PROBLEM-SOLVING TESTS IN MOLECULAR CELL BIOLOGY

József Szeberényi

University of Pécs
Medical School
2015.

ÚJ SZÉCHENYI TERV

Nemzeti Fejlesztési Ügynökség
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MAGYARORSZÁG MEGÚJUL

A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

Technical assistance

Zita Árvai

Mónika Vecsernyés

With the permission of the International Union of Biochemistry and Molecular Biology.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

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MAGYARORSZÁG MEGÚJUL

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ELECTROPHORETIC BEHAVIOR OF HEMOGLOBIN VARIANTS

Terms to be familiar with before you start to solve the test

*Hemoglobins * erythrocytes * reticulocytes amino acids * α and β globin chains * electrophoresis * isoelectric point * sickle cell anemia*

The experiment

Hemoglobin A (**HbA**), the predominant adult hemoglobin in our red blood cells is a vital protein responsible for the transport of oxygen. It is synthesized in immature reticulocytes and carries out its function in mature erythrocytes. It consist of two α and two β globin chains and one hem molecule bound to each of these subunits. **Hemoglobinopathies** represent a large group of inherited diseases caused by mutations in α and β globin genes. Two of the most common hemoglobin variants are **HbS** (expressed in red blood cells of patients that suffer from **sickle cell anemia)** and **HbC** (causing a milder condition). In both disorders, the mutation affects codon 6 in β globin mRNA: the glutamic acid (Glu) at position 6 in the β globin chain is replaced by valine (Val) or lysine (Lys) in HbS and HbC, respectively (the formulae of these amino acids are shown in Figure 1.

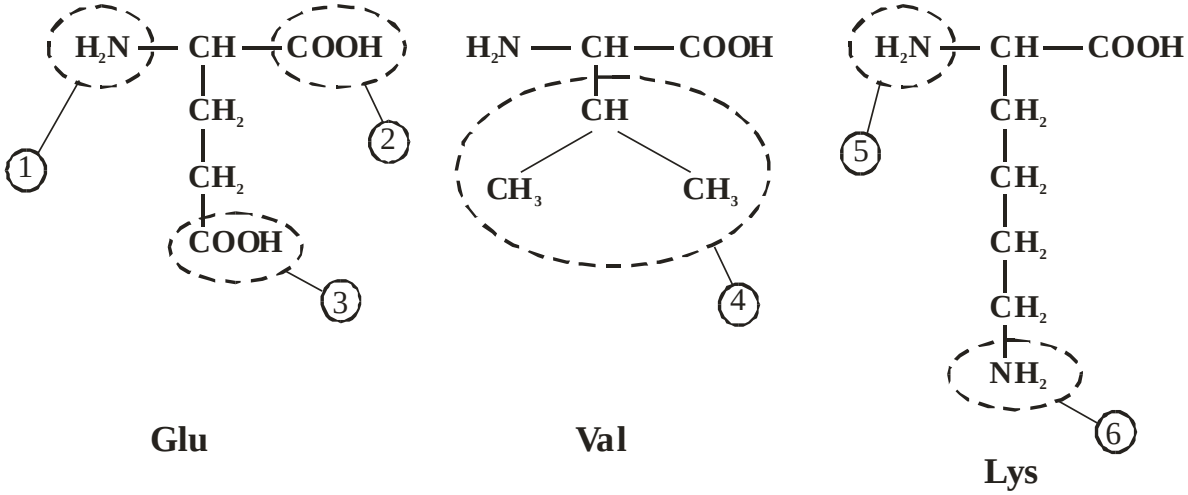

Figure 1: The chemical formula of glutamic acid, valine and lysine.

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.____ After the incorporation of these amino acids into the globin chain, which of these groups can be involved in peptide formation?

A. Group 1
B. Group 2
C. Group 3
D. Groups 1 & 2
E. All three groups

2.____ After the incorporation of these amino acids into the globin chain, which of these groups can acquire a positive charge in aqueous solution?

A. Group 1
B. Group 2
C. Group 5
D. Group 6
E. Groups 1, 5 & 6

3.____ After the incorporation of these amino acids into the globin chain, which of these groups can acquire a negative charge in aqueous solution?

A. Group 1
B. Group 2
C. Group 3
D. Groups 2 & 3
E. Group 4

4.____ After the incorporation of these amino acids into the globin chain, which of these groups is most likely buried in the internal „core” of the hemoglobin molecule?

A. Group 1
B. Group 3
C. Group 4
D. Group 6
E. Groups 3 & 4

Quantitative Comparison

(In this type of question paired statements describe two entities that are to be compared in a quantutative sense. Select
A if A is greater than B;
B if B is greater than A;
C if the two are equal or very nearly equal.)

5.____ A. The isoelectric point of HbA
B. The isoelectric point of HbS

Red blood cell extracts from four individuals with different hemoglobin variants (HbA, HbC and/or HbS) in their erythrocytes were analyzed by gel electrophoresis in the experiment described in this test. After electrophoretic fractionation of the samples the gel was stained with a protein dye (Figure 2).

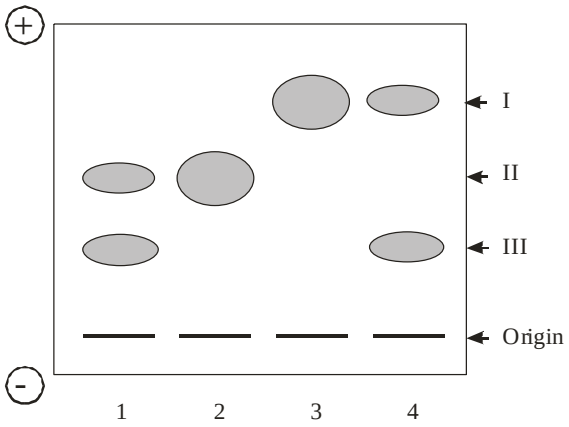

Figure 2. Electrophoretic analysis of red blood cell extracts from four individuals (- and + indicate the positions of the electrodes during electrophoresis). (After Fig. 6.9. in Gelehrter, T.D., Collins, F.S., Principles of Medical Genetics, Williams & Wilkins, Baltimore, 1990.)

Study the figures and solve the following multiple-choice questions. (Note that the single amino acid differences do not affect significantly the molecular masses of HbA, HbS and HbC: the size of these proteins is essentially the same.)

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.)

6.____ Under the electrophoretic conditions used all the hemoglobin variants (I, II and III) were negatively charged.

7.____ The pH of the electrophoretic buffer was the same as the isoelectric point of hemoglobin variant III.

8.____ The isoelectric point of protein I is higher than that of protein III.

9.____ Individuals 1 and 4 are carriers of the HbC mutation.

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.)

10.____ Which of these individuals has/have the worst prognosis?

A. Individual 1
B. Individual 2
C. Individual 3
D. Individual 2 & 3
E. Individual 4

11.____ Which of these individuals has/have only mutant hemoglobin in the red blood cells?

A. Individual 1
B. Individual 2
C. Individual 1 & 2
D. Individual 3
E. Individual 4

12.____ Which of the following statements describes the hemoglobin status of red blood cells in individual 2 best?

A. The structure, function and cellular level of hemoglobin is normal
B. Hemoglobin synthesis in the reticulocytes is normal, but degradation is increased
C. Hemoglobin synthesis in the reticulocytes is reduced, but degradation is normal
D. Hemoglobin is secreted to the plasma by these cells
E. The water solubility of hemoglobin is decreased

Correct anwers

1.	D	7.	B
2.	D	8.	B
3.	C	9.	B
4.	C	10.	B
5.	B	11.	C
6.	A	12.	E

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Szeberényi J. (2004) Problem-solving test: Electrophoretic behavior of hemoglobin variants. Biochem.Mol.Biol.Educ. *32*, 350-351.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

REAL-TIME POLYMERASE CHAIN REACTION

Terms to be familiar with before you start to solve the test

polymerase chain reaction, DNA amplification, electrophoresis, breast cancer, HER2 gene, genomic DNA, in vitro DNA synthesis, template, primer, Taq polymerase, 5'→3' elongation activity, 5'→3' exonuclease activity, deoxyribonucleoside triphosphates, DNA structure, proofreading, thermocycler, fluorescence

The experiment

In traditional **polymerase chain reaction (PCR)** the analysis of the amplified DNA region takes place after 30-40 cycles the product is studied by electrophoresis and DNA staining. The advantage of **real time PCR** is that the process can be monitored during the reaction: the extent of DNA amplification can be determined after each PCR cycle. Several different methods have been developed for this, the principle of one of the most popular techniques **(TaqMan reaction)**[1] is described in the following experiment.

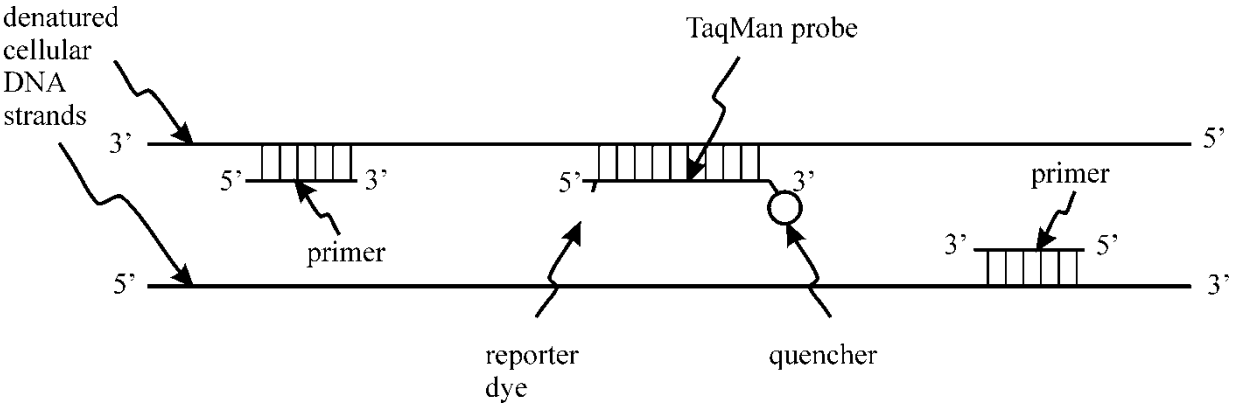

Figure 1. The principle of TaqMan method (details in the text).

A tumor was removed from the breast of a patient. Genomic DNA was isolated from the tumor and the surrounding normal tissue and PCR reaction was performed using identical amounts of the two DNA samples. Reaction mixtures contained the following components.

DNA template (the genomic DNA samples); many copies of *2 primers* specific for a region of the HER2 gene (their binding to the template is shown in Fig. 1);

many copies of a *TaqMan probe*, an oligonucleotide binding to one of the template strands in the region flanked by the two primers (a fluorescent *reporter dye* is attached to the 5'-end and a *quenching molecule* to the 3'-end of the probe, inhibiting the fluorescensce of the reporter);

Taq polymerase (heat-resistant DNA polymerase with 5'→3' elongation and 5'→3' exonuclease activities);

the *four dexoyribonucleoside triphosphates* (dATP, dGTP, dCTP, dTTP).

Using your knowledge of bacterial DNA replication solve the following multiple-choice questions (MCQs).

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: 5'→3' elongation activity of Taq polymerase
B: 5'→3' exonuclease activity of Taq polymerase
C: Both of them
D: Neither of them

1.____ Generates phosphodiester bonds.

2.____ Cleaves.

3.____ Cleaves hydrogen bonds.

4.____ Is primer-dependent.

5.____ Degrades the primers into mononucleotides in the mixture described above.

6.____ Degrades the TaqMan probe into mononucleotides in the mixture described above.

7.____ Has a proofreading function.

Reaction mixtures were incubated in a thermocycler capable of monitoring fluorescence. Fig. 2 shows relative fluorescence values measured after each cycle using the breast tumor (A) and normal (B) DNA sample.

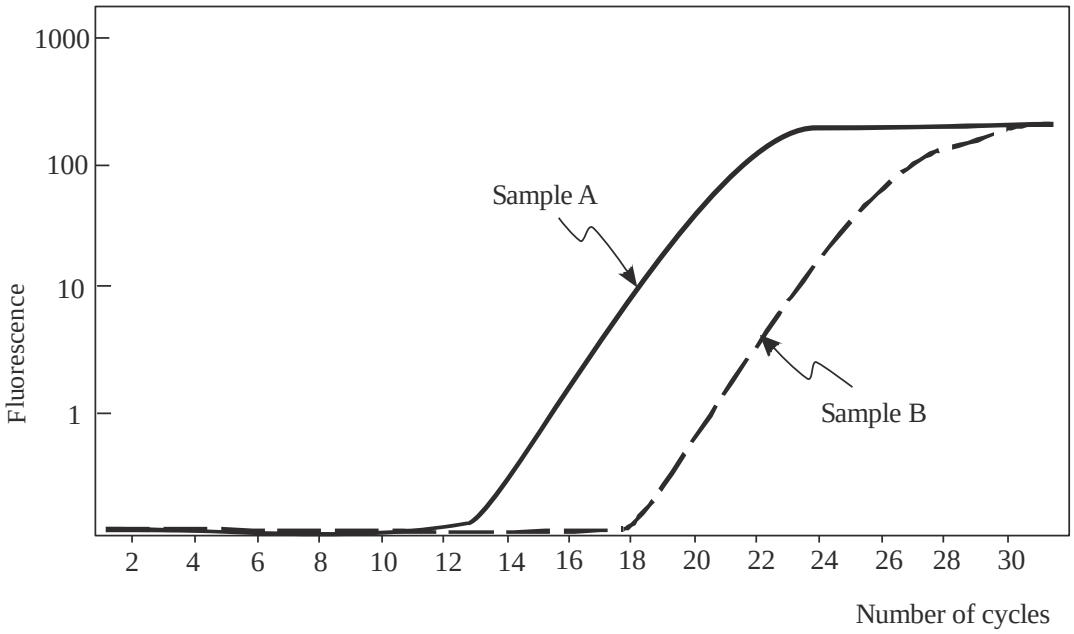

Figure 2. Real-time PCR performed with a breast cancer (A) and a normal (B) genomic DNA sample from the same patient (details in the text).

Study the figure and solve the following MCQs.

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.)

8.____ As PCR reactions proceed, at one point fluorescence increases in both mixtures. What process can explain this?

