Sociedade Brasileira de Bioquímica e Biologia Molecular - SBBq
Edital de Seleção PMBqBM 02/2024

Prova: Suficiência em Língua Inglesa

Candidato: _____

FOLHA GABARITO

Preencha com X a alternativa correta.

	A	B	C	D
1		X		
2				X
3	X			
4				X
5	Questão discursiva			
6				X
7			X	
8		X		
9				X
10	Questão discursiva			

Questões discursivas

5) Captura eficaz do mRNA com carga negativa (ou carregado negativamente).
10) A inibição da via do mTOR por rapamicina ativa a autofagia.

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Prova B: Suficiência em Língua Inglesa

Candidato (nome legível):

_____

CPF: _____

Orientações para a Prova de Suficiência em Língua Inglesa

1. Duração e Formato da Prova
   - A prova tem duração máxima de **2 horas** e é composta por questões de interpretação de texto.
   - A prova é presencial e possui caráter eliminatório, com resultado de "aprovado" (percentagem de acertos igual ou superior a 50%) ou "reprovado" (caso não atinja o percentual mínimo).

2. Regras de Consulta
   - É permitido o uso de **dicionário impresso** apenas, sendo vedada a consulta a qualquer outro tipo de material, mídias ou anotações pessoais.

3. Uso de Equipamentos Eletrônicos
   - Todos os aparelhos eletrônicos, incluindo celulares e dispositivos digitais, devem ser **desligados** e **guardados** antes do início da prova.

4. Preenchimento do Gabarito
   - Preencha a **folha de gabarito** com atenção e sem rasuras. Respostas rasuradas ou duplas não serão consideradas.

5. Devolução dos Materiais
   - Ao término da prova, devolva tanto o caderno de prova quanto o gabarito ao responsável.

6. Finalização e Saída da Sala
   - Ao finalizar, informe o responsável pela prova e permaneça no seu lugar até que receba orientações para a saída.

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1	A	B	C	D
2	A	B	C	D
3	A	B	C	D
4	A	B	C	D
6	A	B	C	D
7	A	B	C	D
8	A	B	C	D
9	A	B	C	D

Questões discursivas

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TEXT 1

The Nobel Prize in Physiology or Medicine 2023 was awarded jointly to Katalin Karikó and Drew Weissman "for their discoveries concerning nucleoside base modifications that enabled the development of effective mRNA vaccines against COVID-19"

Adapted from: The Nobel Prize in Physiology or Medicine 2023. NobelPrize.org. Nobel Prize Outreach AB 2024. Tue. 15 Oct 2024.
<https://www.nobelprize.org/prizes/medicine/2023/summary/>

The concept of using mRNA for vaccination and *in vivo* delivery of therapeutic proteins was first proposed over 30 years ago, but several hurdles had to be overcome to make this a clinical reality. Early experiments demonstrated that *in vitro* transcribed mRNA stimulates undesired inflammatory responses and inefficient protein production in cells and tissues. A turning point was the discovery by Karikó and Weissman demonstrating that mRNA produced with modified nucleoside bases evades innate immune recognition and improves protein expression. These findings, combined with the development of efficient systems for in vivo mRNA delivery, stabilization of the SARS-CoV-2 spike antigen, and unparalleled investments by industry and governments, led to the approval of two highly successful mRNA-based COVID-19 vaccines in late 2020.

A significant advancement in delivering nucleic acids into cells was achieved by Pieter Cullis's lab at the University of British Columbia, where ionizable cationic lipids were developed. These lipids could be maintained in a positively charged or neutral form depending on the pH of the environment. Forming these lipid nanoparticles (LNPs) at low pH had the benefits of cationic lipids in efficiently entrapping negatively charged mRNA within the vesicles. However, when delivered in vivo and exposed to physiological pH, the lipids lost their charge, which had several benefits including lower in vivo toxicity. Notable, the delivery of nucleic acids was further optimized through the T-connector that could generate dense lipid nanoparticles made of four components: an ionizable cationic lipid, a helper lipid, cholesterol, and polyethenylene glycol (PEG). More efficient ionizable cationic lipids were identified in large-scale screening programs in several biotech companies. Consequently, lipid nanoparticles now enable safe and efficient in vivo delivery of nucleic acids, including mRNA, into human cells. This advance is of great importance for clinical applications of nucleic acid-based technologies.

