Moonlighting proteins—an update

Constance J. Jeffery*

Received 12th January 2009, Accepted 13th January 2009
First published as an Advance Article on the web 3rd February 2009
DOI: 10.1039/b900658n

A growing number of diverse proteins are being identified that moonlight. Moonlighting proteins comprise an interesting subset of multifunctional proteins in which the two functions are found in a single polypeptide chain. They do not include proteins that are multifunctional due to gene fusions, families of homologous proteins, splice variants, or promiscuous enzyme activities. This review summarizes recent discoveries that add to the list of known moonlighting proteins. They include several different kinds of proteins and combinations of functions. In one case, a novel DNA binding function was found for a biosynthetic enzyme through a proteomics microarray project. The review also summarizes recent X-ray crystal structures that provide clues to the molecular mechanisms of one or both functions, and in some cases how a protein can switch between functions. In addition, the possibility that many proteins with intrinsically unstructured regions might also moonlight is discussed.

Introduction

In recent years the idea of one gene–one protein–one function has been superseded by the knowledge that many proteins have multiple functions. One intriguing type of multifunctional proteins is moonlighting proteins. Moonlighting proteins have two different functions within a single polypeptide chain.[1] This class of multifunctional proteins does not include proteins where the two functions are the result of gene fusions, families of homologous proteins, splice variants, or promiscuous enzyme activities. They also do not include proteins that have the same function in different cell types or subcellular locations. Some of the first proteins that were found to moonlight were crystallins, which are found in the lens of the eye. The crystallins in several mammals, geckos, birds, and other species are the same proteins as several cytosolic enzymes.[2,3] Other moonlighting proteins have been found to be involved in cellular functions and biochemical pathways important in cancer and other diseases, as well as in basic physiological functions such as cell motility, angiogenesis, transmembrane transport, DNA synthesis or repair, chromatin and cytoskeleton structure, and protein, amino acid, sugar, or lipid metabolism (reviewed in ref. 1 and 4–6). Knowing more about these proteins could help in understanding which types of additional proteins might also have a second function, which would be useful in determining the function(s) of the thousands of proteins identified through the human genome project and other genome projects. It could also be important in selecting a target protein for drug development; for example, in some cases it would be important to only inhibit one function of a moonlighting protein because loss of the second function could lead to toxicity. This review will describe several additional proteins that were recently found to moonlight and new X-ray crystal structures of moonlighting proteins that have helped in elucidating the molecular mechanisms for one or both functions and, in some cases, how the protein switches between functions (Table 1).

Laboratory for Molecular Biology, Department of Biological Sciences, MC567, University of Illinois, 900 S. Ashland Ave., Chicago, IL 60607, USA. E-mail: cjeffery@uic.edu; Fax: +1 312 996 3168; Tel: +1 312 413 2691

Constance Jeffery

Constance Jeffery obtained her BS degree at the Massachusetts Institute of Technology and her PhD degree at the University of California at Berkeley, where she studied the E. coli *aspartate receptor. She did postdoctoral research at Brandeis University, where she learned X-ray crystallography and coined the term "moonlighting proteins." She joined the faculty of the University of Illinois at Chicago in 1999. She continues to work on the structure and function of moonlighting proteins and transmembrane proteins. A current research focus is multidrug resistance transmembrane transporters.*

New examples of moonlighting proteins have been found

In the past few years, several labs have reported new examples of moonlighting proteins, and an additional function for a protein already known to moonlight. For all of these proteins, there is biochemical, mutagenic, or other evidence to support the presence of at least two separate functions. Moonlighting proteins are a diverse set of proteins that includes several enzymes, chaperones, transcription factors, and proteins with many other types of functions (reviewed in ref. 1 and 4–6).