A: TaqMan probe molecules are degraded into nucleotides
B: TaqMan probe molecules are degraded into smaller oligonucleotides
C: TaqMan probe molecules are released from the template as intact oligonucleotides
D: TaqMan probe molecules serve as primers for Taq polymerase
E: TaqMan probe molecules are incorporated into the newly synthesized DNA strands

Quantitative Comparison

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

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

9.____ A: The number of free primer molecules in sample **A** after cycle 4
B: The number of free primer molecules in sample **A** after cycle 10

10.____ A: The number of free primer molecules in sample **A** after cycle 10
B: The number of free primer molecules in sample **B** after cycle 10

11.____ A: The number of free TaqMan probe molecules in sample **A** after cycle 10
B: The number of free TaqMan probe molecules in sample **B** after cycle 10

12.____ A: The number of free TaqMan probe molecules in sample **A** after cycle 18
B: The r number of free TaqMan probe molecules in sample **B** after cycle 18

13.____ A: The number of reporter dye/mononucleotide complexes in sample **A** after cycle 18
B: The number of reporter dye/mononucleotide complexes in sample **B** after cycle 18

14.____ A: The number of amplified HER2 fragments in sample **A** after cycle 20
B: The number of amplified HER2 fragments in sample **B** after cycle 20

15.____ A: The number of amplified HER2 fragments in sample **A** after cycle 30
B: The number of amplified HER2 fragments in sample **B** after cycle 30

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.)

16.____ Why there is no detectable fluorescence in the samples after the first few PCR cycles?

A. Because Taq polymerase degrades the TaqMan probe molecules
B. Because Taq polymerase degrades the primers
C. Because there is no DNA synthesis
D. Because the quenchers block the fluorescence of all reporter dye molecules
E. Because the fluorescence detector is not sensitive enough

17.____ Why fluorescence does not increase after cycle 30 in either samples ?

A. Because all primer molecules have been used
B. Because all TaqMan probe molecules have been used
C. Because all template molecules have been used
D. A and B
E. A, B and C

18.____ What happened to the HER2 gene in the breast tumor cells?

A. Its copy number increased approximately 30-fold
B. Its copy number increased approximately 5-fold
C. Its copy number decreased approximately 30-fold
D. Its copy number decreased approximately 5-fold
E. Its expression decreased approximately 5-fold

Correct anwers

1.	A	8.	A	15.	C
2.	B	9.	A	16.	E
3.	D	10.	B	17.	D
4.	A	11.	B	18.	A
5.	D	12.	B		
6.	B	13.	A		
7.	D	14.	A		

[1] This test is based on a product description of Applied Biosystems (Foster City, California, USA; www.appliedbiosystems.com).

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Szeberényi J. (2009) Problem-solving test: Real-time polymerase chain reaction. Biochem.Mol.Biol.Educ. *37*, 250-252.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

TARGETED GENE DISRUPTION

Terms to be familiar with before you start to solve the test

mutation, vector, plasmid, origin of replication, promoter, introns/exons, open reading frame, transfection, circular and linear DNA, DNA integration, homologous recombination, DNA replication, gene expression, heterozygote, homozygote.

The experiment

Mutational inactivation of a specific gene is the most powerful technique to analyze the biological function of the gene. This approach has been used for a long time in viruses, bacteria, yeast, fruit fly, but looked quite hopeless in more complex organisms. Targeted inactivation of specific genes (also known as knock-out mutation) in mice is arguably one of the most significant achievements in modern biology. The following test describes the procedure developed in the laboratory of one of the Nobel laureates of 2007, Mario Capecchi [1] and the reader is expected to interpret the principle of K.O. mutation by solving the multiple-choice questions (MCQs).

In this procedure part of the gene to be targeted (designated gene X) is inserted into a knock-out vector (Fig. 1). This vector is a plasmid that does not contain a mammalian origin of replication, but carries two selectable markers. A *neo*^{R} gene that will make the cells resistant to the highly toxic protein synthesis inhibitor Geneticin is inserted into one of the protein coding exons of the targeted gene. The *neo*^{R} gene is supplied with a strong mammalian promoter.

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.____ What can be the consequence of inserting *neo*^{R} gene into the gene X exon?

A. The replication of the targeting vector is stimulated in mammalian cells
B. The expression of protein X is stimulated
C. The reading frame of mRNA X is disrupted
D. A and B
E. A and C

2.____ What is the significance of linking a mammalian promoter to *neo*^{R} ?

A. To make *neo*^{R} expressed in the cells used for gene targeting
B. To inhibit the expression of gene X
C. To stimulate the expression of gene X
D. A and B
E. A and C

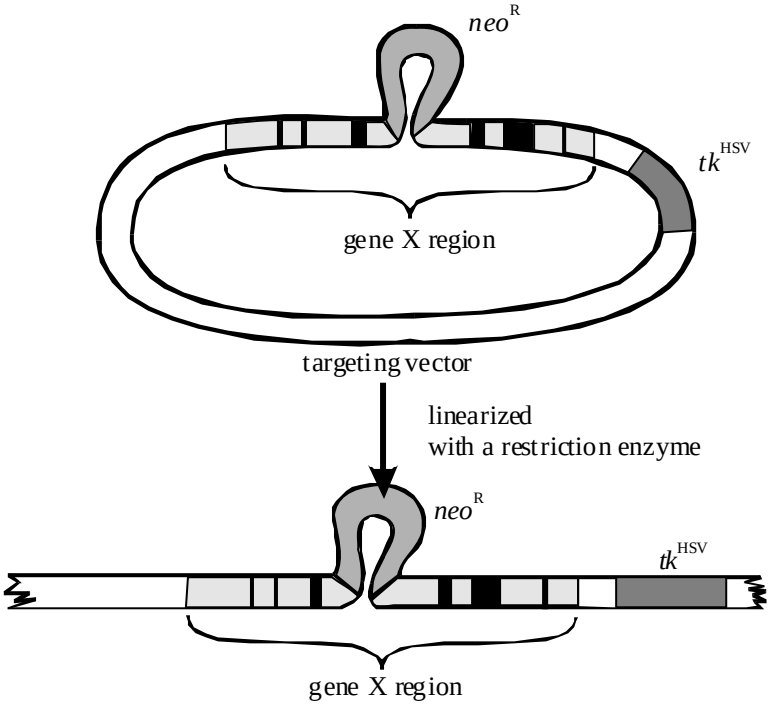

Figure 1. The structure of circular (above) and linearized (below) targeting vector (black bars, exons; light gray blocks, introns of gene X).

The second selection marker in the targeting vector is *tk*^{HSV}, a thymidine kinase gene of herpes simplex virus that, if expressed in mammalian cells, causes cell death when treated with the antiviral drug Gancyclovir.

Mouse cells in a culture are then treated with many linearized copies of the targeting vector under conditions that help the transfer of DNA into the cells. (Interactions between the foreign DNA and the host cell genome described below are more efficient with linear than with circular plasmid DNA.) Many cells do not take up DNA or if they do the foreign nucleic acid is quickly degraded in them. In some cells, however, the targeting vector interacts with the genome of the cells. Two genetic events can take place (Fig. 2). In most cases the linear DNA is randomly integrated into double-stranded breaks of the genome (Fig. 2A). Much less frequently sequences of gene X in the K.O. vector find their genomic counterparts, get into physical contact with them and homologous recombination takes place between the two sequences (Fig. 2B). If recombination happens on both sides of the *neo*^{R} gene, this region is transferred into the genomic gene X, while the corresponding normal sequences will become part of the plasmid. A key step in targeted gene disruption is to distinguish between these two genetic events.

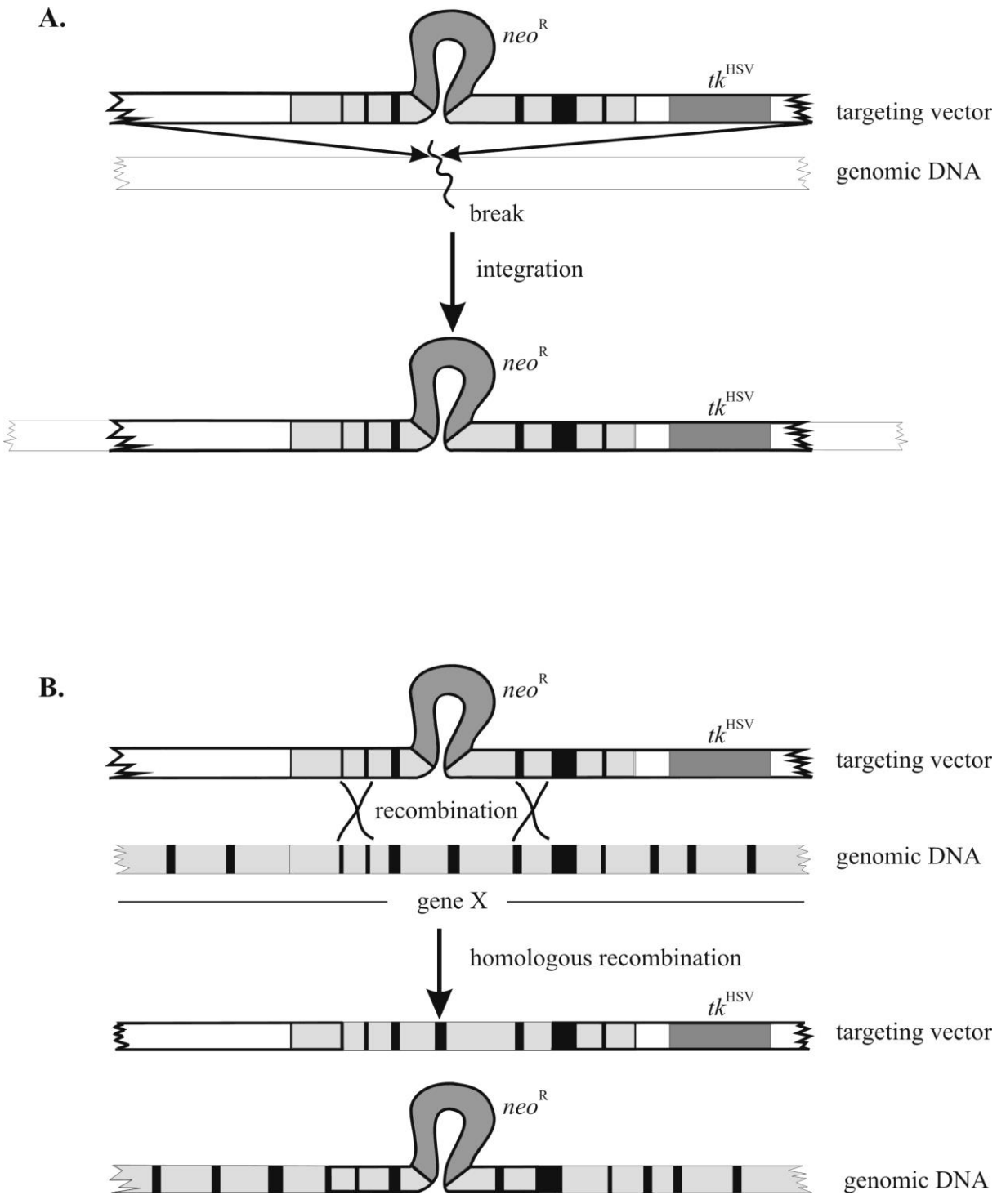

Figure 2. Random integration of the targeting vector (A) and homologous recombination between the targeting vector and gene X (B).

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. Cells with random integration (as shown in Fig. 2A)
B. Cells with homologous recombination (as shown in Fig. 2B)
C. Both of them
D. Neither of them

3.____ Replicate the *neo*^{R} gene during the S phase of the cell cycle.

4.____ Express the *neo*^{R} gene.

5.____ Degrade and loose the *tk*^{HSV} gene rapidly.

6.____ Express the *tk*^{HSV} gene only transiently.

7.____ The function of gene X is inhibited in these cells.

A. Geneticin treatment
B. Gancyclovir treatment
C. Both of them
D. Neither of them

8.____ Kills cells that did not take up foreign DNA during transfection.

9.____ Kills cells in which foreign DNA is degraded in the cytoplasm.

10.____ Kills cells in which the targeting vector randomly integrated into the genome (as shown in Fig. 2A).

11.____ Kills cells with homologous recombination between the targeting vector and gene X (as shown in Fig. 2B).

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.)

12.____ What are the characteristic features of cells surviving double selection with Geneticin and Gancyclovir?

A. They contain the targeting vector in integrated form only
B. They contain targeting vector sequences as a result of both integration and homologous recombination (as shown in Fig. 2)
C. They are heterozygous gene X knock-outs (gene $X^{+/-}$)
D. They are homozygous gene X knock-outs (gene $X^{-/-}$)
E. B and D

Correct anwers

1.	C	7.	B
2.	A	8.	A
3.	C	9.	A
4.	C	10.	B
5.	B	11.	D
6.	B	12.	C

REFERENCE

[1] S. L. Mansour, K. R. Thomas, M. R. Capecchi (19889) Disruption of the proto-oncogene *int-2* in mouse embryo-derived stem cells: a general strategy for targeting mutations to non-selectable genes. *Nature* **336**, *248-352*.

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Szeberényi J. (2008) Problem-solving test: Targeted gene disruption. Biochem.Mol.Biol.Educ. *36*, 299-301.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

THE EFFECT OF BISULFITE TREATMENT ON GENOMIC DNA

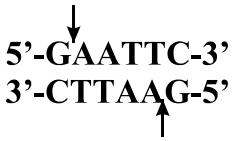
Terms to be familiar with before you start to solve the test

*Polymerase chain reaction (PCR) * primer * promoter * restriction endonucleases * agarose gel electrophoresis * ethidium bromide staining * DNA methylation * Taq polymerase * single nucleotide polymorphisms (SNPs)* CpG islands * tumor suppressor genes * protooncogenes * epigenetic regulation*

The experiment

The assay described in this test was designed to detect an important regulatory DNA modification. Genomic DNA samples from a normal tissue (samples 2 and 3 in Fig. 1) or a tumor (samples 4 and 5) were divided into two aliquots: samples 3 and 5 were treated with bisulfite (that converts unmethylated cytosines into uracils while methylated cytosines remain unchanged), samples 2 and 4 were left untreated. Polymerase chain reaction (PCR) was performed with the two DNA samples using a pair of primers specific for the promoter region of a gene. The PCR products were digested with *Eco*RI restriction endonuclease, the samples were fractionated by agarose gel electrophoresis and stained with ethidium bromide (Fig. 1).

(The recognition site of *Eco*RI is:



The arrows indicate the cleavage sites.)

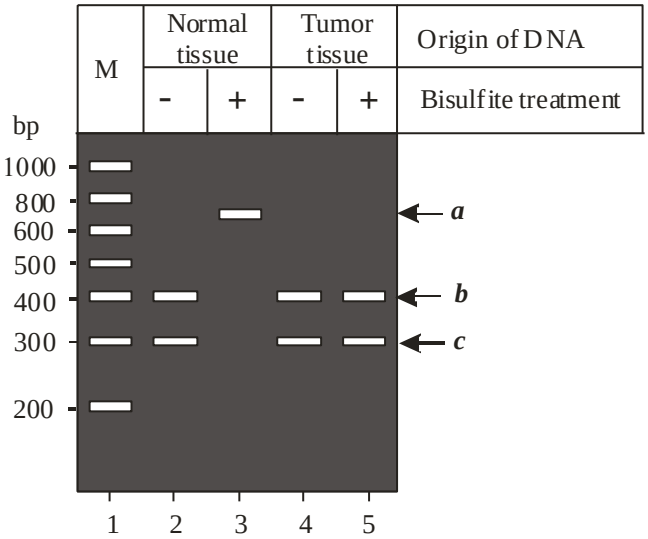


Figure 1: Agarose gel electrophoresis of the DNA samples (for details see the text; M, size marker; bp, base pair).

