

University of Groningen

## Moonlighting proteins

Huberts, Daphne; van der Klei, I.J.

*Published in:*  
Biochimica et Biophysica Acta-Molecular Cell Research

*DOI:*  
[10.1016/j.bbamcr.2010.01.022](https://doi.org/10.1016/j.bbamcr.2010.01.022)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2010

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*  
Huberts, D., & van der Klei, I. J. (2010). Moonlighting proteins: An intriguing mode of multitasking. *Biochimica et Biophysica Acta-Molecular Cell Research*, 1803(4), 520-525.  
<https://doi.org/10.1016/j.bbamcr.2010.01.022>

### Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

### Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*



## 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

## ARTICLE INFO

## Article history:

Received 20 November 2009  
 Received in revised form 28 January 2010  
 Accepted 29 January 2010  
 Available online 6 February 2010

## Keywords:

Multifunctional protein  
 Enzyme  
 Moonlighting  
 Gene sharing

## 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.

© 2010 Elsevier B.V. All rights reserved.

## 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  $\epsilon$ -crystallin turned out to be lactate dehydrogenase [2], whereas turtle  $\tau$ -crystallin is the glycolytic enzyme  $\alpha$ -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](mailto:I.J.van.der.Klei@rug.nl) (I.J. van der Klei).

**Table 1**

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

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

**Table 1 (continued)**

Protein	Organism	Functions	Ref.
<i>Prokaryotes</i>			
Thioredoxin	<i>Escherichia coli</i>	Anti-oxidant T7 DNA polymerase subunit	[17]
<i>Protists</i>			
<b>Aldolase*</b>	<b><i>Plasmodium vivax</i></b>	<b>Glycolytic enzyme</b> <b>Host-cell invasion</b>	[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  $\gamma$ -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  $\gamma$ -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.

## 2.4. Cytochrome *c*

The mitochondrial intermembrane space protein cytochrome *c* is part of the electron transport chain. However, upon release into the cytosol the protein is important in apoptosis. Cytosolic cytochrome *c* forms a complex with the apoptotic protease-activating factor 1 (Apaf-1), which is the start of a signaling cascade that results in apoptotic cell death [29]. The redox and pro-apoptotic functions of cytochrome *c* are completely independent [30,31]. It is possible to create a mutant cytochrome *c* variant that functions normally in respiration, but which is unable to bind to Apaf-1. Vice versa cytochrome *c* does not need to have a functional redox capacity to induce an apoptotic response. Hence, cytochrome *c* also has the characteristics of a true moonlighting protein.

## 2.5. STAT3

Recent data indicate that mammalian STAT3 represents a genuine moonlighting protein [32]. STATs are signal transducers and activators of transcription [33]. Phosphorylated STATs translocate to the nucleus, where they bind to the promoter regions of a specific set of genes to regulate their expression. The hormone leptin activates STAT3, which is important for the regulation of whole-body energy intake and metabolism. Wegrzyn et al. [34] showed that a portion of the cellular STAT3 protein is localized to mitochondria, where it plays a role in oxidative phosphorylation. In the absence of STAT3 mitochondrial oxidative phosphorylation is significantly reduced due to a decrease in the activities of complexes I and II of the electron transport chain. Because the levels of complex I and II proteins were normal in these cells, STAT3 most likely has no role in the transcriptional regulation of these proteins. This was further supported by the observation that transcriptionally inactive STAT3 variants were able to restore mitochondrial function. In addition, a STAT3 mutant has been identified that is unable to restore mitochondrial function, but still possessed transcriptional activity. Hence, inactivation of the function of STAT3 in transcriptional regulation does not influence its role in mitochondrial respiration and vice versa.

## 3. General features of moonlighting proteins

Many of the currently known moonlighting proteins are highly conserved enzymes, also called ancient enzymes. Especially enzymes involved in sugar metabolism appear to moonlight [2,3,35–38]. It has been suggested that as many as 7 out of the 10 proteins in the glycolytic pathway and 7 out of the 8 enzymes of the tricarboxylic acid (TCA) cycle have a moonlighting function [11,39].

Why moonlighting functions are so frequently identified in highly conserved proteins is still very speculative. Possibly this is related to the fact that highly conserved proteins are present in many different organisms and hence there is a higher chance that moonlighting functions are identified for one of these proteins as compared to proteins that are present in only a few species [9,40]. Moonlighting functions also seem to occur more often in proteins that are constitutively expressed at relatively high levels [41]. For these proteins the development of a novel function is not restricted to specific conditions.

