BEXBAN 136(3) 213-317 (2003)

ISSN 0007-4888

Russian Original Vol. 136, No. 3, September, 2003

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

(BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY)

TRANSLATED FROM RUSSIAN

Effect of Regulatory Peptides on Gene Transcription

V. Kh. Khavinson*, L. K. Shataeva*, and A. A. Chernova**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 328-330, September, 2003
Original article submitted July 11, 2003

Experimental studies of geroprotective activity of synthetic oligopeptides and conformational analysis of the tetrapeptide Epithalon allowed us to hypothesize that regulatory oligopeptides directly initiate transcription of genes for vitally important proteins. Sequences of nucleotide pairs that can serve as binding sites for tetrapeptide Epithalon were identified in the promoter regions of retinal genes F379, telomerase, and RNA polymerase II.

Key Words: *peptides; geroprotectors; Epithalon; transcription factors; DNA*

Aging is accompanied by inactivation of chromatin and deceleration of protein synthesis. Our recent experiments showed that natural regulatory peptides (RP) and their synthetic analogues possess tissue-specific activity, play a role in chromatin activation, and normalize the rhythm of protein synthesis in tissue culture [3,5,6]. It should be emphasized that RP markedly prolong the lifespan in experimental animals [1,3-5,10]. The complex of epiphysial peptides (Epithalamin) and synthetic tetrapeptide Epithalon synthesized on the basis of acid composition of Epithalamin are of particular interest. The study of the effect produced by Epithalon on gene expression in mouse heart showed that this RP by more than 2-fold increases expression of some genes [2]. A large body of evidence indicates that RP specifically activate protein synthesis. However, the molecular mechanisms underlying the influence of RP on gene transcription and protein synthesis are poorly understood.

Gene transcription is initiated by high-molecular-weight proteins (transcription factors, TF) and involves DNA-dependent RNA polymerase II [8]. Biosynthesis of RNA polymerase II does not occur without initiation of its transcription. Moreover, it was hypothesized that the total lifespan of cell populations correlates with the length of telomeres and, consequently, telomerase activity [8]. Initiation of gene transcription is a key event in protein synthesis during aging. At the same time, the mechanism of RP participation in the initiation of transcription of various vitally important genes, including genes for telomerase and RNA polymerase II, remains unclear.

Here we studied the mechanism of the specific molecular interaction between synthetic tetrapeptide Epithalon (Ala-Glu-Asp-Gly) and promoter regions of genes, whose transcription is important for geroprotective activity.

MATERIALS AND METHODS

Using CS Chem3Dpro software we determined energetically favorable conformations of the tetrapeptide Ala-Glu-Asp-Gly. The length and maximum width of its energetically favorable conformation in water (homogenous environment) were 15.5 and 8.5 Å.

In a saline solution and during multipoint interaction with DNA this tetrapeptide gain an unfolded and elongated shape similar to β-structure, which provides maximum conformational flexibility of the side groups in the peptide chain and allows maximum number of intermolecular interactions.

In previous studies the number of amino acid repeats in regulatory proteins was estimated using original software [7]. A similar approach was used to determine nucleotide repeats in promoter regions of retinal genes F379 (large subunit of RNA polymerase II and telomerase) [11,12,15].

*St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences; **Center for Mathematical Biology, Institute of Mathematics, Oxford University, Great Britain. *Address for correspondence:* khavinson@gerontology.ru. V. Kh. Khavinson

