ISSN 2079-0864, Biology Bulletin Reviews, 2013, Vol. 3, No. 6, pp. 451–455. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © V.Kh. Khavinson, A.Yu. Solov'ev, S.I. Tarnovskaya, N.S. Lin'kova, 2013, published in Uspekhi Sovremennoi Biologii, 2013, Vol. 133, No. 3, pp. 310–316.

Mechanism of Biological Activity of Short Peptides: Cell Penetration and Epigenetic Regulation of Gene Expression

V. Kh. Khavinson[a, b], A. Yu. Solov'ev[b], S. I. Tarnovskaya[b], and N. S. Lin'kova[b]

[a] *Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia*
[b] *St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia*
e-mail: miayy@yandex.ru

Abstract—Data on various aspects of the molecular and cellular mechanism of the biological activity of short peptides used as potential drugs were analyzed. Based on the published data and our own results, a possible mechanism of the penetration of short peptides into the cytoplasm and cell nucleus is considered. In addition, the possibility of the involvement of short peptides in the mechanisms of the epigenetic regulation of gene expression via their complimentary interaction with promoter regions of genes in DNA is substantiated.

Keywords: short peptides, penetration into the cell, epigenetic regulation of gene expression
DOI: 10.1134/S2079086413060042

INTRODUCTION

Short peptides that consist of no more than 20 amino acid residues with molecular weights less than 3.5 kDa are signaling molecules involved in the regulation of homeostasis at different levels of the organization of living matter. I.P. Ashmarin identified endogenous regulatory peptides as part of an "intricate system of specialized signaling molecules and information carriers between the cells of the body" (Ashmarin, 2002).

The design of drugs based on short peptides, peptide biotechnology, is a topical area of modern molecular biology and pharmacology. The term "peptide biotechnology" appeared more than 50 years ago, but an active development of this trend has only been observed in the last two decades. The first synthetic peptide, oxytocin, was created by American biochemist Vincent Du Vigneaud in 1953, for which he won the Nobel Prize in chemistry in 1955 (Du Vigneaud et al., 1953).

In Russia, the design of drugs based on short peptides has been actively developed since the 1970s at the Kirov Military Medical Academy and St. Petersburg Institute of Bioregulation and Gerontology (Khavinson, 2001; Khavinson et al., 2005); Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences (Ivanov et al., 2005; Deigin et al., 2007); Institute of Molecular Genetics, Russian Academy of Sciences (Myasoedov et al., 2011); and Zakusov Institute of Pharmacology (Ostrovskaya et al., 2007).

Based on the results of long-term research, it was concluded that the activity of short peptides is selective or tissue-specific (Khavinson, 2001; Anisimov et al., 2010; Khavinson et al., 2012). Next, a model explaining the development of pathological processes was proposed, according to which the key role is played by disturbances in peptidergic regulation. The correction of these disturbances by the additional administration of short peptides into the body should lead to the regression of the pathological process and normalization of the disturbed functions. Thus, the main advantages of the low-molecular-weight peptides in comparison to the high-molecular-weight protein regulators were established. The former exhibit high biological activity and tissue specificity and have no species specificity or immunogenicity (Khavinson, 2001). The ability of endogenous peptides to have prolonged effects suggests that at least some of them can modulate gene activity, which may also apply to the synthetic peptide compounds (Vanyushin, 2004; Khavinson et al., 2005).

Despite the high efficiency of drugs based on short peptides, the mechanism of biological activity has long remained controversial for a number of issues, such as whether peptides can penetrate into the cell, and what are the principles of their transmembrane transfer and intracellular regulation of homeostasis? Only today, due to the development of epigenetics and peptide biotechnology, a scheme of the interaction of peptides with the cell membrane and DNA that explain their high biological activity can be proposed.

