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Protein isoforms. Origin, structure and functions

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Many proteins in mammalian organism exist as isoforms. These isoforms can be encoded by different genes or produced by alternative splicing of one gene. Despite rapid instrumental progress in the isoform identification, the reasons for their existence and specific functions remain largely unknown. During recent years, attention of researchers was mostly concentrated on spliced isoforms, while the variants of the same protein coded by different genes can play an essential role in different cell processes. This review presents examples of different potential functions of the protein isoforms coded by different genes. Molecular background which could provide a difference between highly homologous protein variants is discussed with an example of isoforms translation elongation factor 1A (eEF1A).

Keywords: protein isoforms, eukaryotic translation, eEF1A

1. Origin of the protein isoforms

The protein isoforms are closely related gene products which can perform both similar and quite different biological functions. The isoforms may differ by biological activity, regulatory potential, intercellular distribution, different spatio-temporal expression *etc.* The protein isoforms are either products of the same gene or the family of genes originated from a single predecessor.

In the first case, a single gene produces several mRNAs by separation and subsequent re-joining of the pre-mRNA exons. Generation of alternative exons is achieved by i) tandem duplication with subsequent divergence of exons; ii) translocation of exons into novel gene context; iii) mutations in the intron or flanking sequence with creation of N-terminal, C-terminal or internal exons [1]. The alternate splicing of equivalent homological exons may have evolutionary importance, as the exchange of homological exons may lead to fine tuning of functions of the corresponding protein. On the contrary, a bulk of the alternative splicing isoforms is predicted to arise due to the large insertions or deletions, or non-homologous substitutions. However, the precise proteomic experiments revealed only a small fraction of predicted alternative isoforms. It appears that

the vast majority of annotated alternative transcripts may not ever be translated into proteins [2].

In the second case, the multiple copies of the same gene are generated by at least three mechanisms: unequal crossing over during meiosis; tandem duplications resulting from DNA replication errors, or translocation of a gene copy to another chromosome [1].

Evolutionary development of multicellular organisms gave a ground to the appearance of the protein isoforms with specialized functions. There are a number of examples of the different proteins isoforms fulfilling similar, diverse and sometimes antagonistic functions. We focus the review on the functional diversity of the protein isoforms coded by different genes.

2. Dissimilar functions of the same protein isoforms

A family of scaffold proteins IQGAP (IQ Motif Containing GTPase Activating Protein) comprise three evolutionary conserved isoforms [3]. All three isoforms participate in controlling the dynamics of cytoskeleton, intracellular signaling and provide a large amount of protein-protein interactions. However, IQGAP1 is expressed in all tissues, IQGAP2 is present mostly in liver whereas the expression of IQGAP3 is limited to brain, which indicates a likelihood of unique functions of every isoform [4]. All three isoforms interact with actin [5–7] and calmodulin [5, 8, 9]. However, for every isoform the specific protein partners were revealed: IQGAP3 binds only ERK1 [10], while IQGAP1 interact with both ERK1 [11] and ERK2 [12]. The interaction of IQGAP3 with annilin was shown, whereas both IQGAP1 and IQGAP2 could not interact with this protein [13]. In living cells, the inhibition of the mRNA coding for IQGAP2 or IQGAP3, contrary to IQGAP1, negatively influenced both the growth and length of axons [4]. Interestingly, a number of known protein partners of IQGAP1 is much greater than for IQGAP2 and IQGAP3 [3]. The functional meaning of such difference awaits a special investigation.

A family of plakophilins comprises three homologous isoforms. Until recently, plakophilins were considered to be mostly the structural proteins, the desmosomal components, which increase cell adhesion due to binding to intermediate filaments of the cytoskeleton [14] Now it is known that plakophilins possess also a scaffold function, controlling a variety of cellular processes and participating in the development of carcinogenesis, cardiomyopathy, hereditary diseases *etc.* [15, 16] This family is a good example of the unique and sometimes functionally antagonistic character of the protein isoforms. In particular, the isoforms differ by cellular localization and kinetics of desmosome formation de novo and are controlled by different mechanisms [17, 18]. Importantly, plakophilin 3 prevents the formation of hyperadhesive desmosomes in a protein kinase C alpha-dependent manner, even in the presence of plakophilin 1 which normally stimulates their formation contributing to the stable intercellular co-adhesion [19].