Karikó and Weissman continued their careful studies of different types of RNA and the work resulted in a breakthrough publication in 2005. The study described the influence of mRNA base modifications on the cytokine response by dendritic cells (DC). They showed that eukaryotic mRNA and tRNA, in which base modifications are abundant, did not stimulate a cytokine response while prokaryotic and in vitro-transcribed mRNA did. They further showed that the incorporation of pseudouridine (Ψ), 5-methylcytidine (m5C), N6-

methyladenosine (m6A), 5-methyluridine (m5U), or 2-thiouridine (s2U) into in vitro transcribed mRNA abrogated activation of inflammatory responses (as cytokine production) when these mRNAs were added to dendritic cells. The incorporation of m6A and s2U almost completely abrogated recognition by TLR3, while TLR7 and TLR8 activation could be evaded using m6A, s2U, m5C, m5U, and Ψ. Importantly, only modifications of uridines (m5U, s2U, and Ψ) abolished DC activation (Figure 1).

Deamination increases the proportion of uridines in the RNA, which Karikó and Weissman had demonstrated was critical for DC activation. Later work showed that the use of N1-methylpseudouridine (m1Ψ), alone or in combination with m5C, further improved the mRNA platform both in terms of reducing recognition of innate immune receptors and increasing protein expression, the latter was in part explained by an increased ribosome occupancy on m1Ψ-containing mRNA. Today, m1Ψ is the most common modified base used in mRNA vaccine production, including in the two COVID-19 vaccines approved in late 2020.

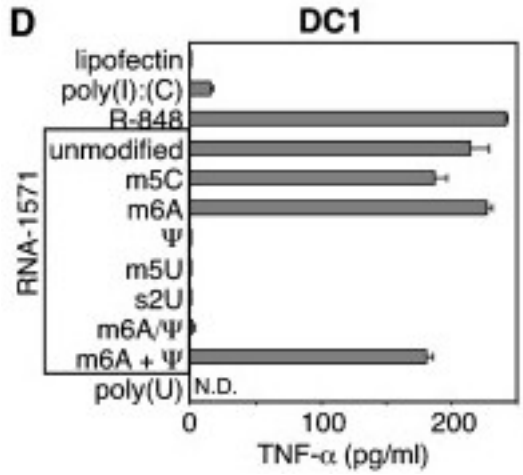

Figure 1: Cytokine production (TNF-ά) by RNA-Transfected DCs with or without nucleoside base modifications. Modified nucleosides present in RNA-1571 are noted. (Figure adapted from Karikó *et al.* Immunity 2005).

Use Text 1 to answer the questions below (1 to 5).

1. Nos experimentos iniciais com o mRNA transcrito in vitro, qual foi um dos grandes desafios observados?
   a) Lack of investment
   b) Poor protein synthesis
   c) Difficulty in performing trials
   d) Public distrust

2. Qual aspecto da tecnologia de mRNA foi aprimorado pela descoberta de Karikó e Weissman?
   a) Vaccine transportation
   b) DNA replication

c) Protein folding
d) Immune system evasion

3. Qual efeito a modificação 5-metilcitidina induz na produção de citocina em células dendríticas (DC1)?

a) It reduces TNF-α production more than unmodified mRNA.
b) It decreases TNF-α levels compared to other modifications.
c) It results in slightly higher TNF-α levels than s2U modification.
d) TNF-α levels are similar to those produced by m6A modification.

4. Qual modificação no mRNA induz aumento na tradução por aumentar a ligação de ribossomos?

a) N6-methyladenosine
b) 2-thiouridine
c) N6-methyladenosine
d) N1-methylpseudouridine

5. Qual a vantagem do uso de lipídios catiônicos em nanopartículas lipídicas? (responda em português)
Captura eficaz do mRNA com carga negativa (ou carregado negativamente) nas vesículas.

Text 2.

Autophagy Captures the Nobel Prize

Adapted from: Tooze, Sharon A. et al. Cell, Volume 167, Issue 6, 1433 - 1435

Autophagy, a lysosome-mediated intracellular degradation pathway, was discovered in the late 1950s. However, today is recognized as a major quality-control pathway and metabolic regulation system in the cell. In essence, autophagy proceeds when a selected part of the cytosol is engulfed by a double membrane, forming the so-called autophagosome, which later fuses with lysosomes, digestive organelles of the cell, where a number of catabolic enzymes facilitate the breakdown of the cargo, enabling recycling of the generated metabolites. Autophagy can be driven by unspecific metabolic stimuli, such as starvation, hypoxia, redox stress, mitochondrial dysfunction, and infection, and others highly specific signals facilitating the removal of damaged or superfluous proteins or organelles, thereby serving cellular homeostasis needs. The road to the discovery of the molecular basis of autophagy by Yoshinori Ohusumi is a story about importance of basic science, long-term dedication, and persistence that yielded unprecedented discoveries with broad significance to biology and medicine.

