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Epigenetic Aspects of Peptide-Mediated Regulation of Aging

**V. Kh. Khavinson[a, b], A. Yu. Solov'ev[b], D. V. Zhilinskii[b],
L. K. Shataeva[b], and B. F. Vanyushin[c, d]**

[a] *Pavlov Institute of Physiology of the Russian Academy of Sciences, nab. Makarova 6, St. Petersburg, 199034 Russia*
[b] *St. Petersburg Institute of Bioregulation and Gerontology, pr. Dinamo 3, St. Petersburg, 197110 Russia*
[c] *Belozerskii Research Institute of Physico-Chemical Biology*
[d] *Moscow State University, Moscow, 119991 Russia*

Abstract—Endogenous peptides in the cyto- and nucleoplasm are formed upon the specific proteasomal degradation of nuclear proteins. These peptides are formed by short blocks of amino-acid residues with charged side groups and therefore a high local concentration of electrostatic charge of either sign is characteristic of them. These peptides are capable of complementary binding to certain short nucleotide sequences in DNA strands. This binding can cause a significant weakening of the interstrand bonds in the double helix of DNA and therefore stimulate the splitting of strands, which is necessary for gene transcription and replication. Aging is always accompanied by a decrease in the degree of genome methylation. The age-related decrease of the degree of methylation of nucleotide repeat sequences in the genome promotes the site-specific binding of short peptides to DNA, which hinders the hydrolysis of non-methylated DNA fragments by endonucleases. The available experimental data on the peculiarities of binding to methylated DNA are indicative of the involvement of short peptides in the epigenetic regulation of aging processes.

Keywords: epigenetics, endogenous peptides, complementary binding, DNA methylation.

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Investigation of the structure–function relationships characteristic of bioactive compounds became established as a fundamental problem already at the beginning of the development of biochemistry. Biological information transfer from parents to progeny is known to be mediated by DNA replication, with the gene distribution following Mendel's laws. As well, changing environmental factors may affect the dominant feature or the characteristics of the organism (phenotype) and this effect can spread as an adaptation mechanism and persist in the progeny. However, the molecular mechanisms of the emergence and conservation of these changes upon the conservation of the genotype were not subjected to systematic study until recently. This situation is well illustrated by the following quote: "One must not forget that there are powerful regulatory systems in the body (both in the genome itself and among the cell systems) that control gene functioning. These signals are superposed onto genetic information and often provide their own answer to the fundamental question: to be or not to be? Even a perfect set of genetic parameters may remain hidden if the epigenetic factors are unfavorable (the Greek prefix *epi*- means *above*)" [3].

Epigenetics is a scientific discipline that studies the inherited properties of an organism that are not directly related to changes in the nucleotide sequence of DNA itself. In particular, this discipline addresses the mechanisms of genome modification that affect the course of gene activation or its activity and therefore underlie certain phenotypic effects.

Comparison of the genomes of organisms that belong to different phenotypic groups has shown that the process of evolution is associated not only with an increase in gene number and length, but also, and even more importantly, with the regulation and coordination of its activity becoming more complicated. The present review addresses certain questions of the origin of endogenous regulatory oligopeptides that enter the cell nucleus from the cytoplasm and participate in the regulation of transcription by interacting with DNA, including the gene promoter sites, directly.

The two approaches to analyzing the structure of peptide chains are the following: morphological analysis of protein macromolecules and the analysis of the amino-acid sequences of polypeptide chains.

Structural analysis provides a general characteristic of the "architecture" of polypeptide molecules. It allows for the detection of structural domains, which are certain densely packed chain fragments connected to the adjacent chain stretches and are more flexible and less ordered, in the macromolecule [56]. Structural modules can be considered a subclass of domains. The structure of highly mobile nuclear proteins can be considered an example [48].

Comparison of the conserved chain fragments and similar functional characteristics of the molecules allows one to refer certain proteins to groups. For

Table 1. The contents of proteins that contain charged clusters and blocks in the proteomes of different species, % [42]

species	Positively charged		Negatively charged		Mixed	
	clusters	blocks	clusters	blocks	clusters	blocks
H. sapiens	6.2	0.4	9.5	2.3	11.5	2.3
Drosophila	6.7	0.2	14.6	2.0	12.2	2.5
S. cerevisiae	4.6	0.0	8.5	1.2	12.3	2.8
Prokaryotes	<1.9	<0.2	<2.6	<0.3	<5.8	<1.6

Table 2. The number of DNA-binding transcription factors in various species [34]

Species	Number of transcripts in the proteome	Number of proteins containing DNA-binding domains
E. coli	4280	267
S. cerevisiae	6357	245
C. elegans	31877	1463
H. sapiens	32036	2604

example, the G-protein coupled receptors (muscarinic, opioid, adrenoreceptors, etc.) form a family with distinct signaling and pharmacological properties. Comparison of the structure of multiple proteins isolated from different sources allows for the detection of common features, i.e. conserved stretches of the polypeptide chain, in proteins that fulfill the same or related functions. These islands of stability in the sea of mutational variation are usually referred to as structural motifs, and sometimes as blocks or segments. Analysis of the amino-acid sequence is currently used for classifying proteins and referring them to certain families [59].

