ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, Suppl. 1, pp. S36-S59 © Pleiades Publishing, Ltd., 2025.
Russian Text © The Author(s), 2025, published in Uspekhi Biologicheskoi Khimii, 2025, Vol. 65, pp. 55-90.

REVIEW

Moonlighting Proteins of Human and Some Other Eukaryotes. Evolutionary Aspects

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Received February 7, 2024
Revised March 27, 2024
Accepted April 2, 2024

Abstract—This review presents materials on formation of the concept of moonlighting proteins and general characteristics of different similar proteins. It is noted that the concept under consideration is based on the data on the existence in different organisms of individual genes, protein products of which have not one, but at least two fundamentally different functions, for example, depending on cellular or extracellular location. An important feature of these proteins is that their functions can be switched. As a result, in different cellular compartments or outside the cells, as well as under a number of other circumstances, one of the possible functions can be carried out, and under other conditions, another. It is emphasized that the significant interest in moonlighting proteins is due to the fact that information is currently accumulating about their involvement in many vital molecular processes (glycolysis, translation, transcription, replication, etc.). Alternative hypotheses on the evolutionary origin of moonlighting proteins are discussed.

DOI: 10.1134/S0006297924602855

Keywords: moonlighting proteins, protein polymorphism, multiple protein functions, glycolytic enzymes, ribosomal proteins, proteins of chromatin

INTRODUCTION

In modern biochemistry notions about proteins, one of the main research objects of this science, are developing constantly. In particular, already at the end of XX century it was shown that expression of the exon-intron genes of eukaryotes often occurs with alternative splicing, and, as a result, one gene is capable of synthesizing several sometimes very different protein products. It was found out later that these macromolecules are subjected to individual or multiple posttranslational modifications, which further expand the set of products of functioning of a single gene. Moreover, accumulated data indicate that in different human individuals (and other eukaryotic organisms) polypeptide chains determined by the same gene could have slight differences, such as single amino acid replacements due to the so-called point mutations in this gene. All this knowledge and other available data resulted in introduction of the notion of proteoforms to the scientific literature in 2013 [1].

The ability of single genes to produce a set of proteoforms with certain structural and functional features has been considered as a consequence of continuing evolutionary processes facilitating acquiring of many important traits by different organisms such as, for example, resistance to environmental stresses [2, 3].

At the same time, the data have been accumulated that proteoforms in general are involved in such phenomenon as protein polymorphism, which is characterized as existence in humans and other mammal of several protein molecules with certain common features, but with clearly pronounced differences, which often are crucial for their functions [4]. Furthermore, it has been suggested in numerous studies that the existence of polymorphic proteins is the result of evolution from the so-called last universal common ancestor, to mammals and humans, for example [5, 6].

Investigation of multifunctional proteins could be considered as an important direction in the modern studies of proteins. Almost three thousand of reviews

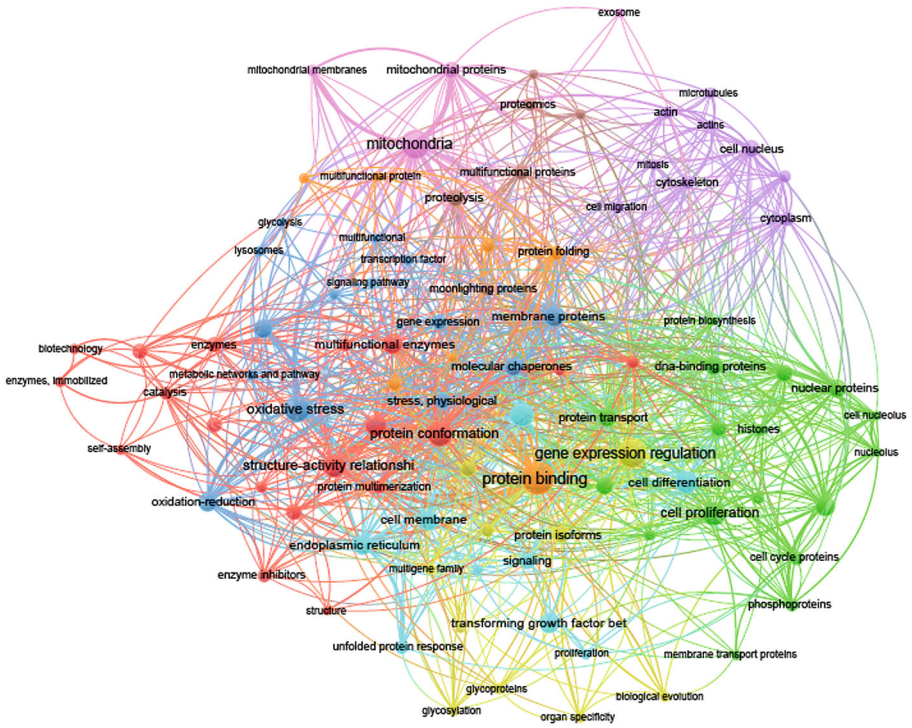

Fig. 1. Graphic representation of bibliometric map created with the help of VOSreviewer program, which covers the subject area limited to the words “multifunctional protein, review” and contains information on materials available in PubMed database published during 2014-2024. Thematic clustering was carried out automatically based on the most often used key words. In the graph center the node “moonlighting proteins” is highlighted.

on the features of such proteins exhibiting different main functions (enzymatic, transport, regulatory, and others) have been published in the recent decade, which also have other ‘additional’ functions. These publications have been annotated in the PubMed database, which is for many years supported by the National Center for Biotechnology Information (NCBI) of the USA. General overview of these publication could be obtained with the help of bibliometric analysis performed with the help of the program VOSreviewer.

VOSreviewer is a free computer program capable of constructing graphic images of bibliometric maps of certain subject areas [7-9]. In our case the subject area was limited to the words ‘multifunctional protein, review’. VOSreviewer provides visualization of the subject area based on analysis of the selected publications presented as a network between the sources in a two-dimensional space. The formed network consists of ‘nodes’ and ‘edges’ with nodes representing frequency of publications with certain key words and edges existence of thematic associations between the nodes.

Graphic representation of a bibliometric map including materials from PubMed database published in the period from 2014 to 2024 is presented in Fig. 1. Visualization was performed based on the most often used key words (around 100) using thematic clustering.

As can be seen in Fig. 1, there are 9 clusters (with ‘nodes’ marked in different colors). In the center of the created map the node has been identified shown as “moonlighting proteins”. This term was introduced to the scientific literature more than twenty years ago [10] and was used to describe a special group of multifunctional proteins.

In the second decade of XXI century these studies have been advancing significantly. It has been noted by many authors that some aspect of investigation of “moonlighting proteins” are associated with the fundamental principle of modern molecular biology and emphasized that existence of such proteins corresponds not to the formula “one gene – one function”, but to the formula “one gene – two functions” [11-13]. Consequently, based on the abovementioned information, the goal of this review was analysis of the results of investigation of properties of “moonlighting proteins” of human and of some other eukaryotes with emphasis on evolutionary aspects.

EMERGENCE AND DEVELOPMENT OF THE CONCEPT OF MOONLIGHTING PROTEINS IN EUKARYOTES

Publications in the end of 1980s reporting existence in some organisms of certain genes with products

exhibiting not one, but at least two significantly different functions manifested depending on, for example, cellular or extracellular localization, could be considered as a prelude to formation of the concept of "moonlighting proteins". In particular, it was demonstrated that the known cytoplasmic enzymes (lactate dehydrogenase, enolase) are capable play a role of structural crystallins in an eye lens [14, 15]. According to the presented data the indicated enzymes and isoforms of crystallins have identical amino acid sequences.

Later Constance J. Jeffery [10] in her review summarized the available data on such proteins and suggested a term "moonlighting proteins" to describe these proteins. This unusual term originates from an English slang word "moonlighting" referring to a side job outside of normal working hours or to a practice of theft cattle or crop destruction at night times.

At present, increasing interest in moonlighting proteins could be explained by the fact that numerous publications are emerging on their involvement in vital molecular processes (see, for example [16-18]). However, a number of issues associated with moonlighting proteins remain unresolved during the recent decades; in particular, it is poorly understood when, how, and why such additional molecular functions appeared. Although it has been suggested that this property could evolve in the course of evolution [10, 19-21]. On the other hand, there is an alternative point of view according to which the ancient proteins initially had several functions, which were gradually lost during evolution leaving the protein with only one function, enzymatic, for example [18].

