

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

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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.

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).

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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	<p>von Willebrand factor A domain-containing protein 3B (vWA3B)</p> <p>SRYADGLFPQFHRAEDGRVYNLTANSELIY SRYADGLFPRFYTAEDGRLYNLTAKSELIY SRYADGLFPQIYTAEDGRLYNLTAKTELIC</p> <p>Tubulin-specific chaperone A</p> <p>MKAEDGKNYAIKKQAEILQESQTMIPDCQR MKAEDGENYAIKKQAEILQESRMMIPDCQR MKAEDGENYAIKKQAEILQESRMMIPDCQR</p>
Bronhogen	<p>Fascin (Protein Phf14 in rat)</p> <p>MDEVEWMHRHPKAEDLRIGLISWAGTYLTF MAEVDWIHRHPKAEALRVGLISWAGTYLTF MAEVDWIHRHPKAEDLRVGLISWAGTYLTF</p> <p>Transcription factor AP-4</p> <p>EGIGSPDIWEDEKAEDLRREMIELRQQLDK EGIGSPDIWEDEKAEDLRREMIELRQQLDK EGIGSPDIWEDEKAEDLRREMIELRQQLDK</p> <p>Uncharacterized protein (human Nck-associated protein 5-like protein homolog)</p> <p>EPESLNISKLMAKAEDLRRALEEEKAYLSR EAESLNISKLMAKAEDLRRALEEEKAYLSR EAESLNISKLMAKAEDLRRALEEEKAYLSR</p> <p>1,25-dihydroxyvitamin D₃ 24-hydroxylase, mitochondrial</p> <p>EIQSVLPENQMPRAEDLKKMPYLKACLKES No proteins close by structure were found in data base for rat and mouse EIQSVLPDNQTPRAEDVRNMPYLKACLKES</p> <p>Leucine-rich repeat serine/threonine-protein kinase 1 (LRRK1)</p> <p>KGSRVAKNGVIRAEDLRMLLVGTGFTKQT KGSRVAKNGVIRAEDLRMLLVGTGFTQQT KGSRVAKNGVIQAEDLRMLLVGTGFTQQT</p>

Table 1 continued.

Peptides	Proteins
	<p>Dynein heavy chain 2, axonemal</p> <p>YEIIPHYVVNVAGRAEDLRRILRENLLLWARD</p> <p>FETPHYVMNVADRAEDLRRILRENLLLWARD</p> <p>FETPHYVMNVAERAEDLRRILRENLLLWARD</p>
Testagen	<p>General transcription factor IIF (TFIIF) subunit 2</p> <p>ANHQYNI EYERKKKEDGKRARADKQHVLDM</p> <p>ANHQYNI EYERKKKEDGKRARADKQHVLDM</p> <p>ANHQYNI EYERKKKEDGKRARADKQHVLDM</p> <p>Serrate RNA effector molecule-like protein, или Serrate RNA effector molecule homolog, или Arsenite-resistance protein 2 (SRRT, ARS2)</p> <p>GDGERKAGDKDDKEDGKQAENDGSNDDKT</p> <p>GDGERKVNDKDEKEDGKQAENDSNDDKT</p> <p>GEGERKANDKDEKEDGKQAENDSNDDKT</p> <p>Lipoxygenase-like protein domain-containing protein 1, or Lipoxygenase homology domains 1 (LOXHD1)</p> <p>EFLFLCGRWLSLKKEDGRLERLFYEKEYTG</p> <p>EFLFLCGRWLSLKKEDGRLERLFYEKEYTG</p> <p>EFLFLCGRWLSLKKEDGRLERLFYEKEYTG</p> <p>Patatin-like phospholipase domain-containing protein 7 (PNPLA7), или NTE-related esterase (NRE)</p> <p>YIVLSGRLRSVIRKEDGKKRLVGEYGLRDL</p> <p>YIVLSGRLRSVIRKDDGKKRLAGEYGRGDL</p> <p>YIVLSGRLRSVIRKDDGKKRLAGEYGRGDL</p>
Livagen	<p>Prostaglandin reductase 1 (PGR1), или leukotriene B4 dehydrogenase</p> <p>GGRRERGQEEEEKEDAKKEKGRSLMMVRA</p> <p>No proteins close by structure were found in data base for rat and mouse</p> <p>Short stature homeobox protein 2, или homeobox protein Og12X, или paired-related homeobox protein SHOT</p> <p>PRLTEVSPELKDRKEDAKGMEDEGQTKIKQ</p> <p>PRLTEVSPELKDRKEDAKGMEDEGQTKIKQ</p> <p>PRLTEVSPELKDRKDDAKGMEDEGQTKIKQ</p>

Table 1 continued.

