CHAPTER 20

Short Peptides Regulate Gene Expression, Protein Synthesis and Enhance Life Span

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20.1 Introduction

There is accumulated evidence that many of the so-called "diseases of aging", including cancer, are caused by dysregulated immune functions and decreased organism resistance to infections.[1] Peptide extracts of thymus and peptides isolated from thymus were the first preparations proposed for correction of immunodeficiency.[2,3] The origin of short regulatory peptides in the organism became obvious after the discovery of protein degradation in proteasomes.[4,5] The same high-molecular proteins can be differentially hydrolyzed resulting in various short peptides. The peptides produced show different biological functions as compared to the original macromolecules.[6]

Karlin and Altschul demonstrated in their work that protein macromolecules contain several types of recurrent blocks of amino-acid residues with charged side chains. Such blocks are mostly observed in nucleoproteins. Among them, there are transcription factors, centromere proteins and high

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mobility group.[7] Proteasome hydrolysis of these nucleoproteins can provide a sufficient amount of peptides with charged side chains. These and some other investigations gave rise to the development of the peptide bioregulation concept.[8,9] This concept suggests that low-molecular peptides are involved in intercellular transfer of information encoded in the amino acid sequence and conformation modifications, thus facilitating regulation of proliferation, differentiation and intercellular interaction.[8,9] Peptide bioregulators were isolated from different tissues. Their major function consists of normalizing the functions of the organs from which they have been isolated. They can also substitute and/or complement biologically active compounds secreted in this morphological structure.[8]

Apart from immunity dysregulation, aging causes other alterations on the cellular level, for example, accumulation of mutations in somatic cells.[10] Although the rate of accumulation of age-specific changes is determined genetically,[11] there are a number of exogenous factors that accelerate this process. Oxidative stress is considered to induce both cell and body aging.[12] Proteins and DNA are known to be damaged by reactive oxygen species (ROS). There is substantial evidence of the role of DNA oxidative damage in organismal senescence.[12–14]

Aging-associated accumulation of somatic mutations is accompanied by the decreasing of DNA repair level, which leads to growing incidence of pathologies including cancer.[15] Higher concentration of damages in heterochromatin regions as compared to active (euchromatic) regions of the DNA can be explained by the fact that reparation can occur only in the DNA regions that are involved in active transcription and are accessible for reparation enzymes.[16] This corresponds to intensive DNA reparative synthesis in the G2 cycle with more active chromosome heterochromatinization, as compared to in the G1 cycle.[17] The frequency of sister chromatid exchanges (SCE) confirms age-related reduction of reparation level. The SCE level in fibroblasts and lymphocytes of the elderly (60–70 y.o.) was found to be lower than that of the younger donors (30–40 y.o.), regardless of their gender.[18] Thus chromosome heterochromatinization and related decrease in the DNA reparation intensity is considered to be a key factor in organismal aging.[19]

Various experimental models have been used for studying preparations with a protective effect against aging and carcinogenesis.[20] In several animal studies, short peptides were demonstrated to be promising in anti-aging medicine. Their geroprotective and anticarcinogenic effects are believed to be mediated by their immunomodulatory and antioxidative properties.[21] The peptides were shown to lead to the reduction of the level of age-related chromosome aberrations (ChA)[22] and to affect the chromosome heterochromatinization,[19] thus retarding the aging process. They can influence the expression of various genes,[23] which is determined by specific short peptides–DNA binding.[24,25] Safety of long-term administration is one of the main advantages of peptide therapy. These properties make them promising candidates for clinical application in old and senile patients.[26] Further investigation of peptide bioregulators appears to be very perspective in modern gerontology, bearing in mind their capacity to inhibit senescence and restore functions of the aging organism.

20.2 Isolated Peptide Complexes

One of the first polypeptide preparations isolated from the calf thymus was Thymalin.[27,28] This polypeptide was able to restore disturbed immunological responsiveness, improve cell metabolism and stimulate cell immunity, regeneration and haemopoiesis (in case of their suppression). It displayed geroprotective properties and increased mean life span in experimental animals.[14,29,30] The important feature of polypeptide preparations is their anti-carcinogenic activity. This property of Thymalin was reported in experiments on induced and spontaneous carcinogenesis. Rats with 7,12-dimethyl-benzantracene (DMBA)-induced carcinogenesis treated with Thymalin revealed decreased tumour incidence by 24% and reduced the number of mammary adenocarcinomas 3.8-fold as compared to the control animals.[21] Administration of Thymalin to irradiated mice and rats for ten days, twice daily, decreased the number of malignant neoplasia. At the same time, the mice exposed to fraction irradiation and treated with Thymalin showed a 3.5-fold decrease in the number of tumors as compared to the irradiated control (Table 20.1).