Although many moonlighting functions reside in highly conserved proteins, the presence of such a function cannot be predicted based on homology. For example, the yeast *Saccharomyces cerevisiae* contains two pyruvate carboxylases, which each share about eighty percent sequence identity with pyruvate carboxylase from *H. polymorpha*. Despite the high similarity between these proteins, neither one of the pyruvate carboxylases from *S. cerevisiae* can perform the moonlighting function of *H. polymorpha* pyruvate carboxylase in the assembly/translocation of peroxisomal AO [42]. Pyruvate carboxylase from the methylotrophic yeast *P. pastoris* also shares about eighty percent

sequence identity with pyruvate carboxylase from *H. polymorpha*. In contrast, it is capable of fulfilling the same moonlighting function as its counterpart in *H. polymorpha* [23,43].

## 4. Evolution of moonlighting proteins

It has been speculated that moonlighting possibly evolves as a way to expand the functional capabilities of an organism without the burden of an expanding genome [9]. However, this is not very likely as in many organisms large parts of the genome do not seem to have a function and thus there may be little selection pressure to limit genome size [44].

Gancedo [12] presented a more plausible explanation and referred to the tinkerer's way of evolution [45], which means that there is no end goal in evolution and that novel functions only develop by adapting existing ones. If a particular novel function results in an advantage for the organism, this function will be selected for during evolution.

For the development of a moonlighting function, a protein is proposed to have some innate compatibility for a new function [46]. Additional mutations, which augment the protein's ability to perform this novel function, are then subsequently selected for.

There are indications that a very limited number of mutations is sufficient to introduce a new moonlighting function. A good example is GroEL from *Enterobacter aerogenes*, a bacterium that lives in the saliva of antlions. Antlions are larvae of the Myrmeleontidae family that prey on other insects. They paralyze their prey by injecting a paralyzing toxin produced by salivary bacteria. Yoshida et al. [47] identified the paralyzing toxin in the saliva of *Myrmeleon bore* larvae as a homologue of GroEL, a well known molecular chaperone [48]. *E. aerogenes* GroEL and *E. coli* GroEL differ only by 11 amino acids [47]. Yet *E. coli* GroEL is not toxic, but *E. aerogenes* GroEL is. Mutational analysis revealed that only four amino acids were essential for toxicity. Because none of them were in proximity to the GroEL folding cage, these residues are not involved in the chaperone function of GroEL. Interestingly, a toxic *E. coli* GroEL variant could be constructed by introducing the corresponding residues in this protein. Hence, only four amino acid substitutions were sufficient to introduce a moonlighting function!

## 5. Moonlighting and gene duplication

The stress many people experience when combining two jobs may also be true for moonlighting proteins [49,50]. For instance, the expression pattern required for one function may not be ideal for the other function. Also, a particular mutation may increase the efficiency of one function, but compromise the efficiency of the other one. In these cases it may be advantageous to distribute the original and moonlighting function over two genes by means of gene duplication.

An example is argininosuccinate lyase, the fourth enzyme of the urea cycle. In ducks and ostriches this enzyme is a moonlighting protein as it also serves as a crystallin in the eye lens [8,51]. In chickens, however, the two functions of argininosuccinate lyase protein are performed by two highly homologous proteins. One of these is the enzymatically inactive form of the enzyme and functions as structural crystallin in the eye lens. The other is the enzymatically active enzyme that functions in the urea cycle. Consequently, unlike in ducks and ostriches, two genes are necessary in chickens to fulfil the function of the original one [8].

Another example is galactokinase in the yeast species *Kluyveromyces lactis* and *S. cerevisiae* [52]. In *K. lactis*, this protein catalyzes the first step of the galactose utilization pathway and in addition acts as a transcriptional activator for itself and the other proteins of the galactose pathway [53,54]. Its function as transcriptional activator is independent of its enzymatic function and vice versa as mutagenesis can inactivate either one without affecting the other. In *S. cerevisiae*

the two functions of *K. lactis* galactokinase are however performed by two different proteins, which are highly homologous [55,56]. *S. cerevisiae* galactokinase however has not completely lost its ability to activate transcription of the galactose pathway proteins [57,58].

## 6. Medical relevance

The complex phenotypes of several disorders, may be related to the involvement of moonlighting proteins [11]. Although there is insufficient evidence to support most of these claims, there are well studied examples of moonlighting proteins that could play a role in disease. Two of these are detailed below.

