

Development of Problem-Solving Teaching and Assessment in a Molecular Cell Biology Course

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INTRODUCTION

The University Medical School of Pécs has a rather traditional type of curriculum, with students beginning their medical studies after high school, typically at the age of 18. Approximately 180 and 80 students are accepted to our Hungarian and English programs, respectively. The 6-year medical curriculum consists of 2 years of basic sciences, 1 year of preclinical subjects, 2 years of clinical studies and a final year of clerkship. Molecular cell biology is taught during the two semesters of the first year, and for more than two decades the emphasis has been on improving and assessing problem-solving skills. This article will briefly describe three of the most useful techniques developed over the years, Application Tests, Figure Analysis, and Planning of Experiments.

FEATURES OF THE MOLECULAR CELL BIOLOGY COURSE

Forms of teaching in this course include lectures for the whole class, as well as practicals and seminars for groups of 15-20 students. The main topics covered in this course are listed in Table 1.

Student performance is regularly monitored by tri-weekly midterm tests. At the end of both semesters students are required to pass an examination to be allowed to continue their studies. These exams consist of a multiple-choice question-based written test and an oral examination.

Table 1. Topics in Molecular Cell Biology

Functional Morphology of the Eukaryotic Cell
Nucleic Acid and Protein Synthesis
Regulation of Gene Expression
Tumor Biology
Cytogenetics
Molecular Medicine

Techniques of cell biology and molecular biology continue to become integrated within the clinical subjects making these methods increasingly important for diagnostic and therapeutical purposes. Therefore we strongly believe that medical students should develop problem-solving skills to understand novel molecular approaches to medicine. To this end, we try to apply an experimental approach to our subject. In the last 20 years we have developed problem-based educational exercises to improve and test the creativity of medical students. The main principles of three of our problem-oriented exercises are described below, with illustrative examples given for each. For further details and examples, the reader is referred to earlier publications.¹⁻⁵

Application Tests

Such a multiple-choice question-based test is usually prepared from an original paper,¹ and presents the aim of the study, the experimental setting and the results. The student is expected to draw the right conclusions by answering a set of multiple-choice questions. A good application test thus combines problem-solving with objectivity.

EXAMPLE: The consequences of $\Delta F503$ mutation in the cystic fibrosis gene (based on Yang et al.⁶ and Pind et al.⁷).

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasian populations. It is characterized by increased viscosity of secretions of various exocrine glands, leading to obstruction of the intestine, fibrosis of the pancreas or severe pulmonary infections. Identification and cloning of the CF gene was achieved in 1989, and was found to code for a chloride channel named cystic fibrosis transmembrane conductance regulator (or CFTR). Surprisingly, the most common mutation leading to CF (the $\Delta F508$ mutation) was found to not have much effect on the chloride channel activity of the protein.

The aim of the experiments described in this test was to identify the consequences of the $\Delta F508$ mutation. In the first set of experiments the authors wanted to determine if the cellular lo-

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calization of the mutant protein is normal. They infected a pancreatic cell line expressing no endogenous CFTR protein, with recombinant adenovirus vectors coding for wild-type (wt) or $\Delta F508$ CFTR protein, and studied the intracellular distribution of the exogenous proteins in these cells. They found that while the wt-CFTR protein was mainly present in the cell membrane, the $\Delta F508$ CFTR protein accumulated in the rough endoplasmic reticulum and was absent from the cell surface.

To analyze the metabolism of CFTR, protein cells expressing wt or $\Delta F508$ CFTR were labeled with $[35S]$ -methionine. After brief labeling, unlabelled methionine was added to the culture medium (pulse-chase labeling). At different time points of chase, samples were withdrawn from the cultures, cell extracts were prepared and immunoprecipitated with an anti-CFTR antibody. The experiment was performed in cells treated with ammonium chloride, an inhibitor of lysosomal function, as well.

INSTRUCTIONS: How to Solve the Multiple Choice Questions

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

QUANTITATIVE COMPARISON

In this type of question, paired statements describe two entities that are to be compared in a quantitative sense

- A. if A is greater than B
- B. if B is greater than A
- C. if the two are equal or very nearly equal

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.

Study the autoradiograph of the immunoprecipitates (Figure 1) and solve the questions that follow.

