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Protein species and moonlighting proteins: Very small changes in a protein's covalent structure can change its biochemical function☆

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ABSTRACT

In the past few decades, hundreds of moonlighting proteins have been identified that perform two or more distinct and physiologically relevant biochemical or biophysical functions that are not due to gene fusions, multiple RNA splice variants, or pleiotropic effects. For this special issue on protein species, this article discusses three topics related to moonlighting proteins that illustrate how small changes or differences in protein covalent structures can result in different functions. Examples are given of moonlighting proteins that switch between functions after undergoing post-translational modifications (PTMs), proteins that share high levels of amino acid sequence identity to a moonlighting protein but share only one of its functions, and several "neomorphic moonlighting proteins" in which a single amino acid mutation results in the addition of a new function.
Biological significance: For this special issue on protein species, this article discusses three topics related to moonlighting proteins : Post-translational modifications (PTMs) that can cause a switch between functions, homologs that share only one of multiple functions, and proteins in which a single amino acid mutation results in the creation of a new function. The examples included illustrate that even in an average protein of hundreds of amino acids, a relatively small difference in sequence or PTMs can result in a large difference in function, which can be important in predicting protein functions, regulation of protein functions, and in the evolution of new functions.

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1. Introduction

Over the past few decades, more and more proteins have been identified that perform two or more distinct and physiologically relevant biochemical or biophysical functions that are not due to gene fusions, multiple RNA splice variants, or pleiotropic effects. These moonlighting proteins [1] are found throughout the evolutionary tree, from eukaryotes, including mammals, yeast, worms, and plants, to bacteria, archea and even viruses. Some of the first examples to be discovered were soluble enzymes that were adopted for a second function as structural proteins in the lens of the eye (crystallins) [2,3]. More recently many other examples have been found that include cytosolic enzymes and chaperones that moonlight as receptors on the cell surface or as secreted cytokines, components of the cytoskeleton, transcription factors, translation factors, adhesins or scaffolds. Over a dozen ribosomal proteins have been found to moonlight as transcription factors or translation factors, sometimes as members of other protein complexes

☆ Database linking MoonProt Database, moonlightingproteins.org.
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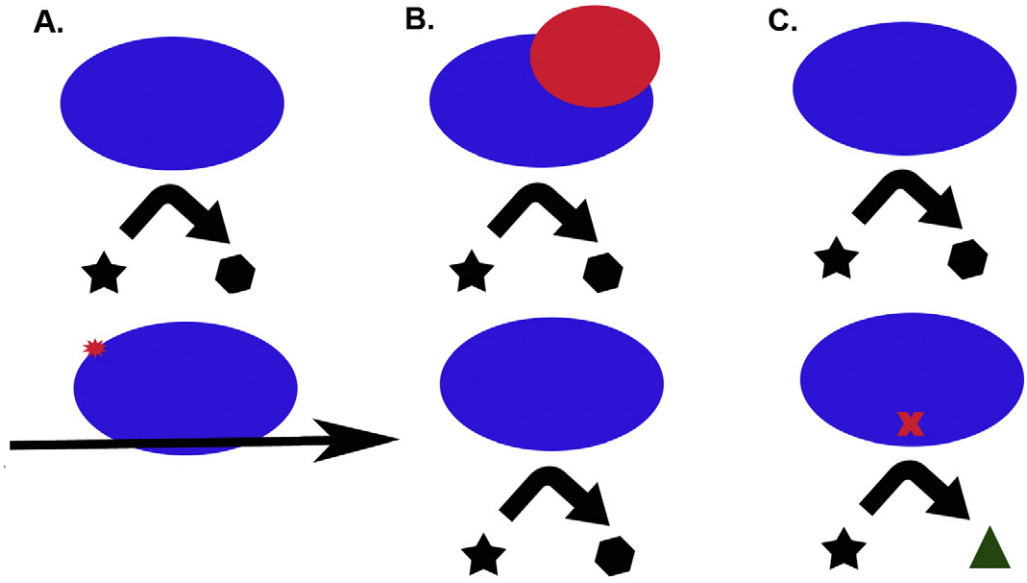

Fig. 1. Small differences between "protein species" can correlate with differences in moonlighting protein functions. A. PTMs can cause moonlighting proteins to toggle between two functions (i.e. phosphorylation (red star) resulting in the switch from an enzyme function in the top panel to a transcription factor function in the bottom panel). B. A homolog of a moonlighting protein might have only one of the two functions (i.e. enzyme function in both proteins, but the protein-protein interaction function is found only in the protein in the top panel). C. A single amino acid substitution (red X) can result in the gain of a neomorphic moonlighting function (i.e. a new enzyme catalytic activity, with a new product (green triangle)).