Different participation of very similar isoforms in cell signaling is demonstrated by three closely related human genes *Kras* (4A, 4B), *Hras* and *Nras*, the products of which are the main members of Ras subfamily of GTP-binding proteins. The isoforms are encoded by

three different genes whereas Kras4A and Kras4B are produced due to differential splicing of the exons. The isoforms differ in short C-terminal regions. For example, Kras4B contains the polylysine sequence and a single farnesyl modification [20]. The isoforms differ at least by four C-terminal amino acid residues. This region is found to be responsible for the variation in the lipid post-translation modifications and membrane localization of the ras isoforms. Though the H-, N- and K-ras soforms show similar effector-binding properties they have different biological functions in the development, cell growth and oncogenesis. Quantification of spatiotemporal patterns of Ras isoforms expression during development showed a relative contribution of KRas4B >> NRas ≥ KRas4A > HRas to the total Ras expression with KRas4B typically representing 60–99 % of all Ras transcripts. KRas4A was the most dynamically regulated Ras isoform with significant up-regulation of the expression observed in stomach, intestine, kidney and heart [21]. The functional divergence may be explained, at least partially, by different membrane compartmentalization of the Ras isoforms [22]. Differential distribution of the Ras proteins on cell membranes may be responsible for the unique spatio-temporal models of activation of the effector pathways including the potential and duration of the signal activation [23].

The isoforms of STAT5 protein represent another example of different participation of the protein isoforms in signaling. The STAT (Signal transducers and activators of transcription) proteins are latent cytoplasmic transcription factors coupling the intracellular signals with the target genes expression [24]. STAT5 is directly activated by JAK2 kinase downstream from several cytokine receptors and oncogenic tyrosine kinase BCR-ABL. STAT5 is represented by two proteins: STAT5A and STAT5B, which share 94 % structural homology, but are transcribed from separate genes [25] STAT5A was found preferentially in the mammary tissue while the STAT5B expression was observed mostly in muscle and liver [26]. In the majority of functional tests STAT5A and STAT5B behave similarly [25]. However, downregulation of STAT5B by RNAi essentially inhibited the BCR-ABL-dependent hematopoietic cells proliferation [27]. The STAT5B isoform rather than STAT5A is important for the expression of BCL-XL in the presence of BCR-ABL. Moreover, downregulation of STAT5B rather than STAT5A made the BCR-ABL-positive human cells sensitive to the anticancer drug imanitib. Moreover, the expression of STAT5A and STAT5B showed opposite correlations with drug response gene expression [28].

One more example of a differential role of the isoforms in signaling is Rho-associated kinases ROCK1 and ROCK2, which are activated by RhoGTPase and control the cytoskeletal rearrangements. It was shown that despite more than 90 % homology of kinase domains it is the ROCK2 isoform which plays an exclusive role in controlling plasticity of T-cells and macrophage polarization [29].

The adenine nucleotide translocase hANT which exchanges ADP for ATP in the mitochondrial inner membrane and participates in oxidative phosphorylation, is represented by three homologous genes, the expression of which is tissue-specific and depends on physiologic state of a cell. hANT1 is mainly ex-

pressed in terminally differentiated muscle cells; hANT2 is growth-regulated and is up-regulated in highly glycolytic and proliferative cells; and hANT3 is considered to be ubiquitous and non-specifically regulated [30]. The protein partners of the isoforms were found to be similar, and the absence of the main isoform hANT3 had no impact on the oxidative phosphorylation process [31]. However, the isoforms were found to be de-regulated in human tumors [2]. Recently, it has been shown that cancer cells require both hANT2 and hANT3, depending on their proliferation status: hANT2 when proliferation rates are high, and hANT3 when proliferation slows [30].

Thus, the homologous isoforms of the same protein can play different roles in human cells. Possible molecular background for such difference will be discussed below taking as an example the isoforms of translation elongation factor 1A (eEF1A).