### 6.1. Dihydrolipoamide dehydrogenase (DLD)

Dihydrolipoamide dehydrogenase (DLD) is a mitochondrial enzyme that is a component of at least five different multienzyme complexes [50]. Due to its participation in these enzyme complexes DLD is critical for energy metabolism and redox balance. Deficiencies in DLD activity are associated with severe disorders in infancy, such as an inability to thrive, hypotonia and metabolic disorders. The severity of symptoms is however very variable and depends on the mutation present in the gene.

Under normal conditions DLD is predominantly present as active homodimers, which are in dynamic equilibrium with the inactive monomer [59]. However, under certain conditions, such as acidification of the mitochondrial matrix, the protein is mainly monomeric, resulting in a loss in DLD enzyme activity. Babady et al. [50] showed that mutations that destabilize the homodimer may have an additional effect, namely an increased ability of DLD to function as a protease due to enhanced exposure of a catalytic dyad at the dimer interface. This moonlighting proteolytic activity is independent of its enzymatic function. A single point mutation in the catalytic dyad, S456A, results in the complete loss of proteolytic activity without affecting enzymatic function. This moonlighting function of DLD may negatively contribute to the metabolic defects seen in some DLD patients.

### 6.2. *Mycobacterium tuberculosis* glutamate racemase (MurI)

The pathogenic bacterium *M. tuberculosis* is the primary cause of tuberculosis in humans [60]. This disease is highly infectious and can be lethal, if left untreated. Ciprofloxacin is a broad-spectrum antibiotic, which can be used to combat *M. tuberculosis* [61]. It stimulates the introduction of double-strand DNA breaks upon binding to a DNA gyrase. Unfortunately, however, a moonlighting function of the *M. tuberculosis* MurI protein counteracts the effects of ciprofloxacin. MurI is a glutamate racemase, which is an essential *M. tuberculosis* enzyme involved in cell wall (peptidoglycan) biosynthesis. It catalyzes the conversion of L-glutamate to D-glutamate [62], a peptidoglycan building block. In several bacterial species, including *M. tuberculosis* [63–65], MurI however can also function as DNA gyrase inhibitor, by reducing binding of gyrase to DNA. This second function is independent of its glutamate racemase enzymatic activity, hence it represents a moonlighting function [65].

Overproduction of MurI, enzymatically active or not, protects *M. tuberculosis* against the action of the ciprofloxacin [65], because MurI inhibits binding of gyrase to DNA and subsequent introduction of cytotoxic double-strand DNA breaks.

Moonlighting proteins are also suggested to play a role in host cell invasion [66,67]. Due to their possible role in disease, enhancing or blocking moonlighting functions could be a target for future drug design. Such an approach would however require more knowledge of moonlighting functions in general and their molecular basis.

## 7. Moonlighting proteins in molecular life sciences

The existence of moonlighting functions is a major challenge in genome annotation. Even well studied proteins might harbor additional functions that have yet to be discovered. The discovery of many moonlighting functions has so far been entirely serendipitous [1,23,35,47,50]. A reason for this is that moonlighting functions are generally difficult to predict, because many functions do not depend on well known, conserved protein motifs [23,50]. Additionally, moonlighting functions are often not conserved [42,47,49]. Examples exist where specific proteins have different moonlighting functions in different species. For instance, aconitase in *S. cerevisiae* has a second function in mitochondrial DNA (mtDNA) maintenance [35], while in mammals [68–70] and in *M. tuberculosis* [71] it is involved in iron homeostasis. Aldolase is essential for the assembly and activity of the vacuolar H<sup>+</sup>-ATPase in yeast [36,72], but its actin binding capabilities are important for host cell invasion in *Plasmodium falciparum* and *Plasmodium vivax* [73–75].

In addition to serendipitous discovery, moonlighting proteins have also been discovered using yeast two-hybrid assays [72] and proteomics [76]. A previous review has also suggested mass spectrometry as a tool for identifying novel moonlighting proteins [77]. A novel strategy to directly search for moonlighting functions in enzymes would be to compare the phenotypes of strains in which the enzyme is inactivated by a point mutation with strains in which the entire gene is deleted. In case of a gene encoding a moonlighting protein there should be a discrepancy between both phenotypes.

An important challenge is to understand how a moonlighting protein can perform such diverse functions. One strategy is to identify the residues which are essential for the moonlighting function. In some cases these residues are relatively easy to find. For instance, the toxic *E. aerogenes* GroEL differs only 11 amino acids with the non-toxic *E. coli* GroEL [47]. In this case it is very evident that (some of) these 11 residues must be important for the moonlighting activity. Although more challenging, it would be interesting if the amino acids crucial for the moonlighting function were also pinpointed in other moonlighting proteins.