Analysis of the amino-acid sequences found in protein and peptide sequence databases allows for the use of *in silico* fragmentation of protein chains and comparison of the results with the fragmentation pattern of these proteins observed after specific hydrolysis in vivo, especially if the fragments formed are known to have a specific physiological function in the neuroendocrine system [4]. One distinctive feature of the suggested method for mathematical analysis and estimation of the highest possible number of fragments of naturally occurring proteins is the ability to not consider repeats of the same fragment in the proteome to be analyzed. We consider the presence of multiple oligopeptide repeats in a group of proteins the basis for the estimation of the informational value of oligopeptide blocks, in addition to being a sign of the functional unity of this group [22].

The studies of Karlin that address the peculiarities of the amino-acid residue distribution along the peptide chain demonstrated the existence of several types of repeating amino-acid residue blocks containing charged side chains. Amino acids containing positively or negatively charged side groups (Glu, Asp, Lys, and Arg) form charged blocks and clusters in protein and peptide chains. The existence of charged clusters is indicative of the non-random distribution of charged residues in the peptide chain and of very high local concentrations of opposite charges and the emergence of local dipole moments due to the close apposition of the negatively and positively charged side chains of the amino-acid residues in the block. The content of such blocks and clusters is the highest in the structures of transcription factors and high-mobility nuclear proteins (the difference from other proteins is statistically significant) [35, 41].

EKDKRERD is an example of a charged block sequence (the one-letter code is used here and below for the amino-acid residues); LPEDSAESDAPKRLKP is an example of a charged cluster and HPHSHPHAHPQ is an example of a histidine cluster.

A charged block includes only amino-acid residues with (similarly or differently) charged side chains; a charged cluster contains several hydrophobic amino-acid residues separating these blocks. It should be noted that the distribution of charge density along the peptide chain depends on the pH of the equilibrium aqueous solution, since the degree of side-chain amino and carboxyl group ionization is determined by the pH.

Data on the content of blocks and clusters in the proteomes of four evolutionarily advanced organisms, as well as the mean values for prokaryotes, are summarized in Table 1 [42].

Almost 25% of eukaryotic proteins contain charged clusters and 5% contain charged blocks; these values for prokaryotes equal 10 and 3%, respectively. The number of negatively charged clusters and blocks in the proteins all four classes of organisms is significantly higher than that of the positively charged.

Charged clusters and blocks are found in the most conserved sites of protein macromolecules, including the DNA-binding domains of nuclear proteins. The number of these clusters is the highest in high-mobility proteins, transcription factors, and centromere-associated proteins, as well as in proteins that control the developmental stages in Drosophila. Table 2 illustrates a correlation between the degree of evolutionary advancement of an organism and the number of proteins that contain DNA-binding domains [34].

The DNA-binding domains of transcription factors that are synthesized on the coding fragments of a certain genome are derived from a relatively small set of conserved ancient superfamilies and are highly variable among different phylogenetic groups. One can suppose that a significant share of these groups developed by extensive duplication of transcription factors and their target domains.

Table 3. Charged peptide blocks and clusters in the structure of RNA polymerase II transcription factors (*K*, *Lys*; *R*, *Arg*; *E*, *Glu*; *D*, *Asp*)

Transcription factor	Number of amino-acid residues	Charged clusters and blocks	Reference
*Tf*1	395*aa*	*IEDI AEKL KEDE MEDQ LKDQ LDES*	[40]
*Tf*2	125*aa*	*AEDA KEX2 PKEDKEN VDEL TEDH*	[24]
*Tf*3	622*aa*	*AEDV VERV PEDR VEDF GDKKEP GDES PEDP AREG*	[57]
*Tf*4	961*aa*	*CKEDL NKEL IEKT LEKL GREDQ TDEDDH PDKEP ADEM PEKKL PDREG NEDEG LEKP PDERDI*	[53]
*Tf*6	560*aa*	*VEDI IEKY KEDA SKEF SEDG NKEW LEDQ WEKEV KEX5 EW*	[65]

We detected charged clusters and blocks in the structure of several transcription factors involved in the biosynthesis of RNA polymerase II. Data on the content of clusters and blocks in the amino-acid sequence of transcription factors involved in the biosynthesis of RNA polymerase II are presented in Table 3.

