Problem solving in the time of coronavirus pandemic

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Abstract
Problem solving, multiple-choice question-based educational tools have been used for decades in molecular cell biology courses at the University of Pécs Medical School, Pécs, Hungary. A set of these tests was published in Biochemistry and Molecular Biology Education between 2002 and 2015. Such tests using an experimental approach help students to understand how living cells function. Besides being tools of education, they can be used for examination purposes as well to assess higher levels of intellectual skills (interpretation and problem solving) acquired by the students. The test presented in this paper is based on parts of an original publication in which the authors described seminal observations on the function of a viral protein in the infection process of SARS-CoV-2. The test is aimed at helping the students to understand the methods used in the experiments, to analyze the data and to draw conclusions from them regarding certain aspects of the mechanism of coronavirus infection.

KEYWORDS
coronavirus infection, problem-based learning, problem-solving test, protein synthesis, SARS-CoV-2, viruses

1 | INTRODUCTION
An important principle of medical education is to develop the intellectual skills of students to the possible highest level to make them able to comprehend, analyze, and solve complex medical problems. This principle should be followed not only in clinical training, but in teaching basic sciences as well. A problem-oriented approach was introduced and has been used for decades in molecular cell biology courses at the University of Pécs Medical School. Among other problem-oriented educational tools, a novel type of multiple-choice question (MCQ)-based test, designated application test, has also been extensively used for teaching and examination purposes (for detailed description of these tests, see References 2–4). Such application tests were regularly published in Biochemistry and Molecular Biology Education from 2002 to 2015 (for a list of these papers, see Appendix S1). In the application test presented in this paper, two experiments from an article recently published by Schubert et al5 were used. This multifaceted article was aimed at elucidating the mechanism of coronavirus infection of human cells and the role of a protein (Nsp1) in the process. Understanding these processes is paramount in the development of antiviral drugs and vaccines. According to our opinion helping students to become able to use their factual knowledge in molecular cell biology to interpret such sophisticated experiments should be an outstanding objective of teaching cell biology, biochemistry, immunology, and virology.

2 | THE TEST
2.1 | Terms to be familiar with before the student starts to solve the test
Differential centrifugation; microsomal fraction; ribosomes; density gradient centrifugation; chelator;
Western blotting; agarose gel electrophoresis; the mechanism of translation; expression vectors; cDNA; transfection.

2.2 | Experiment 1

SARS-CoV-2 virus is the causing agent of the COVID-19 pandemic. One of the proteins encoded by its genome, **Nsp1** (= non-structural protein 1) suppresses host innate immune functions responsible for rapid antiviral actions by the infected organism.

In this experiment, the action of Nsp1 protein on host cell ribosomes was studied. Ribosomal subunits were isolated from human embryonic kidney cells by differential centrifugation.

Five-choice completion

(This type of question consists of a question or incomplete statement followed by five suggested answers or completions. Select the **one** best answer.)

1. Which of the following samples generated by differential centrifugation is most enriched for ribosomes?

A. Homogenate  
B. Nuclear fraction  
C. Postnuclear supernatant  
D. Postmitochondrial supernatant  
E. Microsomal fraction

2. What treatment can be used to remove the membranes from the sample of MCQ 1 without causing damage to the ribosomes?

A. Treatment with a mild detergent (e.g., deoxycholate)  
B. Treatment with a strong detergent (e.g., sodium dodecylsulfate)  
C. Treatment with a strong acid  
D. Treatment with an alkaline solution  
E. Treatment with phenol

The purified, membrane-free ribosomes were then treated with ethylenediamine-tetraacetate (EDTA), a chelator, followed by sucrose density gradient centrifugation (Figure 1; a sample without EDTA/−EDTA in the chart/ was also used as control). Gradient fractions were collected and their ultra-violet light absorption at 260 nm was determined. Interpret the sedimentograms by solving the following multiple-choice questions!

Experiment/figure analysis

(The following statements are related to the information presented above. Based on the information given, select:

A. **if the statement is supported by the information given**;  
B. **if the statement is contradicted by the information given**;  
C. **if the statement is neither supported nor contradicted by the information given**.)

3. The concentration of sucrose is higher in fraction 1 than in fraction 20.  
4. Measuring A_{260} was used in this experiment to detect DNA contamination in the ribosome sample.  
5. EDTA caused the dissociation of ribosomal proteins from rRNA.  
6. Divalent cations are required for holding the ribosomal subunits together.  
7. The protein/RNA ratio is higher in the large than in the small subunits.