One of the important characteristics of moonlighting proteins is their ability for switching functions. As a result, in different cellular compartments and outside the cell, as well as under other specific conditions one of the functions could be performed, and under different conditions – another.

"Switching" of functions by moonlighting proteins attract attention of researchers from the very beginning of investigation of this phenomenon until now (see, for example [10, 12, 22]). The mechanisms of "switching" are actively examined, and among those, the mechanisms associated with posttranslational modifications have been considered [22, 23]. Among the conditions causing "switching" of functions specific features of synthesis of moonlighting proteins in the cells with different types of differentiation have been considered, as well as changes in oligomeric composition of the protein (homo- or heterooligomers) associated or not associated with intracellular concentration of a ligand, substrate, cofactor, or product, etc. [24, 18]. Many authors described also various combinations of the variants of "switching" functions.

In this regard, it seems important to note that starting from the end of the XX century and until now the concept is being developed about the so-called intrinsically disordered proteins [25, 26]. Emergence of this concept was facilitated by the success of genomic projects. In particular, it has been established that the major part of gene sequences encodes proteins that do not have the ability for unassisted folding into a globular structure [25]. These intrinsically disordered proteins have long segments of amino acid sequences, which, likely, either unfold in solution, or form non-globular structures with undetermined conformation. Moreover, the idea began to spread that under physiological conditions such proteins (and/or protein segments) do not have one unique three-dimensional structure, but assume several interconverting conformational states [26, 27].

One of interesting reflection of this concept with regards to moonlighting proteins are the studies indicating association between the ability of a single polypeptide chain perform two (or more) significantly different functions and the presence in such polypeptide chains of intrinsically disordered regions [27, 28-30]. It has been suggested that due to ability of the intrinsically disordered regions in these proteins to assume several interconverting conformational states, they have the ability to realize different functions. Ability of some moonlighting proteins to perform both enzymatic and chaperon-like activity, as well as play a role of an enzyme or a regulatory factor could be mentioned as examples [20, 27, 29, 31].

Another important feature of moonlighting proteins worth mentioning is that in the case of introduction of a point mutation to the coding gene, which disrupt one of the functions of this protein, other function could be preserved (see [18, 22, 32, 33] for examples).

Multifunctional proteins, which presumably emerged as a result of fusion of ancestral genes, commonly are not considered as moonlighting proteins [10, 34, 35]. Ability of these proteins to perform several functions usually is associated with the presence in their polypeptide chain of special domains with each of them performing only one function. Nevertheless, is has been assumed that for performing different function different structural elements of polypeptide chain of typical moonlighting proteins could be used.

The increasing interest in moonlighting proteins and numerous unsolved issues related to them was, likely, the reason for publishing at the end of the second decade of XXI century of the special paper by Constance J. Jeffery with a telling title "Protein moonlighting: What is it, and why is it important?" [20]. In this manuscript the author characterizes moonlighting proteins as macromolecules capable of performing "more than one physiologically relevant biochemical or biophysical function within one polypeptide chain".

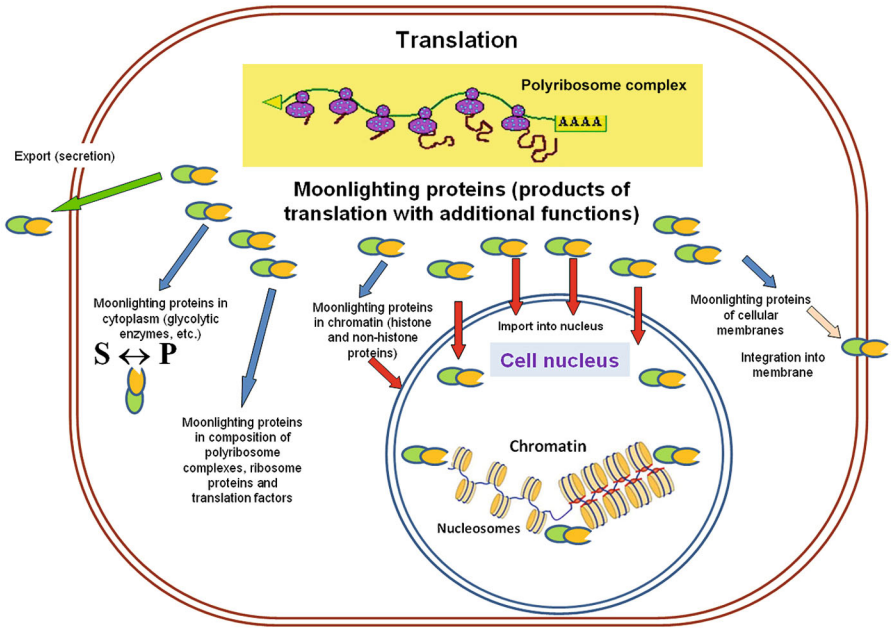

Fig. 2. Schematic representation of general knowledge on biosynthesis and distribution of moonlighting proteins in eukaryotic cells; arrows show distribution of moonlighting proteins in cellular compartments, where they perform the main known function [10, 20, 23, 28, 42, 44].

This definition has been accepted by many researchers [12, 36].

However, it must be also mentioned that some authors emphasize existence of certain problems in using these criteria of moonlighting proteins, especially with regards to subunits of oligomeric protein complexes (see, for example, [37, 38]). Moreover, some authors suggest that if the protein contain several domains, which are all required for performing different functions, these proteins could be also considered as moonlighting proteins [16].

In general, it is obvious that at present the concept on moonlighting proteins has been formed, which is manifested by thousands of publications that include the term “moonlighting proteins” in their content and annotated in the combined databases PubMed and ScienceDirect. Vast majority of these studies are devoted to moonlighting proteins of eukaryotes (from unicellular organisms to multicellular plants and vertebrate animals). Around one third of all publications are devoted to moonlighting proteins of bacteria and significantly lower number of papers are devoted to moonlighting proteins of archaea. Among the paper on eukaryotic moonlighting proteins, majority, obviously, is on human moonlighting proteins.

It has been reported in a number of publications that moonlighting proteins are present practically in all cellular compartments and subcellular structures, as well as in extracellular formations. Schematic representation of the processes of biosynthesis and distribution of moonlighting proteins in eukaryotic cells is shown in Fig. 2.

It has been established in the studies that inside the cell individual moonlighting proteins are often transported from one compartment to another, for example, from cytoplasm to nucleus and back [39], are integrated into mitochondria [40, 41] and cellular membrane [23]. Moonlighting proteins in the extracellular structures are transported there through secretion from certain types of cells [42]. Moreover, some moonlighting proteins have been found in blood flow [43].

Biosynthesis of moonlighting proteins (same as of all other cellular proteins) is realized via translation of respective mRNAs, after that the newly synthesized polypeptide chains are subjected to various posttranslational modifications and begin to perform their main known functions and additional functions. In many cases to begin performing one or another function transport of the protein to various cellular compartment is required such as transport into the cell nucleus or integration into the cellular membrane (Fig. 2).

Considering the abovementioned materials on moonlighting proteins, it seems very interesting to review the available information on eukaryotic proteins involved in some metabolic processes and maintenance of some intracellular structures that emerged in the early stages of evolution. Correspondingly, data on moonlighting proteins among the glycolytic enzymes, as well as moonlighting proteins participating in the processes of translation and those that are components of chromatin and of cellular membranes are presented in the following four sections of this review.

MOONLIGHTING PROTEINS AMONG GLYCOLYTIC ENZYMES

It is known that glycolytic processes occur in the cell cytoplasm and are observed in certain forms in all investigated eukaryotes. Among the proteins characterized as moonlighting, glycolytic enzymes were identified from the very beginning of investigation [10, 14, 15]. Similar glycolytic enzymes often carry out their additional functions outside of the cell cytoplasm in such locations as extracellular structures or cell nuclei. It is important to note that in the third decade of XXI century new information on glycolytic enzymes, which are considered moonlighting proteins, contains to be published [45, 46].

Glycolysis is recognized as one of the most characterized metabolic processes, which sometimes is described as one of the central metabolic pathways [47-49]. Phylogenetic approach to investigation of glycolytic enzymes revealed that this metabolic process is indeed very ancient and existed in live organism already at the time point when eukaryotes diverge from prokaryotes, i.e. around 1800 million years ago [47, 49]. Moreover, there is information that certain reactions similar to glycolytic could occur prior to advent of living cells in prebiotic period [50].