It should be noted that most clusters contain a negatively charged ED nucleus or a bipolar KE/EK nucleus.

The properties of endogenous oligopeptides that were isolated from the extracts of various organs of cattle, including neurohormones, immunostimulators, repair stimulators, and geroprotective peptides, have been in the focus of researcher attention for the past two decades. These studies were of both fundamental and practical significance because many of the tissue-specific peptide preparations isolated from the organs of young animals became widely used in medicine [14].

The origin of the endogenous pool of short regulatory peptides in a young organism became obvious after the discovery of specific ubiquitin-mediated proteasomal degradation of nuclear proteins ("Ubiquitin-mediated proteolysis," the Nobel prize in chemistry in 2004) by Ciechanover, Gerschko, and Rose. Proteasomes are high-molecular weight multisubunit proteoltytic complexes that play the principal role in the ordered degradation of proteins in the cells of evolutionarily advanced organisms [62]. Each proteasome particle contains two chymotrypsin-like, two trypsin-like, and two caspase-like proteolytic centers. These centers are supposedly connected by a network of allosteric interactions that order the sequence of proteolytic events. For example, caspase-like centers (sometimes referred to as postglutamyl peptide hydrolases) cleave bonds formed by the carboxyl group of aspartate residues more efficiently than those formed by the carboxyl group of glutamate residues [47].

Thus, a single high-molecular weight protein can be hydrolyzed in several different ways by the proteasome and this leads to the formation of a range of short peptides. The peptides thus formed can have functions that are completely different from those of the parent macromolecule [33, 39]. As the body ages, proteasomal structure changes and the hydrolytic efficiency of the proteasomes decreases [38]. Short biologically active peptides can form either as a result of specific protein hydrolysis or as a result of specific synthesis i.e., the translation of the so-called small RNAs and RNA fragments formed from the noncoding (regulatory) parts of the genome.

The administration of endogenous tissue-specific polypeptides isolated from young animal tissues into the aging body can probably compensate for the deficiency of the peptide pool caused by proteasome dysfunction. Such oligopeptides display geroprotective properties [27].

Methods for the selective isolation and purification of low-molecular weight peptides from the organs of cattle were developed by Khavinson et al. in the early 1970s. Peptides isolated from the endocrine regulatory organs (thymus and epiphysis) that control the immune system of the body were the first to be analyzed. These thymus and epiphysis extracts and the low-molecular weight peptides isolated from them enhanced immunity to pathogens, displayed antitumor activity, and contributed to the increase of the average lifespan of animals [26, 30, 36]. Short peptides isolated from other tissues also displayed tissue-specific activities and restored various physiological functions in old animals [8, 9].

A range of experiments demonstrated the principal advantages of low-molecular weight peptides, namely, tissue specificity, lack of species specificity, and lack of immunogenicity. These features of regulatory peptides are similar to those of peptide hormones [25, 32]. The information thus obtained was used for the chemical synthesis of a range of short peptides that are analogous to peptides of natural origin [60]. The biological activities of natural and synthetic preparations were shown to be identical. For example, the peptide Glu—Trp enhanced immunity when used in different therapeutic forms [28, 55]. The biological activity of natural and synthetic peptides was similar in standard animal tests performed in rats and mice [29, 31], as well as in tests performed with organotypic tissue cultures [44].

Clinical trials of peptide preparations demonstrated their distinct geroprotective action [6, 7, 43].

Aging is accompanied by multiple changes at the cell level, in addition to the weakening of immunity. For example, the structure of the cell nucleus changes. The DNA–protein complex of the cell nucleus (chromatin) self-organizes into chromosomes upon cell division. In the stationary state the nuclear chromatin is represented by two variants, euchromatin and heterochromatin [49]. Heterochromatin is usually found on the periphery of the nucleus and mostly contains the inactivated part of the genome, i.e., genes blocked by repressors. The volume of heterochromatin in the nucleus increases with aging (on average, from 63 to 80%); this causes the downregulation of protein synthesis in the cell. The synthetic peptide Ala–Glu–Asp–Gly promotes a decrease of heterochromatin content in the nucleus and makes more genes available for transcription factors, this ultimately leading to enhanced transcription and upregulation of protein synthesis [2, 15].

Aging of various eukaryotes (such as fish and mammals) is always accompanied by a global tissue-specific decrease of the 5-methylcytosine content in the DNA, i.e. by a decrease of the genome's methylation level [1, 5, 63], which is more pronounced in the case of repeats than in the case of unique genomic sequences [64]. An age-related decrease of the degree of genomic repeat methylation obviously plays a regulatory role, since DNA methylation is one of the epigenetic regulatory mechanisms of the genetic processes (transcription, replication, recombination, and repair of DNA) and cell differentiation. Unfortunately, the effect of short peptides on DNA methylation is virtually uncharacterized, but peptides are supposed to play a role in the control of DNA methylation [19].

