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Review

Moonlighting proteins: An intriguing mode of multitasking

Daphne H.E.W. Huberts, Ida J. van der Klei *

Molecular Cell Biology, GBB, University of Groningen, Kluyver Centre for Genomics of Industrial Fermentation, P.O. Box 14, 9750 AA Haren, The Netherlands

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ABSTRACT

Proteins are macromolecules, which perform a large variety of functions. Most of them have only a single function, but an increasing number of proteins are being identified as multifunctional. Moonlighting proteins form a special class of multifunctional proteins. They perform multiple autonomous and often unrelated functions without partitioning these functions into different domains of the protein. Striking examples are enzymes, which in addition to their catalytic function are involved in fully unrelated processes such as autophagy, protein transport or DNA maintenance. In this contribution we present an overview of our current knowledge of moonlighting proteins and discuss the significant implications for biomedical and fundamental research.

1. The definition of moonlighting proteins

The first examples of moonlighting proteins were described in the late 1980s, when Piatigorsky and Wistow [1] reported that certain crystallins, structural proteins in the lens of the vertebrate eye, were well known enzymes. For example, duck ε-crystallin turned out to be lactate dehydrogenase [2], whereas turtle τ-crystallin is the glycolytic enzyme α-enolase [3]. A metabolic role of these enzymes in the lens, where they accumulate to very high concentrations, is not likely [4]. Instead they probably have only a structural function in the lens. In line with this assumption is the observation that some crystallins are enzymatically inactive paralogs of these enzymes (see below) [5–7].

To describe the phenomenon of moonlighting, Piatigorsky initially coined the term gene sharing [8], but nowadays moonlighting is the generally used term [9], in analogy to moonlighting people who have multiple jobs. Moonlighting proteins are very special multifunctional proteins, because they perform multiple autonomous, often unrelated, functions without partitioning these functions into different protein domains. Hence, proteins that have multiple functions as a result of gene fusion are excluded. The same is true for proteins that are translation products of different splice variants of the same gene. Another important criterion for a moonlighting protein is the independency of both functions, meaning that inactivation of one of the functions (e.g. by mutation) should not affect the second function and vice versa.

Moonlighting should also not be confused with pleiotropism. Pleiotropic effects generally are the result of inactivation of a single function, which is involved in multiple cellular processes, e.g. a protein that has multiple interaction partners in different pathways or an enzyme which is important in several metabolic pathways. Instead, moonlighting proteins perform multiple functions, which differ mechanistically.

2. Moonlighting proteins: widespread and involved in many processes

As illustrated in Table 1, examples of moonlighting proteins have been described in many species including plants [10], animals [11], yeast [12] and prokaryotes [9,13]. Although most examples of moonlighting proteins have been identified in yeast [12], this is probably only due to the fact that these organisms are extensively studied. The currently known moonlighting functions are extremely diverse and are involved in a large range of biological functions (exemplified in Table 1). To illustrate their widespread occurrence and the diversity in functions, five characteristic examples of moonlighting proteins are detailed below.

2.1. Escherichia coli thioredoxin

The *E. coli* anti-oxidant protein thioredoxin is an example of a prokaryotic moonlighting protein [14]. Upon infection with the bacteriophage T7 *E. coli* thioredoxin forms a complex with T7 DNA polymerase, which results in enhanced T7 DNA replication [15,16], a crucial step in successful T7 infection. Thioredoxin binds to a loop in T7 DNA polymerase, thereby creating a sliding clamp that allows the polymerase to bind more strongly to the DNA [17]. The anti-oxidant function of thioredoxin is fully autonomous and completely independent of its function in T7 DNA replication [18,19], in which the protein most likely fulfils a structural role.

* Corresponding author. Tel.: +31 50 363 2179; fax +31 50 363 8280.
E-mail address: I.J.van.der.Klei@rug.nl (I.J. van der Klei).

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Table 1
A selection of established moonlighting proteins in different kingdoms of life.

