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RESEARCH ARTICLE

Peptide bioregulation of aging: results and prospects

Vladimir N. Anisimov · Vladimir Kh. Khavinson

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Abstract The review comprises the results of author's long-term investigation in the mechanisms of aging and a role of peptide bioregulators in prevention of age-related pathology. A number of small peptides have been isolated from different organs and tissues and their analogues (di-, tri-, tetrapeptides) were synthesized from the amino acids. It was shown that long-term treatment with some peptide preparations increased mean life span by 20–40%, slow down the age-related changes in the biomarkers of aging and suppressed development of spontaneous and induced by chemical or radiation carcinogens tumorigenesis in rodents. Possible mechanisms of the biological effects of small peptides are discussed in the paper. The results of clinical applications of peptide preparation during the period of 6–12 years are presented as well.

Keywords Small peptides · Pineal gland · Thymus · Life span extension · Tumors · Rodents

V. N. Anisimov (✉)
N.N. Petrov Research Institute of Oncology,
Leningradskaya Str., 68, Pesochny-2,
197758 St. Petersburg, Russia
e-mail: aging@mail.ru

V. Kh. Khavinson
St. Petersburg Institute of Bioregulation and Gerontology,
Prospect Dynamo, 3, 197110 St. Petersburg, Russia

Introduction

It is known that specific limit of animal and human lifespan is approximately 30–40% higher than their mean lifespan. It could be referred to the impact of adverse factors causing changes in the gene structure and expression accompanied by disorders in the protein synthesis and organism functioning. The involution of the central organ of immune system—thymus and that of the neuroendocrine system—pineal gland are major manifestations of these disturbances.

To restore functions of thymus, pineal gland and other organs we developed a special method for isolation, refinement and fractionating of low-molecular peptides from extracts of these organs (Khavinson 2004, 2006; Morozov and Khavinson 1991, 1996). Low-molecular peptides isolated from thymus (preparation Thymalin) and pineal gland (preparation Epithalamin) of animals were studied in different biologic models. These peptide preparations contributed to a reliable increase in animals mean lifespan in numerous experiments (Table 1). Of particular importance is a correlation between mean lifespan and the main index of cellular immunity (reaction of blast-transformation of lymphocytes with phytohaemagglutinin) determining T lymphocytes function (Anisimov et al. 1982a, b).

A significant increase in the mean lifespan of animals was evidently caused by a reliable antitumor activity of low-molecular peptides isolated from thymus and pineal gland (Table 2).

Table 1 Effect of peptide bioregulators on life span of rodents

Peptide preparations	Species, strain of rodents	Sex	Age at the start of treatment, months	Life span, % to the control		Effect on spontaneous tumor	Reference
				Mean	Maximum		
Epithalamin	Rats	Female	3.5	+25*	+6	↓	Dilman et al. (1979a, 1979b)
	Rats	Female	15	+6	+9	↓	Anisimov and Khavinson (1991)
	C3H/Sn mice	Female	3.5	+31*	+14	↓	Anisimov et al. (1982a, 1982b)
	SHR mice	Female	3.5	+13	+5	↓	Anisimov et al. (1987)
		Female	3.5	+11*	−2	↓	Anisimov et al. (1989)
		Female	12	+6	−3	↓	Anisimov et al. (1989)
Thymalin	C3H/Sn mice	Female	3.5	+28*	+11	↓	Anisimov et al. 1982a, 1982b
	SHR mice	Female	3.5	+12*	0	↓	Anisimov et al., 1987
		Female	3.5	−2	−6	↓	Anisimov et al. (1989)
		Female	12	+13[a]	0	↓	Anisimov et al. (1989)
Glu-Trp	Rats	Female	4	+2	+14	↓	Anisimov et al., 2000
Lys-Glu	Мышии CBA	Female	6	+3*	+7	↓	Anisimov et al. 2001a, 2001b, 2002a, 2002b
	HER-2neu mice	Female	2	−7	0	↑	Anisimov et al. (2002a, 2002b)
Ala-Glu-Asp-Gly	Rats	Female	4	−8	−6	↓	Vinogradova et al. (2007)
		Male	4	+7[a]	+5	↓	Vinogradova et al. (2008)
	SHR mice	Female	3	+13[a]*	12	↓	Anisimov et al. (2003)
	CBA mice	Female	6	+5*	+42	↓	Anisimov et al. (2001a, 2001b, 2002a, 2002b)
	SAMR-1 mice	Female	2	+5	−8	=	Anisimov et al. (2005)
	SAMP-1 mice	Female	2	+7*	+8	=	Anisimov et al. (2005)
	HER-2/neu mice	Female	2	+13*	+14	↓	Anisimov et al. (2002a, 2002b)
DSIP	SHR mice	Female	3	+17[a]*	+24	↓	Popovich et al. (2003)
	SHR mice	Female	3	+20[a]*	+2	=	Voitenkov et al. (2008)