[for reviews see 4–23]. The online MoonProt Database, which includes information about those moonlighting proteins for which biochemical or biophysical evidence supports the presence of at least two biochemical functions in one polypeptide chain, includes hundreds of moonlighting proteins [24, moonlightingproteins.org], and the list is continuing to grow every year, so it is likely that many other proteins also have additional functions that have not yet been found.

For this special issue on protein species [25,26], this article discusses three topics related to moonlighting proteins that illustrate how small changes or differences in protein covalent structures can result in different functions. Examples are given of moonlighting proteins that switch between functions after undergoing post-translational modifications (PTMs), proteins that share high levels of amino acid sequence identity to a moonlighting protein but share only one of its functions, and several "neomorphic moonlighting proteins" [27] in which a single amino acid mutation results in the addition of a new function (Fig. 1).

2. PTMs can cause a switch between the different functions of a moonlighting protein

Post-translational modifications (PTMs) are widespread in proteins and can be a means of regulating function, including increasing or decreasing the rate of enzyme catalysis or altering information flow through a signaling pathway. In the case of moonlighting proteins, some PTMs have been identified that cause a switch between two of the functions of the protein, in effect serving as a toggle between functions (Fig. 2, Table 1A). These post-translational modifications can be especially important regulatory mechanisms for moonlighting proteins that function in two different pathways and can enable a switch between the activities of the two pathways. Because they can be dynamic and reversible, and are added much more quickly than synthesizing a new protein, PTMs on moonlighting proteins can contribute significantly to a cell's ability to adapt quickly to changes in the environment.

In response to interferon-gamma of human myeloid cells, ribosomal protein L13a becomes phosphorylated, which causes its release from the ribosome [28] (Fig. 2). L13a becomes a translation factor as part of IFN-gamma-activated inhibitor of translation (GAIT complex) consisting of ribosomal protein L13a, glutamylprolyl tRNA synthetase (EPRS), NS1-associated protein-1 (NSAP1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The GAIT complex binds to 3′-UTR noncoding regions of mRNAs for VEGF-A, ceruplasmin, and other inflammation-related proteins to regulate (decrease) their expression and limit or terminate inflammation.

When not serving as part of the GAIT complex, the EPRS amino acyl-tRNA synthetase ligates an amino acid to its cognate tRNA molecule for use in protein synthesis. Phosphorylation of the human EPRS in response to interferon-gamma releases it from the tRNA multi-synthetase protein complex so that it can become part of the GAIT complex [29,30] (Fig. 2). Initial phosphorylation of Ser886 in the linker between the glutamyl rRNA synthetase and prolyl-tRNA synthetase domains of EPRS and a second phosphorylation at Ser999 cause release from the tRNA multi-synthetase complex and enables binding to the NS1-associated protein, followed by binding to ribosomal protein L13a and GAPDH for formation of the GAIT complex.

GAPDH in glycolysis also has an additional function related to those above. rpL13a is unstable and subject to proteolysis once released from the ribosome, but GAPDH serves as a chaperone to extend its half-life. Under oxidative conditions, which can result from the inflammatory response, oxidatively modified LDL (LDLox) can cause a post-translational modification of Cys247 of GAPDH, resulting in S-nitrosylated GAPDH (SNO-GAPDH) [31]. SNO-GAPDH cannot bind to and protect rpL13a from degradation.

Ribosomal proteins rpL10A and S3 (rpS3) have functions in the nucleus when released from the ribosome. rpL10A helps protect plants from geminiviruses [32,33] (Fig. 2). Phosphorylation by NSP-interacting kinase (NIK) directs rpL10A to the nucleus where it interferes with geminivirus proliferation or movement to other cells. rpS3 participates in repair of DNA damage by serving as an endonuclease that acts on damaged DNA. Protein kinase C-delta (PKCdelta) phosphorylation of rpS3 causes it to move to the nucleus to repair damaged DNA [34]. The phosphorylation is only observed in the free, non-ribosomal associated protein, and repair endonuclease catalytic activity increases after phosphorylation. rpS3 also serves as a component of some NF-kappaB transcription factor complexes and provides specificity for binding of the complex to specific DNA sequences and target genes (Fig. 2). For this function, rpS3 becomes phosphorylated at Ser209 by the kinase