PENETRATION OF SHORT PEPTIDES IN CYTOPLASM AND CELL NUCLEUS

At the end of the 20th century, it was found that short peptides synthesized based on Tat protein (a transcription activator of the human immunodeficiency virus (HIV-1) genome) are able to penetrate

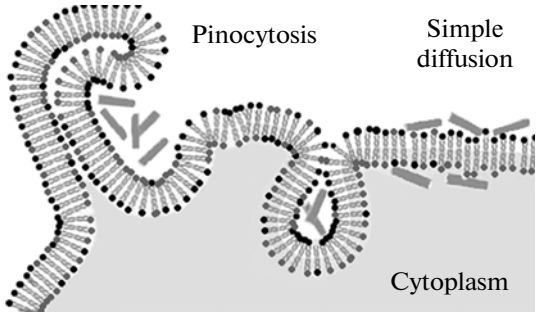

Fig. 1. Scheme of penetration of short peptides into the cytoplasm via pinocytosis and simple diffusion. Negatively charged phosphatidylserine heads are shown in black, and the positively charged phosphatidylcholine and phosphatidylethanolamine heads are shown in gray.

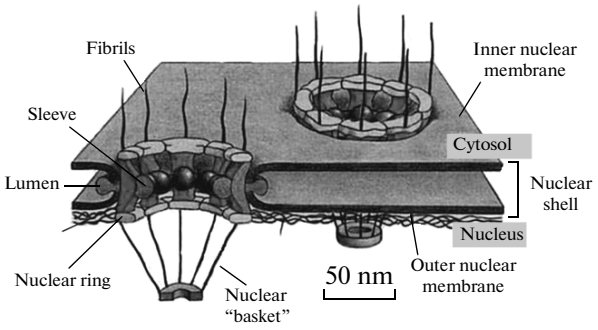

Fig. 2. Schematic representation of nucleopore (according to Alberts et al., 1994).

into cells (Frankel et al., 1988). These peptides were created as transmembrane carriers for drugs, which determined the appearance of the term "cell-penetrating peptides" (CPPs). This group includes the peptides that transport proteins, nucleic acids, and liposomes into the cell (Kyte et al., 1982; Morozov et al., 1998; Lindgren et al., 2000).

Long-term observations have shown that the ability to penetrate into the cell through the membrane is characteristic primarily of alkaline peptides, the structure of which contains an excess of positively charged amino acid residues. The advantage of these peptides is that they can easily overcome the acid glycocalyx layer, which adjoins the cell membrane (Frankel et al., 1988; Futaki et al., 2003; Duchardt et al., 2007). It was shown that synthetic alkaline and amphiphilic peptides containing several lysine residues can not only penetrate into cell, but also form complexes with DNA and RNA, as well as that the binding of these peptides to DNA strengthens its double strand (Kubo et al., 2000). These oligopeptides also belong to the CPP family because they are intended for performing the transport function, i.e., the transfer of biologically active substances through the cell membrane (Morris et al., 2001; Martin et al., 2007; Ferrer-Miralles et al., 2008). In addition to the carrier function, these peptides are able to simultaneously condense DNA, block cell metabolism, and penetrate into the nucleus and bind cell receptors.

The direct interaction of a peptide with the membrane is determined by the electrostatic interaction of the positively charged side groups of arginine and lysine residues with the negative carboxyl groups of phosphatidylserine exposed on the outer side of the cytoplasmic membrane (Denisov et al., 1998). For the negatively charged (carboxyl) side groups of peptides, the binding sites are represented by positively charged groups on phosphatidylcholine and phosphatidylethanolamine. Thus, the main mechanism by which small peptides penetrate across the cytoplasmic membrane may be pinocytosis (Fig. 1).