3. The eEF1A1 and eEF1A2 isoforms of translation elongation factor eEF1A

eEF1A is a translation elongation factor providing the GTP-dependent delivery of aminoacyl-tRNA to the A site of the ribosome. Genome of mammalian cells comprises several eEF1A sequences; however, the only eEF1A1 and eEF1A2 are actively transcribed. Remaining genes are considered retropseudogenes originated from eEF1A1 [32–34]. The EEF1A1 and EEF1A2 genes are localized on 6q14 and 20q13.3 human chromosomes, correspondingly [34]. The coding regions of the corresponding mRNAs are similar by 75%, while the 3' and 5' untranslated regions are completely different [35–37] which opens a way for differential control of the isoforms expression at the post-transcriptional level. The proteins eEF1A1 and eEF1A2 show 97 % homology and 92 % identity. Expression of the isoforms is tissue-specific and mutually-exclusive. eEF1A1 is a major isoform present everywhere in the organism except neurons, muscles, including cardiac muscles [38, 39] and some specialized cells [40]. The importance of tissue-specific expression of eEF1A2 is highlighted by "wasted" mutation in mouse. Normally, during postnatal development of a mouse the eEF1A1 isoform gradually disappears from muscle and neurons being substituted with eEF1A2. "Wasted" mutation is a deletion of the *eEF1A2* locus which makes impossible the expression of eEF1A2. As a result, one can observe in the "wasted" mice newborns the major neurological and immunological impairments, starting with 21st day after birth, with their subsequent death on 28th day. These changes parallel the decrease in the eEF1A1 expression which cannot be compensated by the appearance of eEF1A2 in wasted mouse [40, 41].

eEF1A1 is considered a pro-apoptotic protein whereas eEF1A2 shows anti-apoptotic properties [42, 43]. Importantly, eEF1A2 was shown to appear in a number of human cancers of different localization [44–46]. In some cases, this isoform demonstrates the strong oncogenic potential [45, 47]. The nature of oncogeneity of eEF1A2 is not yet elucidated in detail. It is apparently not related to the gene amplification, mutations in the gene coding region and changes in the gene methylation [48] which suggest that the main contribution should be from a protein molecule *per se*. There are reports on the participation of eEF1A2 in JAK/STAT and AKT signaling in

mouse plasmacitomas [49], PI3K/AKT/mTOR-dependent stabilization of MDM4 [50] and PI3K/Akt/NF-κB signaling in hepatocellular carcinomas [51].

4. Functions of the eEF1A isoforms

Generally speaking, the translational functions of eEF1A1 and eEF1A2 which show tissue-specific and mutually exclusive localization in an organism should not differ much. This notion is supported by in vitro translation studies [52]. However, it does not preclude a variance in the isoforms interaction with other translation components. For instance, affinity of eEF1A2 for tRNA is somewhat higher than eEF1A1 [53]. GDP dissociation rate constant for eEF1A1 is several fold higher than for eEF1A2 [52]. eEF1A1 shows less affinity for EF1Bα, as compared to eEF1A2 [54] which permitted to suggest that eEF1A2 could be more dependent on the nucleotide exchange factors than eEF1A1. Peculiar in this regard is an observation that the ribosomal elongation rate (reverse to ribosomal transit time) is less in neurons where the only eEF1A2 is expressed than that in glial cells where eEF1A1 is an exclusive isoform [55, 56].

Thus, the main translational function of the two isoforms is similar; however, some details of the two isoforms performance during the elongation step may differ. One may speculate that this provides, for example, slower and, supposedly, more precise synthesis of proteins in neurons.

It has become evident that eEF1A plays in cells many non-translational functions as well. It is reported to be involved in the spermatogenesis [57], cell cycle progression [58], chaperon-mediated autophagy [59], protein renaturation [60], apoptosis [61], lypotoxic cell death [62], endogenous proteolysis [63], cytoskeleton rearrangements [64]. It appears that eEF1A may serve as the important hub protein which links together different cellular processes. An important and still unresolved problem is what could be the mechanism of the distribution of eEF1A between all these processes. We suggest that the eEF1A1 and eEF1A2 isoforms may participate in different processes in cell. The physical basis for this is their different spatial organization, an ability to form dimers, different lipophilic properties [65–67], different number and level of post-translational modifications [68–70]. The X-ray structure is only known for eEF1A2 [71]. The different features of the isoforms can be considered as providing a specific landscape for different protein-protein interactions of eEF1A1 and eEF1A2, which, in turn, should be a main contributing factor to their dissimilar distribution between cellular processes.