To analyze the behavior of Nsp1 protein 40S (samples 1 and 2 in Figure 2) and 60S ribosomal subunits (samples 3 and 4) were mixed with Nsp1 protein. Samples 5 and 6 contained Nsp1 protein only (ribosomal subunits were not added). All six samples were loaded on the surface of a 30% sucrose layer in separate centrifuge tubes, high speed ultracentrifugation was performed and
the pellets (samples 2, 4, and 6) and supernatants (samples 1, 3, and 5) were subjected to further analysis: Western-blotting was performed using an antibody that detects Nsp1 (upper panel), as well as agarose gel electrophoresis of RNA isolated from the samples was carried out, followed by nucleic acid staining (lower panel). The rest of the samples served as controls: sample 7 contained purified Nsp1, sample 8 40S subunits and sample 9 60S subunits (without ultracentrifugation).

Study the experimental protocol and Figure 2, and solve the following multiple-choice questions! (use your knowledge on ribosomes and translation!)

Four-choice association

(In this type of question, a set of lettered headings is followed by a list of numbered words or phrases. Select:

A. if the word or phrase is associated with A only;
B. if the word or phrase is associated with B only;
C. if the word or phrase is associated with A and B;
D. if the word or phrase is associated with neither A nor B.)

A. Molecules in band a
B. Molecules in band b
C. Both of them
D. Neither of them

8. Are present in the granular component of the nucleolus.
9. Contain a Shine-Dalgarno sequence.
10. Each ribosome contains one copy of this molecule.
11. Were synthesized in the peripheral chromatin.
12. Have peptidyl transferase activity.
13. Are coded by tandemly repeated genes.

Experiment/figure analysis

(The following statements are related to the information presented above. Based on the information given, select:

A. if the statement is supported by the information given;
B. if the statement is contradicted by the information given;
C. if the statement is neither supported nor contradicted by the information given.)

14. The molecular mass of Nsp1 protein is approximately 25,000 daltons.
15. The Nsp1 protein binds to the small ribosomal subunits.
16. All Nsp1 molecules bound to the 40S subunits in this experiment.
17. The binding of Nsp1 protein leads to the degradation of RRNA.
18. All ribosomal subunits were sedimented to the bottom of the centrifuge tube in this experiment.
19. Free Nsp1 molecules passed through the 30% sucrose layer by the end of centrifugation.

2.3 | Experiment 2

In this experiment, the effect of Nsp1 on protein synthesis of cervical cancer cells (HeLa cells) was studied. A unique type of labeling with puromycin was used.

Puromycin (Figure 3a) is an antibiotic that is able to bind to the A site of ribosomes. If you carefully study its formula—after reviewing the mechanism of protein synthesis—you may figure out the mechanism of action of puromycin. Answering the following questions will help you to do so.

Four-choice association

(In this type of question, a set of lettered headings is followed by a list of numbered words or phrases. Select:}
20. Contains a heterocyclic molecule.
21. It is an α-amino acid.
22. Contains a deoxyribose molecule.
23. Contains a peptide bond.
24. It is a nucleotide.
25. Contains a pyrimidine ring.

Five-choice completion

(This type of question consists of a question or incomplete statement followed by five suggested answers or completions. Select the one best answer.)

26. Puromycin resembles a region of a molecule involved in protein synthesis. Which of the following is this region?

A. Region I of the puromycin molecule  
B. Region II of the puromycin molecule  
C. Both of them  
D. Neither of them

27. Regions I and II in the molecule of MCQ 26 are not connected by a N atom as in puromycin. What bond provides this connection in that molecule?

A. A bond via O atom  
B. A bond via C atom  
C. A bond via S atom  
D. A bond via P atom  
E. Phosphodiester-bond

28. The bond described in MCQ 27 is cleaved during protein synthesis. By what molecule?

A. An initiation factor  
B. An elongation factor  
C. A termination factor  
D. Aminoacyl-tRNA synthase  
E. Peptidyl transferase

29. Puromycin is incorporated into the nascent polypeptide chain. Which of its groups can be used for this?

A. Group a  
B. Group b  
C. Group c  
D. Group d  
E. Group e

30. What is characteristic of the polypeptide chains synthesized in the presence of puromycin?

A. Contain a single puromycin molecule at their N-terminus  
B. Contain a single puromycin molecule at their C-terminus  
C. Contain puromycin at both ends  
D. Their tyrosines are replaced by puromycin  
E. Contain several puromycin molecules at random positions

In the experiment shown in Figure 3b, HeLa cells were transfected with expression plasmids containing no DNA insert (“empty” plasmid, sample 1), cDNA-coding for wild-type (sample 2) or mutant Nsp1 (sample 3). A cell culture was treated with cycloheximide (sample 4; these cells were not transfected). Two days later a 10-min puromycin treatment was performed, postmitochondrial
supernatants were prepared from the four cell cultures, followed by Western-blot analysis using an anti-puromycin antibody. Study the experimental protocol and the figure and answer the following questions!