After the first publications on enzymes participants of glycolysis (1930s-1940s) and until now glycolytic processes occurring in eukaryotes are traditionally described as a Embden–Meyerhof–Parnas pathway (E-M-P). It has been established that as a result of functioning of this pathway under aerobic conditions glucose molecules are converted in the course of ten consequent enzymatic reactions into two pyruvate molecules. Under anaerobic conditions these pyruvate molecules in the eleventh reaction are reduced with the help of lactate dehydrogenase to lactic acid. Detailed description of the reactions in the E-M-P pathway could be found in numerous textbooks on biochemistry (see, for example [51]). Nevertheless, certain features and original schemes of realization of the E-M-P pathway have been published recently (see [18, 49, 52-54] for examples). One of such schemes (with small modifications) is shown in Fig. 3 for convenience of further discussion.

According to the accumulated data many glycolytic enzymes have been found to be moonlighting proteins (and in some organisms practically all, see [21, 55] for examples). It is known that the human glycolytic enzymes and glycolytic enzymes of higher vertebrates exhibit pronounced polymorphism (i.e., they comprise groups of related proteins) [46, 47, 54, 55].

Moreover, structure of the same glycolytic enzymes in different organisms is quite conserved. At the same the additional functions have been discovered not for all isoforms; hence, the question remains whether all similar isoforms should be considered to be potential moonlighting proteins.

Numerous publications are available in the literature on polymorphism and multifunctionality of glycolytic enzymes [18, 21, 55]. That is why it seems inadvisable to describe all glycolytic enzymes and their isoforms with additional functions. Below information on only three group of human glycolytic enzymes (full names are shown in Fig. 3), among which there are isoforms with established additional functions, are considered as examples.

Hexokinases. It has been established that human genome contains at least five genes encoding different enzymes capable of catalyzing reaction of phosphorylation of hexose using ATP [56, 57]. Two of these genes (*HK1*, *HKDC1*) are located in the segment 10q22.1, and the rest – on other chromosomes: *HK2* – in the region 2p12, *HK3* – in 5q35.2, and *HK4* – in 7p13, according

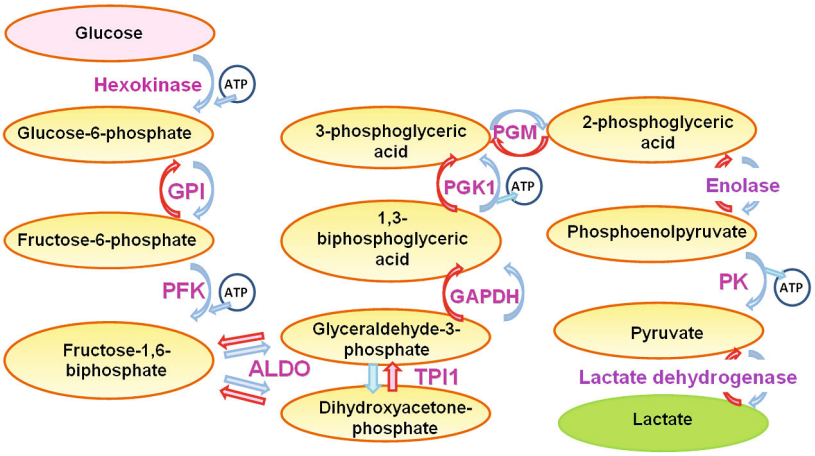

Fig. 3. Generalized scheme of glycolytic breakdown of glucose (Embden–Meyerhof–Parnas pathway), according to [51-53]. Oppositely directed blue and red arrows indicate reversible reactions. Full names are shown for three types of enzymes, which are moonlighting proteins; rest of the enzymes are shown using common designations. Additional explanations are presented in the text.

Table 1. General characteristics of human hexokinases and their various proteoforms, according to UniProt and OMIM NCBI

Accepted names and *symbols of genes*	Numbers in UniProt/OMIM	Quantity of amino acid residues (aa)	Manifestation of polymorphism	
			Natural variants***	PTM****
Hexokinase-1, Brain form hexokinase), *HK1*	P19367/142600, 235700* 605285*	917**	577	3
Hexokinase-2, Muscle form hexokinase, *HK2*	P52789/601125	917	797	3
Hexokinase-3, *HK3*	P52790/142570	923	965	1
Hexokinase-4, glucokinase, *HK4*, *GCK*	P35557/138079, 125851* 125853*	465**	588	no data available
Hexokinase domain-containing protein 1, *HKDC1*	Q2TB90/617221	917**	916	no data available

* Numbers in OMIM NCBI on pathological syndromes associated with mutations in the gene of this protein.

** Several isoforms are known differing in sizes of amino acid sequences:
P19367-1 – 917 aa, P19367-2 – 916 aa, P19367-3 – 921 aa, P19367-4 – 905 aa;
P35557-1 – 465 aa, P35557-2 – 466 aa, P35557-3 – 464 aa;
Q2TB90-1 – 917 aa, Q2TB90-2 – 806 aa, Q2TB90-3 – 736 aa.

*** Natural variants – number of proteoforms usually with single amino acid substitutions, according to UniProt.

**** PTM – number of possible posttranslational modifications of amino acid residues, according to UniProt.

to the data of the database 'Online Mendelian Inheritance in Man' (OMIM, NCBI). Hence, it is obvious that in humans there is a prominent multilocus polymorphisms of hexokinases.

Three of the five indicated genes (*HK1*, *HK2*, *HK3*) provide synthesis of very similar protein products [58, 59]. They have molecular mass of ~100 kDa and their amino acid sequences display 70% identity [60]. These hexokinases contain two similar large domains. The hexokinases 1 and 3 one of the domains (C-terminal) is catalytic, and another (N-terminal) – regulatory (see structures P19367, P52790 in UniProt database) [58], while in the hexokinase 2 both domains are catalytic (see structure P52789 in UniProt). Protein products of two other genes (*HK4* and *HKDC1*) are significantly different in structural and functional characteristics from the first three. At the same time, expression of all cited hexokinase genes could result in formation of numerous proteoforms through various mechanisms. Furthermore, point mutations have been discovered in some hexokinase genes that are associated with certain inheritable diseases. Information on these topics is presented in Table 1.

It has been established that in humans at least protein products of the genes *HK2* and *HK4* have additional functions, i.e. are moonlighting proteins [21, 61, 62]. In particular, it was demonstrated that these enzymes play a role of 'glucose sensors' mediating glucose-stimulated secretion of insulin in pancreatic cells. Furthermore, protein products of the *HK2* gene were shown to have ability for interaction with mitochondrial membranes, as well as they exert cytoprotective effect on the healthy and neoplastic cells [63]. In other words, it was shown that additional functions of individual isoforms of hexokinases could play significant role in the development of various pathological processes.

Enolases. In the early 1970s information began to emerge that several isoforms of enolases, enzymes catalyzing conversion of 2-phosphoglyceric acid into phosphoenolpyruvate, exist in different human tissues (Fig. 3) (see [64] for an example). Later, the respective isoforms differing in their biochemical properties, were designated as isoforms α, β, and γ. It has been established in the process that α-enolase is present in the cells with very different types of differentiation, while β-enolase is specific for muscles, and γ-enolase – for nerve cells [65, 66]. It is generally assumed that in human cells enolase isoforms are usually present as homodimers [54, 66, 67]. However, there are also data on existence of heterodimers with composition αβ and αγ.

Later, practically before the start of extensive human genome investigations, three main genes encoding enolases were identified and mapped: *ENO1* (1p36.23) – encoding α-subunit, *ENO2* (12p13.31) –

encoding γ-subunit; *ENO3* (17p13.2) – encoding β-subunit (according to the OMIM NCBI database]. Inconsistencies in designations of the two indicated genes and their protein products (*ENO2* – encoding γ-subunit and *ENO3* – encoding β-subunit) have developed historically, but are still present. Existence of one more gene in the human genome encoding enolase, *ENO4*, is also known. This gene encodes the sperm-specific isoform of enolase: A6NNW6 UniProt, 131375 OMIM NCBI. The *ENO4* gene is mapped to chromosome 10q25.3. The presented data indicate that multilocus polymorphism of enolases exist in humans.

