lack of significant changes
    (c) Therapeutic vulnerabilities
    (d) Lack of activation of neuronal programs

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Aging is a leading contributor to cognitive decline, but dietary restriction (DR) delays aging across species and slows the progression of neurodegenerative diseases; however, the mechanisms that mediate the protective effects of DR in the brain are not fully understood. Additionally, factors such as natural genetic variation greatly influence response to DR, leading to the concept of precision nutrigeroscience to understand how differences between individuals and across tissues modulate responses to diet and influence healthspan and lifespan. We previously used the *Drosophila* Genetic Reference Panel (DGRP) to better understand the genetic effectors of DR-based lifespan response, and in this current study, we focus on polymorphisms in the gene *mustard* (*mtd*), which associate with DR-dependent longevity. Genetic variants in the human homolog of *mtd*, *Oxidation Resistance 1* (*OXR1*), are associated with cerebellar atrophy, hypotonia, language delay, and seizures, and its overexpression improves survival in a mouse model of amyotrophic lateral sclerosis (ALS). Despite the protective properties of OXR1, its biochemical mechanism remains unknown. Here, we identify the dietary and genetic factors which regulate *mtd/OXR1* and demonstrate its necessity for the maintenance of the retromer complex, which is a heteropentameric complex of proteins necessary for recycling transmembrane proteins and lipids from endosomes to the *trans*-Golgi network or the cell membrane. We further show that *mtd/OXR1* regulates a network of genes that are essential for protection against brain aging and neurodegenerative diseases across flies and humans. [modified from *Nat Commun* (2024). https://doi.org/10.1038/s41467-023-44343-3]

24. What is a significant finding regarding DR and cognitive decline according to the statement?
    (a) DR accelerates aging
    (b) DR has no impact on cognitive decline
    (c) DR delays aging and slows the progression of neurodegenerative diseases
    (d) DR only affects humans, not other species

25. What is the main focus of precision nutrigeroscience in relation to DR?
    (a) Identifying genetic variants associated with cerebellar atrophy
    (b) Understanding how differences in individuals & tissues influence responses to diet
    (c) Exploring the protective properties of OXR1 in neurodegenerative diseases
    (d) Investigating the biochemical mechanism of the retromer complex

26. Which genetic variants were focused on in the study mentioned, particularly those associated with DR-dependent longevity?
    (a) Genes related to language delay
    (b) Polymorphisms in the gene *mtd*
    (c) Genetic effectors of the retromer complex
    (d) Human homologs of *OXR1*

27. What is the identified role of mtd/OXR1 in the maintenance of the retromer complex?
    (a) It accelerates the degradation of the retromer complex
    (b) It is not involved in the regulation of the retromer complex
    (c) It is necessary for the maintenance of the retromer complex
    (d) It hampers the formation of the retromer complex

Part D. (20 points, 2.5 pt for each) Experimental comprehension. Please read the procedures and answer the following questions.

The enzymatic activity of lactase is essential for the catabolic processing of the disaccharide lactose. Here, the activity of

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lactase found in dietary supplements is assayed using a colorimetric assay. In the biochemistry lab class, you are asked to perform the activity assay of lactase and understand the enzyme kinetics. [modified from *jove.com*]

I. To begin the protocol, label a 15-milliliter tube suspension.

II. Crush one lactase tablet containing 200 milligrams of enzyme into an even powder using a mortar and pestle. Place the resulting powder into the labeled tube and re-suspend it in 10 milliliters of 100 millimolar phosphate buffered saline, or PBS. Vortex the solution for one minute to maximize the enzyme extraction.

III. Next, transfer one milliliter from the suspension to a 1.5 milliliter tube and spin the sample for one minute at 10, 000 times G to sediment the solid particles. Transfer 500 microliters of the supernatant to a clean 1.5 milliliter tube labeled lactase extract. Place 390 microliters of 100 millimolar PBS into a 1.5 milliliter tube labeled reaction A. Then add 100 microliters of five millimolar ONPG solution and mix well by vortexing.

