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Introduction

Moonlighting Proteins in Neurological Disorders

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INTRODUCTION

The recognition that there are moonlighting proteins that display more that one function in an organism was reported some 20 years ago, instancing certain structural proteins like crystallins, which exhibit glycolytic activity (*1*). Nowadays, moonlighting is used in a wider sense. Moonlighting proteins comprise proteins with multiple, independent functions, more than one role in an organism, but their characteristics do not originate by gene fusion, splice variants, or post-translational modifications (*2*).

There is currently no generally straightforward method to identify which proteins, encoded by a genome sequence, have multiple functions or to determine whether a protein of interest is a moonlighting protein. However, there are reports, some recently published, in which examples of moonlighting proteins are listed and categorized (*3, 4*). A couple of examples related to this research area are given to illustrate how the moonlighting proteins display multiple functions by different mechanisms and ways.

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is considered a classic example of a protein with moonlighting characteristics. This glycolytic enzyme displays oligomerization- and localization-dependent distinct functions: the tetramer catalyzes the conversion of 3-phosphoglyceraldehyde in the cytosol, whereas the monomer is a nuclear uracil-DNA glycosylase (*5*). The oligomerization state of this enzyme is modulated by the cellular concentrations of adenosine triphosphate (ATP), nicotinamide-adenine-dinuclcotide (NAD^+), and by its interactions with a potential partner, for example, the association of function-related glycolytic enzymes can enhance the pathway efficiency (*6*), or its binding to β-amyloid precursor protein and to its truncated toxic polypeptide, Aβ, affects the functions of both partners (*7*). Cytoskeletal filaments, actin or microtubule systems depending on the cell type, are also targets of GAPDH binding (*8*). In addition, GAPDH also has partners in the nucleus and interacts with RNAs; thus, its functions can be switched by multiple factors and trigger apparently unrelated pathways too (*9*).

Triosephosphate isomerse (TPI) exemplifies a moonlighting feature by its two independent functions; the dual function is originated by inherited mutations (*10*). Mutation causes enzymopathy (TPI deficiency) frequently coupled with neurological deficiency, which is unique among the glycolytic enzymopathies. The mutant, misfolded enzyme can decrease the isomerase activity; however, this effect is compensated at the system level by switching different mechanisms, such as activation of other glycolytic enzymes or their microcompartmentation (*10*). On the other hand, the mutant TPI behaves as a "structure protein," which enters aberrant protein–protein interactions leading to the formation of pathological inclusion via TPI-promoted protein aggregation. Consequently, as we suggested recently, a reappraisal of this disease is necessary as in the case of TPI deficiency not the enzymatic activity, but rather the structural function of the protein is decisive in the etiology of this serious clinical symptom (*11*). Therefore, a moonlighting feature could contribute to the complexity of this disease similar to that suggested in the cases of other metabolic diseases (*3*).

Unlike classical cases, some moonlighting proteins enable switching a physiological function to a pathological one which may put them into a specific subset of moonlighting proteins because their actions are based upon on different mechanisms. This feature is characteristic for the structural unfolded (disordered) proteins probably due to their extensive capacity to adapt different conformations upon binding. These disordered proteins do not have stable, well-defined 3D structures; consequently, they can actively form pathological ultrastructures, such as fibrils, oligomers, or aggregates with distinct toxicity. Although the initiation at the molecular level such as aberrant protein–protein interactions is similar in the cases of etiology of "disordered diseases," yet the outcome, the clinical symptoms, is very different (*12*). We propose that the moonlighting characteristic of the proteins can contribute extensively to the complex phenotype of a given disorder. Thus, the idea that the identification of all activities in the proteome is essential for understanding human diseases should be taken into account.

TPPP/p25, a disordered protein, is enriched in the pathological human brain tissue characteristic of synucleinopathies (*13*). This protein is primarily expressed in oligodendrocytes where

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the tubulin polymerization promoting and microtubule bundling activity of TPPP/p25 are crucial for the development of projections in the course of differentiation of the progenitor cells leading to myelinization, requiring the ensheathment of axons for their normal function in the central nervous system (CNS). However, its function is entirely different when it is co-localized and enriched in neurons or glia cells with α-synuclein forming pathological inclusions such as Lewy bodies.