An important experimental fact that confirms the ability of short peptides to penetrate into the cell was the finding that FITC-labeled di-, tri-, and tetrapeptides can penetrate not only into the cytoplasm, but also into the nucleus and nucleolus of HeLa cells. HeLa cells were incubated with FITC-labeled peptides for 12 h (Fedoreeva et al., 2010). In the samples of cell cultures treated with peptides, fluorescence was detected in the cytoplasm, nucleus, and nucleolus in the form of numerous small granules, whereas in the control samples, the fluorescence was not detected (Fedoreeva et al., 2010). The relative fluorescence intensity of different labeled peptides in the nuclei of HeLa cells varied. Fluorescence intensity was stronger when the cells were incubated with FITC-labeled peptides pinealon and epithalon and weaker after incubation with testagen. Consider the possible mechanism of penetration of short peptides into the nucleus in more detail. The nucleus of eukaryotic cells has a system of transport pores (nucleopores) formed by protein complexes nucleoporins. The inner diameter of nucleopores is about 50 nm; therefore, they are permeable to small, freely diffusing molecules with molecular weights of less than 3.5 kDa (Fig. 2) (Ohno et al., 1998).

Thus, the possibility of the penetration of short peptides with the characteristics listed in the table (charge, size, and hydrophobicity) through the cytoplasmic and nuclear membrane is well substantiated. The transport of substances is determined by the combination of their steric and physicochemical properties.

The table shows that the sizes of the molecules differ only slightly and are considerably smaller than the size of nucleopores. In the case of conventional passive diffusion, less hydrophilic substances diffuse more easily through the lipid bilayer and, at first glance, bronchogen has an advantage. However, the relatively high hydrophilicity (testagen, pancragen, chonluten) is determined by the presence of lysine and arginine residues, which are also amphiphilic moieties capable of enhancing at least the preference of association of a peptide with the membrane, if not its transport.

Main characteristics of short peptides developed at St. Petersburg Institute of Bioregulation and Gerontology

No.	Formula, name	Indications for use	Total charge	Molecular weight, kDa	Hydrophobicity index (Kyte et al., 1982)
1	Timogen (dipeptide)	Immunomodulator (Morozov et al., 1998)	–1	0.333	–4.4
2	Vilon (dipeptide)	Stimulation of tissue regeneration (Khavinson et al., 2000)	0	0.275	–7.4
3	Normophthal (dipeptide)	Regulation of the retina functions (Khavinson et al., 2002)	0	0.275	–7.4
4	Cartalax (tripeptide)	Regulation of functions of joints (Khavinson et al., 2008a)	–2	0.333	–5.2
5	Pinealon (tripeptide)	Regulation of the brain functions (Khavinson et al., 2009)	–1	0.418	–11.5
6	Chonluten (tripeptide)	Regulation of the respiratory system functions (Khavinson et al., 2008c)	–2	0.319	–7.4
7	Vezugen (tripeptide)	Regulation of vascular functions (Khavinson et al., 2010)	–1	0.391	–10.9
8	Epithalon (tetrapeptide)	Regulation of the neuroendocrine system (Khavinson, 2004)	–2	0.390	–5.6
9	Prostamax (tetrapeptide)	Regulation of the prostate functions (Khavinson et al., 2004)	–1	0.488	–12.5
10	Livagen (tetrapeptide)	Regulation of the liver functions (Khavinson, 2006)	–1	0.462	–9.1
11	Cortagen (tetrapeptide)	Regulation of the brain functions (Khavinson et al., 2007)	–2	0.430	–6.8
12	Pancragen (tetrapeptide)	Regulation of the pancreas functions (Khavinson et al., 2009a)	0	0.576	–11.8
13	Cardiogen (tetrapeptide)	Regulation of myocardial functions (Khavinson et al., 2008d)	–1	0.490	–9.7
14	Testagen (tetrapeptide)	Regulation of testicular functions (Khavinson et al., 2008b)	–1	0.448	–11.3
15	Bronchogen (tetrapeptide)	Regulation of bronchial functions (Khavinson et al., 2009b)	–2	0.446	–1.4

POSSIBILITY OF PEPTIDE BINDING TO DNA AND EPIGENETIC REGULATION OF GENE TRANSCRIPTION WITH INVOLVEMENT OF SHORT PEPTIDES

The obtained results allow us to consider the possibility of a direct interaction of short regulatory peptides with DNA. In recent years, molecular modeling is becoming increasingly popular for analyzing nanostructures, which include short peptides (Sokolova, 2012). We have proposed a molecular model of a complementary interaction of peptides and DNA double strand (Fig. 3).