Table 1 Examples of moonlighting proteins

Name	One function	Second function	Reference
ARGONAUTE4	Directing chromatin remodeling factors to a target locus	Cleaving RNA transcripts to produce siRNAs	7
Ubp6	Deubiquinating cysteine protease	Delays the degradation of ubiquinated proteins by the proteasome	8
Tuf	Translation elongation factor	Receptor for host proteins	9
hRoDH-E2	Dehydrogenase/reductase	Transcriptional repressor	10
La protein	Protect RNA from 3′-end digestion	RNA folding chaperone	11
cPrxI	Peroxidase	Chaperone	12
FdGOGAT	Glutamate synthase	Subunit of UDP-sulfoquinovose synthase	13
Amelogenin	Regulates the size and shape of mineral crystallites	Unknown	14
Mitochondrial aconitase	Enzyme in the citric acid cycle	mtDNA maintenance	15
Enolase	Enzyme in glycolysis	Assists mitochondrial import of tRNAsLys	16
Arg5,6	Reductase/kinase	DNA binding	17
Gpx4	Peroxidase	Formation of the mitochondrial capsule during sperm maturation	18,19
U5-52K or CD2BP2	Protein in the spliceosome	Binds CD2 receptor	20
Pth2/Bit1	Peptidyl-tRNA hydrolase 2	Inhibitor of transcription	21
Cytoplasmic aconitase/IRP1	Aconitase	mRNA binding protein	22,23
I-TevI endonuclease	Homing endonuclease	Transcriptional autorepressor	24,25

Different methods have been reported for a moonlighting protein to switch between functions, including secretion into the extracellular space, interactions with DNA or RNA, a change in temperature, a change in the redox state of the cell, a change in oligomeric state of the protein, interactions with different polypeptide chains in different protein complexes, interaction with a membrane, or changes in the cellular concentration of a ligand, substrate, cofactor, or product. These methods are not mutually exclusive since a combination of factors might be employed. Of the new examples, some proteins make use of the general methods seen in previously identified moonlighting proteins to switch between functions, although the details differ, for example how interacting with a different protein partner or cofactor results in a conformational change that then affects function. For some of the new examples it is not yet known what methods are used to switch between functions. In general, the moonlighting proteins described to date include many enzymes, and a few proteins that have a function involving transcription, but overall they are a very diverse group of proteins, and there does not seem to be one overlying factor common to many of the moonlighting proteins described here or previously.

ARGONAUTE4 (AGO4) performs two separate functions in RNA-directed DNA methylation (RdDM).[7] It is an enzyme that cleaves target RNA transcripts to produce siRNAs, and it has a second, non-catalytic function of directing chromatin remodeling factors to a target locus. The separation of the two functions is supported by the observation that some mutations of the catalytic residues result in loss of RNA cleavage activity without loss of the non-catalytic function of the protein.

Ubp6 is a deubiquinating cysteine protease enzyme associated with recycling ubiquitin at the yeast proteasome. It promotes progressive removal of ubiquitin from target proteins. Hanna and coworkers recently demonstrated that it also delays the degradation of ubiquinated proteins by the proteasome.[8] This new activity does not require its enzymatic activity as demonstrated by non-equivalence of null and catalytic point mutations.

Translation elongation factor tuf plays a newly found role in host immune system evasion and tissue invasion in the bacterial pathogen *Pseudomonas aeruginosa*.[9] To perform its function as a translation elongation factor, tuf is located inside the cell, but it is also found on the surface of the bacterial cell. It was recently found that its role on the cell surface is to serve as a receptor for two host plasma proteins, factor H and plasminogen, that are exploited by the bacterium to evade the host's immune system and invade tissues, respectively. Factor H is a cofactor of plasma serine protease factor 1 which is needed for degradation of complement protein C3b. Therefore factor H has a role in preventing induction of an important host response, complement activation, that would help eliminate the invading pathogen. Upon binding to tuf, plasminogen is activated to proteolytically active plasmin. Plasmin then helps degrade the host's extracellular matrix to aid in invasion of host tissues and dissemination of the pathogen.

hRoDH-E2 is a retinoid and hydroxy-steroid dehydrogenase/reductase that uses NADP as a co-substrate. Recent studies indicate it also serves in the nucleus as a transcriptional repressor of promoter activity.[10] It is recruited to a protein complex on the profilaggrin promoter where it affects the function of the DNA-bound Sp1 transcription activator protein. It is not clear if there is a direct interaction between Sp1 and hRoDH-E2, but hRoDH-E2 does not bind directly to DNA. hRoDH-E2 downregulates promoter activity by affecting the amount of acetylation and deacetylation of histones near transcription initiation sites, possibly by affecting recruitment and/or activity of deacetylases and acetylases. The role in transcriptional repression involves protein–protein interactions instead of its catalytic activity, since enzymes with mutations in the active site were still able to repress profilaggrin promoter activity.