## 8. Perspectives

At present it is still difficult to assess how abundant moonlighting proteins are. However, the fast growing number of identified moonlighting functions suggests that it is a general phenomenon in all kingdoms of life. Further research into moonlighting is certainly warranted as it will allow us to better understand the essence of protein function. What allows a protein to perform a particular function?

How do moonlighting functions evolve? Also, moonlighting functions create a whole new level of complexity in the cell. A moonlighting protein may link a metabolic pathway to a signaling pathway in a completely unexpected manner (e.g. very relevant in systems biology approaches). As a result, awareness of moonlighting is very important in many disciplines of life sciences.

Moonlighting is a phenomenon that illustrates nature's ingenuity. It is a source of inspiration that should remind scientists to always keep the unexpected in mind, even on familiar ground.

## Acknowledgements

D.H.E.W.H. is financially supported by the Division of Chemical Sciences of The Netherlands Organisation for Scientific Research (NWO-CW).

## References

- [1] J. Piatigorsky, G.J. Wistow, Enzyme/crystallins: gene sharing as an evolutionary strategy, *Cell* 57 (1989) 197–199.

- [2] W. Hendriks, J.W. Mulders, M.A. Bibby, C. Slingsby, H. Bloemendal, W.W. de Jong, Duck lens epsilon-crystallin and lactate dehydrogenase B4 are identical: a single-copy gene product with two distinct functions, *Proc. Natl. Acad. Sci. U. S. A.* 85 (1988) 7114–7118.
- [3] G.J. Wistow, T. Lietman, L.A. Williams, S.O. Stapel, W.W. de Jong, J. Horwitz, J. Piatigorsky, Tau-crystallin/alpha-enolase: one gene encodes both an enzyme and a lens structural protein, *J. Cell Biol.* 107 (1988) 2729–2736.
- [4] G.J. Wistow, J. Piatigorsky, Lens crystallins: the evolution and expression of proteins for a highly specialized tissue, *Annu. Rev. Biochem.* 57 (1988) 479–504.
- [5] Y. Fujii, K. Watanabe, H. Hayashi, Y. Urade, S. Kuramitsu, H. Kagamiyama, O. Hayaishi, Purification and characterization of rho-crystallin from Japanese common bullfrog lens, *J. Biol. Chem.* 265 (1990) 9914–9923.
- [6] S.I. Tomarev, R.D. Zinovieva, J. Piatigorsky, Characterization of squid crystallin genes. Comparison with mammalian glutathione S-transferase genes, *J. Biol. Chem.* 267 (1992) 8604–8612.
- [7] R.D. Zinovieva, S.I. Tomarev, J. Piatigorsky, Aldehyde dehydrogenase-derived omega-crystallins of squid and octopus. Specialization for lens expression, *J. Biol. Chem.* 268 (1993) 11449–11455.
- [8] J. Piatigorsky, W.E. O'Brien, B.L. Norman, K. Kalumuck, G.J. Wistow, T. Borras, J.M. Nickerson, E.F. Wawrousek, Gene sharing by delta-crystallin and argininosuccinate lyase, *Proc. Natl. Acad. Sci. U. S. A.* 85 (1988) 3479–3483.
- [9] C.J. Jeffery, Moonlighting proteins, *Trends Biochem. Sci.* 24 (1999) 8–11.
- [10] B. Moore, Bifunctional and moonlighting enzymes: lighting the way to regulatory control, *Trends Plant Sci.* 9 (2004) 221–228.
- [11] G. Sriiram, J.A. Martinez, E.R. McCabe, J.C. Liao, K.M. Dipple, Single-gene disorders: what role could moonlighting enzymes play? *Am. J. Hum. Genet.* 76 (2005) 911–924.
- [12] C. Gancedo, C.L. Flores, Moonlighting proteins in yeasts, *Microbiol. Mol. Biol. Rev.* 72 (2008) 197–210 table of contents.