The number of chromosome aberrations is used as a marker of DNA damage in the aging body. Somatic mutations may emerge as a result of the accumulation of stable aberrations and underlie age-related pathologies, including malignant tumors. The antimutagenic and repair-promoting effects of the synthetic peptides Lys–Glu and Ala–Glu–Asp–Gly were confirmed by the decrease of the number of chromosomal aberrations in corneal epithelial cells of mice that exhibit accelerated aging [10, 46].

Incubation of cultured human lung fibroblasts with the peptide Ala–Glu–Asp–Gly at 30°C for 30 min induces the expression of the telomerase gene and telomerase activation, this ultimately leading to a 2.4-fold increase in telomere length. Activation of gene expression is accompanied by a 42.5% increase in the number of cell divisions, leading to the surpassing of Hayflick's limit of cell division [12, 13]. These results correlate with the previously reported maximum increase of the lifespans of animals (by 42.3%) after the administration of this peptide. Importantly, these results imply a direct site-specific interaction of this peptide with promoter sites in the DNA.

The possibility of oligopeptide permeation from the environment into the cell nucleus through the cytoplasm was doubted for many years. Detailed studies of the structure of eukaryotic nuclear membranes demonstrate the existence of a sophisticated system of transport pores (nucleopores) formed by protein complexes, the nucleoporins. The inner diameter of nucleopores is approximately 42 nm; this makes them easily permeable for freely-diffusing low-molecular-weight compounds with a molecular weight lower than 1000 Da [50, 58]. A class of specific lysine- (or arginine-) rich peptides, which is referred to as cell-penetrating peptides (CPP), that are capable of entering the cell by pino-or endocytosis is presently known [52]. These peptides are considered efficient cargo transporters that are capable of delivering nucleic acids and various pharmaceuticals into the cell.

Thus, the possibility of short peptide penetration into cells cannot be doubted.

A study of the intracellular localization of short peptides that carried a fluorescent label was performed recently. HeLa cells were incubated with FITC-labeled peptides for 12 h. Cell preparations were imaged using a fluorescent microscope. Fluorescence was detected in the cytoplasm (as multiple small granules), nucleus, and nucleolus [11]. The relative intensity of fluorescence in the nuclei of HeLa cells varied for different labeled peptides, being quite pronounced in the case of the FITC-labeled peptides Glu–Asp–Arg and Ala–Glu–Asp–Gly, and somewhat lower in the case of the peptide Lys–Glu–Asp–Gly. Thus, short peptides are indeed capable of penetrating the cell nucleus and nucleolus, which enables their interaction with both DNA and RNA (since ribosomal RNA is synthesized in the nucleolus).

The obvious gap between the considerable amount of data that prove the specific effects of regulatory peptides in the activation of gene transcription and the very limited understanding of the biochemical processes that underlie the selective binding of the transcription factor with specific DNA sites has existed for a long time. Computer models of the interaction of each four nucleobases with individual amino acids have been constructed. The interactions were the strongest for the following pairs: Lys–G, Lys–T, Arg–G, Asp–C, and Asp–A [51].

It is necessary to note that the structure of each amino acid has specific spatial and electrochemical features. The same is true for the nucleobases. However, biologically relevant interactions between these two types of molecules occur when they are included into heterogeneous polymeric chains. The specific order of the elements in these chains accounts for the recognition and selective interaction of polypeptides and nucleotides.

The following fundamental properties of amino acids are conserved upon the formation of the peptide bond:

– Optical activity caused by the chiral structure of the amino-acid residues;

– Ionizability of the carboxyl and amino groups, and;

– The ability to form intramolecular hydrogen bonds.

The individual ratio of hydrophilic and hydrophobic side chains in the peptide is determined for each fragment of the amino-acid sequence upon the formation of a peptide molecule from amino-acid residues.

A simple dipeptide is highly polarizable and has a constant dipole moment due to a specific spatial distribution of different electric charges and a dipole moment of 3.5 D characteristic of the peptide bond. These acquired characteristics confer a broader range of energetic states to dipeptides as compared to the individual amino acids. However, this is compensated by a decrease of the number of the spatial degrees of freedom of the system. Each of the individual amino-acid molecules has six degrees of freedom (three translational and three rotational) in solution; therefore two non-interacting molecules have twelve degrees of freedom, while a dipeptide formed by these molecules has only six. Statistical thermodynamics regards this as ordering of the system, which corresponds to an entropy decrease.