The *La protein* had previously been found to perform different activities in tRNA maturation. Huang and coworkers used mutagenesis and activity assays to demonstrate that two different protein surfaces of the La protein were found to

perform these different functions.[11] The helical La motif domain binds to the 3′ UUU-OH of RNA to protect the RNA from 3′-end digestion. Another domain, RRM1, with helices and a beta-sheet, is also needed in a second function as a chaperone to assist in RNA folding.

Yeast cytoplasmic peroxiredoxin I (cPrxI) was found to switch functions with a change in quaternary structure.[12] In response to oxidative stress or an increase in temperature, cPrx reversibly converts from a low molecular weight peroxidase to a higher molecular weight chaperone. The chaperone activity could be important for inhibiting thermal-induced aggregation of other proteins. Jang and coworkers demonstrated that Cys47 is needed for peroxidase activity, but a mutant lacking Cys47 could still confers cells with heat shock resistance.[12]

Ferredoxin-dependent glutamate synthase (FdGOGAT) is another enzyme that was recently found to be adopted into a multiprotein complex where it is likely to have a moonlighting function. In spinach chloroplasts, FdGOGAT is found to be a subunit of UDP-sulfoquinovose synthase (SQD1).[13] SQD1 catalyzes the transfer of sulfite to UDP-glucose in the biosynthesis of the sulfolipid sulfoquinovosyldiacylglycerol. FdGOGAT might have been recruited for this moonlighting function in spinach chloroplasts because its flavin mononucleotide (FMN) cofactor binds sulfite and can possibly be a source of sulfite for the SQD1 enzyme.

Amelogenin is a protein found in mineralizing tooth enamel that was recently found to be expressed in brain and other soft tissues.[14] Normally, finding a protein in a novel location is not sufficient evidence to determine that it is a moonlighting protein. Many proteins perform the same function in more than one cell type, so in most cases evidence of two different functions (*i.e.* multiple catalytic activities, mutagenesis studies demonstrating loss of one but not the other function, *etc.*) are needed to show the protein has two distinct functions. However, in the case of amelogenin, the first known function of the protein and its proposed mechanism are very unusual and it is not clear how it could be performing the same function in soft tissues. Amelogenin regulates the size and shape of the mineral crystallites in mineralizing tooth enamel, apparently by self-assembling into nanosphere-like structures. There appears to be no need for this function in brain and other soft tissues, so it is very likely that amelogenin has a moonlighting function in those tissues.

An additional function has been found for mitochondrial aconitase (Aco1p), an enzyme in the citric acid cycle.[15] Chen and coworkers found Aco1p in the nucleoid, a protein–DNA complex that contains mitochondrial DNA and is essential for mtDNA maintenance under certain growth conditions. They demonstrated that mutations in a cysteine that coordinates the iron–sulfur cluster (4Fe–4S) cofactor required for catalytic function did not affect the enzyme's newly found function. Therefore, its catalytic activity is not required for this second function. The authors proposed that using aconitase in this manner may help the cell coordinate metabolic signals and mtDNA maintenance, and that perhaps mtDNA nucleoids have multiple states that depend on the metabolic condition of the cells. They suggested that aconitase might help coordinate metabolic signals and mtDNA maintenance in derepressed cells and another protein, Abf2p, might be important for mtDNA maintenance in glucose repressed cells.

Another moonlighting function has been found for enolase, a glycolytic enzyme that has already been implicated in several other roles. Enolase catalyzes the dehydration of 2-phosphoglycerate to phosphoenolpyruvate. Entelis and coworkers found that it also has a role in assisting import of tRNAsLys into the mitochondria by aiding in the formation of a preMsk1p–tRNA complex.[16] Interestingly, it appears that enolase can help in formation of the complex without becoming part of the complex. The authors propose that enolase might serve as an RNA folding chaperone by helping the tRNA to obtain the correct conformation to interact with the protein or to stabilize the RNA conformation. Some mutations that affect enolase catalytic function do not affect this second function.

Proteomics has been used to identify a moonlighting function for a metabolic enzyme

Proteomics is the use of large-scale biochemical, genetic, or computational methods to identify, characterize, and determine the functions of thousands of proteins simultaneously.

Specific goals often include identifying the locations and times of gene expression or the interactions of proteins with other molecules. This information can provide valuable clues to protein function. The presence of moonlighting proteins can complicate analysis of the results from proteomics projects because the same protein might have, for example, confusing patterns of expression or multiple unrelated binding partners. At the same time, these large-scale proteomics approaches have great potential for suggesting many other proteins that moonlight (reviewed in ref. 26 and 27). Finding a protein in an unexpected cell type or a novel multiprotein complex can suggest that that protein might have a second function, and many of the proteomics techniques do not require prior knowledge of protein function.