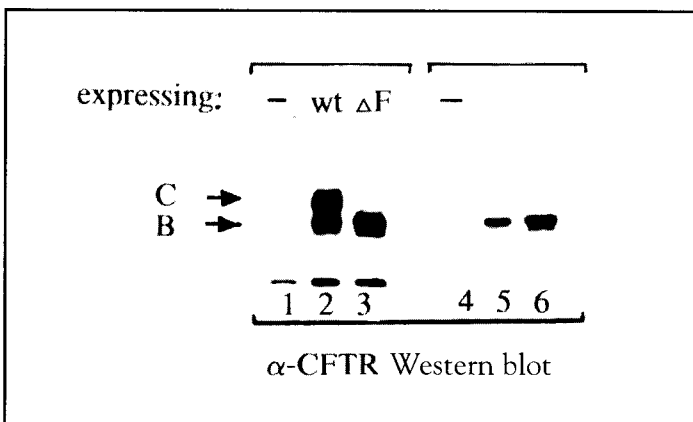


Figure 1. Pulse-chase labelling of wild-type (WT) and mutant ($\Delta F508$) CFTR proteins.

FIGURE ANALYSIS

1. ___ The mutant gene is not transcribed.
2. ___ The mutant mRNA is unable to bind to ribosomes.
3. ___ There is a precursor \rightarrow product relationship between the proteins in bands B and C.
4. ___ Protein C is generated from protein B by a signal peptidase.

QUANTITATIVE COMPARISON

5. ___ A. The lifespan of wt-CFTR protein in the cell
B. The lifespan of $\Delta F508$ -CFTR protein in the cell

FIVE-CHOICE COMPLETION

6. ___ Which of the following statements can explain the difference in electrophoretic mobility between protein B and C?
 - A. Protein C is a dimer stabilized by ionic bonds
 - B. Protein C is a dimer stabilized by hydrogen bonds
 - C. Band C contains an RNP stabilized by ionic bonds
 - D. Band C contains a RNP stabilized by hydrogen bonds
 - E. Protein C is a glycoprotein
7. ___ Evaluate the effect of ammonium chloride. Which of the following statements best describes the role of lysosomes in CFTR metabolism?
 - A. Lysosomes are not involved in CFTR degradation
 - B. Only the glycosylated form of CFTR is degraded in lysosomes
 - C. Only the non-glycosylated form of CFTR is degraded in lysosomes
 - D. Both glycosylated and non-glycosylated forms of CFTR are degraded in lysosomes
 - E. Only the mutant form of CFTR is degraded in lysosomes

In the last part of the experiment the role of calnexin in the metabolism of CFTR was studied. Calnexin is a transmembrane protein of the endoplasmic reticulum that acts as a chaperone. Extracts were prepared from cells not expressing CFTR protein (samples 1 and 4 in Figure 2), and from cells expressing the wt-CFTR (samples 2 and 5) or the mutant CFTR (samples 3 and 6). The extracts were immunoprecipitated with anti-CFTR (samples 1 to 3) or anti-calnexin antibodies (samples 4 to 6). The immunoprecipitates were fractionated by SDS-polyacrylamide gel electrophoresis and Western blotting was performed using an anti-CFTR antibody.

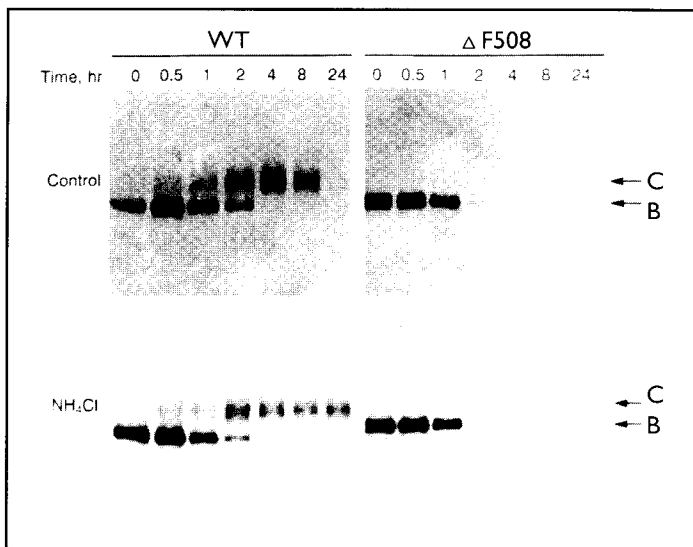


Figure 2. Interaction between calnexin and wild-type (WT) or mutant (Δ F) CFTR proteins.