IV. Add 10 microliters of extract into the reaction A tube and vortex the contents. Observe the reaction mixture often for five minutes and note any colorimetric changes of the solution. Set up two 1.5 milliliter tubes, one labeled reaction B, one labeled control.

V. Add 390 microliters of 100 millimolar PBS to the reaction B tube, 400 microliters of 100 millimolar PBS to the control tube, and then add 100 microliters of five millimolar ONPG to each tube. Mix the contents by vortexing. Add 10 microliters of lactase extract to the reaction B tube, mix by vortexing, and allow the reaction to proceed for one minute at room temperature.

VI. Once one minute has elapsed, add 500 microliters of one molar sodium carbonate to both tubes to inhibit the lactase enzyme by increasing the pH, stopping the reaction. Transfer 500 microliters from each tube into clean spectrophotometer cuvettes and measure the absorbance at 420 nanometers. Set up three control tubes and three reaction tubes and label them four degrees Celsius, room temp, and 37 degrees Celsius.

VII. Following the addition of the enzyme extract, incubate one tube on ice, one at room temperature, and one at 37 degrees Celsius in a preheated water bath for one minute. Then stop the reaction by adding 500 microliters of one molar sodium carbonate to each tube. Measure the absorbance at 420 nanometers for each tube.

VIII. Record the values, subtracting the control value from the reaction value in each case. Using the protocol, the control remains clear while the reaction solution containing extract from the lactase tablet turns yellow as ONPG is hydrolyzed, releasing ortho nitrophenol. Following analysis of samples using the spectrophotometer, production of ortho nitrophenol is lowest when the reaction is incubated on ice and highest when the reaction is carried out at 37 degrees Celsius.

28. What is the first step in the experiments for assaying the enzymatic activity of lactase?
    (a) Crushing a lactase tablet
    (b) Labeling a 15-milliliter tube suspension
    (c) Vortexing the lactase tablet powder
    (d) Spinning the lactase tablet suspension

29. Why is the lactase tablet crushed into an even powder in the protocol?
    (a) To enhance the colorimetric changes
    (b) To maximize enzyme extraction
    (c) To facilitate labeling of the tube
    (d) To improve the efficiency of the spectrophotometer

30. What is the purpose of spinning the lactase suspension in a 1.5-milliliter tube at 10,000 times G?
    (a) To mix the lactase extract
    (b) To inhibit the lactase enzyme
    (c) To sediment solid particles
    (d) To maximize the colorimetric changes

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31. What is added to the reaction A tube before the addition of lactase extract in the protocol?
    (a) ONPG solution
    (b) 100 millimolar PBS
    (c) Sodium carbonate
    (d) Spectrophotometer cuvettes

32. In the protocol, what is the purpose of adding one molar sodium carbonate to both reaction B and control tubes?
    (a) To maximize enzyme extraction
    (b) To enhance the lactase enzyme activity
    (c) To reduce the pH of the reaction
    (d) To stop the reaction and measure absorbance at 420 nanometers

33. Why are three control tubes and three reaction tubes labeled with different temperatures (4 degrees Celsius, room temp, and 37 degrees Celsius) in the protocol?
    (a) To measure absorbance at different wavelengths
    (b) To observe colorimetric changes
    (c) To evaluate the effect of temperature on lactase activity
    (d) To facilitate labeling for spectrophotometer measurement

34. What is the expected observation when comparing the control and reaction tubes after using the spectrophotometer in the protocol?
    (a) Both tubes turn yellow
    (b) Control remains clear, while the reaction turns yellow
    (c) Both tubes remain clear
    (d) Both tubes show colorimetric changes

35. What condition results in the lowest production of ortho nitrophenol, according to the protocol?
    (a) Reaction incubated at room temperature
    (b) Reaction incubated on ice
    (c) Reaction incubated at 37 degrees Celsius
    (d) Reaction with the highest ONPG concentration