Protein	Organism	Functions	Ref.
Animals			
Aconitase	*Homo sapiens*	TCA cycle enzyme Iron homeostasis	[69]
ATF2	***Homo sapiens***	**Transcription factor** **DNA damage response**	[79]
Crystallins*	Various	Lens structural protein Various enzymes	[2,3,8]
Cytochrome *c*	Various	Energy metabolism Apoptosis	[30]
DLD	*Homo sapiens*	Energy metabolism Protease	[50]
ERK2	***Homo sapiens***	**MAP kinase** **Transcriptional repressor**	[80]
ESCRT-II complex*	***Drosophila melanogaster***	**Endosomal protein sorting** **bicoid mRNA localization**	[81]
STAT3	***Mus musculus***	**Transcription factor** **Electron transport chain**	[34]
Plants			
Hexokinase	***Arabidopsis thaliana***	**Glucose metabolism** **Glucose signaling**	[82]
Presenilin	***Physcomitrella patens***	**γ-secretase** **Cytoskeletal function**	[28]
Yeast			
Aconitase	*Saccharomyces cerevisiae*	TCA cycle enzyme mtDNA stability	[35]
Aldolase	*Saccharomyces cerevisiae*	Glycolytic enzyme V-ATPase assembly	[36]
Arg5,6	*Saccharomyces cerevisiae*	Arginine biosynthesis Transcriptional control	[76]
Enolase	*Saccharomyces cerevisiae*	Glycolytic enzyme Homotypic vacuole fusion Mitochondrial tRNA import	[38] [83]
Galactokinase	*Kluyveromyces lactis*	Galactose catabolism enzyme Induction galactose genes	[54]
Hal3	***Saccharomyces cerevisiae***	**Halotolerance determinant** **Coenzyme A biosynthesis**	[84]
HSP60*	***Saccharomyces cerevisiae***	**Mitochondrial chaperone** **Stabilization active DNA ori's**	[85]
Phosphofructokinase	*Pichia pastoris*	Glycolytic enzyme Autophagy peroxisomes	[37]
Pyruvate carboxylase	*Hansenula polymorpha*	Anaplerotic enzyme Assembly of alcohol oxidase	[23]
Vhs3	***Saccharomyces cerevisiae***	**Halotolerance determinant** **Coenzyme A biosynthesis**	[84]
Prokaryotes			
Aconitase	*Mycobacterium tuberculosis*	TCA cycle enzyme Iron-responsive protein	[71]
CYP170A1	***Streptomyces coelicolor***	**Albaflavenone synthase** **Terpene synthase**	[49]
Enolase*	*Streptococcus pneumoniae*	Glycolytic enzyme Plasminogen binding	[67]
GroEL*	***Enterobacter aerogenes***	**Chaperone** **Insect toxin**	[47]
MurI	***Mycobacterium tuberculosis***	**Glutamate racemase** **DNA gyrase inhibitor**	[65]
Thioredoxin	*Escherichia coli*	Anti-oxidant T7 DNA polymerase subunit	[17]
Protists			
Aldolase*	***Plasmodium vivax***	**Glycolytic enzyme** **Host-cell invasion**	[73]

Examples of moonlighting proteins are included that illustrate their widespread occurrence and the large variety in cellular functions. The table excludes examples that are not true moonlighting proteins according to the criteria indicated in the text (e.g. fusion proteins, pleiotropy). The table includes all examples referred to in the text of this review and (in bold) several new examples of moonlighting not mentioned in earlier reviews [9–12,78]. For some examples (marked with *), there is data that strongly suggests that these proteins are genuine moonlighting proteins, but there is no conclusive experimental evidence yet that the multiple functions of these protein are indeed independent.

2.2. Pyruvate carboxylase in methylotrophic yeast

Pyruvate carboxylase is a highly conserved enzyme, which catalyzes the carboxylation of pyruvate into oxaloacetate, thereby replenishing the tricarboxylic acid cycle [20]. Surprisingly, in methylotrophic yeast species, such as *Hansenula polymorpha* and *Pichia pastoris*, pyruvate carboxylase is also essential for proper targeting and assembly of the peroxisomal protein alcohol oxidase (AO). AO, the first enzyme of methanol metabolism [21], is a homo-octameric flavoenzyme [22]. In wild type cells the bulk of this enzyme is present as enzymatically active AO octamers in the peroxisomal matrix. However, in cells lacking pyruvate carboxylase enzymatically inactive, FAD-lacking AO monomers accumulate in the cytosol, indicating that pyruvate carboxylase has a second fully unrelated function in assembly and import of a peroxisomal matrix protein [23]. How pyruvate carboxylase fulfils this function is yet unknown. As prescribed for a genuine moonlighting protein [24], the function in AO import/assembly is fully independent of the enzyme activity of pyruvate carboxylase, because amino acid substitutions can be introduced that fully inactivate the enzyme activity of pyruvate carboxylase, without affecting its function in AO assembly and import. Conversely, mutations are known that fully block the function of pyruvate carboxylase in import and assembly of AO, but have no effect on the enzyme activity of the protein [24].

2.3. Physcomitrella patens presenilin

Presenilin is the catalytic component of the multiprotein γ-secretase enzyme complex [25], which cleaves important proteins such as Notch [26] and amyloid precursor protein (APP), proteins implicated in Alzheimer's disease [27]. Mammalian presenilin is suggested to have several moonlighting functions, but it is difficult to study these functions in mammals. To facilitate the analysis, the moss *P. patens* is used as a model organism, because this organism contains γ-secretase, but not Notch or APP. Upon deletion of the *P. patens* gene encoding presenilin phenotypic abnormalities were found, which strongly suggested that presenilin has a function in the cytoskeletal network of the moss [28]. This novel function is unrelated to the enzymatic activity of presenilin, because enzymatically inactive variants of presenilin could rescue the aberrant morphology. Interestingly an enzymatically inactive version of human presenilin could also rescue the phenotype upon introduction in *P. patens*. This suggests that presenilin may have an evolutionary conserved moonlighting function, which is present in plants as well as in mammals.