Thousands of publications are devoted to investigation of human enolases. In particular, the PubMed search using keywords 'human enolase' produced around 10 thousand of publications, while search in the ScienceDirect database – more than 20 thousand. Based on this enormous amount of information summarized in the UniProt database, it was revealed that the products of expression of the *ENO1* gene (P06733) are present not only in cytosol, but also in cell nuclei, membranes, and outside the cell (blood plasma), where they perform a number of functions in addition to the catalytic one. Considering all accumulated data many researchers characterize the indicated protein products as moonlighting proteins that are capable of binding DNA and regulate expression, play a role of plasminogen receptor, and participate in a number of pathological processes. Moreover, alpha-enolase is designated as an oncomarker, and as a potential target for chemotherapy of some malignant tumors [68].

Recently direct indication appeared that the protein products of the *ENO2* gene could be considered as moonlighting proteins [69]. These are no similar data about the human proteins biosynthesis of which is mediated by the *ENO3* and *ENO4* genes.

Lactate dehydrogenase. Human lactate dehydrogenases (LDH) comprise a set of isoforms, which are homo- or heterodimers formed by one or two subunits, respectively. These enzymes catalyzing the last reaction of anaerobic glycolysis (Fig. 3) have been investigated in great detail, their properties are described in numerous biochemistry textbooks (see [51] as an example), and detailed characteristics of the subunits are presented in the UniProt database (P00338; P07195; P07864). Brief information on these polypeptide chains from the UniProt and OMIM NCBI databases is presented below.

Three genes have been identified in the human genome that encode LDH – *LDHA*, *LDHB*, and *LDHC*. The *LDHA* gene is mapped to the 11p15.4 region. This gene is expressed in many organs, but most active expression is observed in skeletal muscles. Its protein product is named subunit A or M (from the word 'muscle'). The *LDHB* gene encodes subunit designated as subunit B or H (from word 'heart'), it is mapped to the 12p12.2-p12.1 region. Expression of the *LDHB* gene also has been observed in many organs, but sharply dominates in the heart muscle. Moreover, it is known that expression of the *LDHC* gene (synonym *LDHX*, localization – 11p15.5-p15.3) is realized almost exclusively in testes; and the corresponding subunit forms homotetramer of the specific form of LDH.

Hence, functioning of polymorphic human LDHs is characterized by the significant tissue-specificity, which allows using isoforms of LDH as markers of certain types of pathology, for example markers of malignant tumors [70, 71]. At the same time, a number of researchers emphasized certain features of LDH isoforms, which provide basis for considering them not as simple indicators of tumor presence, but consider their involvement into activation of several oncogenic pathways and into the process that make tumors invasive. In other words, isoforms of these enzyme perform functions not associated with enzymatic catalysis.

There are data indicating that the LDH isoforms, products of the *LDHA* gene, are present in the cell nuclei, where they bind DNA, interact with some DNA polymerases, stimulate synthesis of DNA and DNA repair after exposure to UV radiation [72, 73]. Moreover, some authors directly associate protein products of the *LDHA* gene as moonlighting proteins, which play functions of a regulatory factors in the cell nucleus that bind to transcription complexes [74]. It is important to note that according to the data reported by Roseweir et al. [75], nuclear proteoforms determined by the *LDHA* gene (isoform LDH-5) in the patients with colorectal cancer are associated with poor prognosis. Consequently, it could be assumed that significant contribution to the tumor progression could be provided by LDH isoforms serving as nuclear regulatory factors.

According to the UniProt data, protein products of the *LDHB* and *LDHC* genes found in the cell cytoplasm (P07195; P07864) so far have not been detected in the cell nuclei. There are no indications in the PudMed and ScienceDirect databases that the respective isoenzymes exhibit any additional functions. Hence, most likely among all human LDH isoenzymes only protein products of the *LDHA* gene are moonlighting proteins.

Concluding our consideration of the available information on glycolytic enzymes that are considered as moonlighting proteins, it is important to note that recently a detailed review has been published on this topic, in which many other metabolic enzymes have been characterised as moonlighting proteins [18].

MOONLIGHTING PROTEINS PARTICIPATING IN TRANSLATION

Protein biosynthesis, one of the most important manifestation of life, which occurs as a result

of a complex of interconnected molecular processes, quintessence of which is translation of genetic information encoded in the nucleotide sequence of messenger mRNAs into amino acid sequence of the corresponding proteins [76]. Apparatus of translation includes the most conserved cellular proteins and RNAs, which is considered as the most reliable proof of common origin of all forms of life [77, 78]. It has been assumed at the same time that the processes of translation emerged in the very early stages of evolution.

Various aspects of translation are being investigated at present [79, 80]. It has been established that translation in eukaryotes occurs not only cytoplasm, but also in mitochondria, and in plants – also in chloroplasts, where special protein-synthesizing systems with ribosomes exist, which differ significantly from cytoplasmic systems. Although in the third decade of XXI century the protein-synthesizing systems of mitochondria and chloroplasts still attract significant attentions, information considered in this review will be mostly about the studies of eukaryotic ribosomes and translation factors functioning in composition of cytoplasmic polyribosome complexes.

Ribosomal proteins. It is considered that ribosomes comprise the main of mass of polyribosomal complexes, and about 50% of each ribosome composition include several tens of ribosomal proteins (RPs) (see, for example, [76, 81, 82]). RPs are described as individual relatively small polypeptide chains; the eukaryotic 80S ribosomes contain 79-80 types of RPs [83].

At present, after many decades of intensive investigation of RPs in organisms belonging to three so-called main domains of life (prokaryotes, archaea, eukaryotes) a number of important conclusions could be made [79, 80, 82]. In particular, the accumulated data on amino acid sequences and on a number of other characteristics of RPs in bacteria, archaea, and eukaryotes demonstrate high degree of homology between them. This provided the possibility to summarize the available data and suggest an integral system of RPs nomenclature, which was developed by the representatives of 25 scientific organizations [82].

The suggested system stipulates that the homologous RPs are assigned the same name regardless of the species. The name typically includes a capital letter and a number (SX or LY), where the letters symbolize belonging to the large (L) or small (S) subunit, and numbers (X and Y) – integral numbers. In addition, the names were supplemented with special prefixes. Considering that the significant part of RPs was found to be typical for all domains of life (prokaryotes, archaea, eukaryotes), these ribosomal proteins were given the prefix ‘u’ (universal). Prefix ‘b’ was given to those RPs that were found only in bacteria, and prefix ‘e’ – to the RPs of eukaryotes and archaea. To designate RPs of mitochondria and chloroplasts the prefixes ‘m’ and ‘c’ were suggested, respectively (for example, uL2m; uL2c).

The developed nomenclature suited to almost all known at that time RPs (with rare exceptions); and the summarizing table included 39 proteins of small subunits and 61 proteins of large subunits [82].

However, this nomenclature has been used in far from all publications, which creates certain problems for comparative analysis of the reported results, because, for example, in the integral nomenclature system there is a protein named uL16, which among the human of yeast ribosomal proteins is sometimes called L10 [82, 84].

It can be stated in conclusion that the suggested classification reflects various information regarding the facts that the ribosomal proteins belonging to the evolutionary distant groups of organisms display pronounced structural conservatism, which defines the respective functional properties. Presence of structural and functional conservatism among the majority of ribosomal proteins allows suggesting that they existed already at the early stages of evolution [81, 85].

In the process of ribosome biogenesis in eukaryotic cells dozens of RPs are first synthesized in cytoplasm followed by their transport into a nucleus and next to nucleolus [79, 86-88]. Hence, before the start of formation of ribosomal particles, RPs are in a free state located, in particular, in cytoplasm.

In the next step, the eukaryotic ribosome biogenesis, which starts in a nucleolus, continues in a cytoplasm, where immature ribosomal particles bind certain free RPs [79, 86-88]. After that, the completely formed ribosomes are incorporated into polyribosome complexes and perform translation function.

In general, the accumulated data indicate presence of cytoplasmic pool of RPs. Investigation of free cytoplasmic RPs and their functions during several decades was carried out by the scientists from different countries (see [89-91] as examples). As a result, indications emerge that the individual RPs have the so-called ribosomal and extra-ribosomal functions [79, 92-94]. In particular, a table with information on RPs with established extra-ribosomal function or with presumed extra-ribosomal was presented in the review by Wang et al. [94]. This table includes 21 RPs of the small ribosomal subunit and 19 RPs of the large ribosomal unit. It must be emphasized also that in the case of different disruptions of ribosome biogenesis, accumulation of free ribosomal proteins was observed in the cells, which could also perform various extra-ribosomal functions [95, 96].