Study the figure and solve the following multiple-choice questions (MCQs).

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: Band *a*
B: Band *b*
C: Both of them
D: Neither of them

1.____ Consists of fragments with one intact 5′-GAATTC-3′ sequence.

2.____ Consists of fragments with several intact 5′-GAATTC-3′ sequences.

3.____ Consists of fragments with one intact 5′-GAATTT-3′ sequence.

4.____ Consists of fragments with one intact 5′-AAATTT-3′ sequence.

5.____ Consists of fragments with two blunt ends.

6.____ Consists of fragments with one blunt and one sticky end.

7.____ Consists of fragments with two sticky ends.

Experiment Analysis

(The following statements are related to the information presented in the description of the experiment. 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.)

8.____ Both strands of the *Eco*RI recognition site were methylated in the original normal DNA molecule.

9.____ Both strands of the *Eco*RI recognition site were methylated in the original tumor DNA molecule.

10.____ DNA strands containing uracil serve as templates for *Taq* polymerase.

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11.____ *Taq* polymerase has DNA methyl transferase activity.

12.____ Bisulfite treatment of the template DNA inhibited the polymerase chain reaction.

13.____ The *Eco*RI site of the promoter region analyzed in this experiment is flanked by C≡G pairs at both sides.

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.)

14.____ What can be the biomedical significance of this assay?

A. To detect single nucleotide polymorphisms (SNPs) in genomic DNA
B. To analyze the methylation state of the promoters of tumor suppressor genes in cancer cells
C. To study the expression of protooncogenes in normal cells
D. B and C
E. A, B and C

Correct answers

1.	D	8.	D
2.	D	9.	A
3.	A	10.	A
4.	D	11.	C
5.	A	12.	B
6.	B	13.	A
7.	D	14.	B

The test describes a fictitious experiment based on: Zymo Research Catalog, 2006/2007, p42.

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Szeberényi J. (2008) Problem-solving test: The effect of in vitro bisulfite treatment on genomic DNA. Biochem.Mol.Biol.Educ. *36*, 66-67, 2008.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

ANALYSIS OF THE CELL CYCLE BY FLOW CYTOMETRY

Terms to be familiar with before you start to solve the test

*cell cycle * flow cytometry * cell culture * fluorescent DNA dye * diploid and tetraploid cells * mitosis * phases of the cell cycle * growth factors * microtubules * apoptosis * DNA synthesis * proteasome * [^{3}H]thymidine * pulse labeling * histones * protein phosphorylation * M-phase promoting factor (MPF) * cyclins * cyclin-dependent kinases (Cdks) * synchronized culture*

The experiment

Human tumor cell cultures were treated with a drug profoundly affecting the cell cycle. Thereafter the drug was washed out from the cultures (at time zero), and the cells were kept under conditions optimal for growth. At the indicated times cultures of identical cell numbers were stained with a fluorescent DNA dye and then subjected to flow cytometry. Figure 1 shows the flow cytometric curves.

Study the figure and solve the following multiple-choice questions!

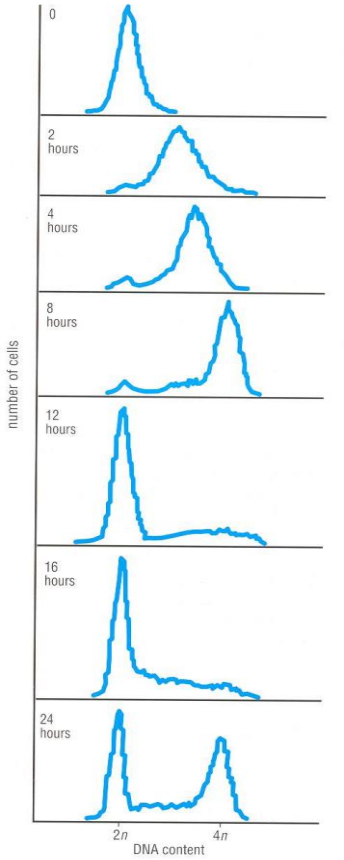

Figure 1. Flow cytometric analysis of human tumor cell cultures (for experimental details see the text). (Taken from D.O. Morgan: The Cell Cycle, with permission.)

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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 treatments could be used to produce the time-zero situation?

A. Treatment with a growth factor
B. Treatment with an inhibitor of microtubule assembly
C. Treatment with an apoptosis-inducing agent
D. Treatment with a DNA synthesis inhibitor
E. Treatment with a proteasome inhibitor

2.____ Which of the cultures would be most heavily labeled after a [^{3}H]thymidine pulse?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. The 24-hour culture

3.____ The cells of which culture contain the largest amount of histones?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. The 24-hour culture

4.____ The cells of which sample contain the highest number of phosphorylated H1 histone molecules?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. There is no difference between the cultures

5.____ The cells of which culture contain the highest levels of MPF activity?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. The 24-hour culture

6.____ The cells of which culture contain the lowest level of cyclin B?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. The 24-hour culture

7.____ At what time did the cells divide?

A. At time zero
B. Between 4 and 8 hours
C. Between 8 and 12 hours
D. At 24 hours
E. There was no cell division during the course of the experiment

8.____ Which of the cultures is the least synchronized?

A. The 0-hour culture
B. The 2-hour culture
C. The 8-hour culture
D. The 12-hour culture
E. The 24-hour culture

9.____ The cells of which culture contain the highest amount of Cdk1, a component of MPF?

A. The 0-hour culture
B. The 8-hour culture
C. The 12-hour culture
D. The 24-hour culture
E. All contain approximately the same amount of Cdk1

10.____ The cells of which culture contain the highest Cdk1 activity?

A. The 0-hour culture
B. The 8-hour culture
C. The 12-hour culture
D. The 24-hour culture
E. All contain approximately the same activity Cdk1

Correct Answers

1.	D	6.	D
2.	B	7.	C
3.	C	8.	E
4.	C	9.	E
5.	C	10.	B

The test is based on Figure 2-18 in David O. Morgan: The Cell Cycle. Principles of Control. Oxford University Press, New Science Press Ltd., London, UK, 2007.

This test was published in Biochemistry and Molecular Biology Education and is presented here with the permission of the International Union of Biochemistry and Molecular Biology.

Szeberényi J. (2007) Problem-solving test: Analysis of the cell cycle by flow cytometry. Biochem.Mol.Biol.Educ. *35*, 153-154.

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α1-ANTITRYPSIN DEFICIENCY: AN EXAMPLE FOR A PROTEIN FOLDING DISEASE

Terms to be familiar with before you start to solve the test

*protein conformation * protein folding * proteases * protein synthesis * protein glycosylation * glycoproteins * N-linked and O-linked oligosaccharides * endoplasmic reticulum * Golgi complex * secretory pathway * microsomes * pulse/chase labeling * SDS-polyacrylamide gel electrophoresis * immunoprecipitation * chaperones * protein translocation*

The experiment

α1-antitrypsin (α1-AT) is an abundant protease inhibitor of human blood plasma synthesized and secreted by liver cells. It is a glycoprotein containing 3 N-linked oligosaccharide chains. A single amino acid substitution (Glu 342 → Lys) results in the autosomal recessive disorder α1-AT deficiency that is characterized by a 90% reduction of α1-AT in the blood. The main consequence of this is an increased activity of elastase enzyme in the lungs leading to the destructive lung disease familial emphysema.

The following test describes a series of experiments designed to analyze the molecular mechanisms involved in the pathogenesis of α1-AT deficiency. Study the description of experiments and the results presented in the figures carefully and solve the multiple choice questions (MCQs).

Before starting to study the experiments, answer this question:

Relationship Analysis

(This type of question consists of a sentence with two main parts: an assertion and a reason for that assertion. Select

A if both assertion and reason are true statements and the reason is a correct explanation of the assertion;
B if both assertion and reason are true statements but the reason is not a correct explanation of the assertion;
C if the assertion is true but the reason is a false statement;
D if the assertion is false but the reason is a true statement;
E if both assertion and reason are false statements.)

1.____ The conformation of the α1-AT protein may be affected by this mutation, BECAUSE this amino acid substitution causes an alteration of charged groups on the surface of the protein.

Figure 1 shows the results of an experiment in which cells expressing wild-type or mutant α1-AT were compared in a pulse/chase/immunoprecipitation experiment using [^{35}S]methionine for labeling (for details see the figure legend).

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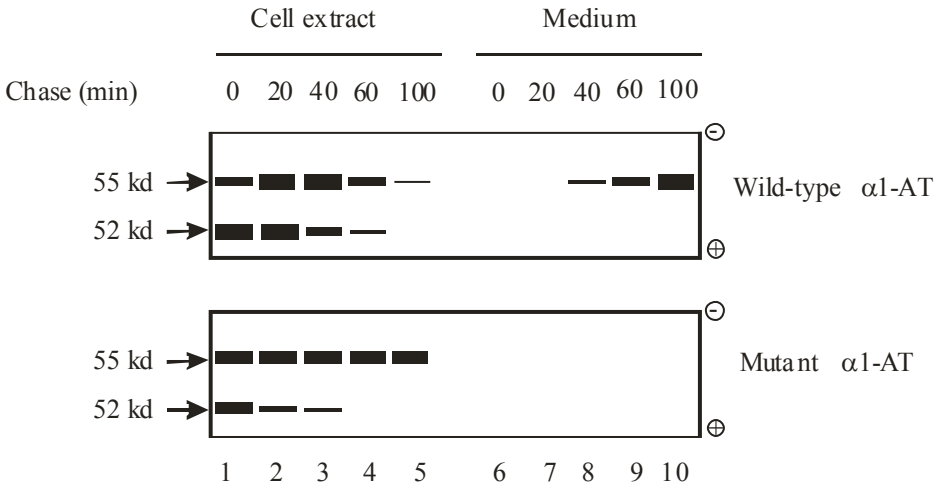

Figure 1. Analysis of the fate of wild-type and mutant α1-antitrypsin produced by cultured human cells. Cell cultures expressing the normal (upper panel) and mutant forms (lower panel) of α1-AT were pulse-labeled with [^{35}S]methionine for 10 minutes and then chase was performed for the intervals indicated in the figure. At the end of each interval cell extracts (samples 1 to 5) and medium (samples 6 to 10) corresponding to the same number of cells were immunoprecipitated using an anti-α1-AT antibody. The immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography. (The position of electrodes during electrophoresis are indicated on the right side of the panels.)

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.)

2.____ What features of α1-AT can be studied in this experimental setting?

A. Its exact amount in the cells
B. Its rate of synthesis and turnover in the cell
C. Its rate of secretion
D. B and C
E. A, B and C

The experiment of Figure 2 was designed to characterize the oligosaccharide moieties attached to α1-AT. To this end endoglycosidase H (endo H) treatment was used: endo H cleaves N-linked oligosaccharides produced in the endoplasmic reticulum (see Fig. 4A), but not O-linked and Golgi-processed N-linked oligosaccharides (experimental details are given in the legend to Fig. 2).

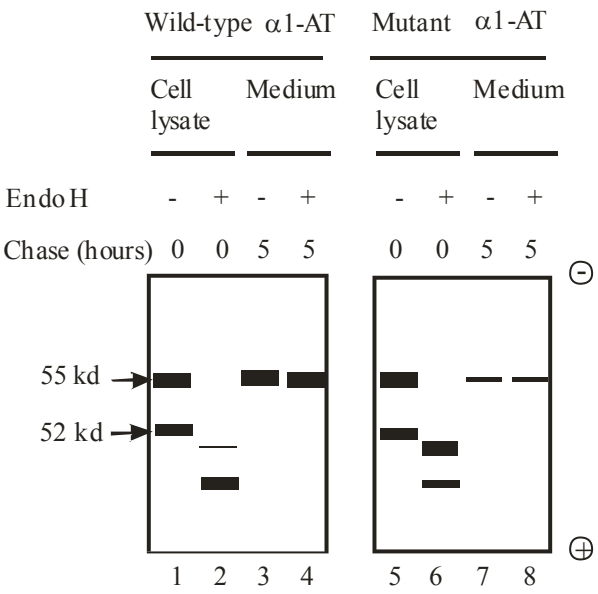

Figure 2. Effect of endoglycosidase H on wild-type and mutant α1-antitrypsin. Cell cultures expressing wild-type (samples 1 to 4) or mutant α1-AT (samples 5 to 8) were pulse/chase-labeled as described in the legend to Fig. 1. Cell lysates and medium samples were subjected to mock digestion (-) or endo H digestion (+) as indicated in the figure, and then to immunoprecipitation analysis with anti-α1-AT.

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. The 52 kilodalton band
B. The 55 kilodalton band
C. Both of them
D. Neither of them

3.____ Corresponds to the free oligosaccharide moieties of α1-AT.

4.____ Contains glycosylated α1-AT.

5.____ Molecules of this band reach the Golgi complex within 10 minutes after termination of translation.

6.____ Molecules of this band are secreted.

Figure 3 shows the results of a functional assay to compare wild-type and mutant α1-AT (details in the legend).

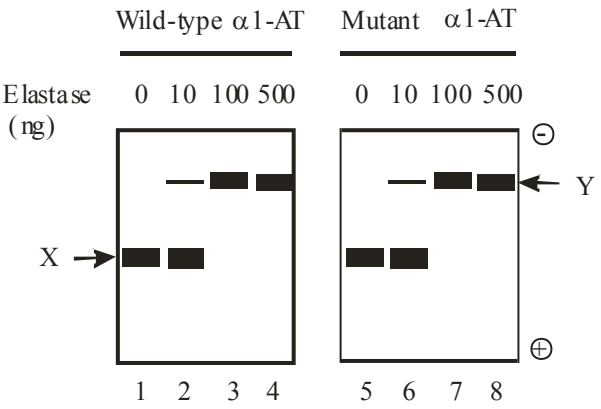

Figure 3. Functional activity of wild-type and mutant α1-antitrypsin. Labeled wild-type (samples 1 to 4) or mutant α1-AT (samples 5 to 8) were incubated with increasing amounts of non-radioactive elastase enzyme as indicated and then the mixtures were analyzed by SDS-PAGE and autoradiography.

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.)

7.____ What is the most likely explanation for the shift of electrophoretic mobility of α1-AT (from band X to band Y) in this experiment?