DSIP delta-speep inducing peptide, Trp-Ala-Gly-Asp-Als-Ser-Gly-Glu

↓, decrease in the incidence and/or multiplicity and/or increase of tumor latency; = , no effect; *, The difference with the controls is statistically significant, $P < 0.05$

[a] Mean life span of last 10% survivors

Small peptides isolated from different organs and tissues as well as their synthesized analogues (di-, tri-, tetrapeptides) revealed a reliable tissue specific (gene specific) effects both in cellular cultures and in experimental in young and old animals (Khavinson et al. 1997; Khavinson 2001).

Peptide tissue specific activity manifested in stimulation of protein synthesis in cells of those organs they had been isolated from. The enhancement of protein synthesis under the effect of the peptide has been registered in young and old animals (Brodsky et al. 2001).

Especially significant appeared restoration of reproductive function system in old female rats subjected to the pineal peptide treatment (Dilman et al. 1979a, b). Persistent estrus and anestrus in rodents, analogous to menopause in women, lowered from the initial 95% down to 52% after the preparation administration, while regular cycles, typical of the normal estrus function, increased from the initial 5% up to 48%. It should be emphasized that in a special experiment initially none of the rats got pregnant after mating. Repeated mating after the administration of the pineal gland peptide entailed pregnancy in 4 out of 16 animals which gave birth to 5–9 healthy off-springs each.

Thus there were ascertained main advantages of low-molecular peptides: they possessed high biologic

Table 2 Effect of peptide bioregulators on induced carcinogenesis in rodents

Peptide preparations	Species, strain of rodents	Carcinogenic agent	Main tumor site	Effect on tumor development	Reference
Epithalamin	Rats	DMBA	Mammary gland	↓	Dilman et al. (1979a, b)
	Rats	NEU, transplacentally	Nervous system, kidney	↓	Alexandrov et al. (1996)
	Rats	X-rays	Malignancies at various sites	↓	Anisimov et al. (1982a, b)
Thymalin	Rats	DMBA	Mammary gland	↓	Anisimov et al. (1980)
	Rats	NEU, transplacentally	Nerous system, kidney	↓	Alexandrov et al. (1996)
	Rats	X-rays	Malignancies at various sites	↓	Anisimov et al. (1982a, b)
	Mice	X-rays	Mammary gland	↓	Anisimov and Khavinson (1993)
Glu-Trp	Rats	^{90}Sr and ^{137}Cs	Mammary gland	↓	Anisimov et al. (1992a, b)
Lys-Glu	Rats	BHBNA	Bladder	↓	Pliss et al. (2001a, b)
	SHR mice	DMH	Colon	↓	Pliss et al. (2005)
	FVB/N mice	HER-2/neu	Mammary gland	↑	Anisimov et al. (2002a, b)
Ala-Glu-Asp-Gly	Rats	Constant light	Tumors at various sites	↓	Vinogradova et al. (2007, 2008)
	Rats	Natural illumination[a]	Tumors at various sites	↓	Vinogradova et al. (2007)
	Rats	DMH	Colon	↓	Anisimov et al. (2002a, b) and Kossoy et al. (2003)
	C3H/He mice	MMTV	Mammary gland	↓	Kossoy et al. (2006)
	FVB/N mice	HER-2/neu	Mammary gland	↓	Anisimov et al. (2002a, b)