The abovementioned information on RPs is summarized and presented schematically in Fig. 4.

Among the extra-ribosomal function of RPs, facilitation of cell proliferation and differentiation, as well as participation in the processes of apoptosis and

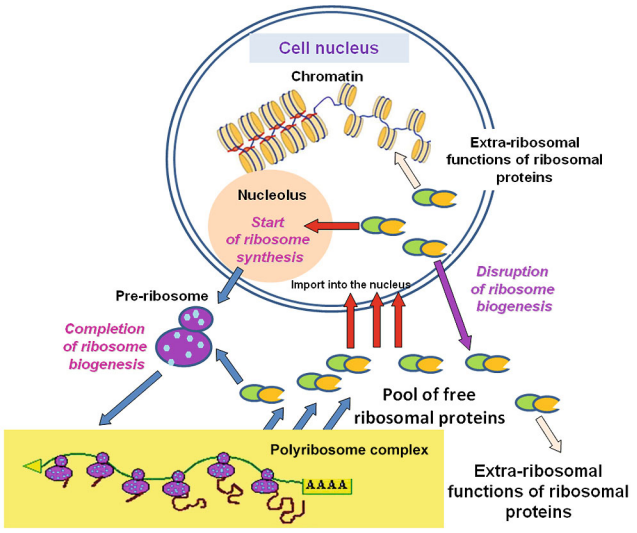

Fig. 4. Schematic presentation of the processes of biosynthesis and transport of ribosomal proteins in eukaryotic cells (based on information reported in [86, 87]). Designations as in Fig. 2.

DNA repair have been mentioned [94]. It has been also mentioned that some of ribosomal proteins perform their extra-ribosomal functions through interaction with certain cytoplasmic and/or nuclear proteins [90, 97]. Furthermore, the ribosome-free state of some ribosomal proteins allows their post-synthetic modifications (such as uS5, for example) and perform various extra-ribosomal functions including activation of p53-dependent or p53-independent pathways as a response to stress. In addition, it has been reported that the ribosomal protein termed L13a (uL13, according to the integral nomenclature) has extra-ribosomal functions in the breast cancer cells [98]. Elucidation of the role of free ribosomal proteins (i.e. their extra-ribosomal functions) in the processes of oncogenesis of various types of tumors could be considered as a key result of such studies [99].

A number of publications of the Russian scientists have been devoted to extra-ribosomal functions of certain ribosomal proteins [100-102]. It was noted in these works that the eukaryotic protein S3 participates in DNA repair, as well as in selective regulation of gene activity, induction of apoptosis, and other molecular processes. In addition, the eukaryotic protein S15 (uS19, according to the integral nomenclature) plays a function outside of the ribosome associated with participation in regulation of tumor suppressor. Moreover, it has been emphasized that mutations in the gene encoding the S15 protein are associated with the development of certain diseases (Diamond–Blackfan anemia, chronic lymphocytic leukemia, Parkinson's disease) [101]. And finally, in the recently published paper Ochkasova et al. [102] considered possible extra-ribosomal functions of RPs in eukaryotes associated with their participation in cell-cell communications via extracellular vesicles including exosomes.

It could be concluded based on the available data that many RPs have established extra-ribosomal functions, and, hence, could be considered as moonlighting proteins (see, for example [79, 96, 103, 104]). Research on the extra-ribosomal activities of RPs is continuing, and recently Mołoń et al. [105] demonstrated that the uL6a plays a key role in the cell response to oxidative and osmotic stress, and could be considered as a moonlighting protein.

Translation factors. It is known that several groups of protein factors participate in functioning of polyribosome complexes, which are required for mediating certain steps of translation (initiation, elongation, and termination). Among those, moonlighting proteins have been identified in different organisms [106, 107]. It has been recognized that at least 12 proteins termed eukaryotic initiation factors (eIF) are responsible for translation initiation facilitating interaction of the small ribosomal subunit (40S) with 5′-untranslated regions (UTR) in mRNA. The canonic cap-dependent translation initiation involved binding of eIF4F with 7-methylguanosine cap (m7G cap) at the 5′-end of mRNA, and eIF4G1 plays a central role acting as multipurpose ribosome adapter connecting other eIFs, such as eIF4E, eIF4A into the specialized initiation complexes [106, 107].

Since the first decade of XXI century, publications started to emerge reporting that some eIFs are present in nuclei, where they perform additional functions associated with regulation of transcription, processing, and mRNA export (see, for example, [106, 108]). In the process it was found out that some eIFs interacting with the 5′-cap of mRNA and regulating global translation have additional functions involving their ability to bind certain proteins outside of polyribosome complexes [109]. It is worth mentioning that there is information that eIF5A, which is considered to be highly conserved in eukaryotes and archaea, also exhibits endoribonuclease activity [110].

Among numerous studies devoted to investigation of eukaryotic translation elongation factors (eEF), there a number of studies indicating that these proteins have additional functions (see, for example, [111-113]). In particular, more than 20 years ago Ejiri S. presented information (2002) [111] in the large review on the role of eEF-1 subunits not only in mediating translation in polyribosome complexes, but also on the ability of these proteins to bind actin, as well as ability to interact in the case of nuclear localization with the zinc-finger protein R1.

It has been demonstrated recently that the eukaryotic translation elongation factor eEF1A2 has cell membrane and organelle membrane binding sites,

and, moreover, its involvement in some molecular processes has been shown [114]. As a result, the cited authors noted that considering new information on the role of eEF1A2 in autophagy, oncogenesis, and virus replication, modern notions on significance of this ancient protein go far beyond its canonical function involving delivery of aminoacyl-tRNA to ribosome.

It seems important to mention the recent review by Negrutskii et al. [113], which summarizes current information on the family of mammalian translation elongation factors eEF1 obtained as a result of many years of work by several generations of scientists. Negrutskii et al. [113] analyzed the data on spatial organization and posttranslational modification of eEF1A1 and eEF1A2, as well as provided examples of their participation in the processes not associated with translation. It was emphasized that although both variants of eEF1A exhibit similar activity in translation, they could differ with regard their additional functions.

According to the opinion of a number of authors, termination is a crucial step of translation; in particular, it is important that the premature termination of translation could result in generation of toxic truncated peptides (see, for example [115]). Three proteins termed release factors and designated as RF1, RF2, and RF3 mediate translation termination. According to the data reported by Chai et al. [116] the eukaryotic release factor RF3 (eRF3) is present both in cytoplasm and in the nucleus, where it presumably participates in morphological organisation of the nucleus. Correspondingly, these authors suggested to consider eRF3 as a polyfunctional protein with additional roles in the process of protein synthesis termination. Later it was demonstrated that eRF3 indeed is a polyfunctional protein that plays a key role in translation termination, and also participates in initiation of mRNA decay and regulation of apoptosis [117].

Hence, there are convincing data indicating that many proteins involved in the processes of translation (which are realized in cytoplasm) are present also in the cell nuclei, where they play various additional functions.

CHROMATIN PROTEINS WITH ADDITIONAL FUNCTIONS

Nuclei of eukaryotic cells are described as large organelles with majority of the space occupied by chromatin containing genomic DNA, histone and non-histone proteins, as well as various types of RNAs [118, 119]. Chromatin is well-known as a rather dynamic formation containing different structural elements (termed ‘regions’, ‘compartments’, ‘territories’, and others, see examples in [119]). It is commonly recognized that chromatin has two main regions: euchromatin and heterochromatin. In general, euchromatin is defined as less dense genome region enriched with transcriptionally active genes, and heterochromatin – as a region consisting of more dense genome regions containing transcriptionally inactive genes [120, 121]. Among the multiple components of chromatin, proteins have been identified that have, in addition to nuclear, other localizations, which in addition to the function of maintaining chromatin structure were shown to have additional functions. In other words, some chromatin proteins are characterized as multifunctional, and some are directly assigned to moonlighting proteins. It is assumed that clear polymorphism of chromatin proteins is a result of directed evolution [122-124].

Histones. The main histones in vertebrates, including humans (H1, H2A, H2B, H3, and H4), are the most well-investigated eukaryotic proteins, which provide basis for chromatin formation via specific interactions with genomic DNA [125-127]. In these interactions four pairs of histones H2A, H2B, H3, and H4, which sometimes are called core histones, form special octameric complexes – nucleosomes. Existing estimates show that DNA molecule wraps around each nucleosome (1.67 turn, 147 bp), and histone H1 presumably plays a role of an internucleosomal linker.