Indeed, some evidence of differential abilities of the isoforms to bind some protein partners has been obtained. eEF1A2 was reported to bind peroxyredoxin 1 helping to protect against oxidative stress [72], and to interact with oncosupressor $p16^{INK4a}$ [73]. eEF1A1 formed complexes with the multifunctiunal Sgt1 protein *in vitro* and *in cellulo* while eEF1A2 did not [74]. eEF1A1 rather than eEF1A2 interacted with calmodulin in Ca^{2+}-dependent way [75]. eEF1A is known to interact with actin [76] probably due to its dimeric form [77]. Interestingly, the eEF1A1 and eEF1A2 isoforms induced the formation of differently shaped actin bundles [75] which could be important for a supposed role of eEF1A2 in oncogenesis.

Summarizing the information on the dissimilarity in eEF1A1 and eEF1A2 interaction with different protein partners one may indicate some important points to consider.

First, as eEF1A2 is found in the excitable tissues (nervous and neuronal) where the processes of the Ca^{2+}-mediated signaling involving a number of Ca^{2+}binding proteins are very active, it has been suggested that eEF1A2 is less sensitive comparing to eEF1A1 to Ca^{2+}-mediated signaling [75]. This makes translation process more prone to the changes in the concentration of Ca^{2+} in these tissues. It has been proposed that one of the reasons of the appearance of the eEF1A2 isoform in evolution and the tissue-specific expression of this isoform is a need to protect mRNA translation in the specialized tissues from the influence of regular changes in the Ca^{2+} concentration observed in these tissues, thus providing a steady level of protein synthesis.

Second, though an oncogenic role of eEF1A2 is still far from being elucidated, one may suggest that eEF1A2 avoids an eEF1A1-adapted control in cancer tissues, thus acting in non-controlled or mis-controlled way. It has been proposed that actin-bundling role of A2 should be specially considered as cancer-related one [75] especially taking into account that the dysregulated actin bundling may play a key role in the metastatic processes [78, 79].

Finally, the evolutionary appearance of the isoforms of different proteins evidences for the existence of an additional level of the control of cellular processes. The mechanisms of isoform-specific regulation are not yet known in detail and their understanding still remains mostly at phenomenological level. Thorough examination of the functions and specific protein partners of the isoforms is needed to uncover the molecular instruments of the cell control which depend on the isoforms variance of cellular proteins.

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Ізоформи білків. Походження, структура та функції

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Велика кількість білків в організмі ссавців існує у вигляді декількох ізоформ. Ці варіанти кодуються різними генами або є сплайсованими модифікаціями продуктів того ж самого гена. Незважаючи на швидкий інструментальний прогрес у ідентифікації ізоформ, причини їх існування та специфічні функції у більшості випадків залишаються достеменно невідомими. Останнім часом увага дослідників здебільшого зосереджена на сплайсованих ізоформах, у той час як різногенні білкові ізоформи можуть відігравати суттєву роль у різних клітинних процесах. У огляді наводяться приклади різних потенційних функцій ізоформ того ж самого білка, які кодуються різними генами. Молекулярне підгрунтя, що може забезпечувати існування такої різниці функцій у високогомологічних білкових ізоформ обговорюється на прикладі останніх досягнень у вивченні ізоформ фактора елонгації трансляції 1А (eEF1A).

Ключові слова: білкові ізоформи, еукаріотична трансляція, eEF1A

Изоформы белков: Происхождение структура и функции

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Многие белки в организме млекопитающих существует в виде нескольких изоформ. Эти изоформы кодируются разными генами или являются продуктами альтернативного сплайсинга одного и того же гена. Несмотря на быстрый инструментальный прогресс в идентификации изоформ, причины их существования и специфические функции в большинстве случаев остаются точно неизвестными. В последнее время внимание исследователей в основном сосредоточено на сплайсированных изоформах, в то время как разногенные белкоые изоформы могут исполнять существенную роль в разных клеточных процессах. В обзоре приводятся примеры разных потенциальных функций изоформ одного и того же белка, кодируемых разными генами. Молекулярные основы существования такой функциональной разницы высокогомологичных белковых изоформ обсуждаются на примере последних достижений в изучении изоформ фактора элонгации трансляции 1A (eEF1A).

Ключевые слова: белквые изоформы, эукариотическая трансляция, eEF1A

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