Complementarity of the mobile conformations is a novel feature acquired by amino acids upon the formation of a peptide chain; this feature merits a more detailed discussion.

The concept of complementarity originates from the early naturphilosophical theories. Two descriptive concepts, "similar to similar" and "opposites attract," were formed during the development of alchemy. The former was based on the experience of substance separation and purification, and the latter, on the observations of chemical transformations that reveal the interaction of chemical elements with opposite properties and the complementation of these properties. The "similar to similar" concept developed into the contemporary theory of hydrophilic and hydrophobic interactions, while the complementarity principle is illustrated by the attraction of positive and negative charges in electrostatics, the alignment of convex and concave surfaces, and the relationship between the elements of an object and is reflection in the mirror (matrix complementarity of early polygraphy). In all these examples "opposites" are represented by unchanging objects.

Biomacromolecules, and peptides in particular, maintain the complementarity of the intermolecular interactions in a rather wide range of conformations. This feature underlies the catalytic activity of enzymes; the "lock-and-key" model of complementary interactions was proposed in the early years of enzymology. The same model was later used in the discussion of the specificity of antigen-antibody binding and selective ligand–receptor interactions.

The model of matrix complementarity of nucleotide pairs was first developed in nucleic-acid research. This model allowed for the demonstration of precise copying of the macromolecular structure, with the parent molecule serving as the matrix for the daughter molecule formation.

The principle of matrix complementarity is simple and elegant: it implies that every element of the ordered molecular structure (electrostatic charge, proton donor or hydrophobic group) is reproduced in another molecule that is complementary to the first molecule.

Matrix complementarity was detected upon in-depth analysis of peptide synthesis, phospholipid bilayer membrane formation, and polymer self-assembly [23].

We have proposed a molecular model of interaction between short peptides and the double strand of DNA in the promoter site of a gene. The molecular model is based on the geometric and chemical complementarity of the amino-acid sequence of the peptide and the nucleotide pair sequence of the DNA. Each nucleotide pair sequence in the double DNA strand forms a unique pattern of proton–donor, proton–acceptor and hydrophobic groups on the surface of the major groove of the DNA double helix. The average distance between the nucleotide pair planes equals 3.4 Å, which is similar to the distance between the α-carbon atoms of a peptide. A short peptide recognizes a specific site in the DNA double helix if the functional group sequence in this peptide is complementary to a sufficiently long nucleotide sequence of the DNA, in other words, if the interaction is specific due to sequence matching [20, 21].

A peptide in the stretched β-conformation can be complementarily bound to the major groove of DNA along the axis of the double helix. Published data on the molecular geometry of the double helix and the peptide β-strand were used to detect the specific nucleotide pair sequence binding the Ala–Glu–Asp–Gly peptide. The screening showed that this tetrapeptide can bind to the major groove of the DNA fragment with the sense strand sequence ATTTG or ATTTC, due to the complementary localization of the functional groups [45].

Experiments with synthetic [poly(dA-dT): poly(dA-dT)] DNA preparations that form a double helix and the Ala–Glu–Asp–Gly tetrapeptide were performed to test the molecular model. Gel chromatography of the solution containing both DNA and peptide proved that the Ala–Glu–Asp–Gly peptide forms a stable intermolecular complex with double-stranded DNA (Fig. 1) [16].

As shown in Fig. 1, some tetrapeptide molecules are eluted together with the high-molecular weight DNA during gel chromatography (elution volume 20 ml), while the elution volume of the free tetrapeptide equals 29 ml.