**Five-choice completion**

(This type of question consists of a question or incomplete statement followed by five suggested answers or completions. Select the one best answer.)

31. Which of the following procedures is called transfection?
   A. Infection of cells with an RNA virus
   B. Infection of cells with a DNA virus
   C. Transfer of DNA into eukaryotic cells
   D. B and C
   E. A, B, and C

32. What elements should be present in an expression plasmid to function in human cells?
   A. Promoter for RNA polymerase II
   B. Shine-Dalgarno sequence
   C. Polyadenylation signal
   D. A and B
   E. A and C

33. What proteins are present in the postmitochondrial supernatant of transfected cells?
   A. Proteins synthesized before transfection
   B. Proteins synthesized after transfection
   C. Puromycin-labeled proteins
   D. A and C
   E. A, B, and C

34. What is the common feature of polypeptides present in the bands of Figure 3b?
   A. Contain puromycin
   B. Were produced by premature termination
   C. Their synthesis was terminated by a stop codon
   D. A and B
   E. A and C

35. Wild-type Nsp1 inhibits protein synthesis in HeLa cells
36. Wild-type Nsp1 does not affect the synthesis of virus proteins
37. The mutation inactivated the Nsp1 protein
38. Cycloheximide blocked protein synthesis in the cells
39. A protein encoded by the “empty plasmid” stimulated translation in the cells

**Five-choice completion**

(This type of question consists of a question or incomplete statement followed by five suggested answers or completions. Select the one best answer.)

40. Which of the following statements best summarizes the take-home message of the experiments presented in this text?
   A. Nsp1 inhibits the response of cells by interfering with the secretion of antiviral proteins
   B. Nsp1 inhibits cellular protein synthesis by directly blocking peptidyl-transferase activity
   C. Nsp1 inhibits cellular protein synthesis by interfering with the function of the small ribosomal subunit
   D. Nsp1 stimulates translation by helping the formation of the 40S initiation complex
   E. Nsp1 stimulates translation by blocking the function of termination factors

**3 | DISCUSSION**

The figures of this test have been presented in small group discussions in an advanced molecular cell biology course for general medicine and dentistry students. The students were involved as much as possible in an interactive manner in the discussion. Elements of this application test were included in the final exam of the course, the majority of the students understood these complicated experimental situations, were able to interpret the results presented in the figures and performed reasonably well on the exam. The summary of exam statistics is shown in Appendix S2.

General medicine and dentistry students study Molecular Cell Biology as a compulsory subject in their first...
year at the Medical School of our university. The mechanism of protein synthesis is an important part of the subject. The primary aim of constructing and using the problem-solving exercise presented in this paper was to help students to understand how certain aspects of translation can be analyzed using methods of molecular biology (they are supposed to know anyway), what is the chemistry of polypeptide chain elongation and peptide bond formation (the bond between the amino acid and its tRNA, peptidyl transferase reaction, the direction of protein synthesis). A second and more general aim was to demonstrate to the students that their knowledge in basic cell biology has a direct link to clinical medicine: understanding the processes by which coronavirus kills the infected cell may help in the rational design of drugs able to interfere with those elements of infection.

The course (called Experiments in Molecular Cell Biology) is an advanced elective course in our curriculum. The 50 students enrolled (out of approximately 400 students in the class) are among those most interested in molecular cell biology. Their performance in the course exam (average grade: 3.9 in our 5-grade grading system; see table in Appendix S2) is well above the performance of the whole class in the compulsory subject Molecular Cell Biology (usually between 2.5 and 3.0). We thus may conclude that most of the students in this course are highly motivated to gain as much as possible from this kind of learning approach and do well in problem solving.

Correct answers and their explanations are presented in Appendices S3 and S4, respectively.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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SUPPORTING INFORMATION
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