The comparison of the spatial arrangement of functional groups on the surface of a major groove of a double-stranded DNA and the side groups of regulatory peptides showed that these polyampholytic structures are capable of multipoint interactions. Examples of this are the models of selective binding of synthetic oligopeptides vilon, livagen, and epithalon. In particular, peptide Ala-Glu-Asp-Gly can bind to a complementary site in DNA in the promoter gene region, causing local separation of strands and thus initiating the process of gene transcription by RNA polymerase II (Khavinson et al., 2003a, 2003b).

It has been experimentally demonstrated that the addition of tetrapeptide Ala-Glu-Asp-Gly into the culture of human lung fibroblasts induces the expression of the telomerase gene, stimulates telomerase activity, and promotes telomere elongation by a factor of 2.4. The activation of gene expression is accompanied by an increase in the number of cell divisions by 42.5%, which demonstrates that the Hayflick limit of cell division is overcome (Khavinson et al., 2003b, 2004).

Thus, some endogenous short peptides can be considered as epigenetic factors that perform natural regulation of gene expression (Khavinson et al., 2012).

The existence of specific binding of peptides to single-stranded oligonucleotides may be of particular importance for the epigenetic mechanism of regulation of gene expression (Fedoreeva et al., 2010). DNA always (or temporarily) contains single-stranded regions, which, in particular, appear during the replication, recombination, and repair of the genome.

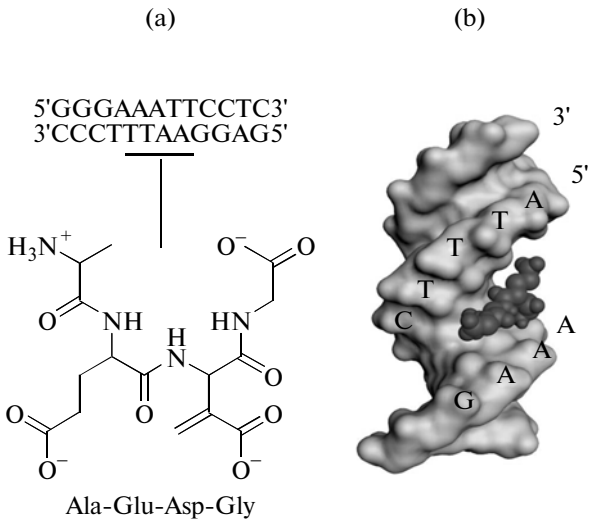

Fig. 3. Molecular model of a complementary interaction of the Ala-Glu-Asp-Gly peptide and the DNA double helix: (a) peptide structure and DNA sequence to which the peptide binds; (b) localization of the peptide in DNA major groove.

These genetic processes can be directionally regulated due to interaction of short peptides with such regions. In addition, the binding of short peptides (e.g., tetrapeptide epithalon) to DNA is accompanied by a local DNA unwinding of DNA strands, which may lead to the appearance of single-stranded targets for binding peptides to DNA. This is of special importance in the case of the possible combined action of different peptides in the cell, when some peptides function as inducers of single-stranded structures in the genome, whereas others are the actual initiating regulatory agents realizing the biological effect.

It was found that short peptides modulate the effect of endonucleases (Fedoreeva et al., 2010). Apparently, this is possible due to the site-specific peptide–DNA binding, which protects DNA from enzymatic hydrolysis. The modulation of the effect of endonucleases by peptides is in turn modulated by histones (histone H1) and, thus, histones of chromatin can definitely affect the binding of short peptides to DNA in the cell (nucleus). At the same time, some peptides (cardiogen) can apparently control the hydrolysis of DNA by endonucleases at the level of the interaction of the peptide with the enzyme.