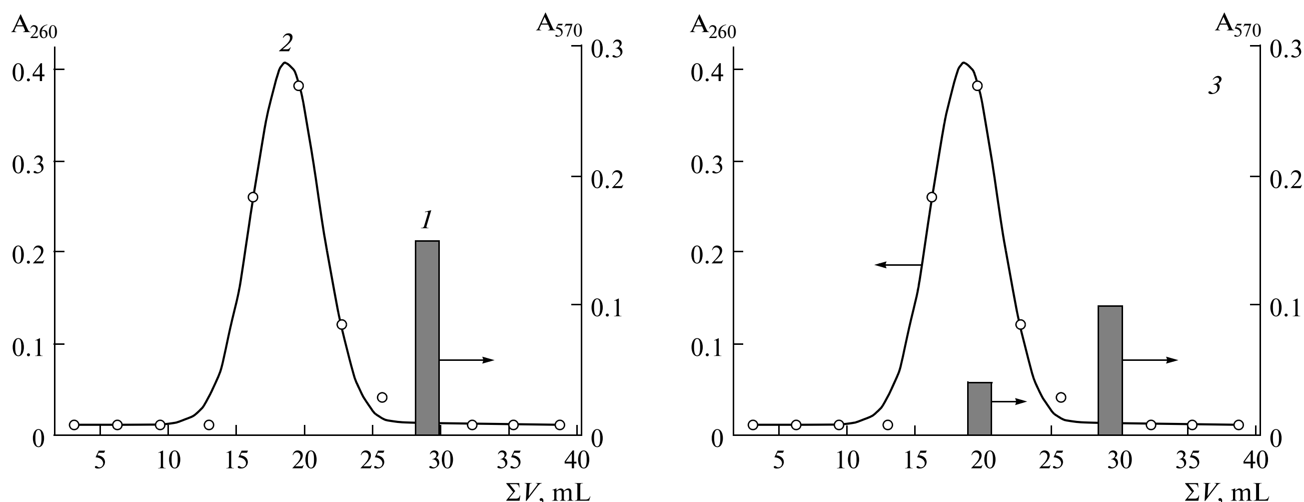

Fig. 1. Gel chromatography of the peptide and DNA on Sephadex G-25 in physiological saline at room temperature: 1, gel chromatogram of the free Ala–Glu–Asp–Gly peptide; 2, gel chromatogram of the double-stranded DNA sample [poly(dA-dT):poly(dA-dT)]; 3, gel chromatogram of the DNA–peptide mixture.

Complementary binding of the peptide with the TATATA nucleotide sequence on the sense chain of the double-stranded DNA involves six hydrogen bonds and one hydrophobic bond between the functional groups of the interacting molecules.

Conformational changes in the peptide-bound DNA were analyzed using UV spectrometry. For this, peptide and DNA solutions were mixed so that the final concentration of the DNA ranged from 20 to 25 μg/mL and that of the peptide ranged from 1.0 to 1.4 μg/mL. It is necessary to note that the complex formation was analyzed at ultralow concentrations of the interacting components.

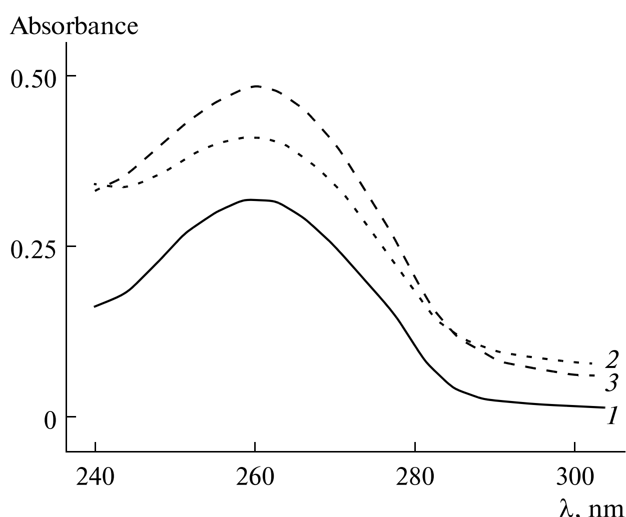

Fig. 2. UV spectra of the solution of the double-stranded DNA (1) and DNA mixtures with the peptide a ratio of one peptide molecule per nine (2) or fifteen (3) nucleotide pairs.

UV spectra of the solutions containing the double-stranded DNA and the peptide are indicative of an increase of molar extinction of DNA at 260 nm (ε_{260}) due to the binding of the regulatory peptide (hyperchromic effect); however, the maximum absorbance wavelength is not shifted. The UV spectra of the free double-stranded DNA (1) and the complexes of peptide and DNA at different component ratios (2, 3) are shown in Fig. 2. Since the Ala–Glu–Asp–Gly regulatory peptide does not absorb ultraviolet light in the wavelength range we investigated, the hyperchromic effect can be explained by the local division of the strands in the DNA double helix due to peptide binding [17].

DNA is known to form a double helix that is held together by hydrogen bonds between nucleobase pairs under normal physiological conditions. Most biological processes involving DNA (such as transcription and replication) require the splitting of strands that form the double helix. For example, the local dissociation of strands that form the double helix is known to precede the transcription of genes by RNA polymerase. The beginning of transcription (matrix RNA synthesis) requires the removal of histones from the DNA double helix and the dissociation of chains that form the double helix in the transcription start site.

In other words, the Ala–Glu–Asp–Gly peptide can bind to the complementary fragment in the promoter site of the gene, inducing local splitting of chains and promoting the initiation of gene transcription by RNA polymerase. This was proven by the initiation of the telomerase gene in somatic cells by the peptide [12, 13].