Prolonged administration of Thymalin to SHR mice starting from 4 months of age resulted in a significant decrease of spontaneous tumor incidence—40% as compared to 55% in the controls. C3H/Sn mice treated with

Table 20.1 Effect of peptides on experimentally induced tumors in rodents.[a,b,c]

Peptides	Animal species/strain	Carcinogenic action	Tumor site/ localization	Tumor incidence%	
				Control	Peptide
Epithalamin complex of pineal peptides	Rats	DMBA	Mammary gland	81	26[d]
		X-ray irradiation	Mammary gland	16	3[d]
Thymalin complex of thymus peptides	Rats	DMBA	Mammary gland	69	18[d]
		X-ray irradiation	Mammary gland	21	3[d]
	C3H mice	X-ray irradiation	Mammary gland	38	14[d]
			Leukemia	46	14[d]
Thymogen/ Glu–Trp (EW)	Rats	Isotopes ^{90}Sr and ^{137}Cs	Any malignant tumors	16	8[d]
Vilon/Lys–Glu (KE)	CBA mice	DMH	Kidney	60	14[d]
Epitalon/ Ala-Glu-Asp-Gly (AEDG)	C3H/He mice	MMTV	Mammary gland	9	5[d]
	Female rats	Constant lighting	Mammary gland	41	27[d]
	Male rats	Constant lighting	Leukemia	12	0

[a]DMBA: 7,12-dimethylbenz(*a*)anthracene;
[b]DMH: 1,2-dimethylhydrazine;
[c]MMTV: mouse mammary tumor virus;
[d]The differences are statistically significant compared to the control by $p < 0.05$.

Thymalin during their life starting from 3.5 months of age exhibited a 2.8-fold decreased spontaneous tumors incidence and a 2.6-fold decreased incidence of mammary adenocarcinomas. At the same time, the experimental mice did not develop leukemia, while in the control group this pathology was registered in 14.3% of female mice (Table 20.1).[31,32]

Long-term administration of Thymalin led to the increase of the life span in mice: mean life span increased by 28% ($p < 0.05$) as compared to the control animals and maximum life span increased by 11% (Table 20.2).

Table 20.2 The effect of peptides on animal life span (LS).

	Life span indices (days)			
Species of animals or mice strain; effect	Mean life span (MLS)	Mean life span of last 10% survivors	Maximum life span	The rate of aging[a] ($days^{-1}$)
Rats				
Control	681 ± 14.5	835	1054	6.8
Epithalamin	852 ± 33.8[d]	1050	1112	3.8
%	+25	+27	+6	−44
SHR mice				
Control	564 ± 22.3	750	843	No differences
Epithalamin	627 ± 20.9[b]	750	827	
%	+11	0	−2	
C3H/Sn mice				
Control	487 ± 29.4	691	776	7.0
Epithalamin	640 ± 33.1[c]	757	885	5.1
%	+31	+20	+14	−27
C3H/Sn mice				
Control	487 ± 29.4	No data	776	No data
Thymalin	623 ± 24.6[b]		863	
%	+28		+11	
Rats				
Control	773 ± 18.4	949	965	7.08
Thymogen	786 ± 26.2	1048	1104	4.12
%	+2	+10	+14	−42
CBA mice				
Control Vilon	685 ± 9.2	737	740	No data
%	694 ± 12.5	761	792	
	+1.3	+3	+7	
SHR mice				
Control	456 ± 29	709	740	4.5
Epitalon	455 ± 31	803	1053	3.2
%	−0.2	+13	+42	−29
CBA mice				
Control	685 ± 9.2	737 ± 1.1	740	6.9
Epitalon	721 ± 11.1[c]	842 ± 58.5[b]	1053	4.1
%	+5.3	+14	+42	−41

[a]The rate of ageing (as per Gompertz equation[45]);
[b]The differences are statistically significant compared to the control by: $p < 0.05$;
[c]$p < 0.01$;
[d]$p < 0.001$.