- [13] C.J. Jeffery, Molecular mechanisms for multitasking: recent crystal structures of moonlighting proteins, *Curr. Opin. Struct. Biol.* 14 (2004) 663–668.
- [14] E.S. Arnér, A. Holmgren, Physiological functions of thioredoxin and thioredoxin reductase, *Eur. J. Biochem.* 267 (2000) 6102–6109.
- [15] D.F. Mark, C.C. Richardson, *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase, *Proc. Natl. Acad. Sci. U. S. A.* 73 (1976) 780–784.
- [16] S. Tabor, H.E. Huber, C.C. Richardson, *Escherichia coli* thioredoxin confers processivity on the DNA polymerase activity of the gene 5 protein of bacteriophage T7, *J. Biol. Chem.* 262 (1987) 16212–16223.
- [17] E. Bedford, S. Tabor, C.C. Richardson, The thioredoxin binding domain of bacteriophage T7 DNA polymerase confers processivity on *Escherichia coli* DNA polymerase I, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 479–484.
- [18] S. Adler, P. Modrich, T7-induced DNA polymerase. Requirement for thioredoxin sulfhydryl groups, *J. Biol. Chem.* 258 (1983) 6956–6962.
- [19] H.E. Huber, M. Russel, P. Model, C.C. Richardson, Interaction of mutant thioredoxins of *Escherichia coli* with the gene 5 protein of phage T7. The redox capacity of thioredoxin is not required for stimulation of DNA polymerase activity, *J. Biol. Chem.* 261 (1986) 15006–15012.
- [20] S. Jitrapakdee, M. St Maurice, I. Rayment, W.W. Cleland, J.C. Wallace, P.V. Attwood, Structure, mechanism and regulation of pyruvate carboxylase, *Biochem. J.* 413 (2008) 369–387.
- [21] I.J. van der Klei, H. Yurimoto, Y. Sakai, M. Veenhuis, The significance of peroxisomes in methanol metabolism in methylotrophic yeast, *Biochim. Biophys. Acta* 1763 (2006) 1453–1462.
- [22] P. Ozimek, M. Veenhuis, I.J. van der Klei, Alcohol oxidase: a complex peroxisomal, oligomeric flavoprotein, *FEMS Yeast Res.* 5 (2005) 975–983.
- [23] P. Ozimek, R. van Dijk, K. Latchev, C. Gancedo, D.Y. Wang, I.J. van der Klei, M. Veenhuis, Pyruvate carboxylase is an essential protein in the assembly of yeast peroxisomal oligomeric alcohol oxidase, *Mol. Biol. Cell* 14 (2003) 786–797.
- [24] P.Z. Ozimek, S.H. Klompaker, N. Visser, M. Veenhuis, I.J. van der Klei, The transcarboxylase domain of pyruvate carboxylase is essential for assembly of the peroxisomal flavoenzyme alcohol oxidase, *FEMS Yeast Res.* 7 (2007) 1082–1092.
- [25] D. Spasic, W. Annaert, Building gamma-secretase: the bits and pieces, *J. Cell Sci.* 121 (2008) 413–420.
- [26] M.E. Fortini, Notch signaling: the core pathway and its posttranslational regulation, *Dev. Cell* 16 (2009) 633–647.
- [27] K.T. Jacobsen, K. Iverfeldt, Amyloid precursor protein and its homologues: a family of proteolysis-dependent receptors, *Cell. Mol. Life Sci.* 66 (2009) 2299–2318.
- [28] A. Khandelwal, D. Chandu, C.M. Roe, R. Kopan, R.S. Quatran, Moonlighting activity of presenilin in plants is independent of gamma-secretase and evolutionarily conserved, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 13337–13342.
- [29] Y.P. Ow, D.R. Green, Z. Hao, T.W. Mak, Cytochrome c: functions beyond respiration, *Nat. Rev. Mol. Cell Biol.* 9 (2008) 532–542.
- [30] Z. Hao, G.S. Duncan, C.C. Chang, A. Elia, M. Fang, A. Wakeham, H. Okada, T. Calzascia, Y. Jang, A. You-Ten, W.C. Yeh, P. Ohashi, X. Wang, T.W. Mak, Specific ablation of the apoptotic functions of cytochrome C reveals a differential requirement for cytochrome C and Apaf-1 in apoptosis, *Cell* 121 (2005) 579–591.