Epigenetics postulates the tissue, subcellular, and age specificity of DNA methylation and suggests that the DNA methylation pattern in the cancer cells is different from that in the normal cells (Romanov et al., 2007; Fedoreeva et al., 2011; Vanyushin et al., 2011). Taking into account these facts, it can be assumed that the same biologically active short peptide may differently bind to DNA depending on its methylation pattern and will differently affect the gene function in various tissues (cells), in the nucleus and mitochondria, in young and old cells, and in both normal and malignant cells.

The phenomenon of the modulation of the effect of endonucleases by short peptides can be part of a global biological law, i.e., peptides that bind to DNA in a site-specific or complementary manner (particularly to the regulatory sequences) should modulate the function of nearly all or many specific proteins that interact with DNA (RNA and DNA polymerases, DNA methyltransferases, DNA base repair enzymes, and many regulatory proteins). According to one of the most probable mechanisms of gene activation by short peptides, peptides selectively bind to gene promoter regions, making them inaccessible to DNA methyltransferases; as a result, the promoter remains unmethylated, which is the crucial element of activation of the majority of genes.

Thus, specific (complementary) peptide–DNA interactions can epigenetically control the genetic functions of the cell. Apparently, this mechanism played a major role at the early stages of the origin of life and evolution.

The experimentally found possibility of penetration of short peptides into the cytoplasm, nucleus, and nucleolus and their involvement in the mechanisms of epigenetic regulation of gene expression provides an explanation for their high biological activity and successful application as therapeutic agents with a physiologically adequate effect on cells.

REFERENCES

Alberts, B., Bray, D., Lewis, J., Raf, M., Roberts, K., and Watson, J.D., *Molecular Biology of the Cell*, New York: Garland Science, 1994, pp. 561–563.

Anisimov, V.N. and Khavinson, V.Kh., Peptide bioregulation of aging: results and prospects, *Biogerontology*, 2010, vol. 11, pp. 139–149.

Ashmarin, I.P., Hormones and regulatory peptides: differences and resemblance of the concepts and functions. The place of hormones among other intercellular sensors, *Ross. Khim. Zh.*, 2002, vol. 49, no. 1, pp. 4–7.

Denisov, G., Wanaski, S., Luan, P. Glaser, M., and McLaughlin, S., Binding of basic peptides to membranes produces lateral domains enriched in the acidic lipids phosphatidylserine and phosphatidylinositol 4.5-bisphosphate: an electrostatic model and experimental results, *Biophys. J.*, 1998, vol. 74, no. 2, pp. 731–744.

Deigin, V.I., Semenets, T.N., Zamulaeva, I.A., Maliutina, Y.V., Selivanova, E.I., Saenko, A.S., and Semina, O.V., The effects of the EW dipeptide optical and chemical isomers on the CFU-S population in intact and irradiated mice, *Int. Immunopharmacol.*, 2007, vol. 7, no. 3, pp. 375–382.

Du Vigneaud, V., Ressler, C., and Trippett, S., The sequence of amino acids in oxytocin, with a proposal for the structure of oxytocin, *J. Biol. Chem.*, 1953, vol. 205, no. 12, pp. 949–957.

Duchardt, F., Fotin-Mleczek, M., Schwarz, H., Fischer, R., and Brock, R., A comprehensive model for the cellular

uptake of cationic cell-penetrating peptides, *Traffic*, 2007, vol. 8, pp. 848–866.

Fedoreeva, L.I., Kireev, I.I., Khavinson, V.Kh., and Vanyushin, B.F., Penetration of short fluorescence labeled peptides into the nucleus in HeLa cells and in vitro specific interaction of the peptides with deoxyribooligonucleotides and DNA, *Biochemistry*, 2010, vol. 76, pp. 1210–1219.