A number of reports demonstrate the tight connection between disordered proteins and neurological disorders; on the other hand, the disordered proteins display moonlighting features by their ability to adapt conformations corresponding their binding to different partner proteins. Despite the diversity in the amino acid sequence of the different proteins involved in these neurological diseases, one of the common themes underlying their pathomechanisms is the unfolded/misfolded protein-induced aggregation that can trigger cascades of events, ultimately resulting in neuronal death with devastating clinical consequences in personality. Proteins with moonlighting ability might switch between functions according to the substantial benefit of the cells.

Moonlighting proteins are suggested to play a role in various diseases in addition to neurological disorders, for example, in host cell invasion (*14*). Consequently, enhancing or blocking moonlighting functions could be a target for future drug design. For that task, more knowledge of moonlighting functions at the molecular level is needed.

This mini-theme issue with different topics in the individual papers will focus on molecular events initiated by moonlighting proteins, the functions of which can vary due to changes in cellular localization, oligomeric state, or cellular concentration of any interacting partners with low molecular mass such as ligands, substrates, cofactors, and so forth, or combinations of these mechanisms.

The critical reviews presented in the following sections are prepared by distinguished scientists working in this area, namely, Professors Carlos Gancedo, Kjell Fuxe, Michael R. Waterman, Debashis Mukhopadhyay, and Constance J. Jeffery.

OVERVIEW OF THE MOONLIGHTING VENTURE

Several months ago Professor Whelan, an Editor-in-Chief of this Journal, kindly invited me to propose a theme for a special issue of this journal. I appreciated the opportunity to take part in this new venture, and prepared a proposal entitled "Moonlighting Proteins in Neurological Disorders." This topic was chosen because I thought it would be interesting for scientists working in very different fields of life sciences and call up new ideas and maybe stimulating research activity related to multifunctional proteins. The motivation was to present critical reviews of invited experts on the topic raised in the "Initiative." These scientists expressed their interests to be involved in this venture and have submitted excellent papers discussing the moonlighting phenomenon from very different aspects. The papers, evaluated by independent referees, are presented in this special issue. This resume ventures to summarize the recent discoveries in the field of moonlighting proteins with multifunctional characteristics, taking into account the messages of the review papers. In fact, this special issue introduces a number of different kinds of proteins and combinations of their moonlighting functions, their abilities to switch between functions by changing cellular localization, expression in a novel cell type, oligomeric state, and cellular concentrations of ligands affecting the features of proteins/enzymes. This resume considers selected examples and concepts as well as makes an effort to extract some general lessons for moonlighting as a "phenomenon" in the protein world.

Although the moonlighting concept was introduced more than two decades ago (*1*), there is no unambiguous definition or terminology of the moonlighting proteins/functions as reflected in the presented review papers as well. According to one of the traditional definitions, the moonlighting proteins comprise proteins with multiple, independent functions, more than one role in an organism, but their characteristics are not originated by gene fusion, splice variants, or post-translational modifications (*2, 9*). The term, moonlighting protein, is used to describe proteins in which several functions can be found in a single strand of amino acids (*4, 14*); however, those proteins that perform the same function in different subcellular compartments are not belong to the family of the moonlighting proteins (*1, 9*). The multifunctional proteins display autonomous sometimes unrelated, markedly different functions such as structural and catalytic ones. They form interactions that are peculiarly characteristic for disordered proteins, and the heterocomplexes affect functions, for example, inhibit catalytic functions or any activities of proteins. One of their characteristic features is that they use regions outside the active site for other functions, such as regulatory and structural ones (*15*). Moonlighting proteins are present in diverse organisms (*14*), and the implications of their function should be considered when trying to explain multiple symptoms of single-gene disorders or to predict consequences of metabolic engineering (see the corresponding article by Flores and Gancedo, pages 457–462, in this special issue).

