DOI 10.1007/s10517-017-3876-x
Bulletin of Experimental Biology and Medicine, Vol. 163, No. 5, September, 2017 IMMUNOLOGY AND MICROBIOLOGY

Peptides (Epigenetic Regulators) in the Structure of Rodents with a Long and Short Lifespan

V. Kh. Khavinson[1,3], D. Yu. Kormilets[2], and A. T. Mar'yanovich[3]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 5, pp. 631-636, May, 2017
Original article submitted August 2, 2016

We have discovered motives of short-chain epigenetically active peptides in some proteins of long-lived African mole rat *Heterocephalus glaber*. These epigenetic regulators are located in the protein structure between lysine and arginine residues, thus facilitating their release in limited proteolysis. Some of these epigenetic regulators are not found in the proteins of short-lived species — Norway rat *Rattus norvegicus* and house mouse *Mus musculus*.

Key Words: *aging; peptides; epigenetic regulation*

Short peptides (peptide epigenetic regulators, PER, PEGRs) promoting recovery of damaged tissues, including those impaired as a result of aging, are created at St. Petersburg Institute of Bioregulation and Gerontology [2,5-7].

African rodent, naked mole-rat *Heterocephalus glaber*, attracts attention of gerontologists because of its longevity, uncommon for its order: its maximum documented lifespan is more than 30 years [8] — 10-fold longer than in species evolutionally close to it — Norway rat (*Rattus norvegicus*) and house mouse (*Mus musculus*). Naked mole-rat is resistant to cancer [3]. The first observations of malignant tumors in two males aged 20 and 22 years have just been presented [4].

Among other explanations of *H. glaber* longevity is a special stability of its proteins [9], implying effective mechanisms of repair of damaged molecules [10]. The naked mole-rat genome has been described and the primary structure of all its proteins is available.

The aim of our study is to clear out whether the protein structure of the long lived rodent has motives corresponding to peptides (PER) and whether these motives structurally differ from those of related short lived species.

[1]St. Petersburg Institute of Bioregulation and Gerontology; [2]S. M. Kirov Military Medical Academy; [3]I. I. Mechnikov North-Western State Medical University, St. Petersburg, Russia. ***Address for correspondence:*** atm52@mail.ru. A. T. Mar'yanovich

MATERIALS AND METHODS

The most probable sites of enzymatic cleavage, as a result of which a regulatory peptide is "cut" from the precursor protein molecule, are combinations of the main amino acids Lys-Arg (KR), Arg-Arg (RR), Arg-Lys (RK), and Lys-Lys (KK) [1]. Using specially designed AMS14-003 software, we carried out search for PER in easily released forms K—PER—K, K—PER—R, R—PER—K, and R—PER—R in the primary structure of all proteins of *H. glaber*, *R. norvegicus*, and *M. musculus* included in the MEDLINE data base (in the form of FASTA — text format for presentation of protein sequences in a single letter code).

RESULTS

We have found the target motives in 17 proteins of *H. glaber* and compared the fragments containing them with those in *R. norvegicus* and *M. musculus* proteins (Table 1).

Bronchogen-containing *H. glaber* protein, classified up to the present time as uncharacterized protein, is 85% homologous (according to our data) to a fragment of human Nck-associated protein 5-like protein (Table 2), which fact suggests calling the naked mole-rat protein "human Nck-associated protein 5-like protein homolog" or human NAP5-LPH (Nck — non-catalytic region of tyrosine kinase adaptor protein).

TABLE 1. Short Peptides (PER) Present in Easily Released Form in the Structure of Long Lived *H. glaber* Rodent and Short Lived *R. norvegicus* and *M. musculus* Rodents