A. Elastase cleaved α1-AT
B. Elastase caused complete degradation of α1-AT
C. Elastase formed a stable complex with α1-AT
D. Elastase removed the oligosaccharides from α1-AT
E. α1-AT induced aggregation of elastase molecules

In the second part of this test experiments are presented in which the effect of a plant alkaloid, castanospermine (CST) was studied on the metabolism of α1-AT. CST inhibits glucosidase I, the enzyme responsible for the removal of the distal glucose molecule of N-linked oligosaccharide precursor (Fig. 4A). Fig. 4B shows the effect of CST on the mutant form of α1-AT.

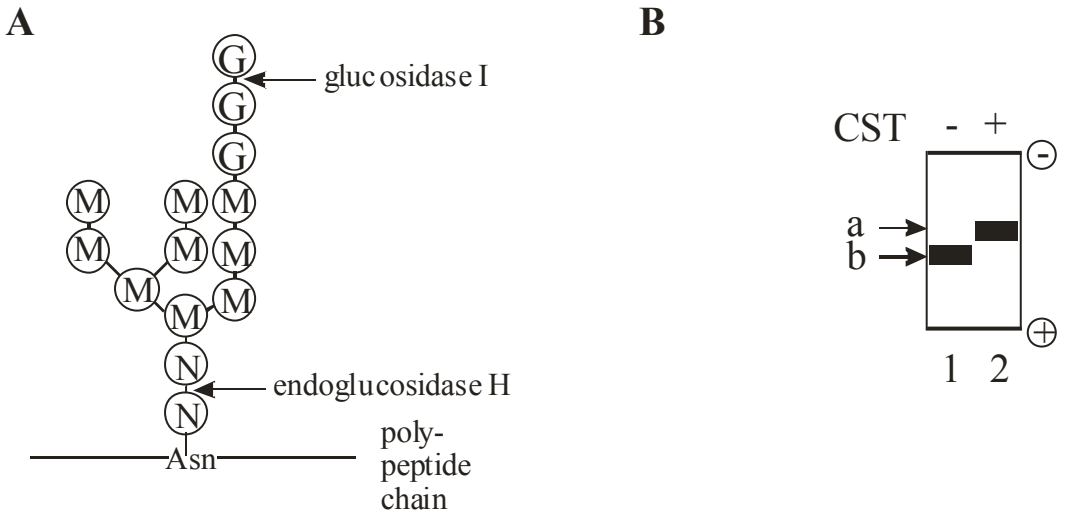

Figure 4. The effect of castanospermine on α1-antitrypsin. (A) The structure of N-linked oligosaccharide precursor and the cleavage sites of glucosidase I and endo H (N, N-acetylglucosamine; M, mannose; G, glucose). (B) The effect of CST. Cells expressing the mutant α1-AT were labeled with [^{35}S]methionine under conditions favoring the labeling of the 55kd protein (see Figs. 1 and 2), in the absence (sample 1) or presence of CST (sample 2). α1-AT was immunoprecipitated and analyzed by SDS-PAGE and autoradiography.

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.)

8.____ What can be the consequence of CST treatment of cells on α1-AT?

A. The isoelectric point of α1-AT is altered
B. The protein becomes more hydrophilic
C. The molecular mass of α1-AT is altered
D. A and C
E. B and C

The effect of CST on α1-AT metabolism was studied in the experiment of Fig. 5 (details in the figure legend).

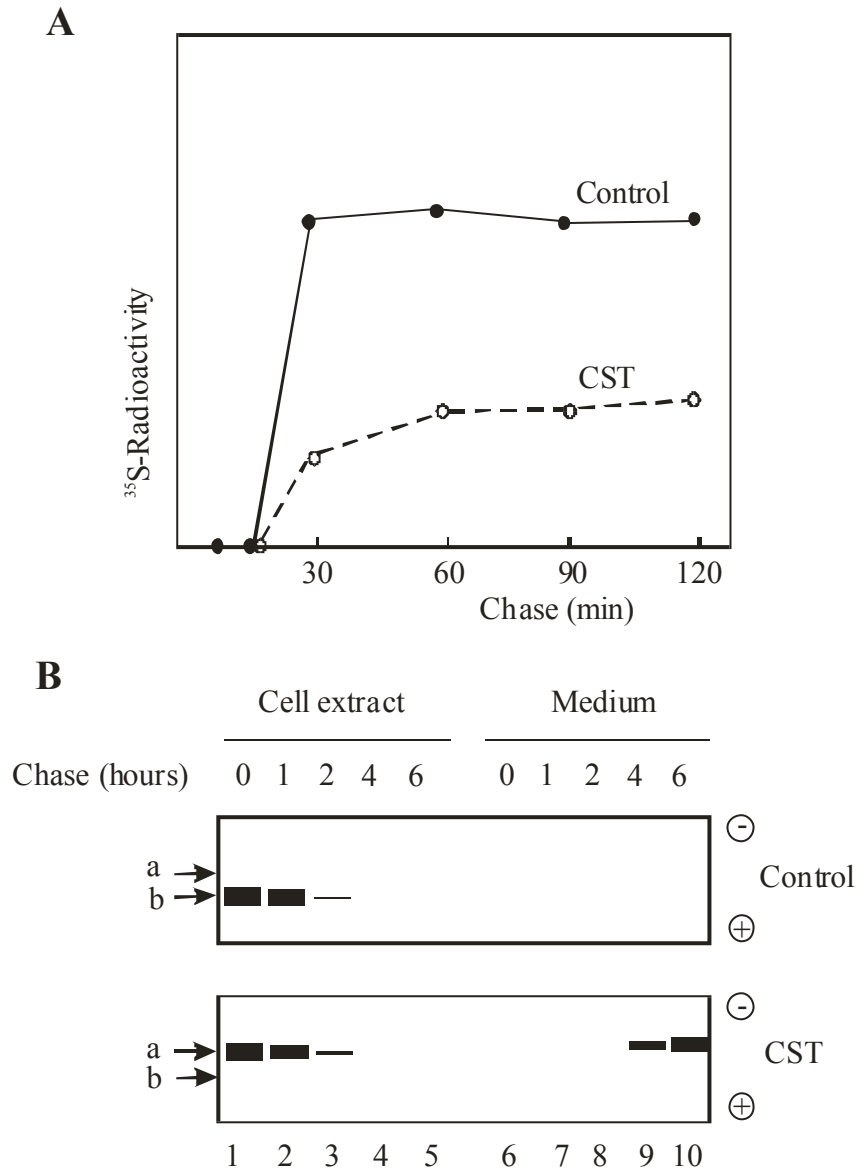

*Figure 5. The effect of castanospermine on the metabolism of wild-type (A) and mutant (B) α1-antitrypsin. Cells were pulse/chase-labeled with [^{35}S]methionine in the presence or absence of CST as indicated. α1-AT was analyzed in cell extracts (B, left panel) or in the medium (A; B, right panel) as described in the legend to Fig.1. (Note that Figs. A and B come from two separate experiments using different cell lines and experimental protocols. Band **a** and **b** in panel B correspond to the bands of Fig. 4B.)*

Fig. 6 presents results from an *in vitro* microsomal translocation experiment designed to study the role of the endoplasmic reticulum membrane chaperone calnexin in the traffic of α1-AT (for experimental details see the legend).

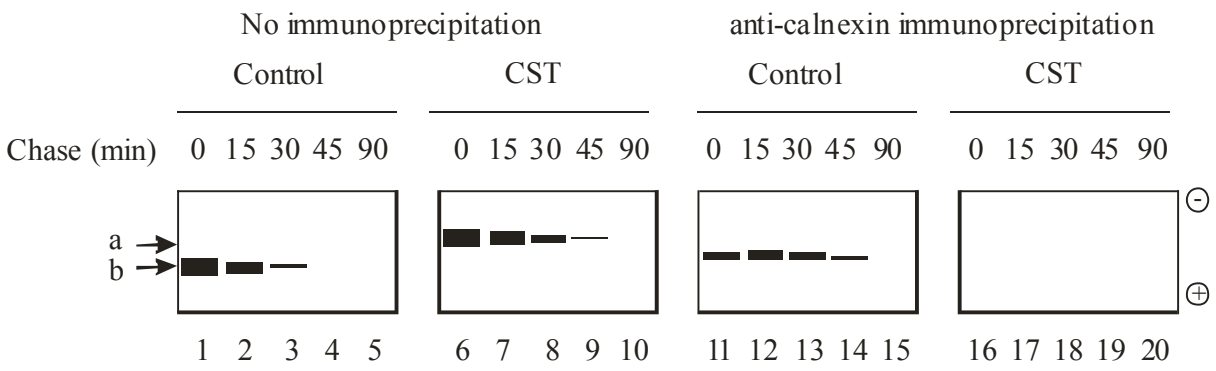

Figure 6. Effect of castanospermine on the fate of mutant α1-antitrypsin in a cell-free microsomal translocation system. Pulse/chase labeling was performed in cell-free protein synthesizing systems containing mutant α1-AT mRNA, [^{35}S]methionine, microsomes and all components required for translation, in the presence or absence of CST as indicated. Microsomes were collected by centrifugation and were run directly (samples 1 to 10) or after immunoprecipitation with an anti-calnexin antibody (samples 11 to 20) in an SDS-polyacrylamide gel. The figure shows the autoradiogram of the gel.

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.)

9.____ What feature of calnexin was studied in this experiment?

A: Its effect on the synthesis of α1-AT
B: Its effect on the glycosylation of α1-AT
C: Its effect on the translocation of α1-AT across the endoplasmic reticulum membrane
D: Its binding to α1-AT
E: All four

Summarize the conclusions of these experiments by solving the following MCQs.

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. Wild-type α1-AT
B. Mutant α1-AT
C. Both of them
D. Neither of them

10.____ Its gene is efficiently transcribed in the cells used in this experiment.

11.____ Its mRNA is efficiently translated.

12.____ It is able to translocate into the endoplasmic reticulum.

13.____ Its glycosylation starts in the endoplasmic reticulum.

14.____ It is mostly retained in the endoplasmic reticulum.

15.____ It is a substrate for glucosidase I.

16.____ It is able to bind to its target protein.

Experiment Analysis

(The following statements are related to the information presented in the description of the experiment. 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.)

17.____ Castanospermine inhibits the translocation of mutant α1-AT across the endoplasmic reticulum membrane.

18.____ The distal glucose of the N-linked oligosaccharide is required for the secretion of α1-AT.

19.____ Glycosylation of α1-AT took place in the cell-free microsomal system.

20.____ Glucosidase I is present in the microsomes.

21.____ Calnexin selectively binds to mutant α1-AT molecules containing the untrimmed oligosaccharide precursor (see Fig. 4A).

22.____ Castanospermine triggers the release of mutant α1-AT from its complex with calnexin.

23.____ Castanospermine stimulates the secretion of mutant α1-AT.

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.)

24.____ Based on the results of this study what would you expect from the treatment of α1-AT deficient patients with castanospermine?

A. Their plasma level of α1-AT would be increased
B. Their lung condition would be improved
C. The mutant α1-AT would disappear from their blood
D. A and B
E. B and C

Correct anwers

1.	A	7.	C	13.	C	19.	A
2.	D	8.	E	14.	B	20.	A
3.	D	9.	D	15.	C	21.	B
4.	C	10.	C	16.	C	22.	A
5.	D	11.	C	17.	B	23.	A
6.	A	12.	C	18.	B	24.	D

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References

[1] V.W. Sasak, J.M. Ordovas, A.D. Elbein, R.W. Berninger (1985) Castanospermine inhibits glucosidase I and glycoprotein secretion in human hepatoma cells. *Biochem. J.* **232**, 759-766.

[2] J.A.J. Burrows, L.K. Willis, D.H. Perlmutter (2000) Chemical chaperones mediate increased secretion of mutant α1-antitrypsin (α1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in α1-AT deficiency. *Proc. Natl. Acad. Sci. USA* **97**, 1796-1801.

[3] N.Y. Marcus, D.H. Perlmutter (2000) Glucosidase and mannosidase inhibitors mediate increased secretion of mutant α1-antitrypsin Z. *J. Biol. Chem.* **275**, 1987-1992.

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Szeberényi J. (2007) Problem-solving test: α1-Antitrypsin deficiency: An example for a protein folding disease. Biochem.Mol.Biol.Educ. *35*, 300-304.

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VECTORIAL TRANSPORT IN INTESTINAL EPITHELIAL CELLS

Terms to be familiar with before you start to solve the test

*polarized cells * vectorial transport * apical and basolateral membrane * tight junction * occluding * transmembrane proteins * passive and active transport * glucose transporter * Na^+/glucose symporter * Na^+/K^+ ATPase * uniporter * antiporter * facilitated diffusion * lateral diffusion*

The Experiment

The main function of the small intestine is to absorb small molecules (sugars, amino acids, nucleosides etc.) from the intestinal lumen and forward them to blood capillaries. The molecular basis of this unidirectional, vectorial transport is based on the polarized nature of the cell membrane: the apical and basolateral regions of the cell membrane contain different transport proteins. The reader is expected to review textbook chapters describing details of the membrane structure of epithelial cells lining the small intestine and then solve the multiple-choice questions (MCQs) presented in the second part of the test. The first MCQs are designed to refresh memories about vectorial transport.

Multiple Completion

(A question or incomplete statement is followed by four numbered completions, one or more of which are correct. Select:

A: if 1, 2 and 3 are correct;
B: if 1 and 3 are correct;
C: if 2 and 4 are correct;
D: if only 4 is correct;
E: if all four are correct.)

1._____ Epithelial cells of the small intestine are held together by tight junctions. What are the characteristic features of these structures?

1. Serve as barriers to prevent mixing of the components of the apical and basolateral membranes
2. Their main components are transmembrane proteins called occludin
3. Seal the intestinal lumen from the extracellular space between epithelial cells
4. These are the main communicative junctions between the epithelial cells

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The most important membrane proteins involved in the vectorial transport of glucose are: glucose transporter, Na^+/glucose symporter and Na^+/K^+ ATPase. What are the main features of these proteins?

Five-choice Association

(This type of question consists of a list of lettered headings followed by a list of numbered words or phrases. For each numbered word or phrase, select the *one* heading which is most closely related to it.)

A: Glucose transporter
B: Na^+/glucose symporter
C: Na^+/K^+ ATPase
D: A and C
E: All three proteins

2. _____ It is a transmembrane protein.

3. _____ It performs facilitated diffusion.

4. _____ Its only function is active transport.

5. _____ It performs both passive and active transport.

6. _____ It is an antiporter.

7. _____ It is localized to the apical membrane.

8._____ It is localized to the basolateral membrane.

9._____ It is a uniporter.

A fictitious experiment is described below in which the effect of a bacterial toxin destroying tight junctions between intestinal cells is studied. Untreated control rats and animals infected with the bacterium are used.