Anti-carcinogenic and geroprotective properties of Thymalin were supposed to be mediated by its ability to prevent aging-associated decrease of cell immunity in female mice.[31]

Pineal gland preparation was shown to manifest anti-oxidant and geroprotective effects in several studies.[33] Old rats with persisting estrus administered with Epithalamin restored the regular estrus cycles, suggesting prevention of the reproductive function.[34] Old male rats administered with Epithalamin exhibited increased levels of luteinizing hormone and testosterone, which also proves the normalizing effect of Epithalamin on reproductive function in old animals.[35] Female rats treated with Epithalamin starting from the age of 15 months exhibited a 1.6-fold decrease in the incidence of neoplasia and a 2.7-fold decrease in the frequency of malignant tumors. Course administration of Epithalamin to C3H/Sn mice starting from 3.5 months of age led to a 2.1-fold decrease in the incidence of tumors of all kinds, including mammary adenocarcinomas (2.9-fold), as compared to the controls.[31] Epithalamin also contributed to decreased incidence and multiplicity of tumors in the model of DMBA-induced carcinogenesis in rats (Table 20.1).[36,37] Epithalamin application in the models of transplantable tumors resulted in the inhibition of metastatic growth and in tumor size reduction.[9,14]

A significant geroprotective potential of Epithalamin was discovered in various animal models: rats,[38] mice,[31] and *Drosophila melanogaster*.[39] All these animals showed an increase in mean life span under the influence of Epithalamin. Maximum life span of rats increased by 3 months: 23% of animals treated with Epithalamin had a longer life span than the most long-lived control rats.

Collectively, these findings suggest that peptide preparations Thymalin and Epithalamin are able to prevent aging and increase life span, as well as inhibit carcinogenesis in various animal species.

20.3 Short Synthetic Peptides

It was discovered that the extracts isolated from the calf thymus contained peptides with molecular weight less than 1000 Da. One of them is dipeptide Glu–Trp with a molecular weight of 333 Da. It was named Thymogen (Glu–Trp).[28] The effects of Thymogen on spontaneous carcinogenesis and life span in rats have been studied.[40] Thymogen was administered throughout the life span of rats starting from 5 months of age. Like Thymalin, this synthetic peptide inhibited malignant tumors development 2.1-fold. A tendency towards mean and maximum life span increase as well as a decreased aging rate in experimental animals was observed, as compared to the controls.[40] Thus, similarly to Thymalin, the synthetic dipeptide Thymogen has significant geroprotective properties.

Another promising short synthetic peptide is the dipeptide Lys–Glu or Vilon (molecular weight 275 Da). It was shown to be able to stimulate the reparative processes.[41] Prolonged administration of Vilon to CBA mice starting from 6 months of age resulted in the increase of their physical activity and

maximum life span, lowering of body temperature and inhibition of spontaneous tumor incidence by 1.5-fold as compared to the control animals. The obtained results prove the safety of chronic administration of Vilon and suggest that its geroprotective properties could be used for prevention of age-related pathologies.[42,43]

The tetrapeptide Ala-Glu-Asp-Gly (molecular weight 390 Da) was synthesized based on the amino acid analysis of Epithalamin. The tetrapeptide obtained was named Epitalon.[44] It showed properties similar to those of Epithalamin: suppression of spontaneous carcinogenesis (Table 20.1) and increase of the life span in experimental animals (Table 20.2).[14] It is important to note that both peptides inhibited aging rate (as per Gompertz equation):[45] Epitalhamin in rats[38] and in C3H/Sn mice,[31] and Epitalon in SHR and CBA mice[14] as compared to the controls.

Epitalon and Epithalamin appeared to be safe alternatives to melatonin in regard to the correction of pineal gland functional insufficiency.[46] Aging leads to decreased production of melatonin, which performs many vital functions.[14,47] Melatonin is involved in the regulation of functions of the central and peripheral nervous systems, endocrine organs and immune system. Decreased melatonin levels caused by the violation of circadian rhythms is considered to be an important factor in reducing life span and causing premature aging and age-related diseases, including cancer.[48,49] Administration of melatonin to experimental animals revealed its geroprotective properties.[14] Melatonin suppressed tumor incidence in chemically or genetically modified animals.[14] Long-term administration of melatonin to CBA mice in spontaneous carcinogenesis models caused the increase of melatonin-mediated malignant tumors (lymphomas) incidence.[50]

Epitalon- and Epithalamin-mediated increases of melatonin levels were recorded in the blood, and also in the pineal gland of old *Macaca mulatta*.[46] Administration of Epitalon to male and female rats stimulated melatonin production during night time, normalized hormonal and metabolic markers and prevented premature aging and tumor development in animals.[51,52]

Thus, administration of short peptides resulted in a number of beneficial effects in different organs and tissues under normal and pathological conditions in experimental studies. However, the mechanisms of their geroprotective and anti-carcinogenic actions are not completely elucidated and require further research.