BHBNA, *N*-butyl-*N*(4-hydroxybutyl)-nitrosamin, *DMBA*, 7,12-dimethyl(a)anthracene, *DMH*, 1,2-dimethylhydrazine, *NEU*, *N*-nitrosoethylurea, *HER-2/neu*, oncogene, *MMTV*, murine mammary tumor virus

[a] Natural light at the Russian North-West (Petrozavodsk)

activity, revealed tissue specificity and were neither species specific nor immunogenic. These features make regulatory peptides similar to peptide hormones.

A detailed study of molecular weight, chemical properties, amino acid composition and sequence of low-molecular peptides isolated from thymus, pineal gland and other organs had been carried out for many years (Morozov and Khavinson 1991, 1996, 1997, 2000). The obtained data were used for chemical synthesis of several small peptides. A comparative analysis showed that biological activity of natural and synthetic preparations was largely identical. Thus, for example, the thymic dipeptide stimulated immunity (Morozov and Khavinson 1996, 2000). Biological activity of natural and synthetic peptides appeared to be similar in standard assays both in vitro and in vivo (Anisimov et al. 1990, 1998a, b, 2000; Khavinson et al. 1997). These results demonstrate prospects for application of these peptides as geroprotectors (Anisimov and Khavinson, 2003, 2009; Khavinson and Anisimov 2009). The necessity of searching for new drugs—geroprotectors dictated the onset of preclinical studies of these preparations on different structural levels.

On the level of the organism in different animals we have registered a significant variety of biologic effects exerted by small peptides especially by peptides of thymus and pineal gland, including proliferative activity and apoptosis (Khavinson and Mylnikov 2000a, b; Khavinson et al. 2000a, b, c, 2001a, b; Anisimov et al. 1994, 1997, 2001a, b, 2002a, b; Khavinson 2002).

On the level of cellular structures, small peptides activate heterochromatin in the cell nuclei in senile patients (Lezhava 1984, 2006).

As it was said above there are two forms of chromatin in the cellular nucleus: light euchromatin

and dense heterochromatin located near nuclear membrane. Gene transcription takes place in the light phase, that's in euchromatin. With aging the amount of heterochromatin in the nucleus increases on average from 63 to 80%. Regulatory peptides entail the increase in the amount of euchromatin in the nucleus. This means that more genes become available for transcription factors, and transcription of gene information goes on more intensively as well as protein synthesis. In other words, the more euchromatin there is in the nucleus the more intensive the protein synthesis in the cell is (Lezhava 1984, 2006; Khavinson et al. 2003).

The capability of peptides to induce polypotent cells differentiation is of special significance (Khavinson 2002). Thus addition of retinal peptides to polypotent cells of *Xenopus laevis* early gastrula ectoderm led to the emergence of retinal and pigment epithelium cells. This outstanding result explains a pronounced clinical effect of the preparation of the retina in patients with retinal degenerations (Khavinson 2002) and in animals with genetically determined retinitis pigmentosa (Khavinson et al. 2002b).

On a chromosome level the number of chromosomes aberrations was used a marker of DNA damages in an aging organism. Somatic mutations can occur due to accumulation of stable aberrations and underlie age-related pathology, including malignancy. Reliable antimutagenic and reparative activity of thymus and pineal gland peptides have been confirmed by a reduction in the number of chromosome aberrations in the bone marrow cells and cornea epithelium cells in animals revealing accelerated aging (Anisimov et al. 2004a).

On the level of gene activity regulation it was established that administration of peptides Lys-Glu and Ala-Glu-Asp-Gly to female transgenic HER-2/neu mice caused a 2–3.6-fold suppression of the gene expression as compared to the control group. This suppression is accompanied by a reliable reduction of the tumor diameter (Anisimov et al. 2002a, b).