Peptides	Proteins
Epitalon	**von Willebrand factor A domain-containing protein 3B (vWA3B)** SRYADGLFPQFHR**AEDG**RVYNLTANSELIY SRYADGLFPRFYT**AEDG**RLYNLTAKSELIY SRYADGLFPQIYT**AEDG**RLYNLTAKTELIC **Tubulin-specific chaperone A** MK**AEDG**KNYAIKKQAEILQESQTMIPDCQR MK**AEDG**ENYAIKKQAEILQESRMMIPDCQR MK**AEDG**ENYAIKKQAEILQESRMMIPDCQR
Bronhogen	**Fascin (Protein Phf14 in rat)** MDEVEWMHRHPK**AEDL**RIGLISWAGTYLTF MAEVDWIHRHPK**AEAL**RVGLISWAGTYLTF MAEVDWIHRHPK**AEDL**RVGLISWAGTYLTF **Transcription factor AP-4** EGIGSPDIWEDE**KAEDLR**REMIELRQQLDK EGIGSPDIWEDEK**AEDL**RREMIELRQQLDK EGIGSPDIWEDEK**AEDL**RREMIELRQQLDK **Uncharacterized protein (human Nck-associated protein 5-like protein homolog)** EPESLNISKLMAK**AEDL**RRALEEEKAYLSR EAESLNISKLMAK**AEDL**RRALEEEKAYLSR EAESLNISKLMAK**AEDL**RRALEEEKAYLSR **1,25-dihydroxyvitamin D_3 24-hydroxylase, mitochondrial** EIQSVLPENQMPR**AEDL**KKMPYLKACLKES No proteins close by structure were found in data base for rat and mouse EIQSVLPDNQTPR**AED**VRNMPYLKACLKES **Leucine-rich repeat serine/threonine-protein kinase 1 (LRRK1)** KGSRSVAKNGVIR**AEDL**RMLLVGTGFTKQT KGSRSVAKNGVIR**AEDL**RMLLVGTGFTQQT KGSRSVAKNGVIQ**AEDL**RMLLVGTGFTQQT

Table 1 continued.

Peptides	Proteins
	Dynein heavy chain 2, axonemal YEIPHYVVNVAGR**AEDL**RILRENLLLVARD FETPHYVMNVADR**AEDL**RILRENLLLVARD FETPHYVMNVAER**AEDL**RILRENLLLVARD
Testagen	**General transcription factor IIF (TFIIF) subunit 2** ANHQYNIEYERKK**KEDG**KRARADKQHVLDM ANHQYNIEYERKK**KEDG**KRARADKQHVLDM ANHQYNIEYERKK**KEDG**KRARADKQHVLDM **Serrate RNA effector molecule-like protein, или Serrate RNA effector molecule homolog, или Arsenite-resistance protein 2 (SRRT, ARS2)** GDGERKAGDKDDK**KEDG**KQAENDGSNDDKT GDGERKVNDKDEK**KEDG**KQAENDSSNDDKT GEGERKANDKDEK**KEDG**KQAENDSSNDDKT **Lipoxygenase-like protein domain-containing protein 1, or Lipoxygenase homology domains 1 (LOXHD1)** EFLFLCGRWLSLK**KEDG**RLERLFYEKEYTG EFLFLCGRWLSLK**KEDG**RLERLFYEKEYTG EFLFLCGRWLSLK**KEDG**RLERLFYEKEYTG **Patatin-like phospholipase domain-containing protein 7 (PNPLA7), или NTE-related esterase (NRE)** YIVLSGRLRSVIR**KEDG**KKRLVGEYGLRDL YIVLSGRLRSVIR**K**D**DG**KKRLAGEYGRGDL YIVLSGRLRSVIR**K**D**DG**KKRLAGEYGRGDL
Livagen	**Prostaglandin reductase 1 (PGR1), или leukotriene B4 dehydrogenase** GGRERRGQEEEEK**KEDA**KKEKGRSLMMVRA No proteins close by structure were found in data base for rat and mouse **Short stature homeobox protein 2, или homeobox protein Og12X, или paired-related homeobox protein SHOT** PRLTEVSPELKDR**KEDA**KGMEDEGQTKIKQ PRLTEVSPELKDR**KEDA**KGMEDEGQTKIKQ PRLTEVSPELKDR**K**D**DAK**GMEDEGQTKIKQ

Table 1 continued.