Histones are assigned to ancient proteins, precursors of which initially emerged, as suggested, in the so-called last universal common ancestor [126, 128]. Later, symbiosis of ancient bacteria with archaea resulted in emergence of eukaryotic cells, in which formation of the set of histones continued; and that provided a possibility of evolution progressing towards increase of genome size [124, 126].

It is known that the exon-intron structure is a specific feature of eukaryotic protein-coding genes, while the similar genes in prokaryotes to not contain introns. However, the intronless genes exist also in higher eukaryotes. In particular, existing estimates show that 3% of the genes in human genome are intronless, and among those 20% are genes encoding histones [122]. In other words, it could be hypothesized that the histone genes in eukaryotes that have come a long way in evolution, preserve some features of the genes-analogues in prokaryotes.

Comparison of the yeast and human genomes could provide some information on certain features of evolution of histone genes on the path from single-cell eukaryotes to multicellular organisms.

It has been reported [129] that the genes encoding histones H2A, H2B, H3, H4 in the *Saccharomyces cerevisiae* genome, are organized into four loci (HTA1-HTB1; HTA2-HTB2; HHT1-HHF1; HHT2-HHF2) each of them on containing two histone genes divergently transcribed from the central promoter.

Table 2. General characteristics of the human histone 1 family, their genes, and polymorphism manifestations according to [130] as well as UniProt and OMIM NCBI databases

Histone designations* (names and synonyms), *symbols of genes*	Numbers in UniProt/OMIM (chromosome localization)	Quantity of amino acid residues	Natural variants***	Number of amino acid residues with PMTs****
H1.0 [Histone H1.0, Histone H1′, Histone H1(0)] *H1-0*, *H1F0*, *H1FV*	P07305**, 142708 (22q13.1)	194	156	6
H1.1 [Histone H1.1, Histone H1a] *H1-1*, *H1F1*, *HIST1H1A*	Q02539, 142709 (6p22.2)	215	352	>20
H1.2 [Histone H1.2, Histone H1c, Histone H1d, Histone H1s-1] *H1-2*, *H1F2*, *HIST1H1C*	P16403, 142710 (6p22.2)	213	596	>50
H1.3 [Histone H1.3, Histone H1c, Histone H1s-2] *H1-3*, *H1F3*, *HIST1H1D*	P16402, 142210 (6p22.2)	221	70	>25
H1.4 [Histone H1.4, Histone H1b Histone H1s-4] *H1-4*, *H1F4*, *HIST1H1E*	P10412, 142220 (6p22.2)	219	123	>30
H1.5 [Histone H1.5, Histone H1a Histone H1b, Histone H1s-3] *H1-5*, *H1F5*, *HIST1H1B*	P16401, 142711 (6p22.2)	226	410	>30
H1.6 [Histone H1t, Testicular H1 histone] *H1-6*, *H1FT*, *H1T*, *HIST1H1T*	P22492, 142712 (6p22.2)	207	47	>10
H1.7 [Histone H1.7, Testis-specific H1 histone, Haploid germ cell-specific nuclear protein 1, Histone H1t2] *H1-7*, *H1FNT*, *HANP1*	Q75WM6, 618565 (12q13.11)	255	305	1
H1.8 [Histone H1.8, Histone H1oo, osH1] *H1-8*, *H1FOO*, *H1OO*, *OSH1*	Q8IZA3*****, 142709 (6p22.2)	346	321	no data
H1.10 [Histone H1.10, Histone H1x] *H1-10*, *H1FX*	Q92522, 602785 (3q21.3)	213	189	>10

* Histone designation according to the new standardized nomenclature [130].

** There are reasons to suggest the possibility of formation of two isoforms P07305-1 and P07305-2 due to posttranslational modification of pre-mRNA of histone H1.0.

*** PTM – number of possible posttranslational modifications of amino acid residues, according to UniProt.

**** Natural variants – number of proteoforms with, as a rule, single amino acid substitutions according to UniProt.

***** According to UniProt data Q8IZA3, there are two isoforms, differing in the size of amino acid sequences: Q8IZA3-1 – 346 aa, Q8IZA3-2 – 207 aa.

It was found out that two genes determining biosynthesis of histone H2A differ slightly from each other; as a result, two isoforms of this protein are formed in *Saccharomyces cerevisiae*. The same situation was observed for the histone H2B. However, the loci HHT1-HHF1 and HHT2-HHF2 contain each a pair of identical genes, encoding identical proteins H3 and H4. In addition, three more histone genes have been identified encoding proteins H1 (HHO1), H2AZ (variant close to H2A), and a special centromeric H3 (CSE4). In total, 11 histone genes were found in the *Saccharomyces cerevisiae* gene [129].

It is important to note that according to the information presented in UniProt database, some histones in yeasts in addition to the main structural functions have additional functions associated with repair of DNA damages (such as P04911 UniProt) or regulation of transcription (Q12692 UniProt).

Unlike in the yeast genome, more than 70 histone genes and a number of pseudogenes were found in the human genome [127, 130]. It was shown that these genes also form four clusters and encode proteins belonging to five respective families (H1, H2A, H2B, H3, H4) [127, 130, 131]. The largest gene cluster

is located on the chromosome 6 (locus HIST1) and three more clusters (loci HIST2, HIST3, and HIST4) – on other chromosomes (according to the OMIM NCBI database). It was found out that the histone genes and encoded proteins comprising respective families are similar in many respects, but far from being identical (for example, according to UniProt database, Table 2).

Moreover, it has been noted that the histone H1.8 is localized both in the cell nucleus and in cytoplasm.

It must be mentioned that different classifications and designations of individual histone genes and their products, histones, exist in the literature. To avoid problems and inconsistencies emerging due to this, several attempts for unification of the used designations have been made; and recently a new standard nomenclature of mouse and human histones has been suggested [130]. This nomenclature including data on such genes in humans and mice was created with participation of the Human Genome Organization Nomenclature Committee (HGNC). Nevertheless, in many sources including UniProt and OMIM NCBI databases variety of the names of histone families are still in use, which must be taken in consideration during analysis of the literature data. General characteristics of the human Histone 1 family, their genes, and manifestations of polymorphisms are presented above in Table 2 as an example.

It follows from the data presented in the UniProt database that all representatives of the H1 histone family play similar functions in the chromatin structure, binding DNA sites located between nucleosomes, and, thus facilitate formation of a macromolecular structure known as chromatin fiber. In addition, the functions of regulators of transcription of specific genes, which are associated with chromatin remodelling and DNA methylation, have been assigned to some H1 histones (see examples of P16403, P16402 in UniProt).

Interestingly enough, some of the H1 histones were suggested to be involved in splicing functions [132, 133]. In particular, the histone H1.5 has the ability to bind DNA at the sites of splicing of short exons in human lung fibroblasts. It was concluded, as a result of investigation, that H1.5 participates in regulation of selection of the splicing site and alternative splicing, i.e., it has additional function [132]. Recently it was shown in the model experiments that the histone H1.2 exhibits high affinity to exons, while H1.3 binds to intron sequences [133]. As a result, due to their effect on elongation performed by RNA-polymerase II, conditions are created for exon skipping and/or intron retention.

It seems important to emphasize that while the representatives of human H1 histone family have similar general functions, they differ significantly in the size of amino acid sequences (Table 2); and some of their genes display certain specificity of expression. According to the data from UniProt database, expression of the majority of the genes of H1 histone family is accompanied by appearance of hundreds of proteoforms that have, as a rule, single amino acid substitutions, as well as various posttranslational modifications of amino acid residues. Correspondingly, it can be assumed that, firstly, numerous isoforms and variants of H1 histones are the products of long evolution, and, secondly, a number of those perform not only structural functions in chromatin composition, but also have additional functions.