20.4 Influence of Short Peptides on Immune and Antioxidant Systems

Short peptides of the thymus may produce a specific effect on immunologic responsiveness, homeostasis and metabolism in case of secondary immunodeficiency. Experimental animals administered with Thymogen for 30 days manifested lymphocyte count increase. Remarkably, Thymogen administration resulted in the increase of T-cell count in thymectomy, with its dose 1000

times less than that of Thymalin.[53] Comparative studies of the immunomodulatory effects of Thymalin and Thymogen on the intensity of immune response in rats immunized with sheep red blood cells showed a more significant effect of Thymogen as compared to Thymalin. Thymogen normalized T- and B-immunity in animals under conditions of experimentally induced immunodeficiency. The molecular mechanism of action of this synthesized preparation on T-lymphocytes is suggested to be based on the activity of calcium (Ca^{2+}) transmembrane exchange, as well as redistribution of intracellular cAMP and cGMP concentration.[54] As a result, these processes can induce gene expression followed by proliferation and differentiation of the relevant lymphocyte populations.[54]

Like Thymogen, Vilon was registered to stimulate cell immunity. Animals administrated with Vilon in concentrations from 10 ng l^{-1} to 100 μg l^{-1} showed an increased level of intracellular Ca^{2+} in thymocytes and macrophagocytes, displaying one of the mechanisms of cell activation. In particular, it leads to the stimulation of T-cell RNA and interleukin-2 (IL-2). Vilon was shown to stimulate mRNA IL-2 synthesis in murine spleen lymphocytes after 5 hours of incubation in cell culture.[54] *In vitro* administration of Vilon entailed significant expression of T- and B-lymphocytes in patients with secondary immunodeficiencies. It has also been shown to stimulate IL-1α, IL-1β, IL-8 and TNF-α production. In thymocytes and epithelial cells, Vilon stimulated expression of the argyrophil proteins associated with the nucleolar organizer region responsible for the synthesis, gathering and transportation of ribosomes into the cytoplasm, predetermining the intensity of protein synthesis in these structures.[53]

Vilon administration to irradiated animals promoted regeneration with a revealed differentiation into cortex and medulla in the thymus.[54] Moreover, hyperplasia of mast cells in the thymus was observed under Vilon's influence. Vilon seems to accelerate the proliferative activity of the irradiation-survivor bone marrow stem cells, which are the precursors of T-lymphocytes and mast cells.[54] Administration of another synthesized peptide, Epitalon, to gamma-irradiated rats contributed to ultrastructural manifestation of pinealocytes secretion strengthening, which had been damaged due to irradiation.[55] These results are suggestive of tissue-specificity of peptide bioregulators. Radiation-induced and age-related alterations are known to have many common features. The effects of Vilon and Epitalon were compared in studies on CBA mice injected with these peptides starting from 6 months of age and up to the end of life. Another group of mice was also treated with melatonin in tap water. As a result, Epitalon suppressed reactive oxygen species effectively in the blood serum and brain tissue of the animals. This effect was accompanied by suppression of lipid peroxidation (LP); the Schiff base decrease in the brain and the decrease in the amount of diene conjugates in the liver were also registered.[14] The effect of melatonin was almost the same. Vilon failed to affect any indexes of the free-radical processes studied. Similarly to melatonin, Epitalon was found to be able to stimulate organism antioxidant activity. In a series of experiments, Epitalon has been demonstrated to be

more efficient *in vivo* than *in vitro*.[56] *Drosophila melanogaster* larva exposure to Epitalon exhibited the reduction of lipid peroxidation intensity and increase in catalase activity in adult flies.[39] A significant antioxidant effect of Epitalon was found in old rats administered with this compound. It significantly suppressed the formation of LP products in blood serum and brain.[57]

Long-term experimental administration of Epitalon to SHR and SAM mice caused decreased chromosome aberrations of bone marrow cells. The most remarkable effect was seen in SAM mutant mice with accelerated aging.[22] The frequency of chromosome aberrations in SAM mice was higher due to DNA damage with reactive oxygen forms, whose production in SAM mice was enhanced.[58] Administration of Epitalon to these mice resulted in statistically significant reduction (by 20–30%) of ChA frequency, which can be associated with activation of antioxidant defense.