The following characteristic types of the moonlighting proteins are considered in the review papers: (i) they accommodate more than one domain displaying unrelated functions meaning that inactivation of one of the functions (*e.g.*, by mutation) should not affect the second function and vica versa, (ii) a single domain is involved in both structural and catalytic functions coupled with conformational changes, (iii) a single domain is involved in multiple functions coupled with structural assemblage such as homo- and heteroassociations, and (iv) their functions could be determined by the modulation of the microenvironment (*16, 17*). In fact, the concept that there is a growing appreciation that some proteins displaying more than one function has profound implications both for understanding the vital machinery of cells at the molecular level and for designing new biotechnology applications using modified cells (see the corresponding article by Flores and Gancedo, pages 457–462, in this special issue). These issues are particularly relevant to yeast wherein moonlighting proteins/functions have been identified in its various metabolic pathways such as glycolysis, the tricarboxylic acid cycle (TCA) cycle, galactose metabolism, amino acid, CoA,

inositol biosynthesis, and their functions are well established; thus, a large variety of yeast mutant species is available (see the corresponding article by Flores and Gancedo, pages 457–462, in this special issue). All these yeast species are important as model organisms in basic research as well. However, glycolytic and TCA cycle enzymes with moonlighting functions have been identified in various organisms. Glyceraldehyde-3-phosphate dehydrogenase, which functions distinctly depending on its homo- and hetero-associations or its intracellular localization, is perhaps the most established moonlighting protein (see the corresponding articles by Jeffery, pages 489–494, and Flores and Gancedo, pages 457–462, in this special issue). Phosphoglucose isomerase independently of its classic glycolytic function displays independent functions, and it binds to cell surface receptors aiding the differentiation of B-cell precursors to mature antibody-secreting cells (see the corresponding article by Jeffery, pages 489–494, in this special issue). This and other observations concern the concept that the moonlighting proteins appear to be highly conserved enzymes; yet, their moonlighting functions are practically impossible to predict.

Moonlighting functions of enzymes involved in very different, unrelated cellular metabolisms are well documented, and some of them are introduced in this special issue. Cytochrome P450 170A1, a bifunctional protein catalyzes monooxygenase activity and terpene synthase activity, having two distinct active sites. In addition, the P450 localizing in the inner mitochondrial membrane takes an active part in transferring electrons through a two-component soluble reductase system (see the corresponding article by Zhao and Waterman, pages 473–477, in this special issue). These and many other data illustrate that the moonlighting function can create a whole new level of complexity in the cell that may link a metabolic pathway to a signaling pathway by several different manners.

The G-protein-coupled receptors (GPCRs) are key players in a complex pathway with their beautiful moonlighting functions. They exhibit a common structural motif consisting of different membrane spanning regions. Receptor occupation promotes interaction between the receptor and the G protein on the interior surface of the membrane. This induces an exchange of guanidine diphosphate (GDP) for guanidine triphosphate (GTP) on the G protein α subunit and dissociation of this subunit from the $\beta\gamma$ heterodimer. In the central nervous system, GPCRs form heteromers owing structural plasticity; receptor–receptor interactions associated with their conformational changes with high propensity toward an unstructured conformation leading to signal integration (*18–21*). These potentially plastic domains can undergo interactions particularly with domains of other receptors and receptor interacting proteins forming clusters of membrane-associated proteins. The interaction of receptors with accessory proteins forming complexes at the cytoplasmic face, denoted "signalsome complexes," affects signaling efficiency/specificity. The formation of a highly dynamic ultrastructure resulting from allosteric interactions is nicely demonstrated in the Borroto-Escuela paper through the example of acetylcholine receptor family: the same amino acid strand could bind in one state to a certain G protein and in a different state to another signaling protein resulting in multiple signaling function producing dynamic multitasking actions. The formation of the GPCR-receptor tyrosine kinase (RTK) heteromer with its allosteric feature leads to changes in agonist/antagonist binding in addition to the binding of signaling proteins developing moonlighting function that may have special relevance for neurodegeneration (see the corresponding article by Borroto-Escuela et al., pages 463–472, in this special issue).