UV spectra of the DNA and peptide solutions, as well as those of the mixtures, were registered at peptide concentrations that ranged from 1 to 70 μM. Since the

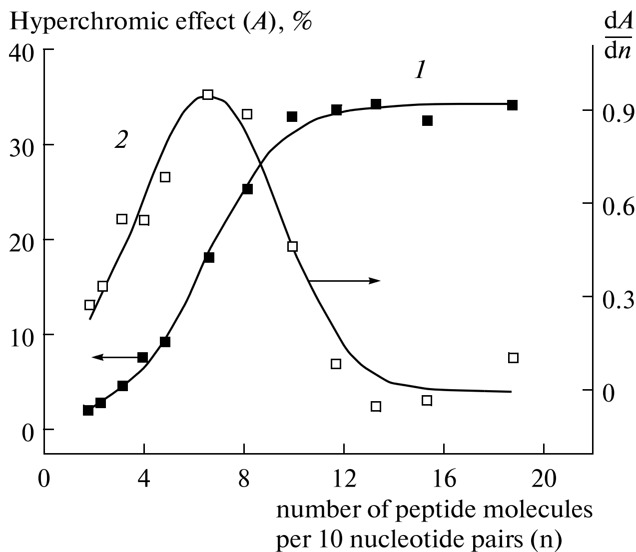

Fig. 3. Correlation between the hyperchromic effect (A) and the number of peptide molecules (n) per ten nucleotide pairs in the DNA (1) and between the first derivative of the hyperchromic effect dA/dn and the number of peptide molecules added (2).

molecular weight of the DNA preparation we used ($S_{20,w} = 12$) is at least two orders of magnitude higher than that of the peptide, the DNA concentration was expressed in moles of base pairs (bp) per unit volume and ranged from 28.5 to 32 μM.

The correlation of the hyperchromic effect A (an increase of OD_{260}) and the number of the peptide molecules added (n) per 10 bp of DNA is illustrated by Fig. 3 [16].

The concentration dependence detected is a saturable isotherm. The initial part of the isotherm reflects the selectivity of peptide binding to the DNA, while the maximum value of the derivative (dA/dN) defines the concentration range (six peptide molecules per ten base pairs) of the strongest multisite binding of the DNA and peptide. The binding of the two polymer molecules occurs due to the hydrophobic interactions between the methyl groups of alanine and thymine and the polar interactions of the three carboxyl groups of the peptide with three adenine residues, namely with the proton-accepting ^{7}N atoms and the proton-donor amino groups at the ^{6}C carbon atom. This binding causes a local splitting of the strands that form the double helix in adjacent DNA fragments. The energy that is required for this process is compensated by the strong polar interactions of the nucleobases with the side chains of the glutamic and aspartic acid residues of the tetrapeptide.

Oligopeptide binding to DNA induces both local changes in the structure of the double helix and an integral change of the strength of all interstrand bonds, ultimately leading to a change of temperature of heat denaturation of the DNA [37].

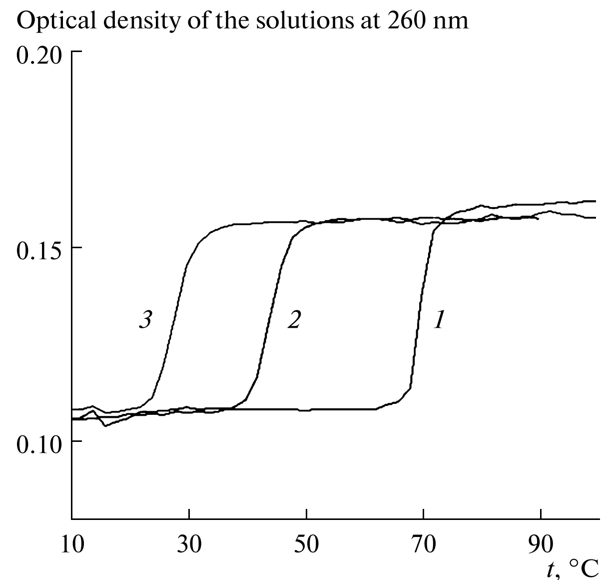

Fig. 4.

The phenomenon of the heat denaturation of DNA is currently used for the study of the selective interactions between base pairs (bps) in short double helices of 10–15 bp in length. As well, the melting curve of the DNA is used for the assessment of the energy of DNA binding to the nuclear proteins of the high mobility group (HMG) enriched in glutamate and aspartate residues [54].

We analyzed the melting of free high-molecular weight DNA and compared it to the melting of DNA in the presence of glutamic acid or the tetrapeptide in a saturating concentration. Dissociation of the chains of free double-stranded DNA, which is also known as helix melting or helix-bundle conformational transfer, occurs upon an increase in temperature and is accompanied by a hyperchromic effect [17]. The melting curves for a free double-stranded synthetic DNA [poly(dA-dT):poly(dA-dT)] (1) and its complexes with glutamic acid (2) or the tetrapeptide Ala–Glu–Asp–Gly (3) are shown in Fig. 4.