The effect of Epitalon on the number of sister chromatid exchanges (SCE) in lymphocyte culture of humans aged 75–88 was studied by cytogenetic methods. Addition of Epitalon to lymphocyte culture resulted in a 1.4-fold increase in SCE frequency ($p < 0.001$), as compared to the control.[59] Vilon under similar conditions increased SCE frequency to a greater extent than Epitalon and showed a 1.9-fold increase as compared to the control ($p < 0.001$).[60] According to early studies, metabolic processes do not take place in the heterochromatin or heterochromatinized chromosome regions.[61] Thus, SCE frequency increase induced by Vilon indicates decondensation (deheterochromatinization) of the chromosome region condensed with aging followed by the release of functionally inhibited genes located therein.[62] The same research also discovered the ability of both short peptides to activate ribosome genes, as evidenced by the increase of nucleolar organizer regions (NOR) in acrocentric chromosomes, deduced by Ag-staining method,[63] as compared to the control.

Generally, the ability of short peptides to normalize or improve humoral and cellular immunity, reinforce antioxidant defense of the body and affect heterochromatinization—one of the aging factors—is an essential component of the geroprotective mechanism of the short peptides.

20.5 The Influence of Short Peptides on Gene Expression

In research based on DNA microarray technology, the impact of Vilon and Epitalon on gene expression has been observed. In this study, the levels of mRNA of 15 247 genes in mouse heart before and after Vilon and Epitalon administration were studied.[23] Epitalon modulated the expression levels of 98 genes; Vilon changed the expression of 36 genes. Combined treatment with Vilon and Epitalon changed the expression of 114 genes. Among the affected genes, there were genes involved in oncogenesis. Vilon and Epitalon inhibited the expression of genes such as mouse Mybl1 (myeloblastosisoncogene-like1) and proto-oncogene Bcl-3, respectively. Chronic administration

of Vilon and Epitalon to female transgene mice led to a 1.9- and 3.7-fold decrease of gene HER-2/neu expression in mammary tumor, as compared to the control group. Moreover, Epitalon reduced the maximum size of mammary tumor and the diameter of lung metastases.[64]

Epitalon-treated culture of human lung fibroblasts manifested the induction of telomerase gene expression, telomerase activity and elongation of telomeres.[65] Activation of telomerase gene expression was accompanied by a 43% increase in the number of cell divisions.[66,67] These results are in accordance with our earlier data demonstrating the impact of particular peptide bioregulators and their complexes on gene expression.[21]

In the rat hypothalamic neurons, Vilon also was shown to stimulate the expression of c-fos gene known to be involved in the organism's stress response. Treatment with Epitalon also led to increased c-fos gene expression in the pineal gland of rats.[9]

One of the essential features of short peptides is their ability to influence cytokines synthesis. The expression of interleukin-2 (IL-2) in lymphocytes is known to decrease with aging.[68] The impact of Vilon on IL-2 gene expression in mouse spleen lymphocytes was studied by *in vitro* hybridization. Lymphocytes were stimulated with Con-A mitogen. Five-hour incubation with Vilon led to increased mRNA synthesis in both lymphoid cells stimulated with Con-A, and in non-stimulated cells. Prolonged Vilon incubation (for 20 hours) promoted IL-2 expression.[69] The effect of Epitalon on subcortex functions has also been found. Administration of Epitalon stimulated IL-2 gene expression in various hypothalamic structures under low stress conditions.[67] In general, our data provide evidence for immunomodulating and stress-protective capabilities of short peptides.[9,67]

The abovementioned experimental data on the mechanisms of action of the short peptides bring us to the conclusion concerning their important role in supporting immune, nervous, endocrine and other systems of the organism throughout the process of aging. These peptide preparations are able to inhibit the development of age-related pathologies, including cancer, thus preventing premature aging. It motivated us to examine their potential for treatment and prevention of age-related diseases in the elderly.

20.6 Application of Peptide Bioregulators in Elderly Patients

The experimental studies of peptide preparations in different animal models proved the safety of those preparations and revealed a wide spectrum of their beneficial effects, making reasonable the application of peptide preparations in humans. Most of the studies were conducted among elderly people and patients with premature aging.