Fedoreeva, L.I. and Vanyushin, B.F., CNG site-specific and methyl-sensitive endonuclease WEN1 from wheat seedlings, *Biochemistry*, 2011, vol. 76, no. 6, pp. 651–657.

Ferrer-Miralles, N., Vázquez, E., and Villaverde, A., Membrane-active peptides for non-viral gene therapy: making the safest easier, *Trends Biotechnol.*, 2008, vol. 26, no. 5, pp. 267–275.

Frankel, A.D. and Pabo, C.O., Cellular uptake of the tat protein from human immunodeficiency virus, *Cell*, 1988, vol. 55, pp. 1189–1193.

Fuchs, S.M. and Raines, R.T., Pathway for polyargenine entry into mammalian cells, *Biochemistry*, 2004, vol. 43, pp. 2438–2444.

Futaki, S., Goto, S., and Sugiura, Y., Membrane permeability commonly shared among arginine-rich peptides, *J. Mol. Recognit.*, 2003, vol. 16, no. 5, pp. 260–264.

Ivanov, V.T. and Yatskin, O.N., Peptidomics: a logical sequel to proteomics, *Expert. Rev. Proteomics*, 2005, vol. 2, no. 4, pp. 463–473.

Khavinson, V.Kh., Tissue-specific effects of peptides, *Bull. Exp. Biol. Med.*, 2001, vol. 132, no. 8, pp. 807–808.

Khavinson, V.Kh., US Patent 6727227, 2004.

Khavinson, V.Kh., US Patent 7101854, 2006.

Khavinson, V.Kh., Bondarev, I.E., and Butyugov, A.A., Epithalon peptide induces telomerase activity and telomere elongation in human somatic cells, *Bull. Exp. Biol. Med.*, 2003b, vol. 135, no. 6, pp. 590–592.

Khavinson, V.Kh., Bondarev, I.E., Butyugov, A.A., and Smirnova, T.D., Peptide promotes overcoming of the division limit in human somatic cell, *Bull. Exp. Biol. Med.*, 2004, vol. 137, no. 5, pp. 503–506.

Khavinson, V.Kh., Grigoriev, E.I., Malinin, V.V., and Ryzhak, G.A., US Patent 7851449, 2010.

Khavinson, V.Kh., Grigoriev, E.I., Malinin, V.V., and Ryzhak, G.A., EEC Patent 2024388, 2009.

Khavinson, V.Kh., Khokkanen V.M., Trofimova S.V., and Malinin, V.V., RF Patent 2177801, 2002.

Khavinson, V.Kh. and Malinin, V.V., *Gerontological Aspects of Genome Peptide Regulation*, Basel, Switzerland: Karger AG, 2005.

Khavinson, V.Kh., Malinin, V.V., Grigoriev, E.I., and Ryzhak, G.A., US Patent 7491703, 2009a.

Khavinson, V.Kh., Malinin, V.V., and Ryzhak, G.A., EA Patent 010724, 2008a.

Khavinson, V.Kh., Malinin, V.V., and Ryzhak, G.A., EA Patent 010735, 2008b.

Khavinson, V.Kh., Morozov, V.G., Malinin, V.V., and Grigoriev, E.I., US Patent 7189701, 2007.

Khavinson, V.Kh., Ryzhak, G.A., Grigoriev, E.I., and Ryadnova, I.Yu., EEC Patent 1758922, 2008c.

Khavinson, V.Kh., Ryzhak, G.A., Grigoriev, E.I., and Ryadnova, I.Yu., US Patent 7625870, 2009b.

Khavinson, V.Kh., Ryzhak, G.A., Grigoriev, E.I., and Ryadnova, I.Yu., EEC Patent 1758923, 2008d.

Khavinson, V.Kh., Serv, S.V., and Morozov, V.G., US Patent 6139862, 2000.