It was revealed that addition of tetrapeptide Ala-Glu-Asp-Gly to the cultural medium of human lung fibroblasts induced telomerase gene expression and contributed to a 2.4-fold lengthening of telomeres. Activation of gene expression is accompanied by a growing number of cellular divisions (by 42.5%), which is the evidence of Hayflick's limit overcoming (Khavinson et al. 2004a, b).

The effect of treatment with di- and tetrapeptides (Lys-Glu, Glu-Trp, Ala-Glu-Asp-Gly, Ala-Glu-Asp-Pro) on the expression of 15,247 genes was studied with the employment of DNA-microarray technology in heart and brain of CBA mice (Anisimov et al. 2004a, b). In this experiment, there were used clones from the library of the National Institute on Aging, USA. This experiment provided unique data on alteration in the expression of different genes under the effect of peptide preparations. An important conclusion driven from the experiment was that every peptide specifically regulates particular genes. Results of this experiment testify to the existing mechanism of peptide regulation of gene activity. It was also registered that dipeptide Lys-Glu, showing immunomodulating activity, regulates gene interleukin-2 expression in blood lymphocytes (Khavinson et al. 2000b).

On the molecular level, there was an obvious gap between multiple evidence of specific effects, caused by regulatory peptides in activation of gene transcription (Khavinson et al. 2002a, c; Anisimov and Khavinson 2003; Sibarov et al. 2002 Anisimov et al. 2003; Djeridane et al. 2003; Kossoy et al. 2003; Khavinson and Morozov 2003; Labunets et al. 2004a, b), and limited schemes of the process underlying the selective binding of the transcription factor with specific DNA sites. Meanwhile non-specific binding of proteins with the DNA double helix was proved using physicochemical methods (Riadnova et al. 2000). Activation of gene transcription in cells of higher organisms as a rule needs dozens of macromolecular activators and transcription factors.

We proposed a molecular model of interaction between regulatory peptides and DNA double helix in gene promoter region of (Fig. 1a, b; Khavinson et al. 2005, 2006).

Geometrical and chemical complementarity of peptide amino acid sequence and DNA nucleotide pairs sequence was assumed as a basis for the molecular model. Regulatory peptide recognizes a specific site in the DNA double helix if its own amino acid sequence is complementary to the DNA nucleotide sequence for a sufficient length. In other words, their interaction is specific due to matching sequences.

Each sequence of the DNA double helix nucleotide pairs forms a unique pattern of functional groups on the surface of the DNA double helix major groove. A peptide in the unfolded β-conformation can