Considering the membrane structure of small intestinal cells, what differences do you expect to see between the two groups of animals? Solve the following MCQs!

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Relationship Analysis

(This type of question consists of a sentence with two main parts: an assertion and a reason for that assertion. Select:

A: if both assertion and reason are true statements and the reason is a correct explanation of the assertion;
B: if both assertion and reason are true statements but the reason is not a correct explanation of the assertion;
C: if the assertion is true but the reason is a false statement;
D: if the assertion is false but the reason is a true statement;
E: if both assertion and reason are false statements.)

10._____The toxin reduces the concentration of glucose in the epithelial cells, BECAUSE the Na^+ concentration is lower than normal in the intestinal lumen of infected animals.

11._____ Blood sugar levels are higher than normal in the infected animals, BECAUSE the Na^+K^+ pump is more active in their intestinal cells.

Quantitative Comparison

(In this type of question paired statements describe two entities that are to be compared in a quantitative sense. Select:

A: if A is greater than B;
B: if B is greater than A;
C: if the two are equal or very nearly equal.)

12._____ A: The density of Na^+/glucose symporter molecules in the apical membrane of intestinal cells in the infected animals
B.: The density of Na^+/glucose symporter molecules in the basolateral membrane of intestinal cells in the infected animals

13._____ A: The rate of glucose influx across the intestinal apical membrane in control animals
B: The rate of glucose influx across the intestinal apical membrane in infected animals

14._____ A: The rate of glucose outflux across the intestinal basolateral membrane in control animals
B: The rate of glucose outflux across the intestinal basolateral membrane in infected animals

15._____ A: The rate of glucose outflux across the intestinal apical membrane in control animals
B: The rate of glucose outflux across the intestinal apical membrane in infected animals

16._____ A: The rate of active Na^+/K^+ transport across the intestinal apical membrane in control animals
B: The rate of active Na^+/K^+ transport across the intestinal apical membrane in infected animals

Correct Answers

1.	A	6.	C	11.	E	16.	B
2.	E	7.	B	12.	C		
3.	A	8.	D	13.	A		
4.	C	9.	A	14.	A		
5.	B	10.	C	15	B		

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Szeberényi J. (2006) Problem-solving test: Vectorial transport in intestinal epithelial cells. Biochem. Mol. Biol. Educ. *34*, 449-451.

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EXPRESSION CLONING OF THE ERYTHROPOIETIN RECEPTOR

Terms to be familiar with before you start to solve the test

*cytokines * cytokine receptors * cDNA library * cDNA synthesis * poly(A)$^+$ RNA * primer * template * reverse transcriptase * restriction endonucleases * cohesive ends * expression vector * promoter * Shine-Dalgarno sequence * poly(A) signal * DNA helicase * DNA ligase * topoisomerases * [^{125}I] labeling * transfection * mock transfection * SDS-polyacrylamide gel electrophoresis * β-mercaptoethanol * autoradiography*

The experiment

Erythropoietin (EPO) is a cytokine produced by certain kidney cells that stimulates the production and differentiation of red blood cells. The following test describes experiments [1] performed to clone the cDNA of murine erythropoietin receptor (EPO-R) and to express it in suitable human host cells.

An expression cDNA library was constructed in this study using mouse erythroleukemia cell total poly(A)$^+$ RNA. The first step in library construction is cDNA synthesis.

Multiple Completion

(A question or incomplete statement is followed by four numbered completions, one or more of which are correct. Select

A if 1, 2 and 3 are correct;
B if 1 and 3 are correct;
C if 2 and 4 are correct;
D if only 4 is correct;
E if all four are correct.)

1.____ Besides total cytoplasmic poly(A)$^+$ RNA, which of the following components are required in the reaction mixture to synthesize a collection of cDNAs containing copies of EPO-R cDNA?

1. Oligo(dT)
2. Reverse transcriptase
3. The four deoxyribonucleoside triphosphate
4. RNA polymerase II

cDNAs synthesized in this mixture were converted into double-stranded DNA molecules supplied with Xho restriction endonuclease specific cohesive ends and ligated into the Xho site of a mammalian expression plasmid.

2.____ What sequences should be present in this plasmid if it is to express inserted cDNAs in human cells?

1. A mammalian promoter upstream of the Xho site
2. A Shine-Dalgarno sequence upstream of the Xho site
3. A poly(A) signal downstream of the Xho site
4. A Shine-Dalgarno sequence downstream of the Xho site

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.)

3.____ What enzyme should be added to the mixture of double-stranded cDNA and Xho-cut plasmid to generate a collection of recombinant plasmids?

A. DNA helicase
B. DNA ligase
C. Topoisomerase I
D. B and C
E. A, B and C

The recombinant plasmids were transfected into human cells and a clone was selected that bound radioactively labeled EPO under tissue culture conditions. Figure 1 shows the results of an experiment in which [^{125}I] EPO binding was compared using the original mouse cell culture (A), human cells transfected with an empty plasmid (B) or the human cell clone isolated from the experiment (C). [^{125}I] EPO binding (Y axis) to the cells was measured at different concentrations of the labeled hormone (see X axis), in the absence (●—●) or presence of 100 nM unlabeled ("cold") EPO (○—○).

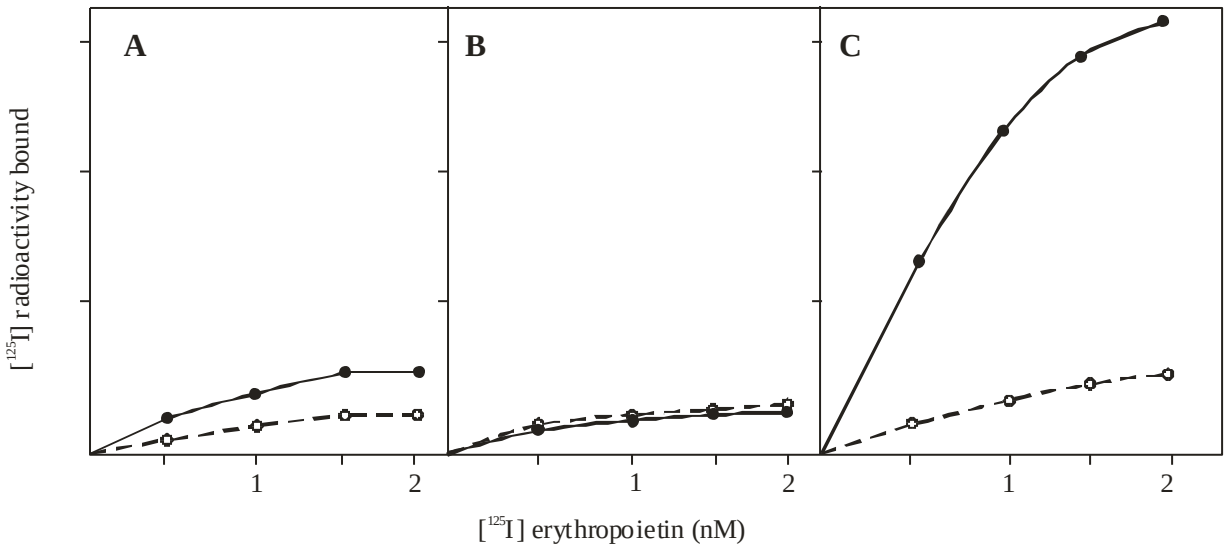

Figure 1. Binding of [^{125}I] erythropoietin to mouse erythroleukemia cells (A), mock-transfected human cells (B) and to human cells of the clone isolated as described in the text (C).

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.)

4.____ What was the aim of using unlabeled EPO in this experiment?

A. To increase specific binding of [^{125} I] EPO
B. To decrease non-specific binding of [^{125} I] EPO
C. To distinguish specific and non-specific binding of [^{125} I] EPO
D. To stimulate EPO signaling
E. C and D

5.____ How can specific binding of [^{125} I] EPO be determined? (The symbols of Fig. 1 are used in this question.) Specific binding equals to

A. Radioactivity corresponding to ●
B. Radioactivity corresponding to ○
C. Radioactivity corresponding to ● plus ○
D. Radioactivity corresponding to ● minus ○
E. Radioactivity measured in the medium in the presence of "cold" EPO

6.____ What can be determined by measuring specific [^{125} I] EPO binding?

A. Cell surface localization of EPO-R
B. EPO-R density
C. The rate of synthesis of EPO-R
D. A and B
E. A, B and C

Quantitative Comparison

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

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

7.____ A. Specific EPO binding to mouse erythroleukemia cells
B. Non-specific EPO binding to mouse erythroleukemia cells

8.____ A. Specific EPO binding to mock-transfected human cells
B. Non-specific EPO binding to mock-transfected human cells

9.____ A. EPO-R density on mouse erythroleukemia cells
B. EPO-R density on the transfected human clone of Fig. 1C

In the second part of the experiment cultures of the cells in Fig.1C were incubated with 1 nM [^{125} I] EPO in the presence (samples 2 and 4) or in the absence (samples 1 and 3) of 100 nM "cold" EPO (Fig. 2). After incubation the media were discarded and the cells were washed with fresh medium that did not contain EPO. The cultures were subsequently incubated in the presence (samples 1 and 2) or absence (samples 3 and 4) of the cross-linking agent disuccinimidyl suberate. Cell membrane fractions were isolated and then subjected to SDS-polyacrylamide gel electrophoresis in gels containing β-mercaptoethanol. The gels were autoradiographed using an X ray film.

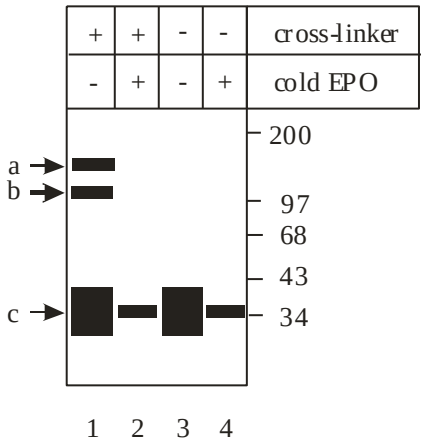

Figure 2. Cross-linking of radiolabeled erythropoietin to its receptor expressed in human cells (numbers on the right indicate molecular weights in kilodalton; for experimental details see the text).

Figure Analysis

(The following statements are related to the information presented in the description of the experiment. 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.)

10.____ The gel regions corresponding to bands *a*, *b* and *c* all contain EPO.

11.____ The gel regions corresponding to bands *a*, *b* and *c* all contain EPO-R.

12.____ Most of the radiolabeled EPO molecules bound to specific receptor sites on the cells.

13.____ Most of the radiolabeled EPO molecules were covalently cross-linked to EPO-R molecules.

14.____ Native EPO-R molecules consist of several subunits.

15.____ Band *a* corresponds to EPO-EPO-R complexes held together by disulfide bonds.

Correct Answers

1.	A	9.	B
2.	B	10.	A
3.	B	11.	B
4.	C	12.	A
5.	D	13.	B
6.	D	14.	C
7.	C	15.	B
8.	B		

Reference

[1] A.D. D'Andrea, H.F. Lodish, G.G. Wong (1989) Expression cloning of the murine erythropoietin receptor. *Cell* **57**, 277-285.

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Szeberényi J. (2008) Problem-solving test: Expression cloning of the erythropoietin receptor. Biochem.Mol.Biol.Educ. *36*, 236-238.

Supported by a grant from the European Union (TÁMOP-4.1.1.C-13/1/KONV-2014-0001).

THE YEAST TWO-HYBRID SYSTEM

Terms to be familiar with before you start to solve the test

*transcription factors * enhancer * promoter * RNA polymerase * expression plasmid * terminator * histidine * transfection * cell culture * fusion proteins * RNA splicing*

The experiment

The following test describes the principle of a powerful technique of molecular biology: the yeast two-hybrid system.

Yeast cells contain a transcription factor, GAL4, required for the expression of the β-galactosidase gene. It consists of a DNA-binding domain that recognizes the specific enhancer sequence in the promoter region of the β-galactosidase gene and an activator domain required for the binding of RNA polymerase II (Fig 1).

Two mammalian proteins, designated X and Y, are studied in the following fictitious experiment using the yeast two-hybrid system. Expression plasmids functioning in yeast cells

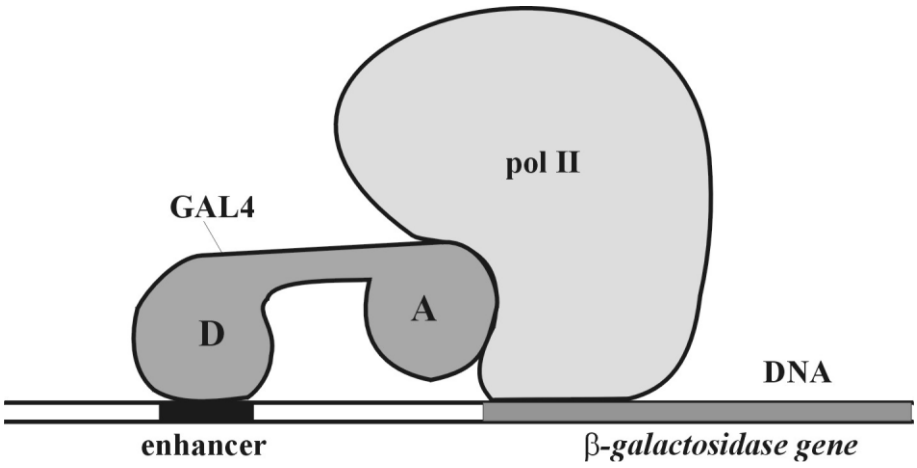

Figure 1. The role of GAL4 in the transcription of the β-galactosidase gene (D, DNA binding domain; A, activation domain; pol II, RNA polymerase II).

were used in the experiment (Fig. 2). They contained a promoter and a terminator region specific for yeast cells. One plasmid (designated DX plasmid) contained a fusion gene consisting of a cDNA coding for the DNA binding domain of GAL4 and a cDNA coding for protein X. The other plasmid (AY plasmid) contained a hybrid gene constructed from cDNA sequences coding for the activation domain of GAL4 and protein Y. A third, so-called reporter plasmid was also used in the experiment: it contained the HIS3 gene as a reporter gene hooked to the enhancer/promoter region of the β-galactosidase gene. The three plasmids were transfected into a culture of mutant yeast cells that contained neither GAL4, nor HIS3 ($GAL4^-$, $HIS3^-$ mutant). The cells were spread on culture dishes with solid media lacking or containing histidine as indicated in Table I.