Situation with the human core histones is much more complicated that with the H1 histones. A whole spectrum of the genes that determine biosynthesis of a large sets of isoforms and variants of histones H2A, H2B, H3, H4 were found in the human genome. These data continued to be detailed. In particular, information on 17 human genes encoding H2A histones has been reported in 2020 [134], while more than 26 of such gene have been identified in the 2022 study (although, some of them have been noted as ‘variants of histone H1 or H3’) [130]. Description of functions of many human core histones in the UniProt database, such as, for example, description of the histone H2A type 2-B (Q8IUE6 UniProt) includes the following: “Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries, which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code…” In other words, it is emphasized that these proteins have, in addition to the main structural function, a number of additional functions. It seems reasonable to mention that thousands of publications have been devoted only to the function, which is termed ‘histone code’ (see, for example [135]).

It is known that histones are synthesized in cytoplasm and, next, are transported through nucleolemma to nucleus [136]. Nuclear import of histones is realized with the help of proteins from the family of karyopherin nuclear transport receptors, also known as importins. Hence, after synthesis histones remain for some time outside of chromatin and, obviously, could perform extra-chromatin functions.

It has been reported in some publications that free histones are present in the cell cytoplasm. In particular, already at the end of XX century Zlatanova et al. [137] reported existence of a cytoplasmic pool of H1 histones, mentioning, at the same time, that no core histones were found in the cytoplasm. However, recently information emerged on the presence

of histones H3 and H4 in the cell cytoplasm, where they bind with specific importins before being transported to the cell nucleus [138, 139]. Presence of main histones (H1, H2A, H2B, H3, H4) in the composition of plasma membranes has been mentioned in a number of studies, where possible existence of additional extra-chromatin functions of histones has been suggested [127, 140, 141]. Moreover, histones were found in mammals outside of the cells in blood flow, where they play role of inflammation factors directly damaging endothelial cells and also cells in various organs [142].

In general, it seems very likely that some histones play not only structural and regulatory functions in chromatin composition, but also could play various roles outside of chromatin.

Non-histone chromatin proteins. The term non-histone chromatin proteins (NHCP) has been suggested more than 50 years ago to describe a large group of proteins capable of binding chromatin, but differing in structure and functions. Three families of high-mobility proteins are commonly assigned to NHCPs, which are designated as HMGA, HMGB, HMGN (from high mobility groups A, B, N (see examples in [143-145]). Proteins of these families are considered as structural components and chromatin architectural factors; however, they interact differently with genome DNA and histones.

A number of proteoforms belong to the HMGA family, which are products of expression of two genes (*HMGA1* and *HMGA2*). According to the data on P17096 and P52926 in the UniProt database, functioning of each gene leads to formation of dozens of proteoforms due to point mutations, alternative splicing, and post-synthetic modifications. The available data indicate that the corresponding proteins are capable of binding with A + T-rich sites of genomic DNA, participate in remodeling of chromatin, interact with transcription factors, therefore providing regulation of expression of different genes, and other.

It is important to note that the protein products of the *HMGA1* gene have been found in the nucleus and nucleoplasm, as well as in cytoplasm (see P17096 UniProt).

Available data indicate that the proteins of HMGA family perform their functions mainly in the course of embryonic development, when expression of the specified genes is sufficiently high, which is followed by low expression in normal adult tissues or complete absence of it [146]. However, proteins of the HMGA family are present in significant amounts in the human tumor tissues, which is considered as a sign of their involvement in the processes of carcinogenesis and is associated with poor prognosis.

Accumulated information on the proteins of HMGA family provide basis for designating them as multifunctional proteins [147]. Moreover, it has been discovered recently that the breast cancer cells secrete the product of expression of the *HMGA1* gene capable of functioning as a growth factor outside of the cell; this allowed to assign this protein to moonlighting proteins [42].

Proteins of the family of human HMGB are encoded by four genes (*HMGB1*, *HMGB2*, *HMGB3*, *HMGB4*), expression of each of them results in formation of several proteoforms. In particular, protein products of the *HMGB1* gene include four verified variants with 26 post-synthetic modifications (P09429 UniProt). They are localized in different cellular compartments (nucleus, cytoplasm, cellular membranes, and others), where they perform different functions. Moreover, secretion of these proteins into extracellular medium and their functioning there as a cytokine was demonstrated. Respectively, according to a number of authors, protein products of the *HMGB1* gene are moonlighting proteins [148].

Five genes encoding the HMGN proteins were found in the human genome with their properties described in the UniProt database (*HMGN1* – P05114, *HMGN2* – P05204, *HMGN3* – Q15651, *HMGN4* – O00479, *HMGN5* – P82970). Expression of the cited genes results in formation of several proteoforms due to post-synthetic modifications. However, so far only two isoforms determined by the *HMGN3* gene were found. It has been also observed that proteoforms determined by the three genes are localized in different cellular compartments: *HMGN1*, *HMGN2* in cellular nuclei, nucleoplasm and cytoplasm, and *HMGN3* – in cell nuclei, nucleoplasm, and mitochondria.

The HMGN proteins are synthesized in all cells of vertebrates. During functioning they bind with nucleosomes, as well as with the regulatory sites of chromatin, including enhancers and promoters [144]. There are also direct data on multifunctionality of the products of expression of the *HMGN1* gene in mammals [149].

Chromatin and transcription factors. At present a large array of data has been accumulated on interaction of chromatin with hundreds of transcriptional factors resulting in certain structural rearrangements and changes in chromatin. Proteins capable of recognizing and binding to the specific DNA sequences thus regulating transcription are considered as transcription factors (see, for example, [123]). It has been assumed that specificity of the transcription factor binding to DNA is determined, on the one hand, by the presence in their polypeptide sequence of specific DNA-binding domains, and on the other hand, by availability in the polynucleotide sequences of DNA of sets of related short sequences (motifs or sites) recognized by the specific factors [123].

There are direct data demonstrating that certain transcription factors in addition to their canonic

functions have other functions. In particular, the transcription factor ATF5 (AMP-dependent transcription factor, ATF-5, according to Q9Y2D1 UniProt) capable of stimulation or suppression of expression of certain genes, is also required for realization of different stages of mitosis. It participates as a moonlighting protein in assembly of centrosomes, special organelles in eukaryotic cells [150].

Among the transcription factors there are proteins with unordered structure capable of oligomerization and interaction with other proteins or low molecular weight ligands while performing their functions (activation or repression of genes), such as, for example, YY1 [151]. According to the available data YY1 binds to the specific DNA motif present in the regulatory elements of many genes. Respectively, this factor is a pleiotropic regulator of a number of cellular processes – cell proliferation, differentiation, and apoptosis.

Furthermore, it has been shown that some of the similar transcription factors, as well as individual subunits of oligomeric transcriptional complexes are involved in the processes of carcinogenesis and, hence, are characterized as oncoproteins [151, 152]. For example, the human c-Fos protein, while localized in the cell nucleus, forms a strong but non-covalent complex with the transcription factor JUN/AP-1 and participates in regulation of gene transcription (according to the data on P01100 in UniProt). However, its presence was observed also in endoplasmic reticulum and in cytoplasm. The c-Fos protein with non-nuclear localization was shown to be able activating synthesis of cytoplasmic lipids in the cell of central nervous system and support neuronal activity; hence, this provided a basis for assigning it to moonlighting proteins [152].

The transcription factor EB (according to P19484 UniProt – Class E basic helix-loop-helix protein 35, TFEB, bHLHe35), which specifically recognizes and binds the 5′-GTCACGTGAC-3′ sequences in DNA molecules, is also assigned to moonlighting proteins. This sequence is present in the regulatory sites of many genes facilitating biogenesis and functioning of lysosomes. Obviously, nuclear localization of the transcription factor EB is required for realization of its main functions; however, significant fraction of this protein is present in cytoplasm, where it is in composition of the specific complex associated with lysosomes [153]. And finally, different data were summarized in a recent review demonstrating that the transcription factor EB is capable of changing its localization, and, in the process, it acts not only as a transcription factor, but performs additional functions [38].

It could be stated in conclusion that many human proteins, which are in composition of chromatin or interact with chromatin during functioning, have additional functions. Some of them are already considered as moonlighting proteins, and a number of others could, likely, be considered as such based on formal characteristics.

MOONLIGHTING PROTEINS IN CELLULAR MEMBRANES

Modern notions on origins of life concentrate specifically on the steps mediating transition from prebiotic stage to formation of cellular forms due to appearance of cell membranes (see, for example [154, 155]). It is assumed that the fundamental conditions for this should be emergence of the source of amphiphilic compounds capable of assembling into membrane compartments. Consequently, model studies are being conducted on self-assembly of simple membranes of protocells as a prerequisite for evolution of cellular metabolism and, practically, emergence of life on the early Earth [155].