The research was conducted among 106 patients (69 ± 2 years of age) with ischemic heart disease (IHD) and signs of premature aging: blood lipid disorders, low tolerance to carbohydrates, functional decrease of reproductive

functions and detoxifying liver function, osteoporosis, mental and physical capacity decrease.[70] All patients were randomly allocated into control and study groups. The patients of the control group received symptomatic therapy while those in the study group were treated with Thymalin in addition to symptomatic treatment, which was administered intramuscularly at a dose of 10 mg every 2–3 days with a total of 5 injections for the whole course and an interval of 5–6 months between the courses. The research lasted for a period of 30 months, during which the patients received 6 courses of Thymalin. An increase in the physical activity threshold by 14% after the first course of injections was found. This was evidenced by the increased ascent along a ramp from 3.4 to 4.8 floors and with decreased fatigue.[70]

Moreover, a significant increase in maximal oxygen intake (MOI) was revealed under the influence of Thymalin during threshold load, which indicates the expansion of the oxygen transport system functionality of the organism.[71] In general, a positive effect of Thymalin was observed in 53% of patients, whereas the same effect was registered only in 7% of the patients in the control group. This slight improvement of MOI registered in patients of the control group could be attributed to the symptomatic therapy they received, while the statistically significant MOI increase in the study group is related to Thymalin treatment.[70]

Thymalin-treated patients exhibited normalized blood lipid markers, *i.e.* significant decrease in cholesterol levels, beta-lipoprotein cholesterol and atherogenicity index. Patients with a high atherogenicity index prior to treatment (over 4) showed a normal level (below 3.5) after the course of Thymalin treatment. At the same time, administration of Thymalin to patients with high levels of circulating immune complexes resulted in their significant decrease, which is believed to be important for reducing the risks of vascular wall damage in IHD patients. Generally, Thymalin-treated patients demonstrated better memory, mood and working capacity. Most patients also exhibited better stress resistance. A lower number of catarrhal diseases and their shorter duration were also observed. No new cases of coronary heart disease, hypertension, heart failure and cardiac arrhythmias were reported during the Thymalin administration period. Thymalin-treated patients demonstrated a decrease in mortality rate during the study period—6.6% as compared to 13.6% in the control group.[70]

A similar research with Epithalamin administration in 46 elderly patients with coronary artery disease and premature aging of the cardiovascular system was conducted. Epithalamin was injected at a dose of 10 mg every 2–3 days (total of 5 injections per one course) for a period of 30 months.[70] The functional age analysis taken prior to the Epithalamin course showed at least a 5 year higher age, as compared to the chronological one, which is clear evidence of premature aging. The first courses of Epithalamin injections resulted in a significant decrease in the functional age of the patients by an average of 7.2 years. Upon completion of a 3 year observation period, the functional age did not differ significantly from initial figures, while the chronological age of the patients non-treated with Epithalamin increased by

3 years.[70] Prior to the treatment undertaken, all elderly patients exhibited lipid profile disorders: low concentrations of high-density lipoproteins, total cholesterol and beta-lipoprotein levels increased, rise in atherogenicity levels (4.0). Epithalamin administration for one year led to a significant decrease of total cholesterol and beta-lipoprotein, as well as the atherogenicity index (3.5). At the same time, the control (conventional therapy) group showed deterioration in the lipid composition. Epithalamin administration also resulted in visual memory and mental capacity improvements, as evidenced by fulfillment of an experimental psychological task in a shorter time period.

The patients in both groups were monitored continuously upon completion of 3 years of peptide therapy. According to the results of a 15 year-long observation of patients in both groups, 66.7% of patients were alive in the Epithalamin-treated group, while only 40% were alive in the control group. A statistically significant decrease of mortality in the Epithalamin-treated patients was demonstrated by the Kaplan–Meier method.[72] The long-term administration of the pineal gland peptide preparation significantly (1.8 times) reduced the number of deaths associated with cardiovascular diseases. Specifically, myocardial infarction- and stroke-caused deaths were observed in 46.2% of the patients in the Epithalamin group, as compared to 83.3% in the control group.

The research also showed that administration of peptide preparations to elderly people can also affect the melatonin-secretion function.[26] Both peptides have a comparable impact on the concentration of melatonin in the blood plasma at night (3:00). The same effect was achieved by administering a significantly lower course dose of Epitalon (0.1 mg) as compared to Epithalamin (50 mg), which indicates the higher biological activity of the synthetic tetrapeptide.