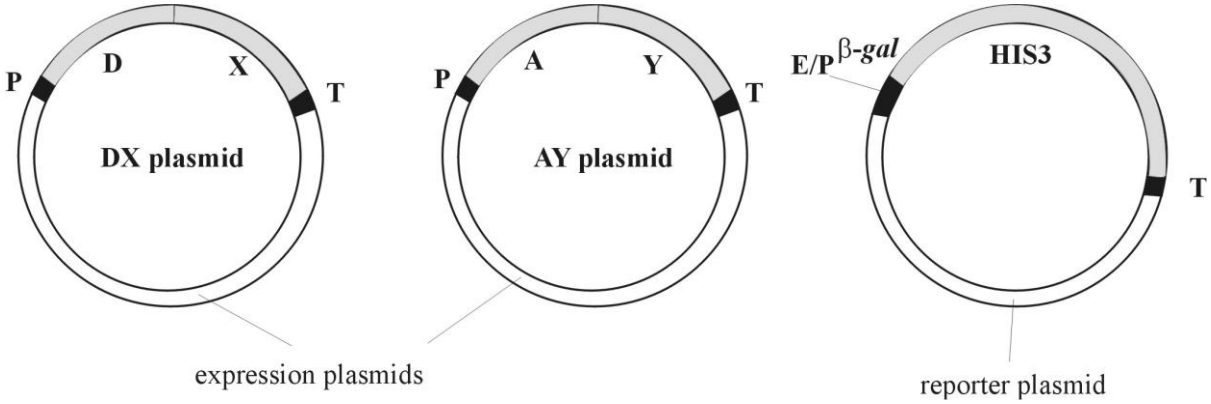

Figure 2. Plasmid constructs used in the experiment. (P, promoter; T, terminator; E/P$^{\beta\text{-}gal}$, the enhancer/promoter region of the β-galactosidase gene; A and D, cDNAs coding for the activation and DNA binding domain of GAL4, respectively; X and Y, cDNAs coding for protein X and Y, respectively).

Table I. Transfection of GAL4$^-$, HIS3$^-$ *yeast cells with various plasmid constructs under different culturing conditions. (The constructs are described in Fig. 2, experimental details are given in the text.)*

Transfection with			Histidine in the medium	Number of colonies on the culture plates
DX plasmid	AY plasmid	reporter plasmid		
-	-	-	+	many
-	-	-	-	none
-	-	+	-	none
+	-	+	-	none
-	+	+	-	none
+	+	+	-	several

Study the experimental strategy, evaluate the results and solve the following multiple-choice questions.

Experiment Analysis

(The following statements are related to the information present in the description of the experiment. 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.)

1.____ Histidine is an essential amino acid in yeast.

2.____ The HIS3 gene codes for an enzyme required for histidine biosynthesis.

3.____ The HIS3 gene of the reporter plasmid is constitutively expressed in the transfected mutant yeast cells.

4.____ The half-life of DX protein is shorter than that of GAL4.

5.____ Histidine can not be taken up by $HIS3^-$ cells.

6.____ Yeast cells are unable to splice pre-mRNAs of mammalian origine.

7.____ X and Y proteins act as transcription factors in their mammalian host cells.

8.____ The β-galactosidase enhancer does not function in the $HIS3^-$ mutant cells.

9.____ The DX and AY fusion proteins can substitute the missing GAL4 activity in the transfected cells.

10.____ The DX fusion protein is able to bind to the β-galactosidase enhancer.

11.____ The AY fusion protein is able to activate RNA polymerase II.

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.)

12.____ What molecular event can be studied by the yeast two-hybrid system?

A. The regulation of β-galactosidase gene expression
B. The regulation of HIS3 gene expression
C. The role of exogenous proteins (X and Y) in gene regulation
D. The role of exogenous proteins (X and Y) in RNA polymerase II function
E: *In vivo* interaction between exogenous proteins (X and Y)

Correct answers

1.	A	7.	C
2.	A	8.	B
3.	B	9.	A
4.	C	10.	A
5.	B	11.	A
6.	C	12.	E

Reference

Spector, D.L., Goldman, R.D. and Leinwand, L.A. (editors): Cells – A Laboratory Manual. Chapter 69: Two-hybrid System/Interaction Trap. Cold Spring Harbor Laboratory Press, 1997.

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Szeberényi J. (2006) Problem-solving test: The yeast two-hybrid system. Biochem.Mol.Biol.Educ. *34*, 306-307.

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ANALYSIS OF THE MECHANISM OF ACTION OF AN APOPTOSIS INDUCING COMPOUND

Terms to be familiar with before you start to solve the test

*apoptosis * Bcl-2 protein family * anti-apoptotic Bcl-2 proteins * pro-apoptotic multi domain Bcl-2 proteins * BH3-only proteins * intrinsic (mitochondrial) pathway of apoptosis * cell fractionation * targeted gene disruption * Western blotting * cytochrome c * loading control * caspases * apoptosome*

The Experiment

Most cancer cells loose their ability to undergo programmed cell death by apoptosis. Proteins involved in the regulation and execution of cell death are therefore promising targets of antitumor drug development. Among these members of the Bcl-2 protein family are particularly important. They come in three forms: anti-apoptotic Bcl-2 members (e.g. Bcl-2, Bcl-x_L), pro-apoptotic multi domain Bcl-2 family proteins (e.g. Bax, Bak) and BH3-only proteins (e.g. Bad, tBid; BH3 stands for Bcl-2 homology 3, this domain is common in all proteins of the family). They regulate the permeability of the outer mitochondrial membrane, thereby the intrinsic (mitochondrial) pathway of apoptosis.

A putative anticancer agent, designated compound 106, was tested *in vitro* on isolated mitochondria in the study described in the following test [1]. In order to be able to interpret the results of the following experiments, review the mitochondrial pathway of apoptosis in an appropriate textbook (e.g. Ref. 2).

The experiments presented in this test were performed using mitochondria isolated from murine embryonic fibroblasts.

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 procedures is most suitable to isolate a pure mitochondrial fraction from these cells?

A: Low-speed centrifugation of the homogenate
B: Medium-speed centrifugation of the homogenate
C: High-speed ultracentrifugation of the homogenate
D: Medium-speed centrifugation of the postnuclear supernatant
E: High-speed ultracentrifugation of the postnuclear supernatant

Experiment 1

The mitochondrial fraction was prepared from Bak$^-$Bax$^-$ cells (in which these genes had been knocked out by targeted gene disruption) and aliquots of the mitochondrial samples were incubated without treatment (sample 1 in Fig. 1), with added Bax protein (samples 2 to 6), tBid protein (sample 3) and increasing amounts of compound 106 (samples 4 to 6) as indicated in Figure 1. After incubation, mitochondria were pelletted by centrifugation, proteins were solubilized in a suitable buffer and Western blot analysis was performed using anti-Bax and anti-Tom 40 antibodies. (Tom 40 is a structural protein of the outer mitochondrial membrane.)

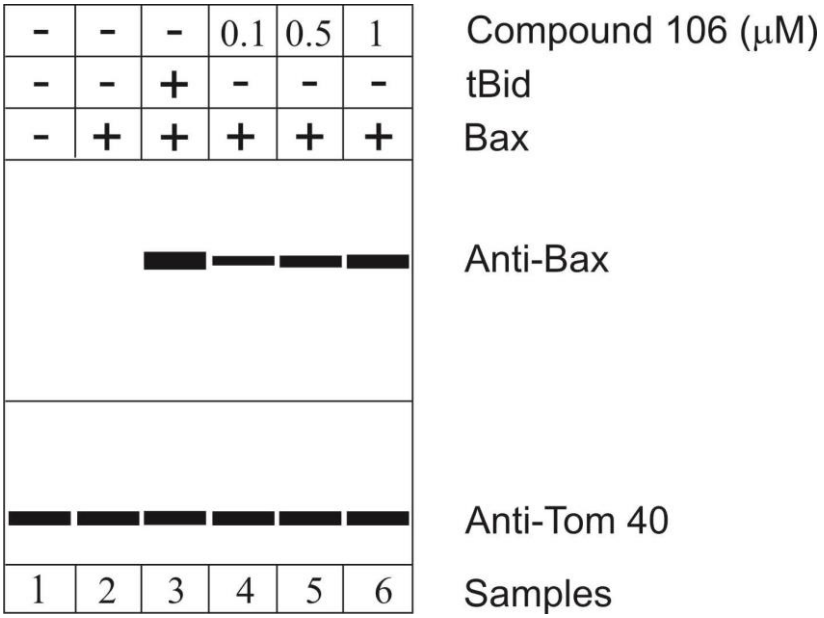

Figure 1. Treatment of mitochondria of Bak$^-$Bax$^-$ cells with Bax and tBid proteins and compound 106 (for experimental details see the text.)

Experiment 2

Mitochondria isolated from Bak$^-$Bax$^-$ cells were treated with the proteins Bax (samples 3 to 14), tBid (samples 5-6 and 11-12), Bcl-x_L (samples 9 to 14) and compound 106 (samples 7-8 and 13-14) as indicated in Fig. 2 (samples 1-2 served as controls). Mitochondria were sedimented by centrifugation and protein extracts of the pellets (P) and supernatants (S) were subjected to Western blot analysis using anti-cytochrome c and anti-Tom 40 antibodies (Fig. 2).

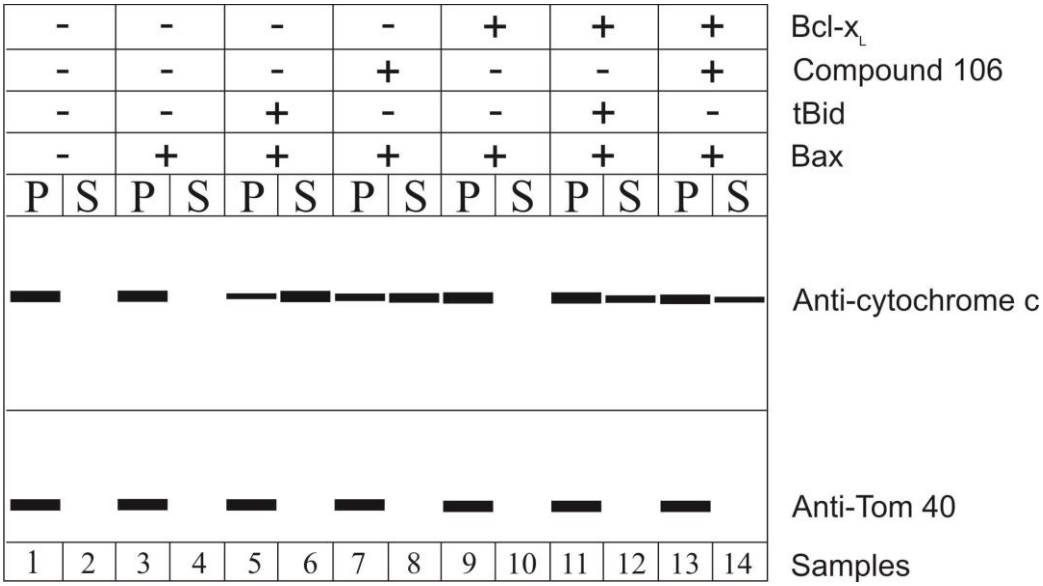

Figure 2. Treatment of mitochondria of Bak$^-$Bax$^-$ cells with Bax, tBid and Bcl-x$_L$ proteins and compound 106 (P, pellet, S, supernatant; for experimental details see the text.)
Experiment 3

A similar experiment was performed using mitochondria from Bak$^+$Bax$^-$ mouse embryonic fibroblasts. Mitochondria were left untreated (samples 1-2 in Fig. 3) or incubated with tBid protein (samples 3-4) or compound 106 (samples 5 to 10). Samples were processed as in Experiment 2; Fig. 3 shows the results of Western blotting.

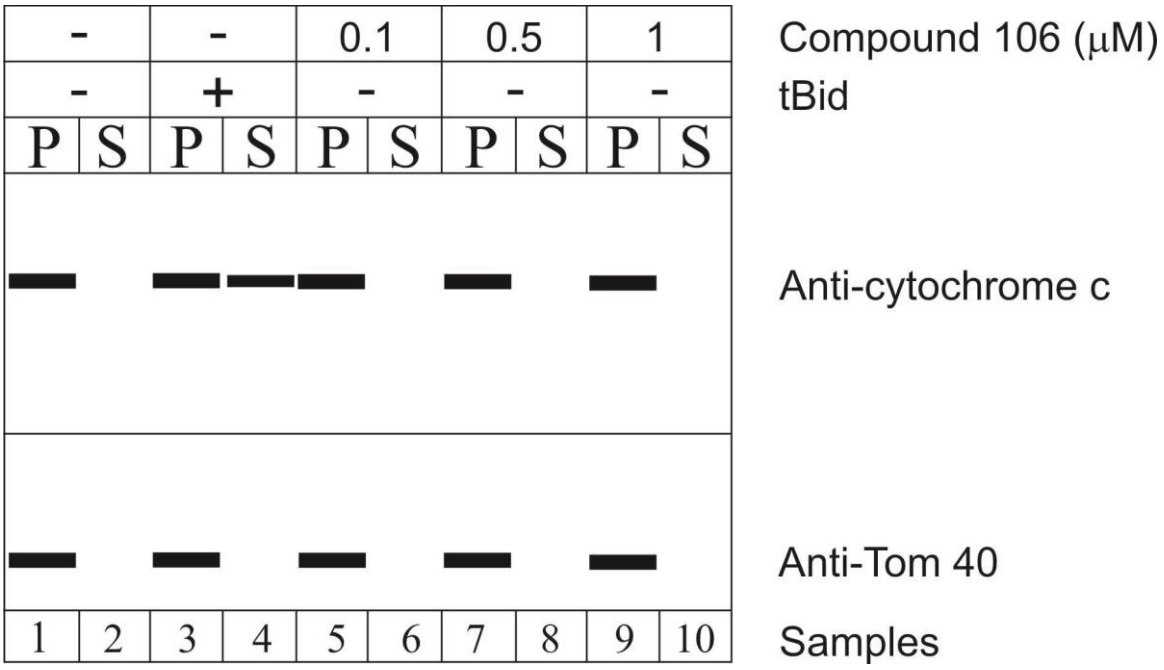

Figure 3. Treatment of mitochondria of Bak$^+$Bax$^-$ cells with tBid and compound 10 (for experimental details see the text.)

Study the experiments and solve the following multiple-choice 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.)

2.____ What was the aim of using anti-Tom 40 antibody in these experiments?

A: It was used as a loading control
B: To check the integrity of mitochondrial structure
C: To check if mitochondrial fractions are free of cytosolic contaminations
D: A and B
E: A, B and C

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.____ Murine embryonic fibroblasts overexpress Bcl-x_L.

4.____ Compound 106 stimulates the translocation of Bax protein into the mitochondria.

5.____ The effect of compound 106 on mitochondria depends on the presence of tBid.

6.____ tBid enhances the action of compound 106.

7.____ Tumor cells expressing the Bax protein are expected to be sensitive to the apoptotic effect of compound 106.

8.____ Tumor cells overexpressing the Bcl-x_L protein are expected to be sensitive to the apoptotic effect of compound 106.