Peptides	Proteins
Cardiogen	**Dehydrogenase/reductase SDR family member 4 (DHRS4)** GLSVTGTVCHVGK**AEDR**KQLVATAVKLHGG GLSVTGVVCHVGK**AEDR**EKLVNMALKLHQG GLSVTGIVCHVGK**AEDR**EKLITTALKRHRG **Eukaryotic translation initiation factor 3 subunit A (EIF3A)** LRSERDEVSSWRR**AEDR**KDDRAEERDPPRR LRSEREEASSWRRTD**DR**KDDRTEERDPPRR LRSEREEASSWRRTD**DR**KDDRTEERDPPRR
Prostamax	**MAP7 domain-containing protein 2 (in rat – Brain-enriched E-MAP-115-like protein, in mouse MAP7 domain-containing protein 2 isoform 2)** KRTRKSDVSPEVK**KEDP**KVEIQPVVCVENK KRTRKSDASLEVK**KEDP**KVEIQPLPDVENK KRTRKSDASLEVK**KEDP**KVELQPPPDVENK

Note. Primary structures: upper lines: *H. glaber*, middle lines: *R. norvegicus*, lower lines: *M. musculus*. Coincidence of the rat and mouse amino acid residues with those of naked mole-rat is shown with a gray color. Motives corresponding to PER are shown with bold letters, the adjacent lysine (K) and arginine (R) residues are underlined. Primary structures of tubulin-specific chaperone A and prostaglandin reductase-1 are presented completely, other proteins presented as fragments (*n*=30) including the target short peptides.

TABLE 2. *H. glaber* Uncharacterized Protein (Fragment, *n*=1003)

XSSGPNCAPGSSSSSSSDEAGDPNEAPSPDTLLGALARRQLNLGQLLEDTESYLQAFLAGAAG
PLNGDHPGPGQSSSPDQAPPQLSKSKGLPKSAWGGGTPEAHRPGFGATSEGQGPLPFLSMFMG
AGDAPLGSRPGHPHSSSQVKSKLQIGPPSPGEAQGPLLPSPARGLKFLKLPPTSEKSPSPGGP
QLSPQLPRNSRIPCRNSGSDGSPSPLLARRGLGGGELSPEGAQGLPTSPSPCYTTPDSTQLRP
PQSALSTTLSPGPVVSPCYENILDLSRSTFRGPSPEPPPSPLQVPTYPQLTLEVPQAPEVLRS
PGVPPSPCLPESYPYGSPQEKSLDKAGSESPHPGRRTPGNSSKKPSQGSGRRPGDPGSTPLRD
RLAALGKLKTGPEGALGSEKNGVPARPGTEKTRGPGKSGESAGDMVPSIHRPLEQLEAKGGIR
GAVALGTNSLKQQEPGLMGDPGARVYSSHSMGARVDLEPVSPRSCLTKVELAKSRLAGALCPQ
VPRTPAKVPTSAPSLGKPNKSPHSSPTKLPSKSPTKVVPRPGAPLVTKESPKPDKGKGPPWAD
CGSTTAQSTPLVPGPTDPSQGPEGLAPHSAIEEKVMKGIEENVLRLQGQERAPGAEVKHRNTS
SIASWFGLKKSKLPALNRRTEATKNKEGAGGGSPLRREVKMEARKLEAESLNISKLMAK**AEDL**
RRALEEEKAYLSSRARPRPGGPAPGPNTGLGQVQGQLAGMYQGADTFMQQLLNRVDGKELPSK
SWREPKPEYGDFQPVSSDPKSPWPACGPRNGLVGPLQGCGKPPGKPSSEPGRREEMPSEDSLA
EPVPTSHFTACGSLTRTLDSGIGTFPPPDHGSSGTPSKNLPKTKPPRLDPPPGVPPARPPPLT
KVPRRAHTLEREVPGIEELLVSGRHPSMPAFPALLPAAPGHRGHETCPDDPCEDPGPTPPVQL
AKNWTFPNTRAAGSSSDPLMCPPRQLEGLPRTPMVRIAAEERERTREQEGVMWGDQFLQ

Note. Gray background shows amino acid residues common for this protein in naked mole-rat and human NAP5-LPH. Motives corresponding to PER are shown with bold letters, the adjacent lysine (K) and arginine (R) residues are underlined.

TABLE 3. Coincidence/Noncoincidence of Short Peptide (PER) Structures in Proteins of Rodents with a Long (H. glaber) and Short Lifespan (*R. norvegicus*, *M. musculus*)