- [31] R.M. Kluck, L.M. Ellerby, H.M. Ellerby, S. Naiem, M.P. Yaffe, E. Margoliash, D. Bredesen, A.G. Mauk, F. Sherman, D.D. Newmeyer, Determinants of cytochrome c pro-apoptotic activity. The role of lysine 72 trimethylation, *J. Biol. Chem.* 275 (2000) 16127–16133.
- [32] M.G. Myers Jr., Cell biology. Moonlighting in mitochondria, *Science* 323 (2009) 723–724.
- [33] C. Schindler, D.E. Levy, T. Decker, JAK-STAT signaling: from interferons to cytokines, *J. Biol. Chem.* 282 (2007) 20059–20063.
- [34] J. Wegrzyn, R. Potla, Y.J. Chwae, N.B. Sepuri, Q. Zhang, T. Koeck, M. Derecka, K. Szczepanek, M. Szlag, A. Gornicka, A. Moh, S. Moghaddas, Q. Chen, S. Bobbili, J. Cichy, J. Dulak, D.P. Baker, A. Wolfman, D. Stuehr, M.O. Hassan, X.Y. Fu, N. Avadhani, J.I. Drake, P. Fawcett, E.J. Lesnefsky, A.C. Larner, Function of mitochondrial Stat3 in cellular respiration, *Science* 323 (2009) 793–797.
- [35] X.J. Chen, X. Wang, B.A. Kaufman, R.A. Butow, Aconitase couples metabolic regulation to mitochondrial DNA maintenance, *Science* 307 (2005) 714–717.
- [36] M. Lu, D. Ammar, H. Ives, F. Albrecht, S.L. Gluck, Physical interaction between aldolase and vacuolar H<sup>+</sup>-ATPase is essential for the assembly and activity of the proton pump, *J. Biol. Chem.* 282 (2007) 24495–24503.
- [37] W. Yuan, D.L. Tuttle, Y.J. Shi, G.S. Ralph, W.A. Dunn Jr., Glucose-induced microautophagy in *Pichia pastoris* requires the alpha-subunit of phosphofructokinase, *J. Cell Sci.* 110 (Pt 16) (1997) 1935–1945.
- [38] B.L. Decker, W.T. Wickner, Enolase activates homotypic vacuole fusion and protein transport to the vacuole in yeast, *J. Biol. Chem.* 281 (2006) 14523–14528.
- [39] J.W. Kim, C.V. Dang, Multifaceted roles of glycolytic enzymes, *Trends Biochem. Sci.* 30 (2005) 142–150.
- [40] L.A. Fothergill-Gilmore, P.A. Michels, Evolution of glycolysis, *Prog. Biophys. Mol. Biol.* 59 (1993) 105–235.
- [41] H.V. Baker, GCR1 of *Saccharomyces cerevisiae* encodes a DNA binding protein whose binding is abolished by mutations in the CTCC sequence motif, *Proc. Natl. Acad. Sci. U. S. A.* 88 (1991) 9443–9447.
- [42] P. Ozimek, P. Kotter, M. Veenhuis, I.J. van der Klei, *Hansenula polymorpha* and *Saccharomyces cerevisiae* Pex5p's recognize different, independent peroxisomal targeting signals in alcohol oxidase, *FEBS Lett.* 580 (2006) 46–50.
- [43] H.R. Waterham, K.A. Russell, Y. Vries, J.M. Cregg, Peroxisomal targeting, import, and assembly of alcohol oxidase in *Pichia pastoris*, *J. Cell Biol.* 139 (1997) 1419–1431.
- [44] T.R. Gregory, Synergy between sequence and size in large-scale genomics, *Nat. Rev. Genet.* 6 (2005) 699–708.
- [45] F. Jacob, Evolution and tinkering, *Science* 196 (1977) 1161–1166.
- [46] A. Aharoni, L. Gaidukov, O. Khersonsky, Q.G.S. Mc, C. Roodveldt, D.S. Tawfik, The 'evolvability' of promiscuous protein functions, *Nat. Genet.* 37 (2005) 73–76.
- [47] N. Yoshida, K. Oeda, E. Watanabe, T. Mikami, Y. Fukita, K. Nishimura, K. Komai, K. Matsuda, Protein function. Chaperonin turned insect toxin, *Nature* 411 (2001) 44.
- [48] K. Braig, Z. Otwinowski, R. Hegde, D.C. Boisvert, A. Joachimiak, A.L. Horwich, P.B. Sigler, The crystal structure of the bacterial chaperonin GroEL at 2.8 Å, *Nature* 371 (1994) 578–586.
- [49] B. Zhao, L. Lei, D.G. Vassilyev, X. Lin, D.E. Cane, S.L. Kelly, H. Yuan, D.C. Lamb, M.R. Waterman, Crystal structure of albaflavone monooxygenase containing a moonlighting terpene synthase active site, *J. Biol. Chem.* 284 (2009) 36711–36719.