9.____ Compound 106 destroys the outer mitochondrial membrane.

10.____ Compound 106 makes the outer mitochondrial membrane leaky.

11.____ Compound 106 stimulates the translocation of cytochrome c from the cytosol to the mitochondria.

The following MCQs can be answered by extrapolating the results of the presented experiments to the whole mitochondrial pathway of apoptosis.

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: $Bak^{+}Bax^{-}$ cells
B: $Bak^{-}Bax^{-}$ cells
C: Both of them
D: Neither of them

12.____ These cells are able to undergo apoptosis.

13.____ Expression of tBid in them leads to caspase 9 activation.

14.____ Treatment of these cells with compound 106 would lead to the formation of apoptosomes.

15.____ Compound 106 triggers caspase 3 activation in these 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.)

16.____ What protein is directly targeted by compound 106?

A: tBid
B: Bax
C: Bcl-x_L
D: Bak
E: Cytochrome c

Correct Answers

1.	D	9.	B
2.	D	10.	A
3.	C	11.	B
4.	A	12.	C
5.	B	13.	C
6.	C	14.	D
7.	A	15.	D
8.	B	16.	B

Reference

[1] Zhao G., Y. Zhon, C.O. Eno, Y. Lin, L. DeLeenw, J.A. Burlison, J.B. Chaires, J.O. Trent, C. Li (2014) Activation of the proapoptotic Bc-l2 protein Bax by a small molecule induces tumor cell apoptosis. Mol. Cell. Biol. 34, 1198-1207.

[2] Cooper G.M., R.E. Hausman (2013) The Cell. A Molecular Appoarch. Sixth Edition. Sinauer Associates, Inc., pp. 681-692.

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Szeberényi J. (2015) Analysis of the mechanism of action of an apoptosis-inducing compound. Biochem.Mol.Biol.Educ. *43*, .

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THE MECHANISM OF ACTION OF A HUMAN PAPILLOMA VIRUS ONCOPROTEIN

Terms to be familiar with before you start to solve the test

*human papilloma virus * cervical cancer * oncoproteins * malignant transformation * retinoblastoma protein * cell cycle * quiescent and cycling cells * cyclin/Cdk complexes * E2F * S-phase genes * enhancer element * proto-oncogenes * tumor suppressor genes * radioactive labeling * immunoprecipitation * SDS-polyacrylamide gel electrophoresis * autoradiography * protein phosphorylation and dephosphorylation * gene induction * agarose beads * centrifugation * Western blot analysis * phases of cell cycle * generation time*

The Experiment

One of the Nobel laureates in Physiology and Medicine of 2008, Harald Zur Hausen, discovered that certain types of the human papilloma virus (HPV) are the causative agents of cervical cancer [1]. HPV type 16 (HPV-16) is among the most common and best studied human oncogenic viruses. Two oncoproteins (E6 and E7) encoded by the viral genome are responsible for the transformation of normal epithelial cells into tumor cells. The experiment presented in this test was designed to analyze the effects of E7 protein at the molecular level, namely, its relationship to the retinoblastoma protein (RB) [2].

Since this research was not aimed at analyzing the behavior of RB protein, in order to understand the mechanism of action of E7 oncoproteins the reader is expected to recall the most important features of RB by solving the following multiple-choice questions (MCQs).

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. RB protein in quiescent cells
B. RB protein in cycling cells
C. Both of them
D. Neithet of them

1.____ Is present in the nuclei.

2.____ Is highly phosphorylated by cyclin/Cdk complexes.

3____ Is complexed to the transcription factor E2F.

4.____ Inhibits the transcription of S-phase genes.

5.____ Is bound to the enhancer elements of S-phase genes.

ÚJ SZÉCHENYI TERV
Nemzeti Fejlesztési Ügynökség www.ujszechenyiterv.gov.hu 06 40 638 638
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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.)

6.____ What are the main characteristics of the RB protein?

A. It is a proto-oncogenic protein
B. It is a tumor suppressor protein
C. It is a regulator of the cell cycle
D. A and C
E. B and C

Fig. 1 shows the results of a preliminary experiment. Cells of a human leukemia cell line (HL-60) were cultured without (samples 1 and 3) or in the presence of phorbol ester (samples 2 and 4), and labeled with [^{35}S]methionine (samples 1 and 2) or [^{32}P]phosphate (samples 3 and 4). Cell extracts were immunoprecipitated with an antibody specific for the retinoblastoma protein (anti-RB), SDS-polyacrylamide gel electrophoresis was performed followed by autoradiography.

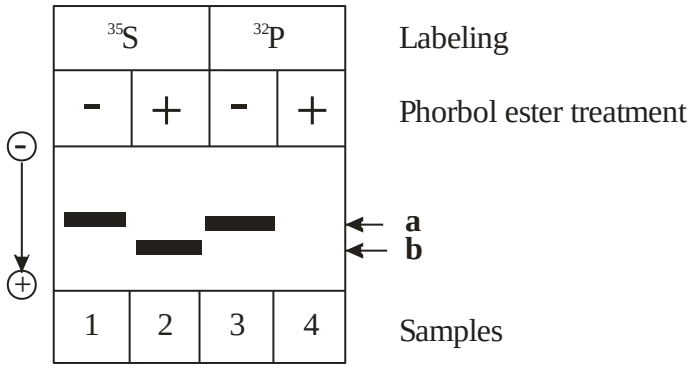

Figure 1. The effect of phorbol ester on the RB protein (for experimental details see the text).

What conclusions can be drawn from this experiment?

Figure Analysis

(The following statements are related to the information presented in the description of the experiment. 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.)

7.____ Band **a** is a degradation product of band **b**.

8.____ All RB protein molecules are phosphorylated in untreated HL-60 cells.

9.____ The RB protein is induced by phorbol ester in HL-60 cells.

10.____ The phorbol ester triggers dephosphorylation of the RB protein in HL-60 cells.

The results of the experiment carried out to analyze the function of the E7 protein are shown in Fig 2. The human leukemia cells were cultured in the absence (samples 1 to 3) or presence of phorbol ester (samples 4 to 6). Cell extracts were prepared and aliquots were incubated with agarose beads to which E7 protein molecules had been covalently attached. After incubation the beads were pelletted by centrifugation and Western blot analysis after SDS-polyacrylamide gel electrophoresis was performed using the anti-RB antibody with total cell extracts (samples 1 and 4), the supernatants (samples 2 and 5), and the sediments (samples 3 and 6).

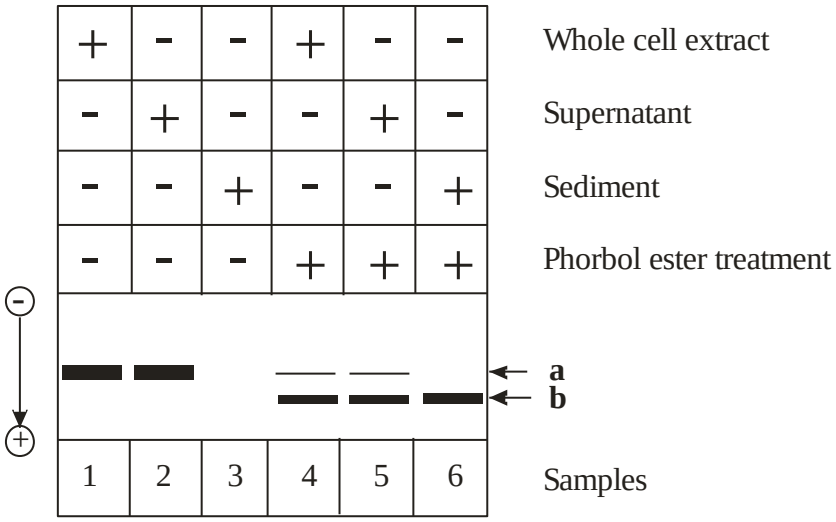

Figure 2. Analysis of cell extracts using E7-agarose beads (experimental details are described in the text).

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.)

11.____ What was the aim of using this E7-agarose bead protocol?

A. To study the expression of RB protein
B. To determine the rate of phosphorylation of RB protein
C. To analyze E7-RB interactions
D. A and B
E. A, B and C

To solve the following questions and describe the effect of E7 protein on the cell cycle you need to combine your knowledge of the function of the RB protein (see MCQs 1 to 6) and the conclusions drawn from the results of this study.

Relationship Analysis

(This type of question consists of a sentence with two main parts: an assertion and a reason for that assertion. Select

A if both assertion and reason are true statements and the reason is a correct explanation of the assertion;
B if both assertion and reason are true statements but the reason is not a correct explanation of the assertion;
C if the assertion is true but the reason is a false statement;
D if the assertion is false but the reason is a true statement;
E if both assertion and reason are false statements.)

12.____ HL-60 cells were in the G_o phase in the absence of phorbol esters, BECAUSE their RB proteins were fully phosphorylated.

13.____ Phorbol ester treatment must have inhibited the proliferation of HL-60 cells, BECAUSE it stimulated the binding of RB protein to the E7 oncoprotein.

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. Phorbol ester treatment of HL-60 cells
B. Expression of E7 oncoprotein in HL-60 cells
C. Both of them
D. Neither of them

14.____ Increases the amount of E2F transcription factor-bound RB protein.

15.____ Increases the expression of S-phase genes.

16.____ Increases the generation time of cells.

17.____ Increases the ratio of cells in the G1 phase of the cell cycle.

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.)

18.____ Let's summarize the effects of E7 protein that can lead to malignant transformation. E7 protein

A. binds and sequesters underphosphorylated RB protein
B. increases the amount of functionally active E2F transcription factor
C. pushes cells into the S-phase
D. increases the rate of cell proliferation
E. stimulates all the above processes

Correct Answers

1.	C	8.	A	15.	B
2.	B	9.	B	16.	A
3.	A	10.	A	17.	A
4.	A	11.	C	18.	E
5.	D	12.	D		
6.	E	13.	B		
7.	B	14.	A		

References

[1] J. Cohen, M. Enserink (2008) Nobel prize in Physiology or Medicine. HIV, HPV researchers honored, but one scientist is left out. *Science*, **322**, 174-175.

[2] Y. Imai, Y. Matsushima, T. Sugimura, M. Terada (1991) Purification and characterization of human papillomavirus type 16 E7 protein with preferential binding to the underphosphorylated form of retinoblastoma gene product. *J. Virol.* **65**, 4966-4972.

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Szeberényi J. (2015) Problem-solving test: The mechanism of action of a human papilloma virus oncoprotein. Biochem.Mol.Biol.Educ. *37*, 118-120.

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SIGNAL TRANSDUCTION IN PHILADELPHIA CHROMOSOME POSITIVE LEUKEMIA CELLS

Terms to be familiar with before you start to solve the test

*reciprocal translocation * proto-oncogene * gene expression * adaptor protein * SH2 and SH3 domains * receptor proteins * tyrosine protein kinases * serine/threonine protein kinases * steroid receptors * Src protein * malignant transformation * second messengers * retroviral oncogenes and oncoproteins * transfection * immunoprecipitation * pre-immune serum * [γ-^{32}P]ATP * SDS-polyacrylamide gel electrophoresis * autoradiograph * Western blot (immunoblot * co-immunoprecipitation * expression vector * cDNA * transient transfection * promoter * Ras protein * transformed foci*

The Experiment

The Philadelphia chromosome is the result of reciprocal translocation between chromosome 9 and 22 in human cells. The unusually short chromosome 22 (Philadelphia or Ph[1] chromosome, named after the city were it was first described) contains a fusion gene between the proximal region of the *bcr* gene and the distal part of the c-*abl* proto-oncogene (originally carried by chromosome 9). Expression of this gene generates a Bcr/Abl fusion protein that is biochemically different from the proto-oncogenic c-Abl protein. The aim of the experiments [1] described in this test was to analyze the signaling mechanisms activated by the Bcr/Abl protein, especially the role of the Grb2 adaptor protein characterized by an SH3-SH2-SH3 domain structure. Before turning to the experiments, recall your knowledge regarding adaptor proteins by solving the following multiple-choice questions (MCQs).

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 statements best describes the role of adaptor proteins?

A. They connect cell surface receptors to intracellular target proteins
B. They connect tyrosine protein kinase receptors to intracellular target proteins
C. They connect tyrosine protein kinase receptors to serine/threonine-specific protein kinases
D. They connect tyrosine-phosphorylated proteins to their target proteins
E. They regulates tyrosine protein kinase activity

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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. SH2 domain
B. SH3 domain
C. Both of them
D. Neither of them

2._____ This domain is the DNA binding region of steroid receptors.

3._____ The v-Src protein carries a similar region.

4._____ Only proteins carrying such domain are able to transform NIH3T3 fibroblast to tumor cells.

5._____ It binds to protein regions phosphorylated on tyrosine side chains.

6._____ It recognizes proline-rich regions of target proteins.

7._____ It is present in second messengers.

8._____ It is present in all retroviral oncoproteins.

In the first part of the experiment (Fig. 1) the adaptor protein Grb2 was studied in chromic myeloid leukemia (CML), acute lymphoid leukemia (ALL) cells and rat fibroblasts transfected with the v-*abl* oncogene (Rat1/v-*abl*). Cell extracts were immunoprecipitated with pre-immune serum (samples 1, 4 and 7 in Fig. 1), anti-Abl (samples 2, 5 and 8) or anti-Grb2 antibodies (samples 3, 6 and 9). The immunoprecipitates were incubated in vitro with [γ-^{32}P]ATP, the proteins were fractionated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by autoradiography.

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.)

9._____ What was the aim of using [γ-^{32}P]ATP in this experimental setting?

A. To study Abl autophosphorylation
B. To study phosphorylation of proteins bound to Abl
C. To study the production of cAMP
D. A and B
E. A, B and C

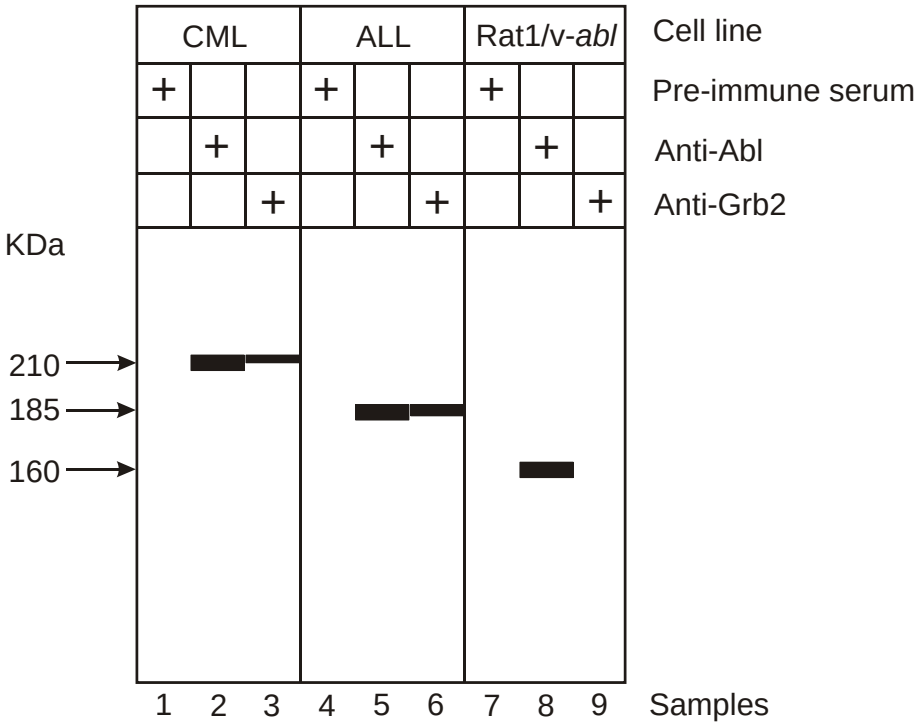

Figure 1. SDS-polyacrylamide gel electrophoresis of immunoprecipitates incubated with [γ-^{32}P]ATP (for details see the text).