Unfolded/disordered proteins, which do not have 3D structure, play crucial roles in the etiology of neurological disorders as their "structures" make it possible to display different conformers with distinct functions caused by mutations and/or epigenetic alterations. In fact, a very limited number of mutations in a protein are sufficient to introduce moonlighting functions; a particular mutation may increase the efficiency of one function, but compromise the efficiency of the other one. In fact, mutation frequently produces pathological moonlighting functions; however, in many cases, it could arise by single or multiple associations of disordered proteins. As I proposed in the Introduction, the phenomenon of switching of physiological function of proteins to pathological ones due to alterations in "circumstances" (mutation, conformation changes, and alteration in the interactions) can be considered as a moonlighting function. Thus, the idea of Dr. Jeffery to introduce a new term for defying this function as "Neomorphic Moonlighting Functions" is highly appreciated (see the corresponding article by Jeffery, pages 489–494, in this special issue). Well-known examples of neomorphic moonlighting are the hallmark proteins of Alzheimer's and Parkinson's diseases, which enter aberrant, nonphysiological interactions resulting in the formation of protein aggregates with pathological consequences.

It is not surprising that the highly flexible disordered proteins can perform more than one function, and their multiple functions could be affected/regulated independently. The fact that inactivation of one of them would not generally affect functions provides some amount of "robustness" of their actions (*14*). A significant fraction of the intrinsically disordered eukaryotic proteins acts as "hubs" to play a dictatorial role in protein–protein interaction networks. The removal of a "hub" protein most frequently causes disruption of the cellular system due to their "hub" functions. In the human network, the occurrences of the disordered proteins are more frequent than that of the properly folded proteins (*22*), which could lead to the formation of pathological inclusions via the aggregation-prone disordered proteins. There are two primary mechanisms by which disorder is used in protein–protein interaction networks: one disordered region binding to many partners and many disordered regions binding to one partner (see the corresponding article by Das and Mukhopadhyay, pages 478–488, in this special issue).

The neurodegenerative diseases are complex, multifactorial disorders where unfolding/misfolding of proteins cause aberrant protein–protein interactions. There are cases when the disease is not characterized by specific mutations, for example, at the nonfamiliar disease types. Most of the causative proteins, however, are intrinsically unstructured which could play pivotal role in these processes. The objective of the Mukhopadhyay's group to evalu-

ate how a small perturbation might lead to disruption of the network and to the disease phenotype is a peculiarly important issue because different neurodegenerative diseases with distinct clinical symptoms can originate from the dysfunction of proteins at different regions of central nervous system. Although there are differences in components and machineries causing disorders, specific protein aggregations and misfoldings trigger a cascade of pathological events such as oxidative damage, mitochondrial dysfunction, impaired bioenergetics, disruption of the Golgi apparatus, and transport, which eventually lead to neurological damages.

In the case of mutation-caused disease, the treatment for the disease is peculiarly difficult when the mutation causes alteration in the function of the moonlighting protein (see the corresponding article by Jeffery, pages 489–494, in this special issue). Consequently, the treatment of only one function of the multifunctional protein may not be enough to treat the disease. As the moonlighting function could also be important for communication within and among the cells by determining various functions, it is an important problem that there is no general method to identify moonlighting proteins by genome sequence. An additional open question is the relationship of the moonlighting function to evolution. In relation to the evolution, it has been suggested that there is no end goal for it; however, novel functions could be developed by adapting existing ones. If the new function has an advantage for the organism, this function will be maintained evolutionary (see the corresponding article by Flores and Gancedo, pages 457–462, in this special issue). Therefore, much work has to be done to understand the too complex features of the moonlighting proteins.

Finally, I very much appreciate the excellent contributions of all participants in this new venture. I thank the referees who reviewed and improved the papers with their suggestions. I am grateful for Professor Whelan for providing me the opportunity to be involved in the new venture, more importantly for his stimulating and helpful advice, and Sandra Black for her great work organizing all procedures over several months at the editorial office.

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