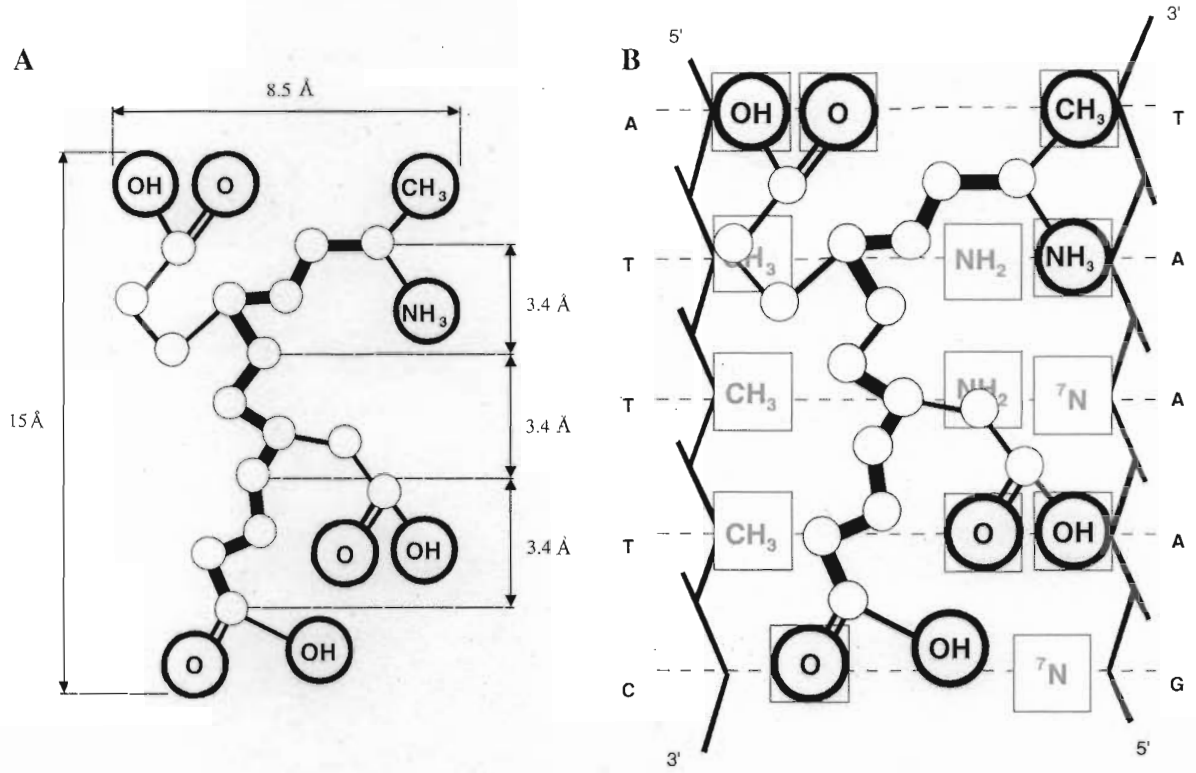

Fig. 1 **a** Unfolded peptide Ala-Glu-Asp-Gly conformation (plan projection). There are shown end and side functional groups, capable of complementary interaction with DNA. $-NH_3$ proton donors groups, =O proton acceptors groups, $-CH_3$ hydrophobic (methyl) group, *Bold line* represents main peptide chain. **b** Scheme of complementary interaction of tetrapeptide Ala-Glu-Asp-Gly with DNA double helix ("DNA-tetrapeptide" complex on the promoter segment of telomerase gene)

complimentary fit into the in the DNA major groove along the double helix axis. We used data on molecular geometry of the DNA double-helix and peptide β-thread from scientific publications in order to identify nucleotide pairs sequence for specific binding of the DNA and peptide Ala-Glu-Asp-Gly. The screening conducted showed that this tetrapeptide can be located in the DNA major groove with the ATTTG (or ATTTC) nucleotide sequence on the main chain in compliance with the complementarity of disposition of their functional groups (Riadnova et al. 2000).

For experimental testing of the molecular model there were used synthetic preparations: DNA [poly-(dA-dT):poly(dA-dT)] (double helix) and tetrapeptide Ala-Glu-Asp-Gly. Gel chromatography helped to prove that peptide Ala-Glu-Asp-Gly forms stable intermolecular complex with the DNA double helix (Khavinson et al. 2006).

Complementary binding of the peptide with nucleotides sequence on the leading strand TATATA of the double helix can be conducted by six hydrogenous and one hydrophobic bonds between functional groups of the both participants.

Under normal physiological conditions DNA exists in the form of a double helix two polymeric chains of which are kept together by hydrogenous bindings between pairs of bases of each chain. Most of the biological processes with DNA participation (transcription, replications) need the double helix to undergo disjunction into separate strands. In particular, it is known, that local separation of double helix strands precedes gene transcription by RNA polymerase. For the transcription onset (synthesis of the matrix RNA) the DNA double helix has to be freed from histones, and in the place where the matrix RNA synthesis starts, the strands of the double helix should be disintegrated.

Concentration dependent hyperchromic effect (increased optic density 260 nm) was found by spectrophotometery of solutions containing synthetic DNA double helix and tetrapeptide Ala-Glu-Asp-Gly.

The hyperchromic effect points out a partial destruction of hydrogen bonds between nucleotide pairs of the double helix and local separation of its strands (allosteric conformational changes; Khavinson et al. 2006). In vitro experiments show that a small peptide of the definite structure and amino acid sequence can participate in activation of genes transcription on the stage of strands disjunction in the DNA double helix. Biochemical aspect of this phenomenon consists in similarity of structure and amino acid sequence of a regulatory peptide and a specific segment of the peptide chain of the macromolecular transcription factor.