FIVE-CHOICE COMPLETION

8. What was the aim of this experimental setting?

- To determine the exact location of CFTR in the cell
- To determine the exact location of calnexin in the cell
- To determine if CFTR and calnexin are complexed in the cell
- To determine the rate of synthesis of CFTR
- To determine the rate of synthesis of calnexin

9. What is the main conclusion of the immunoprecipitation-Western blot experiment?

- The wt-CFTR is unable to bind to calnexin
- The Δ F508-CFTR is unable to bind to calnexin
- Only the glycosylated forms of CFTR bind to calnexin
- Only the non-glycosylated forms of CFTR bind to calnexin
- Calnexin does not bind CFTR

10. Based on these experiment, what would be the best way to cure CF cells?

- Expression of the calnexin-binding domain of Δ F508 in these cells
- Expression of the protein-binding domain of calnexin in these cells
- Expression of calnexin in these cells
- Inhibition of N-linked glycosylation in these cells
- Inhibition of lysosomal function in these cells

CORRECT ANSWERS

1)B, 2)B, 3)A, 4)B, 5)A, 6)E, 7)B, 8)C, 9)D, 10)A

Figure Analysis

In this exercise a figure and its legend is presented to the students. The legend contains all the pertinent information required to understand the experimental situation. After analyzing

the figure, students are asked to evaluate its results and draw conclusions by answering open-ended questions.

EXAMPLE: The biochemical activity of SV40 large T antigen (based on reference 8)

A.



B.

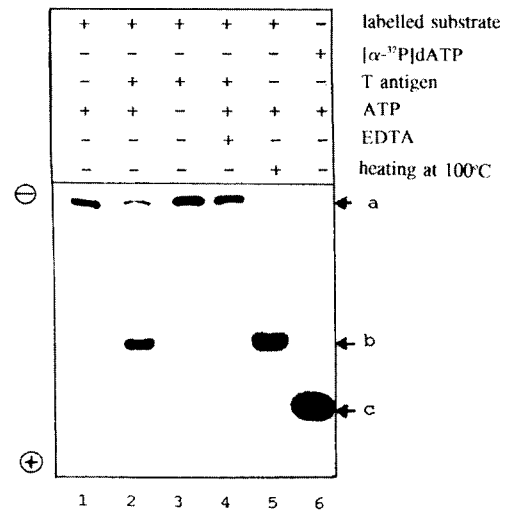


Figure 3. Identification of the enzyme activity of SV40 large T antigen.

Figure 3 shows the results of an experiment in which the enzymatic activity of the SV40 virus large T antigen was identified. The substrate was prepared as follows. The synthetic oligonucleotide duplex shown in panel A was incubated in vitro with dCTP, [α - 32 P]dATP and DNA polymerase I and the labeled DNA was separated from the other components of the mixture. The labeled substrate was then incubated in vitro under the conditions indicated in panel B and the samples were fractionated by polyacrylamide gel electrophoresis and subjected to autoradiography. (- and + indicate the position of electrodes during electrophoresis.) Study the figure and answer the following questions.

- Describe the substrate produced by labeling.
- What molecules correspond to bands a, b and c?
- What enzyme activity is carried by the T antigen? What conditions are required for the enzyme to function?

EXPECTED ANSWERS

1. Under the experimental conditions the synthetic oligonucleotide served as a template-primer complex in the in vitro DNA synthesis reaction. Three labeled A-nucleotides and an unlabeled C-nucleotide were attached to the 3'-end of the lower strand. The substrate thus consisted of an unlabeled longer strand

and a radioactively labeled 19-mer.

2. Band a corresponds to the duplex (it disappears after heat-denaturation; sample 5); band b contains single-stranded 19-mer molecules (band a shifts to b after heat denaturation); band c contains the mononucleotide dATP.

3. The T antigen has the same effect as heating; it has helicase activity (compare samples 2 and 5). It requires ATP and divalent cations to separate complementary DNA strands (compare samples 2, 3 and 4).

Planning Experiments

In this type of exercise the students are presented with a scientific problem and are asked to design an experimental approach to study the problem.

EXAMPLE: Plan an experiment to determine the degree of amplification of the N-myc gene in a human neuroblastoma tumor

EXPECTED ANSWER: (Please note that in most cases more than one correct solution to the problem exists.)

DNA should be prepared from the tumor sample and subjected to Southern blot analysis using probes specific for the N-myc gene and for a gene known to be a single-copy gene. If hybridization conditions – size and specific activity of the probes – are identical for the two genes, copy number of the N-myc gene can be determined by comparing the intensities of the hybridization bands.

CONCLUSIONS

We have found these three forms of student exercises to be useful educational tools first, because they make the process of teaching and learning more interesting, and therefore, presumably, more efficient. Application Tests are best suited for self-instructed learning, while Figure Analysis and Planning of Ex-

periments are ideal for small-group discussions. Using such exercises in a seminar format allows for a wide range of scientific facts and terms to be presented in a creative, rather than routine way. And secondly, we use such techniques – in combination with methods measuring factual knowledge – for examination purposes to assess higher quality student performance.

Although these educational methods are described for a course on molecular cell biology, similar exercises can be prepared and used in all fields of the natural sciences, including clinical subjects.

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