- [50] N.E. Babady, Y.P. Pang, O. Elpeleg, G. Isaya, Cryptic proteolytic activity of dihydrolipoamide dehydrogenase, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 6158–6163.
- [51] S.H. Chiou, C.H. Lo, C.Y. Chang, T. Itoh, H. Kaji, T. Samejima, Ostrich crystallins. Structural characterization of delta-crystallin with enzymic activity, *Biochem. J.* 273 (Pt 2) (1991) 295–300.
- [52] M. Rubio-Texeira, A comparative analysis of the GAL genetic switch between not-so-distant cousins: *Saccharomyces cerevisiae* versus *Kluyveromyces lactis*, *FEMS Yeast Res.* 5 (2005) 1115–1128.
- [53] J. Meyer, A. Walker-Jonah, C.P. Hollenberg, Galactokinase encoded by GAL1 is a bifunctional protein required for induction of the GAL genes in *Kluyveromyces lactis* and is able to suppress the gal3 phenotype in *Saccharomyces cerevisiae*, *Mol. Cell Biol.* 11 (1991) 5454–5461.
- [54] C.T. Hittinger, S.B. Carroll, Gene duplication and the adaptive evolution of a classic genetic switch, *Nature* 449 (2007) 677–681.
- [55] D. Lohr, P. Venkov, J. Zlatanova, Transcriptional regulation in the yeast GAL gene family: a complex genetic network, *FASEB J.* 9 (1995) 777–787.
- [56] A. Platt, H.C. Ross, S. Hankin, R.J. Reece, The insertion of two amino acids into a transcriptional inducer converts it into a galactokinase, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 3154–3159.
- [57] P.J. Bhat, J.E. Hopper, Overproduction of the GAL1 or GAL3 protein causes galactose-independent activation of the GAL4 protein: evidence for a new model of induction for the yeast GAL/MEL regulon, *Mol. Cell Biol.* 12 (1992) 2701–2707.
- [58] C.A. Sellick, T.A. Jowitt, R.J. Reece, The effect of ligand binding on the galactokinase activity of yeast Gal1p and its ability to activate transcription, *J. Biol. Chem.* 284 (2009) 229–236.
- [59] N.L. Klyachko, V.A. Shchedrina, A.V. Efimov, S.V. Kazakov, I.G. Gazaryan, B.S. Kristal, A.M. Brown, pH-dependent substrate preference of pig heart lipoamide dehydrogenase varies with oligomeric state: response to mitochondrial matrix acidification, *J. Biol. Chem.* 280 (2005) 16106–16114.
- [60] D.B. Young, M.D. Perkins, K. Duncan, C.E. Barry III, Confronting the scientific obstacles to global control of tuberculosis, *J. Clin. Invest.* 118 (2008) 1255–1265.
- [61] K. Drlita, X. Zhao, DNA gyrase, topoisomerase IV, and the 4-quinolones, *Microbiol. Mol. Biol. Rev.* 61 (1997) 377–392.
- [62] T. Lundqvist, S.L. Fisher, G. Kern, R.H. Folmer, Y. Xue, D.T. Newton, T.A. Keating, R.A. Alm, B.L. de Jonge, Exploitation of structural and regulatory diversity in glutamate racemases, *Nature* 447 (2007) 817–822.
- [63] M. Ashiuchi, E. Kuwana, K. Komatsu, K. Soda, H. Misono, Differences in effects on DNA gyrase activity between two glutamate racemases of *Bacillus subtilis*, the poly-gamma-glutamate synthesis-linking Glr enzyme and the YrpC (Murl) isozyme, *FEMS Microbiol. Lett.* 223 (2003) 221–225.
- [64] S. Sengupta, M. Shah, V. Nagaraja, Glutamate racemase from *Mycobacterium tuberculosis* inhibits DNA gyrase by affecting its DNA-binding, *Nucleic Acids Res.* 34 (2006) 5567–5576.
- [65] S. Sengupta, S. Ghosh, V. Nagaraja, Moonlighting function of glutamate racemase from *Mycobacterium tuberculosis*: racemization and DNA gyrase inhibition are two independent activities of the enzyme, *Microbiology* 154 (2008) 2796–2803.