Different peptides we analyzed bind to single- or double-strained oligonucleotides or DNA [11]. This type of DNA–peptide binding is referred to as allosteric interaction of biopolymers. We have demonstrated that the effects of peptides on the fluorescence of FAM-labeled oligonucleotides and DNA complexes with ethidium bromide vary and depend on the primary structure of the peptides. Stern–Follmer constants for the quenching of the fluorescence of labeled single- and double-stranded deoxyribooligonucleotides of various structure vary considerably and depend on the primary structure of the peptides [11]. This is indicative of specific interactions between various short peptides and nucleic structures. Peptides are able to discriminate between certain nucleotide

sequences and between sequences that differ only by the degree of cytosine methylation upon the binding. The constants of fluorescence quenching allow for the conclusion that epithalon, testagen, pinealon, and bronchogen preferably bind to CNG-containing deoxyribooligonucleotides (cytosine residues in the CNG sites are targeted by eukaryotic DNA methylases), with epithalon, testagen, and pinealon preferring CAG-containing structures, and bronchogen preferably binding to CTG-containing structures.

In addition to lowering the stability of the DNA double helix, the binding of short peptides to the double helix can modulate the hydrolytic activity of endonucleases. For example, the binding of the Ala–Glu–Asp–Gly peptide suppresses the hydrolysis of nonmethylated DNA by the WEN2 endonuclease, while in the presence of the Ala–Glu–Asp–Leu peptide the DNA is cleaved into fragments of about 140 nucleotides in length. In other words, peptide binding to certain DNA sites can affect the efficiency of enzymes that compete for the same sites, endonucleases among them [18]. The specific binding of peptides to single-stranded oligonucleotides we detected [19] can have a special significance. DNA always contains (transiently appearing) single-stranded sites, which are especially numerous during the replication, recombination, or repair of the genome. Interaction of short peptides with these particular strands can be involved in the direct control of these genetic processes. As well, the binding of short peptides (epithalon) to the double helix of DNA is accompanied by the local unwinding of the DNA helix, resulting in the formation of single-stranded target sites for peptide binding to DNA. This is especially important for the simultaneous action of multiple peptides in the cell, with certain peptides inducing the formation of single-stranded structures in the genome and enabling other peptides to initiate the regulatory process underlying a biological effect.

We discovered tissue, subcellular, and age-related specificity of DNA methylation [61] and were the first to demonstrate the difference between the character of DNA methylation in normal and cancer cells [5]. Taking these facts into account, we state that the same bioactive short peptide can bind to DNA in different ways, which depend on the methylation state of the DNA; therefore, the effects of this peptide on gene function can be different in different tissues (cells), in the nuclei and mitochondria, in young and aged cells, and in normal and malignant cells. Almost all of these hypotheses have been confirmed by experiments. We assume that the (site-specific) peptide binding to DNA affects the functioning of various DNA-modifying proteins (RNA and DNA polymerases, DNA methyltransferases, endonucleases, enzymes of DNA repair systems, various regulatory proteins, transcription factors, etc.) that compete for the same binding sites. For example, we have recently shown that short peptides modulate the functioning of CNG-specific eukaryotic endonucleases [18]. Some hexapeptides are known to be specific structural ligands for protein-free Holliday junctions and therefore are able to block recombination. We proposed one of the most plausible mechanisms of gene activation by short peptides [18]: selective binding of the peptide to the CNG or CG promoter sites can render these sites unavailable for DNA methyltransferases; therefore, the promoter remains unmethylated. This plays a decisive role in the activation of most genes.

These experiments illustrate the principle of economy followed by the living organisms: nothing is unnecessarily complicated. For example, short peptides that form upon endogenous limited proteolysis of the protein molecules or are synthesized in the cell can be involved in gene activation and the regulation of DNA hydrolysis by specific endonucleases. The polyfunctional effect of short peptides on gene expression may result in the change of dominance in paired alleles (i.e., make the role of the recessive allele more important) and modify the developmental variation of the same feature in ontogenesis. The epigenetic mechanisms of genome regulation probably involve a certain sequence (cascade) of biochemical reactions, which accounts for the virtual irreversibility of the biological processes and the conservation of the vector of the evolutionary development. To summarize, the site-specific (allosteric) interactions of peptides and DNA can be involved in the epigenetic control of cell function and they are likely to have played an important role in the expression of genetic information at the earliest stages of the emergence of life, as well as in the course of evolution.

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