Khavinson, V.Kh., Shataeva, L.K., and Bondarev, I.E., A model of interaction between regulatory peptides and DNA double helix, *Usp. Sovrem. Biol.*, 2003c, vol. 123, no. 5, pp. 467–474.

Khavinson, V.Kh., Shataeva, L.K., and Chernova, A.A., Effect of regulatory peptides on gene transcription, *Bull. Exp. Biol. Med.*, 2003a, vol. 136, no. 3, pp. 288–290.

Khavinson, V.Kh., Solovyev, A.Yu., Zhilinski, D.V., Shataeva, L.K., and Vanyushin, B.F., Epigenetic aspects of aging peptide regulation, *Usp. Gerontol.*, 2012, vol. 25, no. 1, pp. 11–16.

Kubo, T. and Yokoyama, R., Structure and affinity of DNA binding peptides, *Nucleic Acids Symp. Ser.*, 2000, no. 44, pp. 49–50.

Kyte, J. and Doolittle, R.F., A simple method for displaying the hydropathic character of a protein, *J. Mol. Biol.*, 1982, vol. 157, no. 1, pp. 105–132.

Lindgren, V. and Hallbrink, M., Cell-penetrating peptides, *Trends Pharmacol. Sci.*, 2000, vol. 21, pp. 99–103.

Martin, M.E. and Rice, K.G., Peptide-guided gene delivery, *AAPS J.*, 2007, vol. 9, no. 1, pp. 18–29.

Morozov, V.G. and Khavinson, V.Kh., US Patent 5814611, 1998.

Morris, M.C. and Depollier, J., A peptide carrier for the delivery of biologically active proteins into mammalian cells, *Nat. Biotechnol.*, 2001, vol. 19, pp. 1173–1176.

Myasoedov, N.F., Andreeva, L.A., Lyapina, L.A., Ulyanov, A.M., Shubina, T.A., Obergan, T.Y., Pastorova, V.E., and Grigorieva, M.E., The combined antidiabetogenic and anticoagulation effects of tripeptide Gly-Pro-Arg as estimated in the model of persistent hyperglycemia in rats, *Dokl. Biol. Sci.*, 2011, vol. 438, pp. 135–137.

Ohno, M., Fornered, M., and Mattaj, I.W., Nucleocytoplasmic transport: the last 200 nanometers, *Cell*, 1998, vol. 92, no. 2, pp. 327–336.

Ostrovskaya, R.U., Gruden, M.A., Bobkova, N.A., Sewell, R.D., Gudasheva, T.A., Samokhin, A.N., Seredinin, S.B., Noppe, W., Sherstnev, V.V., and Morozova-Roche, L.A., The nootropic and neuroprotective prolinecontaining dipeptide noopept restores spatial memory and increases immunoreactivity to amyloid in an Alzheimer's disease model, *J. Psychopharmacol.*, 2007, vol. 6, pp. 611–619.

Romanov, G.A., Naumkina, E.M., Ashapkin, V.V., and Vanyushin, B.F., Methylation of GCGG sites of the patatin promoter is organ-specific and inversely correlates with its activity, *Dokl. Biochem. Biophys.*, 2007, vol. 417, pp. 327–330.

Sokolova, O.S., Shaitan, K.V., Grizel, A.V., Popinako, A.V., Karlova, M.G., and Kirpichhikov, M.P., Three-dimensional structure of human voltage-gated ion channel Kv10.2 studied by electron microscopy of macromolecules and molecular modeling, *Russ. J. Bioorg. Chem.*, 2012, vol. 38, no. 2, pp. 152–158.

Vanyushin, B.F., Materialization of epigenetics or insignificant changes of DNA with great consequences, *Khim. Zhizn'*, 2004, no. 2, pp. 32–37.

Vanyushin, B.F. and Ashapkin, V.V., DNA methylation in higher plants: past, present and future, *Biochim. Biophys. Acta*, 2011, vol. 1809, no. 8, pp. 360–368.

Translated by M. Batrukova