It is assumed that the membrane proteins embedded into lipid bilayer (transmembrane proteins, TMPs) play a vital role in membrane stability and for performing many cellular functions, because they are capable of interacting both with environment and intracellular components. Polypeptide chains of TMPs could be arranged in such a manner that they cross the lipid bilayer only once (single-pass membrane proteins), and, as a consequence, their N-terminal part is exposed the extracellular medium, while the C-terminal – to cytoplasm (type I) or *vice versa* (type II) [156]. TMP with their polypeptide chain spanning the lipid bilayer two or more times are also known (multipass membrane proteins) [156, 157].

Polypeptide chains of the TMPs designated as single-pass membrane proteins usually contain several domains with each of them performing its own specific function. In other words, these proteins are multifunctional, but based on the existing nomenclature they are not assigned to moonlighting proteins. At the same time, it has been demonstrated that some multipass membrane protein are localized not only in the structure of cellular membranes, but also in cytoplasm, such as, for example, epithelial membrane protein 2 (EMP2) according to the data on P54851 in UniProt. Moreover, it has been noted that this small protein in addition to membrane functions have other functions, in particular, associated with regulation of functioning of some signalling pathways and involvement in carcinogenesis [158].

Among the transmembrane proteins characterized as moonlighting proteins, some attention has been drawn to the so-called ectoenzymes, which are membrane-bound enzymes with a catalytic center located outside the cell [23]. In particular, it is known that one of such enzymes, aminopeptidase N (CD13),

which is a Zn^{2+}-dependent metalloproteinase catalyzing hydrolysis of various peptides, also could play a role in amino acid transport, participate in angiogenesis, and serve as a receptor of human coronavirus (see information on P15144 in UniProt). Human TMPs that are Zn^{2+} transporters, which are capable of performing additional functions, have been also considered as moonlighting proteins [159, 160]. It has been noted that the Zn^{2+} transporters in mammals including humans, comprise a rather large family of proteins formed in the process of long evolution with representatives of this protein family located in different cellular membranes. They are capable of forming oligomeric complexes participating in numerous signaling processes. More than ten genes encoding Zn^{2+} transporters were identified in the human genome. Examples of diversity of properties and cellular localization could be found in the UniProt database for the Zn^{2+} transporter ZnT-1 (synonym – Proton-coupled zinc antiporter SLC30A1, Q9Y6M5 in UniProt).

In could be stated in conclusion that considering the fact that membrane proteins could comprise more than 30% of all human proteins (according, for example, to [156]), it is likely that the available information on those that should be considered as moonlighting proteins will increase.

SPECIALIZED DATABASES ABOUT MOONLIGHTING PROTEINS

Accumulation of knowledge on moonlighting proteins in the second decade of XXI century, which was accompanied with the development of postgenomic science and bioinformatics, motivated creation of specialized databases for moonlighting proteins.

In particular, in the end of 2014 a work has been submitted for publication by the scientists from University of Illinois (USA) describing the specialized database containing information of moonlighting proteins named MoonProt. This work was published at the beginning of 2015 [161]; it included information on 200 experimentally verified moonlighting proteins. It is important to mention that among the authors of this paper was Constance J. Jeffery. Later versions of the MoonProt (version 3.0) contained annotations on the properties of more than 500 moonlighting protein from different organisms [35].

Practically simultaneously with the American scientists, a group of researchers from Spain created and used another database named MultitaskProtDB, which contained summarized data on several hundreds of know moonlighting proteins [36, 162]. According to the opinion of the developers, the MultitaskProtDB opened the possibilities for identification of such proteins through analysis of their structural and functional characteristics from numerous other databases with the help of bioinformatics. The version of MultitaskProtDB-II from 2018 contained information on 694 moonlighting proteins [162]. The team of researchers that created this database included scientists from Uruguay in addition to the researchers from Spain. The list of sources analyzed by the creators of the MultitaskProtDB-II database has been expanded significantly. Moreover, it included new information from the publications presented in the PubMed database, as well as in the new versions of the UniProt database. The developers paid special attention also to the information from a number of other databases including the Online Mendelian Inheritance in Man (OMIM), Human Gene Mutation Database (HGMD), the Therapeutic Target Database (TTD), ands the DrugBank database. The list of the used bioinformatics tools also was expanded.

In the second decade of XXI century a specialized database was created in France on Extreme Multifunctional proteins in human including moonlighting proteins, which was named MoonDB [163, 164]. In this work special attention was paid to the studies devoted to investigation of protein–protein interactions, which produced new data on specific interactomes of moonlighting proteins. As a consequence, the developers identified 430 extremely multifunctional proteins combining various network information with information from annotations of the proteins form different databases (including, for example, OMIM NCBI). The second version of the MoonDB 2.0 database (http://moondb.hb.univ-amu.fr/) included information not only on the human moonlighting proteins, but also on similar proteins from some eukaryotes with genomes completely sequenced [164]. In addition, interface of the second version of MoonDB was completely redesigned and improved, and the existing annotations were cross-referenced with the UniProt database.

Next, publication of the Chinese researchers should be noted, which developed a specialized PlantMP database that include information on moonlighting proteins in plants [165]. This database included materials on 110 known moonlighting proteins in plants, as well as on 10 proteins considered to be likely moonlighting, and 27 of presumed moonlighting proteins. PlantMP uses identifiers and designations from the UniProt database, which opens up the possibilities for searching canonical and additional functions of the proteins. Moreover, the PlantMP database also presents references to the publications in the PubMed database. The developers expressed their opinion that organization of all materials on the moonlighting proteins of plants on one platform would help the researchers to collect both processed and unprocessed data on particular plants including information on molecular functions and structural features that are required

to formulate a hypothesis in fundamental and applied science and for biotechnological innovations.

And finally, recently another report has appeared on the specialized database developed in Italy named MultifacetedProtDB, which included extensive information on human moonlighting proteins [166]. This new database included information not only on moonlighting proteins, but also on many other multifunctional proteins. Obviously, the MultifacetedProtDB could allow optimizing investigations of moonlighting proteins and multifunctional proteins. The MultiFacetedProtDB can be accessed at: https://multifacetedprotdb.biocomp.unibo.it/.

It seems that creation of various databases integrating existing information on moonlighting proteins could open wide opportunities for targeted investigation of these proteins in different multifactorial biological processes such as signaling and metabolism regulation, gene expression, and cell–cell communications, which is extremely important for elucidation of pathogenesis of many diseases.

CONCLUSIONS

The presented analysis indicates that, at present, investigation of moonlighting proteins comprises a separate scientific area examining issues of both theoretical and applied nature associated, for example, with diagnostics of socially significant diseases.

Thus, for theoretical biochemistry, of considerable interest is the formation of ideas about due to what, when and how special multifunctional proteins appeared, consisting of one polypeptide chain, but possessing several functions, which in their structure do not have different domains to provide these functions, i.e., moonlighting proteins. One of the possible explanations could be information on intrinsically unordered proteins, whose tertiary structure capable of significant changes depending on environmental conditions. The phenomenon of switching functions in moonlighting proteins at least in some cases is likely associated with this type of changes. However, it requires further verification whether this mechanism is universal or widespread.

It seems important to discuss theoretical issue on evolution of additional functions in moonlighting proteins. Already in the early publications (for example, [10]) the opinion has been expressed that additional functions could be the result of molecular evolution. However, an alternative point of view also exists, according to which ancient proteins were intrinsically multifunctional, and evolution resulted in emergence of proteins with the single specialized functions. This point of view is shared by the author of this paper.

Many studies on moonlighting proteins that are considered as a special group of multifunctional proteins focus on investigation of molecular basis of pathogenesis of widespread diseases including malignant tumors. It is assumed that potential prognostic markers and/or molecular targets could be identified as a result, which could be used for the development of novel effective treatment strategies. For similar purposes, in several countries (USA, Spain, France, China, Italy) have created and are using specialized databases containing information about moonlighting proteins.

In conclusion, it should be stated that the collected information could provide promise that investigation of moonlighting proteins would expand and in our country in the nearest future, which could facilitate creation of the specialized database in Russia.

Funding. This work was supported by ongoing institutional funding. No additional grants to carry out or direct this particular research were obtained.

Ethics approval and consent to participate. This work does not contain any studies involving human and animal subjects.

Conflict of interest. The author of this work declares that he has no conflicts of interest.

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