Coinciding structures	Protein	PER in proteins
PER and terminal Arg/Lys	Fascin	Lys-Bronhogen-Arg (KAEDLR)
	Transcription factor AP-4	Lys-Bronhogen-Arg (KAEDLR)
	Uncharacterized protein (human Nck-associated protein 5-like protein homolog)	Lys-Bronhogen-Arg (KAEDLR)
	General transcription factor IIF (TFIIF) subunit 2	Lys-Testagen-Lys (KKEDGK)
	Serrate RNA effector molecule-like protein, or Serrate RNA effector molecule homolog or Arsenite-resistance protein 2 (SRRT, ARS2)	Lys-Testagen-Lys (KKEDGK)
	Lipoxygenase-like protein domain-containing protein 1, or Lipoxygenase homology domains 1 (LOXHD1)	Lys-Testagen-Lys (KKEDGK)
	MAP7 domain-containing protein 2	Lys-Prostamax-Lys (KKEDPK)
Only PER	von Willebrand factor A domain-containing protein 3B (vWA3B)	Epitalon (AEDG)
	Tubulin-specific chaperone A	
	Epitalon (AEDG)	
	Dehydrogenase/reductase SDR family member 4 (DHRS4)	Cardiogen (AEDR)
3 of 4 PER amino acids	Eukaryotic translation initiation factor 3 subunit A (EIF3A)	Cardiogen (XEDR)
	Patatin-like phospholipase domain-containing protein 7 (PNPLA7), or NTE-related esterase (NRE)	Testagen (KXDG)

Table 3 presents data on the structural similarity of PER in long lived and short lived rodent species.

The motives corresponding to PER are found in the rodent protein composition, including the regions adjacent to lysine and arginine residues facilitating peptide release as whole molecules during partial proteolysis. The structure of the above motives in the molecules of some proteins of long lived *H. glaber* species differs significantly from that in short lived *R. norvegicus* and *M. musculus*: in eukaryotic translation initiation factor 3; in patatin-like phospholipase domain-containing protein 7, and in dehydrogenase/reductase SDR family member 4 Differences in the structure of von Willebrandt factor and tubulin-specific chaperone A are less significant.

Short peptides, identical to PER, are present in the protein structure of *H. glaber*. These short peptides are located between lysine and arginine residues, which facilitates significantly the release of these peptides in limited proteolysis. Some of these peptides, found in the naked mole-rat proteins, are not found in the proteins of short lived species Norway rat and house mouse. The structure and function of these proteins deserve special attention in further studies of the longevity phenomenon.

REFERENCES

1. Mar'yanovich AT. General theory of peptide regulation of physiological functions: the blood-brain barrier and evolution of connections between the brain and periphery. St. Petersburg, 2014. Russian.
2. Khavinson VKh, Solov'ev AYu, Tarnovskaya SI, Lin'kova NS. Mechanism of biological activity of short peptides: Cell penetration and epigenetic regulation of gene expression. Biol. Bull. Rev. 2013;3(6):451-455.
3. Buffenstein R. Negligible senescence in the longest living rodent, the naked mole-rat: insights from a successfully aging species. J. Comp. Physiol. B. 2008;178(4):439-445.
4. Delaney MA, Ward JM, Walsh TF, Chinnadurai SK, Kerns K, Kinsel MJ, Treuting PM. Initial case reports of cancer in naked mole-rats (Heterocephalus glaber). Vet. Pathol. 2016;53(3):691-696.
5. Khavinson VKh, Malinin VV. Gerontological Aspects of Genome Peptide Regulation. Basel, 2005.

6 Khavinson VKh. Peptides, genome, aging. Adv. Gerontol. 2014;27(2):257-264.
7. Khavinson VK, Solov'ev AY, Zhilinskii DV, Shataeva LK, Vanyushin BF. Epigenetic aspects of peptide-mediated regulation of aging. Adv. Gerontol. 2012;2(4):277-286.
8. MacRae SL, Croken MM, Calder RB, Aliper A, Milholland B, White RR, Zhavoronkov A, Gladyshev VN, Seluanov A, Gorbunova V, Zhang ZD, Vijg J. DNA repair in species with extreme lifespan differences. Aging (Albany NY). 2015;7(12):1171-1184.
9. Pérez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Andziak B, Yang T, Edrey Y, Friguet B, Ward W, Richardson A, Chaudhuri A. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc. Natl Acad. Sci. USA. 2009;106(9):3059-3064.
10. Yu C, Li Y, Holmes A, Szafranski K, Faulkes CG, Coen CW, Buffenstein R, Platzer M, de Magalhães JP, Church GM. RNA sequencing reveals differential expression of mitochondrial and oxidation reduction genes in the long-lived naked mole-rat when compared to mice. PLoS One. 2011;6(11):e26729.