Administration of Thymogen and Vilon was effective in the treatment of different diseases and pathologies accompanied by immune disorders. Administration of Thymogen to elderly patients with secondary immunodeficiencies contributed to normalization of immune markers in 83.6% of cases, as well as to metabolic processes and coagulation improvement.[28,54] Intranasal administration of Thymogen for prevention of influenza and acute respiratory infections (ARI) in patients of all ages, including the elderly, helped to decrease their incidence 3–4-fold, reducing the number of toxic forms by over 30 times.[54] Another synthetic peptide, Vilon, was very effective in the treatment of decreased cellular immunity and phagocytosis-associated diseases. Application of Vilon in addition to conventional therapy to surgical patients accelerated the process of tissue regeneration and restoration of the body functions. Administration of Vilon in elderly and old patients with chronic generalized periodontitis contributed to shortening of the pathological process duration due to the reduction of the periodontal pocket depth, as compared to the patients in the control group.[73]

Thus, administration of peptide preparations is beneficial for the quality of life of elderly patients. Peptides seem to be able to retard age-related functional decline, to improve long-term prognosis and to decrease cardiovascular

mortality. Based on the safety, confirmed in experimental studies, peptide preparations can be recommended both for premature aging prevention and for primary or adjunctive therapy in case of various diseases.

20.7 Prospective Cellular and Molecular Mechanism of Action of Short Peptides

It is essential for understanding specific effects of the peptide behavior in the cell and intracellular structures to reveal binding of short peptides with specific DNA sites. In our research, fluorescently labeled short peptides penetrated into cells and intracellular structures.[74] In the HeLa cells, the most intensive fluorescence of the labeled peptides was observed in the nucleus and nucleoli, while the least intensive was observed in the cytoplasm. Investigation of interaction of fluorescence-labeled deoxyribooligonucleotides with short peptides showed that peptides with different primary structures bind with one and the same deoxyribooligonucleotide differently. By using the specific oligonucleotides (FAM–deoxyribooligonucleotides), it has been revealed that Epitalon binds primarily with oligonucleotides that include more cytosine (C) than guanine (G) residues. The constant of binding of Epitalon with FAM–CGC CGC CAG GCG CCG CCG CGC (12 C residues) was almost 2-fold higher than that with FAM–GCG CGG CGG CGC CTG CGC CGC (10 C residues). Introduction of 5-methylcytosine residue into the nucleotide sequence independent of C or G content increased the binding of oligonucleotides with Epitalon. Thus, the binding of peptide Ala-Glu-Asp-Gly is sensitive to the cytosine methylation status of oligonucleotides. Epitalon was shown to preferably bind with single stranded oligonucleotide, containing methylated cytosine.[74] As is commonly known, cytosine DNA methylation is the most extensively studied epigenetic genome modification playing a significant role in stable changes of gene activity upon cell differentiation and aging in mammals.[75–77]

Consequently, there are specific sites for binding of a peptide with a particular amino acid sequence and oligonucleotide with a particular nucleotide sequence. The short peptide may bind to the DNA in various ways depending on its methylation nature; obviously it will cause different effects on the gene functions in various tissues/cells—young and old, normal and cancerous *etc.*[67] Our study shows that unlike the temperature of melting of the DNA double helix (+69.5 °C), in the DNA–tetrapeptide (Ala-Glu-Asp-Gly) system, the melting point occurs at a significantly lower temperature (+28 °C) and is characterized by smaller changes in free energy and an approximately 2-fold decrease in the enthalpy and entropy values.[78] This fact demonstrates that the thermodynamically simplified way of the DNA–peptide complex separation at lower temperature settings is typical of the biochemical processes occurring in living organisms. It also suggests that the mechanism of DNA–Epitalon interaction is based upon the natural mechanism of functioning of a living organism.

A model of complimentary binding of the synthesized peptide Epitalon (Ala-Glu-Asp-Gly) with a DNA double helix was developed.[79] The Ala-Glu-Asp-Gly peptide was found to be located in the major groove of the DNA double helix. A special feature of this model is that the tetrapeptide in the major groove interacts simultaneously with the functional groups of the bases of both DNA strands.[79] The binding between the peptide and the ATTTC sequence in a DNA strand is supported by available data, confirming the appearance of this particular sequence in the promoter region of the telomerase gene.[80] Such interaction of the Ala-Glu-Asp-Gly peptide and the ATTTC sequence can likely explain the geroprotective properties of Epitalon.[81]