In the 1980s, there was no understanding of the molecular mechanisms underlying autophagy to begin to understand what proteins and lipids were required for autophagy in mammalian cells. However, Ohsumi approached this problem by studying autophagy in yeast, in which the lysosome-like vacuoles are end points of the autophagy pathway. He identified autophagic bodies in nitrogen-deprived yeast *S. cerevisiae* and was the first to report that nutrient deficiency induced autophagic degradation in yeast. A key point in his approach was the use of vacuolar protease inhibitors to allow for accumulation of intermediate autophagic bodies, the inner membrane-bound content of the double membrane autophagosome, inside the vacuole. Ohsumi's lab characterized these structures by transmission electron microscopic and determined which proteases were required for their degradation. This seminal observation established the system and facilitated the first genetic screen for yeast autophagy mutants that was carried out in proteinase-deficient yeast cells to enable detection of the short-lived autophagic bodies. The mutants isolated were originally called "apg" (autophagy). Apg1-1 mutants were identified by their defect in accumulation of autophagic bodies, and the Ohsumi lab observed that they lost viability much faster under conditions of nutrient deprivation. They identified the first autophagy genes and showed that there are at least 15 needed in yeast. By 2003, the number of autophagy genes required for survival in yeast grew to 27 and the nomenclature for the genes designated *APG*, *AUT*, *CVT*, *GSA*, *PAG*, *PAZ*, and *PDD* was unified to *ATG*. Today, the number of autophagy-related genes (*ATG*s) has grown to around 40 in yeast with 15 constituting the core machinery of autophagosome formation and the others being required for modulating the core machinery or selective modes of autophagy.

The identification of the yeast *ATG* genes triggered an almost explosive increase in knowledge not only regarding the mechanistic details of the autophagosomal cargo uptake and degradation in the vacuole/lysosome but also concerning the regulation of the entire process, its physiological meaning, and involvement in various diseases. At a molecular level, progress on understanding the regulation of autophagy began with the characterization of

Apg1 (Atg1), as a novel type of Ser/Thr kinase. Its kinase activity was found to be essential for autophagy, since genetic reconstitution with 'kinase-dead' mutant ATG1 cannot rescue the autophagy-deficient phenotype of ATG1 mutant yeast. Moreover, the phosphorylation status of Atg1p was shown to be up-regulated by nutrient starvation. Lastly, the inhibition of mTOR by rapamycin induces autophagy in yeast and mammalian cells, which demonstrates that TOR signalling is upstream of the Atg protein.

In the past two decades, autophagy has been connected to multiple human diseases including cancer, neurodegenerative diseases, aging, metabolic disorders, inflammation, infectious diseases, and others and this accumulated knowledge is channeled toward targeting autophagy in disease treatments. In addition, an overwhelming evidence emerged conceptualizing autophagy not only as an unspecific catabolic process providing energy and nutrients under starvation conditions, but also as a guardian of cellular homeostasis and integrity. A central function of autophagy in the mammalian system is cleaning the cell interior from unwanted and potentially harmful cellular components that disturb cellular homeostasis. Selective autophagy pathways that target specific non-functional cellular components, such as misfolded protein aggregates or dysfunctional mitochondria, but also intruders such as intracellular bacteria, have moved into the focus of autophagy researchers.

Use Text 2 to answer the questions below(6 to 10).

6. As condições abaixo causam aumento de atividade autofágica, exceto:
a. Formation of reactive oxygen species
b. Nutrient deficiency
c. Bacterial colonization
d. Cell division

7. Qual foi a observação do grupo de pesquisa de Ohusumi nas leveduras mutantes para a proteinase?
a. The mutants showed an enhanced autophagic response
b. The group described more 40 genes associated to autophagosome formation
c. Upon starvation, these mutants died faster than wild-type strains.
d. A double-membrane cup-like structure that engulfs autophagic cargo is formed under nutrient deprivation

8. Qual experimento demonstrou que a atividade da quinase ATG1 era importante para a ativação da autofagia?
a. Chemical inhibition of proteases
b. Re-expression of ATG1 with no kinase activity in yeast
c. Mutation of mTOR proteins
d. Genetic sequencing of the autophagic genes

9. De acordo com o texto, qual a principal função da autofagia?
a. To promote metabolic enzymes accumulation
b. To control cell polarity, chemotaxis and integrity
c. To induce cell apoptosis

d. To degrade and recycle proteins and organelles

10. Qual a associação entre a via de mTOR e a autofagia? (responda em português).
A inibição da via do mTOR por rapamicina ativa a autofagia.

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9				X
10				

Questões discursivas

5) Captura eficaz do mRNA com carga negativa (ou carregado negativamente).
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