In the next experiment (Fig. 2) CML cell extracts were incubated with pre-immune serum (sample 1), anti-Abl (sample 2) or anti-Grb2 antibody (sample 3), the immunoprecipitates were fractionated by SDS-PAGE and Western-blot analysis was performed using the anti-Grb2 antibody.

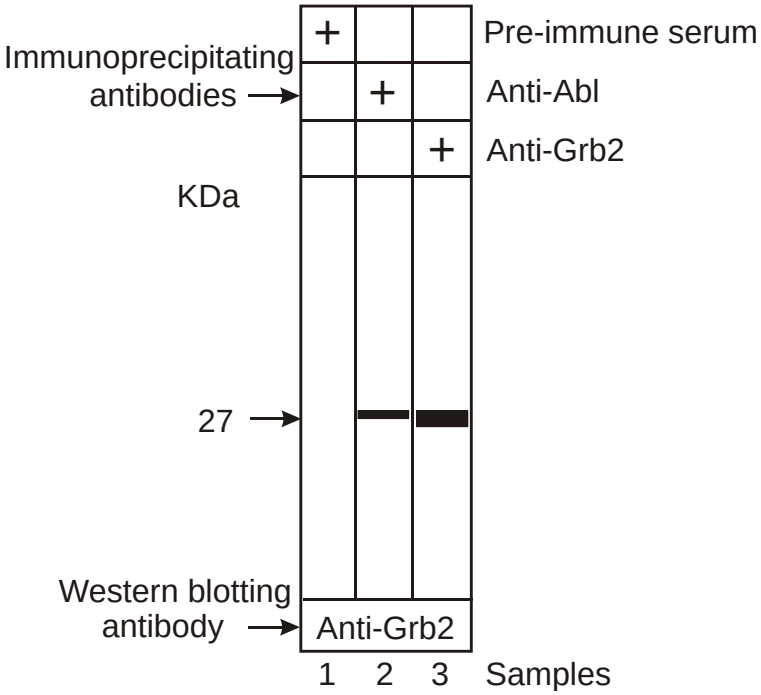

Figure 2. Immunoprecipitation/Western blotting experiment using an extract from CML cells (for details see the text).

In the experiment of Fig. 3 a similar co-immunoprecipitation strategy was followed. Cells transfected with expression vectors containing a *bcr*/*abl* fusion cDNA (samples 1 to 3) or a c-*abl* proto-oncogenic cDNA (samples 4 to 6) were used. Cell extracts were immunoprecipitated with control serum, anti-Abl or anti-Grb2 antibody as indicated in Fig. 3 and then immunoblotting was performed using the anti-Abl antibody.

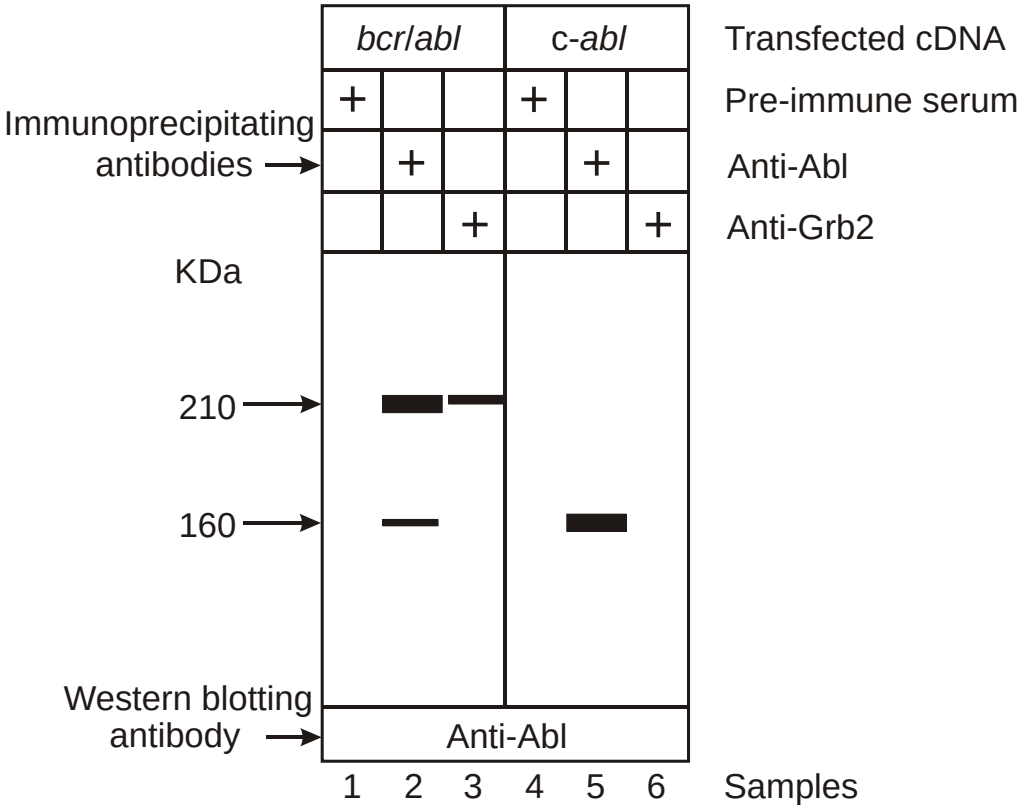

Figure 3. Co-immunoprecipitation experiment using extracts from bcr/abl and c-abl-transfected cells (for details see the text).

Finally, the significance of tyrosine at position 177 in the Bcr-region of the fusion protein was analyzed. A mutant cDNA was generated that, at this position, coded for phenylalanine (+
designated *bcr/abl* Y177F). Co-immunoprecipitation experiments were performed using extracts from cells transfected with wild-type *bcr*/*abl* or *bcr*/abl Y177F cDNA, using the antibodies shown in Fig. 4.

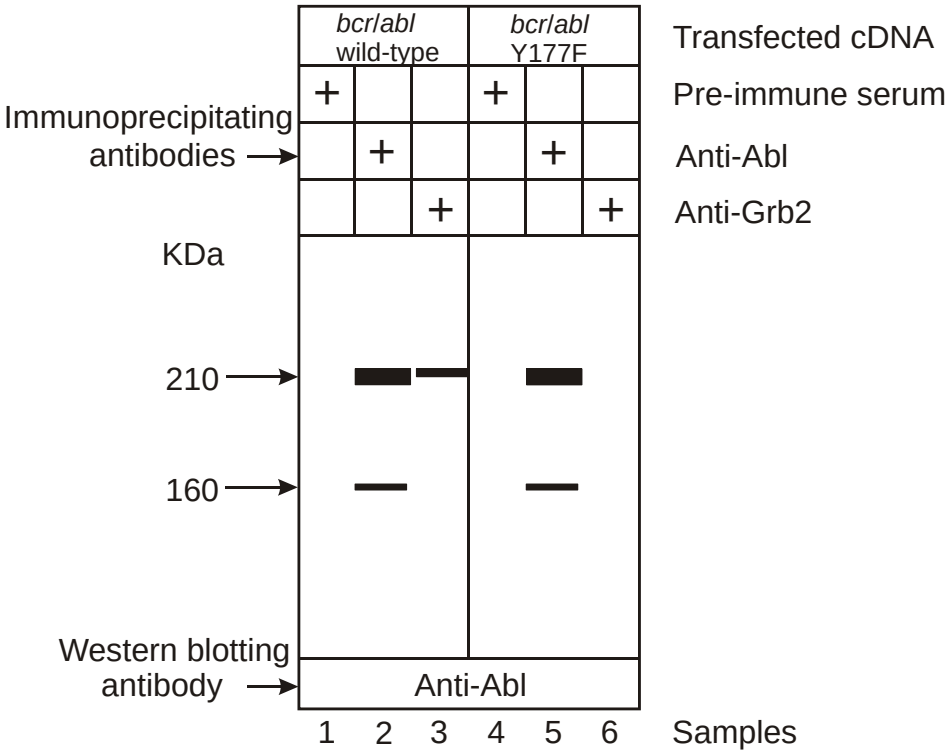

Figure 4. Co-immunoprecipitation experiment using extracts from wild-type bcr/abl and bcr/abl Y177F-transfected cells (for details see the text).

The effect of *bcr*/*abl* fusion gene on gene expression was studied in a transient transfection experiment (Fig. 5). Cells were transfected with a plasmid carrying the cDNA of a reporter gene, the gene coding for chloramphenicol acetyltransferase (CAT), expressed from a promoter containing a Ras-responsive element. Expression of the reporter gene can be easily measured and quantitated. The reporter plasmid was co-transfected into rat fibroblasts with an empty plasmid (sample 1 in Fig. 5) or with expression vectors containing cDNAs of wild-type *bcr*/*abl* (sample 2), wt-*bcr*/*abl* together with an inhibitory mutant *ras* gene (sample 3) or the mutant *bcr*/*abl* Y177F (sample 4). Figure 5 shows the CAT-activities in the transient by transfected cell cultutes.

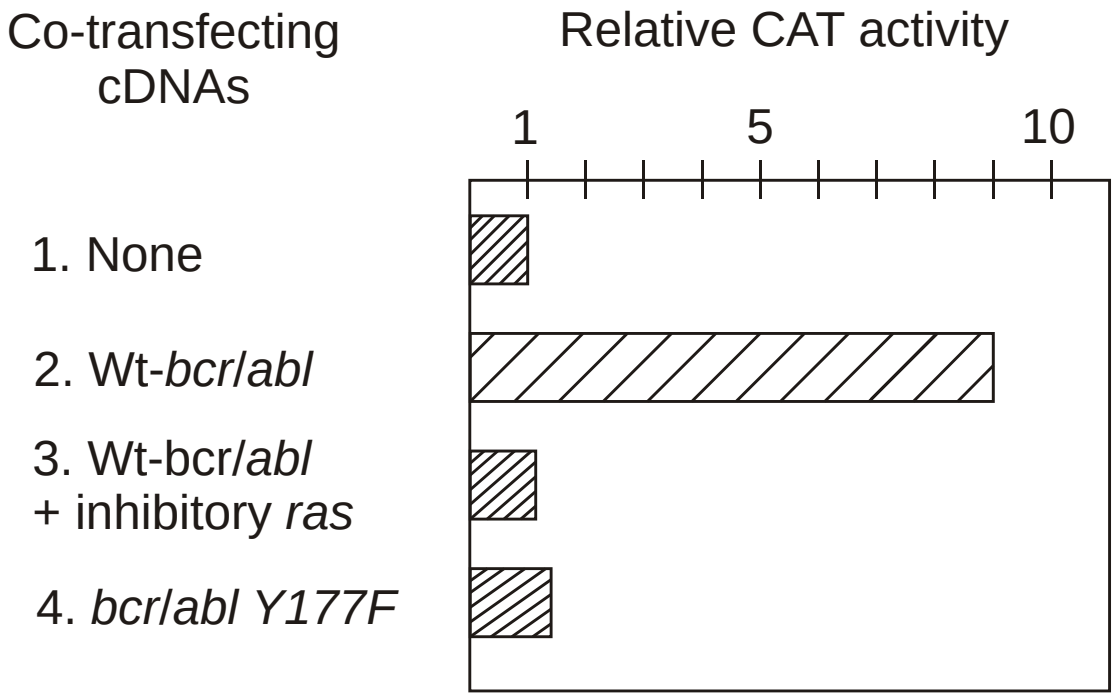

Figure 5. CAT activities transfected with various expression plasmids (for details see the text).

The biological activities of wt-*bcr*/*abl* and *bcr*/*abl* Y177F constructs were also tested in transfection experiments: they produced 37 and 0 transformed foci, respectively, using rat fibroblast cultures.

Study the results of the experiments and solve the following MCQs.

Quantitative Comparison

(In this type of question paired statements describe two entities that are to be compared in a quantitative sense.
Select: **A: if A is greater than B;**
B: if B is greater than A;
C: if the two are equal or very nearly equal.)

10._____ A. The molecular mass of Bcr/Abl protein
B. The molecular mass of c-Abl protein

11._____ A. The molecular mass of Bcr/Abl protein
B. The molecular mass of Grb2 protein

12._____ A. Ras•GDP/Ras•GTP patio in Ph^1-positive leukemia cells
B. Ras•GDP/Ras•GTP patio in normal leukocytes

13._____ A. ERK activity in Ph^1-positive leukemia cells
B. ERK activity in normal leukocytes

Experiment Analysis

(The following statements are related to the information presented in the description of the experiment. 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 Bcr/Abl fusion protein contains an SH1 domain.

15._____ Bcr/Abl phosphorylates Grb2 protein in an *in vitro* reaction.

16._____ Bcr/Abl and Grb2 form a complex *in vivo*.

17._____ Bcr/Abl and v-Abl proteins use the same signaling pathway to cause malignant transformation.

18._____ Chromosomes break at different sites during the translocations cauring CML and All.

19._____ The transforming potential of the Bcr/Abl fusion protein depends on its ability to bind Grb2.

20._____ Gene activation by the Bcr/Abl protein is caused by the stimulation of the Ras pathway.

Correct Answers

1.	E	7.	A
2.	A	8.	B
3.	C	9.	A
4.	C	10.	A
5.	A	11.	E
6.	B	12.	D

Reference

[1] A.M. Pendergast, L.A. Quilliam, L.D. Cripe, C.H. Bassing, Z. Dai, N. Li, A. batzer, K.M. Rabun, C.J. Der, J. Schlessinger, M.L. Gishizky (1993): BCR-ABL-Induced Oncogenesis Is Mediated by Direct Interaction with the SH2 Domain of the GRB-2 Adaptor Protein. *Cell*, **75**, 175-185.

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