Based on the analysis of physical and chemical characteristics of the DNA-peptide complex, a three-dimensional model of complimentary interaction between Ala-Glu-Asp-Gly and the ATTTC sequence was created (see Figure 20.1).[24,25,57] A synthetic nucleic acid preparation was used for studying the interaction between oligopeptides and double stranded DNA. It is a synthetic analogue of the binding site of transcription factors (TATA box, which is usually found in the binding sites of RNA-polymerase II) in the promoter regions of many eukaryotic genes.[82] On the surface of the major groove of the DNA synthetic preparation double helix a group of

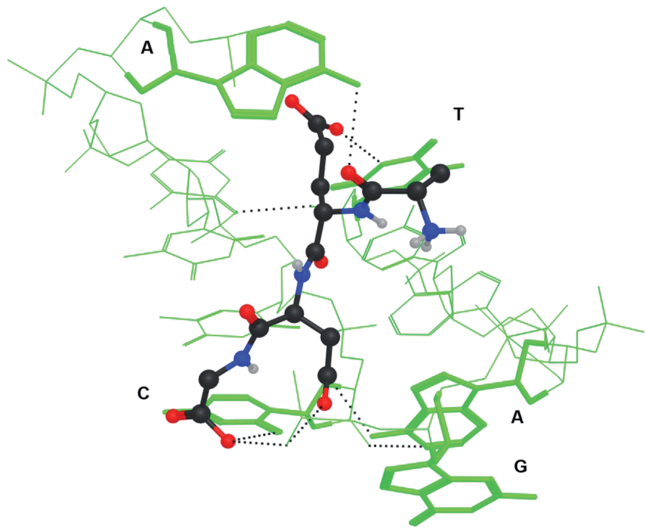

Figure 20.1 The interaction of the AEDG peptide with nitrogen bases of DNA (ATTTC sequence). The dotted line indicates hydrogen bonds between the atoms of peptides and DNA; bold green lines indicate nitrogen bases of DNA forming hydrogen bonds with the peptide. The DNA molecule is indicated in green; letters indicate nitrogen bases (A: adenine, T: thymine, G: guanine, C: cytosine). In the peptide molecule, blue is used for nitrogen atoms, red for oxygen atoms, grey for carbon atoms, and light grey for polar hydrogen atoms.

nucleobases occurs that can interact with the Ala-Glu-Asp-Gly groups. It was found that binding of six nucleotide pairs with TATATA of the DNthe A leading strand can be performed *via* an additional hydrogenous and one hydrophobic bond.[24,78] Thus, a regulatory peptide is believed to be able to bind with a complementary site on the gene promoter region, causing local separation of strands and thereby initiating the process of RNA polymerase gene transcription.

20.8 Conclusion

Physiologically active peptides, including short peptides, represent biologically active compounds that can modulate various cellular and molecular processes. Peptide compounds are essential for invention of new pharmaceutical products.[83] Peptide preparations are highly active, non-toxic and have no side effects, which comprise their main advantages as pharmaceuticals. Short peptides possess pronounced anticarcinogenic and geroprotective properties. In experimental studies in animals the peptides revealed the ability to decrease the risk of spontaneous and induced neoplasia and to enhance lifespan by 20–40%.[14] In general, these properties are determined by the peptides' capability to influence the immune system of the organism, thus preventing aging.[21,54] The peptides possess pronounced antioxidant potential: Vilon reduces the ROS level in *D. melanogaster* mitochondria; Epitalon inhibits the chemoluminescence level and enhances general antioxidant activity in mice blood serum.[14] Epitalon also has an inhibitory effect on the level of age-related chromosome aberrations in mice.[22] Short peptides activate heterochromatin in the cytoblasts of elderly patients and promote activation of genes repressed as a consequence of age-associated heterochromatinization of the euchromatic region of chromosomes.[19] Recognition of the short peptides' ability to influence the expression of various genes was essential for understanding of their role in the aging processes.[23]

Small peptides (di-, tri- and tetra-peptides) revealed the capability of complementary interaction with the DNA-specific binding sites on the promoter segment of genes, inducing separation of double helix strands and RNA polymerase activation. Discovery of the phenomenon of peptide activation of gene transcription allows determination of the mechanism to maintain physiological functions, which is based on the complementary interaction of DNA and regulatory peptides.[24,79]

Application of peptide bioregulators in humans for preventive purposes resulted in a significant restoration of the main physiological functions and a substantial mortality decrease in different age groups for a period of 6–12 years.[26]

Further investigation of the mechanisms of peptide geroprotective action can likely provide new avenues for peptidergic regulation of aging, prevention of premature aging, age-associated pathology and an increase in the period of active human longevity.

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