0007-4868/03/1363-0268$25.00

RESULTS

Most nontranscribed region in the studied genes contain numerous repeats of similar nucleotide sequences, whose length does not exceed 1 turn of the double-stranded DNA (6-10 b. p.). These regular nucleotide sequences in the promoter region can serve as specific sites for selective binding of RP (*e.g.*, Epithalon). Double-stranded DNA and unfolded peptide chain are characterized by certain metric complimentary: the length of the peptide chain corresponding to one amino acid is 3.47 Å, the distance between base pairs in the DNA chain is 3.4 Å, the width of Epithalon is 8.5 Å, therefore, this substance can integrate only into the major groove of DNA with a width of 9.5 Å [8]. It provides conditions for multisite intermolecular interaction between Epithalon and DNA that involves polar and hydrophobic groups of both molecules. Polar interactions of RP with double-stranded DNA depend on electrostatic relationships between carboxylic groups of the peptide, amino groups of adenine and cytosine, and hydrogen bonds between carboxylic groups of the peptide and ^{7}N nitrogen atom of adenine and guanine [9,13]. Hydrophobic interactions in the system are determined by side chains of the peptide and methyl groups of thymine. Rearrangement of nucleotide pairs allows identification of the sequence at which functional groups of nucleotides in the DNA major groove are complementary to the side chains in Epithalon. We found that the nucleotide sequence **ATTTG** optimally corresponds to the position of side groups in Epithalon. A specific feature of these relationships is that RP in the major groove interact with functional groups in both chains of double-stranded DNA (Fig. 1). Multiple repeats of this sequence of nucleotide pairs were found in the promoter regions of studied genes (Table 1). Flexibility of the N-terminal amino group in Epithalon allows this tetrapeptide to interact complementarily not only with the nucleotide sequence ATTTG, but also with the sequence CTTTG. The presence of nucleotide repeats in the promoter region of genes improves their accessibility for RP.

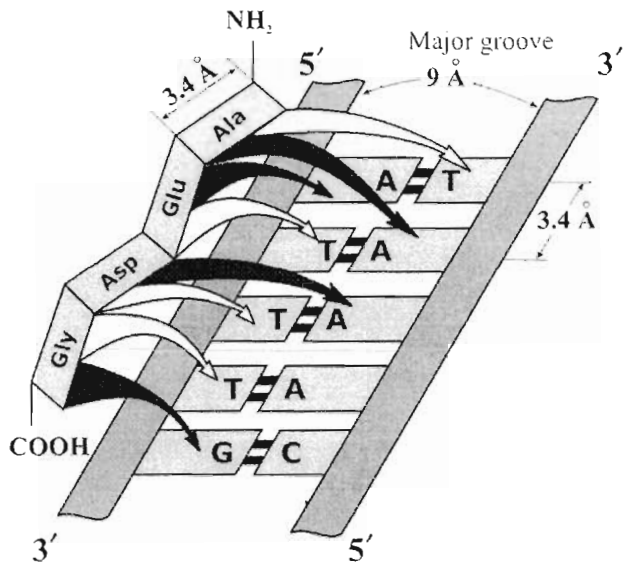

Fig. 1. Multisite interaction of the tetrapeptide Epithalon with functional groups of the nucleotide sequence ATTTG in the major groove of double-stranded DNA. Light arrows: hydrogen bonds; dark arrows: hydrophobic bonds.

Taking into account the structure of the nuclear membrane, it is possible to propose a mechanism of RP transport from the cytoplasm into the nucleus. About 10% of the nuclear membrane surface is occupied by nucleopores with a diameter of 90×150 Å. High-molecular-weight TF are transported into the cell nucleus by protein complexes of these pores. These complexes transport or not transport specific macromolecules, but allow free diffusion of water and water-soluble substances with a molecular weight below 5 kDa [14]. Therefore, oligopeptides can diffuse into the nucleus and interact with active chromatin.

Our results suggest that RP act as activators and agonists of TF. The site-specific interaction of RP in the major groove of DNA is a primary signal for binding of TF to the promoter.

TABLE 1. Complementary Binding of Epithalon (Ala-Glu-Asp-Gly) to Gene Promoter Regions

Epithalon functions	Binding site for Epithalon in the gene promoter region	References*
Activation of telomerase gene transcription	**ATTTG**$_{6}$ TAAAC	[15]
Activation of RNA polymerase II gene transcription	**ATTTG**$_{3}$ TAAAC	[12]
Activation of retinal gene F379 transcription	**CTTTG**$_{2}$ GAAAC	[11]

Note. Bold font for the binding site: major chain of DNA; subscript: number of repeated nucleotide blocks in the gene promoter region. *Reference to data for the gene promoter region.

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