Thus, the studies of peptides biological activity on different structural levels and of physicochemical processes of their interaction proved an indubitably high physiologic activity of peptide regulators. Major conclusion reads that peptides are capable of regulating gene expression. Pre-clinical trials demonstrated high biological activity and safety of synthesized peptides (Khavinson 2001; Khavinson et al. 2001a, b, c, 2002a, b, c, d, 2006). Thus, the administration of peptides Lys-Glu or Ala-Glu-Asp-Gly to animals contributes to a reduced incidence of tumors and an increase of mean lifespan (Tables 1, 2). Peptide Ala-Glu-Asp-Pro stimulates nerve regeneration (Turchaninova et al. 2000), peptide Lys-Glu-Asp-Trp decreases blood glucose level in animals with experimental diabetes mellitus (Khavinson et al. 2007a, b, c).

Taking into consideration a reliable biological activity of peptides we found it reasonable to study the effect of regulatory peptides in monkeys. Restoration of the melatonin level up to normal following the administration of the peptide preparation to old monkeys was among our significant findings. The same old monkeys revealed a restoration to normal indices of a daily rhythm of secretion of the main hormone of adrenal gland—cortisol (Khavinson et al. 2001a, b, c).

Effect of the peptide bioregulators in human

Taking into consideration the encouraging data testifying to high geroprotective activity of both natural tissue specific and synthetic peptide preparations we have been concentrating our attention on studies of geroprotective activity of peptides in old and senile people in recent years (Khavinson 2002; Khavinson and Morozov 2003; Labunets et al. 2004a, b; Korkushko et al. 2004; Anisimov and Khavinson 2005; Khavinson and Malinin 2005; Goncharova et al. 2005; Kozina et al. 2007). Thus, annual treatment course with thymus and pineal preparations led to a reliable decrease in mortality (Table 3), to improvement of brain function and that of immune, endocrine, cardio-vascular systems, increased density of osseous tissue (Khavinson 2002; Khavinson and Morozov 2003; Labunets et al. 2004a, b; Korkushko et al. 2004). It is noteworthy that application of preparation of the thymus led to a twofold decrease in frequency of acute respiratory disease (Khavinson and Morozov 2003). The restoration of nocturnal

Table 3 Effect of treatment with peptide preparation on mortality rate in elderly and old subjects

Group of subjects	Indices	Control (administration of polyvitamins)	Administration of the pineal gland preparation	Administration of the complex of thymus and pineal gland preparations
Elderly people	Number of subjects	48	46	No studies
(60–74 years)	Initial mean age (years)	69.3 ± 2.2	71.1 ± 1.4	
	Mortality rate in the course of 8 years (%)	13.6	8.5*	
	Mortality rate in the course of 12 years (%)	44.1	22.3*	
Old people	Number of subjects	22	24	20
(75–89 years)	Initial mean age (years)	80.2 ± 1.6	81.5 ± 2.1	82.1 ± 2.3
	Mortality rate in the course of 6 years (%)	81.8	45.8*	33.3*

* $P < 0.05$ as compared to the control

melatonin secretion level in patients subjected to administration of preparation of the pineal gland is of special significance (Labunets et al. 2004a, b). These results suggest good prospects for tackling demographic issues (Khavinson and Mikhailova 2007).

Conclusion

Involution of the main organs and tissues of the organism accompanied by a decrease of protein synthesis in cells underlies the process of aging. Peptide preparations isolated from organs of young animals when administered into an old organism are capable to induce protein synthesis followed by restoration of the main functions.

It was shown that long-term application of peptides, both isolated from the organs and synthesized from the amino acid, in animals (as a rule starting from the period after maturity) leads to a reliable increase in their mean lifespan by 20–40% and reaching a species limit.