Peptides	Proteins
Cardiogen	<p>Dehydrogenase/reductase SDR family member 4 (DHRS4)</p> <p>GLSVTGTVCHVGK<u>AEDRK</u>QLVATAVKLHGG GLSVTGVVCHVGK<u>AEDREK</u>LVNMALKLHQG GLSVTGI VCHVGK<u>AEDREK</u>LITALKRHRG</p> <p>Eukaryotic translation initiation factor 3 subunit A (EIF3A)</p> <p>LRSERDEVSSWRR<u>AEDRK</u>DDRAEERDPPRR LRSEREEASSWRR<u>DDRK</u>DDRTEERDPPRR LRSEREEASSWRR<u>DDRK</u>DDRTEERDPPRR</p>
Prostamax	<p>MAP7 domain-containing protein 2 (in rat – Brain-enriched E-MAP-115-like protein, in mouse MAP7 domain-containing protein 2 isoform 2)</p> <p>KRTRKSDVSP<u>EVK</u><u>KED</u>PKVEIQPVVCVENK KRTRKSDASLEVK<u>KED</u>PKVEIQPLPDVENK KRTRKSDASLEVK<u>KED</u>PKVELQPPDVENK</p>

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
AGDAPLGSRPGHPHSSSQVSKLQIGPPSPGEAQGPLLSPARGLKFLKLPPTSEKSPSPGGF
QLSPQLPRNSRIPCRNSGSDGSPSPLLARRGLGGGELSPEGAQGLPTSPSPCYTTPDSTQLRF
PQSALSTTLSPGPVVSPCYENILDLSRSTFRGSPPEPPPSPLQVPTYQPLTLEVPQAPEVLRS
PGVPPSPCLPESYPYGSFQEKSLDKAGESPHPGRRTPGNSSKKPSQSGRRPGDPGSTPLRD
RLAALGKLTGPEGALGSEKNGVPARPGTEKTRGPGKSGESAGDMVPSIHRPLEQLEAKGIR
GAVALGTNSLKQQEPGLMGDPGARVYSSSHSMGARVDLEPVSPRSCLTKVELAKSRLAGALCPQ
VPRTPAKVPTSAPSLGKPNKSPHSSPTKLPKSPTKVVPRPGAPLVTKESPKPKDKGKPPWAD
CGSTTAQSTPLVPGPTDPSQGPEGLAPHS AIEEKVMKGIENVLRLQGQERAPGAEVKHRNTS
SIASWFGKSKLPALNRRTEATKNKEGAGGGSPLRREVKMEARKLEAESLNISKLMKAEDL
RRALEEEKAYLSRARRPRPGGPAPGPNGLGQVQQLAGMYQGADTFMQQLNVRVDGKELPSK
SWREPKEPYGDFQPVSSDPKSPWPACGPRNGLVGPLQCGCKPPGKPSSEPGREEMFSEDSLA
EPVPTSHFTACGSLTRTLDSGIGTFPPPDHGSSGTPSKNLPKTKPRLDPPPVPVPPARPPPLT
KVPRAHTLEREVPGIEELLVSGRHPSPAPFALLPAAPGHRGHETCPDDPCEDPGPTPPVQL
AKNWTFPNTRAAGSSSDELMCPRQLEGLPRTPMVRIAAEERERTREQEGVMWGDQFLQ

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)
Only PER	MAP7 domain-containing protein 2	Lys-Prostamax-Lys (KKEDPK)
	von Willebrand factor A domain-containing protein 3B (vWA3B)	Epitalon (AEDG)
	Tubulin-specific chaperone A	
3 of 4 PER amino acids	Epitalon (AEDG)	
	Dehydrogenase/reductase SDR family member 4 (DHRS4)	Cardiogen (AEDR)
	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.

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