- [66] M. Podobnik, R. Tyagi, N. Matange, U. Dermol, A.K. Gupta, R. Mattoo, K. Seshadri, S. S. Visweswariah, A mycobacterial cyclic AMP phosphodiesterase that moonlights as a modifier of cell wall permeability, *J. Biol. Chem.* 284 (2009) 32846–32857.
- [67] V. Pancholi, V.A. Fischetti, alpha-enolase, a novel strong plasmin(ogen) binding protein on the surface of pathogenic streptococci, *J. Biol. Chem.* 273 (1998) 14503–14515.
- [68] W.E. Walden, A.I. Selezneva, J. Dupuy, A. Volbeda, J.C. Fontecilla-Camps, E.C. Theil, K. Volz, Structure of dual function iron regulatory protein 1 complexed with ferritin IRE-RNA, *Science* 314 (2006) 1903–1908.
- [69] C.C. Philpott, R.D. Klausner, T.A. Rouault, The bifunctional iron-responsive element binding protein/cytosolic aconitase: the role of active-site residues in ligand binding and regulation, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 7321–7325.
- [70] M.C. Kennedy, L. Mende-Mueller, G.A. Blondin, H. Beinert, Purification and characterization of cytosolic aconitase from beef liver and its relationship to the iron-responsive element binding protein, *Proc. Natl. Acad. Sci. U. S. A.* 89 (1992) 11730–11734.
- [71] S. Banerjee, A.K. Nandyala, P. Raviprasad, N. Ahmed, S.E. Hasnain, Iron-dependent RNA-binding activity of *Mycobacterium tuberculosis* aconitase, *J. Bacteriol.* 189 (2007) 4046–4052.
- [72] M. Lu, L.S. Holliday, L. Zhang, W.A. Dunn Jr., S.L. Gluck, Interaction between aldolase and vacuolar H<sup>+</sup>-ATPase: evidence for direct coupling of glycolysis to the ATP-hydrolyzing proton pump, *J. Biol. Chem.* 276 (2001) 30407–30413.
- [73] J. Bosch, C.A. Buscaglia, B. Krumm, B.P. Ingason, R. Lucas, C. Roach, T. Cardozo, V. Nussenzweig, W.G. Hol, Aldolase provides an unusual binding site for thrombospondin-related anonymous protein in the invasion machinery of the malaria parasite, *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 7015–7020.
- [74] C.A. Buscaglia, I. Coppens, W.G. Hol, V. Nussenzweig, Sites of interaction between aldolase and thrombospondin-related anonymous protein in plasmodium, *Mol. Biol. Cell* 14 (2003) 4947–4957.
- [75] J. Wang, A.J. Morris, D.R. Tolan, L. Pagliaro, The molecular nature of the F-actin binding activity of aldolase revealed with site-directed mutants, *J. Biol. Chem.* 271 (1996) 6861–6865.
- [76] D.A. Hall, H. Zhu, X. Zhu, T. Royce, M. Gerstein, M. Snyder, Regulation of gene expression by a metabolic enzyme, *Science* 306 (2004) 482–484.
- [77] C.J. Jeffery, Mass spectrometry and the search for moonlighting proteins, *Mass Spectrom. Rev.* 24 (2005) 772–782.
- [78] C.J. Jeffery, Moonlighting proteins—an update, *Mol. Biosyst.* 5 (2009) 345–350.
- [79] A. Bhoumik, S. Takahashi, W. Breitweiser, Y. Shiloh, N. Jones, Z. Ronai, ATM-dependent phosphorylation of ATF2 is required for the DNA damage response, *Mol. Cell* 18 (2005) 577–587.
- [80] S. Hu, Z. Xie, A. Onishi, X. Yu, L. Jiang, J. Lin, H.S. Rho, C. Woodard, H. Wang, J.S. Jeong, S. Long, X. He, H. Wade, S. Blackshaw, J. Qian, H. Zhu, Profiling the human protein–DNA interactome reveals ERK2 as a transcriptional repressor of interferon signaling, *Cell* 139 (2009) 610–622.
- [81] U. Irion, D. St Johnston, bicoid RNA localization requires specific binding of an endosomal sorting complex, *Nature* 445 (2007) 554–558.
- [82] B. Moore, L. Zhou, F. Rolland, Q. Hall, W.H. Cheng, Y.X. Liu, I. Hwang, T. Jones, J. Sheen, Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling, *Science* 300 (2003) 332–336.
- [83] N. Entelis, I. Brandina, P. Kamenski, I.A. Krasheninnikov, R.P. Martin, I. Tarassov, A glycolytic enzyme, enolase, is recruited as a cofactor of tRNA targeting toward mitochondria in *Saccharomyces cerevisiae*, *Genes Dev.* 20 (2006) 1609–1620.
- [84] A. Ruiz, A. Gonzalez, I. Munoz, R. Serrano, J.A. Abrie, E. Strauss, J. Arino, Moonlighting proteins Hal3 and Vhs3 form a heteromeric PP2C with Ykl088w in yeast CoA biosynthesis, *Nat. Chem. Biol.* 5 (2009) 920–928.
- [85] B.A. Kaufman, J.E. Kolesar, P.S. Perlman, R.A. Butow, A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in *Saccharomyces cerevisiae*, *J. Cell Biol.* 163 (2003) 457–461.