It was revealed that small peptides (di-, tri- and tetrapeptides) are capable of complementary interaction with the DNA specific binding site on the promoter segment of genes, inducing disjoining of double helix strands and RNA polymerase activation. Discovery of the phenomenon of peptide activation of gene transcription points out the natural mechanism of organism to maintain physiologic functions, which is based on the complementary interaction of the DNA and regulatory peptides. This process is fundamental for the development and functioning of the living substance (Figs. 2, 3), while aging is an evolutionary determined biologic process of age-related changes in gene structure and expression.

Application of peptide bioregulators in humans for preventive purposes led to a significant rehabilitation of the main physiological functions and a reliable mortality decrease in different age groups during the period of 6–12 years.

It should be emphasized that this approach to the prevention of aging is based not only on experimental and clinical data, but also on technological developments having world novelty (Anisimov 2008; Khavinson et al. 2006, 2007a, b, 2008a, b).

The authors and their teams believe that the whole complex of 35-year experimental and clinical studies may serve a vital contribution to the advancement of a scientific heritage of the prominent Russian

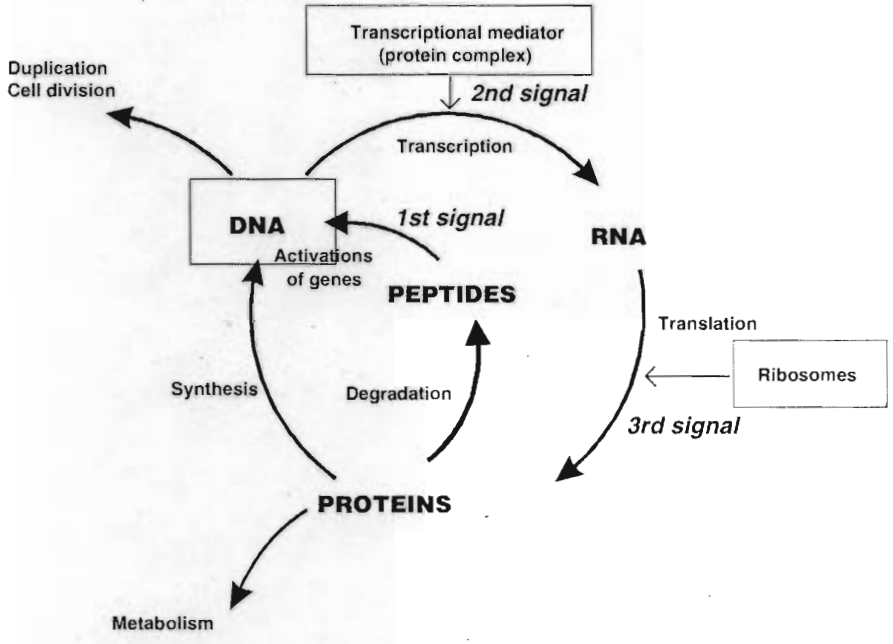

Fig. 2 The role of peptides in the cycle of DNA, RNA and protein biosynthesis

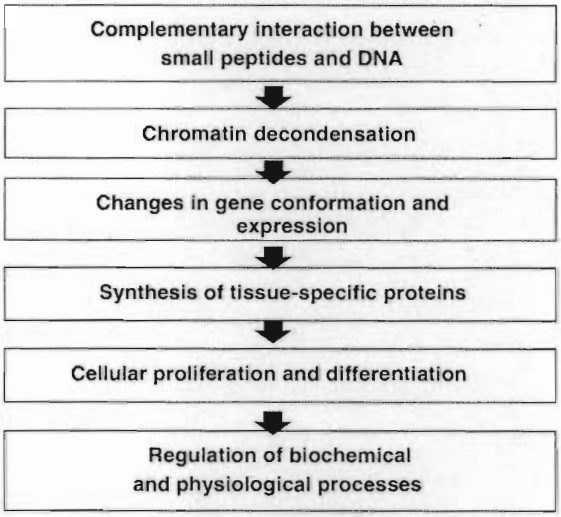

Fig. 3 Mechanism of peptide regulation of the living matter development

scientist I. I. Mechnikov in the field of gerontology and will be to the benefit of people, especially for those of old and oldest old age.

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