Hall and coworkers recently discovered a moonlighting function of a metabolic enzyme using a yeast proteome microarray.[17] They screened the microarray with labeled DNA probes to look for previously unknown DNA binding activities. Arg5,6(*N*-acetyl gamma-glutamyl phosphate reductase/acetylglutamate kinase), a mitochondrial enzyme catalyzing two steps in the biosynthesis of ornithine, a precursor of arginine, was identified. Chromatin immunoprecipitation and binding studies confirmed that Arg5,6 binds to specific DNA fragments *in vivo*. Deletion of Arg5,6 results in altered transcript levels of nuclear and mitochondrial target genes.

It is important to note that Hall and coworkers performed a variety of studies to demonstrate that the protein has the second function, because not every protein found by proteomics methods to be in multiple locations or binding to multiple partners is moonlighting. Additional experiments are generally needed to determine if an unusual location or novel binding partner is actually due to a moonlighting function and is not just a false positive in a proteomics assay.

New X-ray crystal structures of moonlighting proteins have become available

High resolution structures of moonlighting proteins have provided valuable information about one or both molecular

mechanisms and in some cases how a protein switches between functions (reviewed in ref. 28). Overall, though, of the thousands of proteins in the Protein Data Bank, there are relatively few structures for proteins that have been shown to moonlight. In some cases there are structures in the PDB for homologues or orthologues of moonlighting proteins, but homologues or orthologues don't always possess both functions. For example, the duck delta1 and delta2 crystallins share 94% amino acid sequence identity and similar three-dimensional structures.[29] Only the delta2 isoform is argininosuccinate lyase, which is also found in non-lens tissues and catalyzes the breakdown of argininosuccinate to arginine and fumarate.[30] Recently, several new structures of moonlighting proteins have been solved and added to the information about how these proteins function.

Gpx4 phospholipid hydroperoxide glutationone peroxidase is an enzyme that has a second function involving protein–protein interactions; when it is a polymer it is involved in formation of the mitochondrial capsule during sperm maturation. A recent X-ray crystal structure solved at high resolution (1.55 Å) aided in identifying the catalytic triad in the active site pocket that is needed for the enzymatic function as well as possible surface amino acids that are involved in polymerization.[18] The crystal structure was complemented by mutagenesis experiments that helped confirm the identification of the catalytic triad and also provided information about which three surface Cys might be involved in polymerization.

The *52K Protein (U5-52K* or *CD2BP2) of the 20S U5 snRNP in the spliceosome* is an example of a protein with two different binding sites for two different proteins in different functions. It interacts with the 20S U5 U5-15K protein in the spliceosome. It is also a binding protein for the transmembrane CD2 receptor in the immune response. A previously reported crystal structure of the GYF domain of the U5-52K protein in complex with the cytoplasmic tail of the CD2 receptor showed that U5-52K binds to a proline-rich motif on the CD2 receptor.[19] This interaction involves a deep cleft in the GYF domain (Fig. 1A). A new X-ray crystal structure of a complex of the U5-52K GYF domain with the U5-15K protein shows the U5-15K protein binds at different site on the U5-52K GYF domain than the site used for binding the CD2 receptor (Fig. 1B).[20]

Peptidyl-tRNA hydrolase 2/Bcl-2 inhibitor of transcription 1 (*Pth2*/*Bit1*) releases tRNA from covalent peptidyl–tRNA complexes that accumulate due to premature dissociation of mRNA templates during translation. It also plays a role in apoptosis through a protein–protein interaction with a transcriptional co-repressor. De Pereda and coworkers solved an X-ray crystal structure of the catalytic domain (amino acids 63–179) of human Pth2/Bit1 at 2.0 Å resolution.[21] The crystal structure with sequence alignments was valuable in predicting amino acids that might be involved in catalysis.

Two recent X-ray crystal structures of *cytoplasmic aconitase*/*iron regulatory protein* 1 (IRP1) have aided in the understanding of its mechanisms and how it switches between functions. Aconitase uses an iron–sulfur [4Fe–4S] cluster to catalyze the conversion of citrate to isocitrate. A change in the availability of iron in the cell can cause assembly or disassembly of the iron–sulfur cluster. Without the iron–sulfur

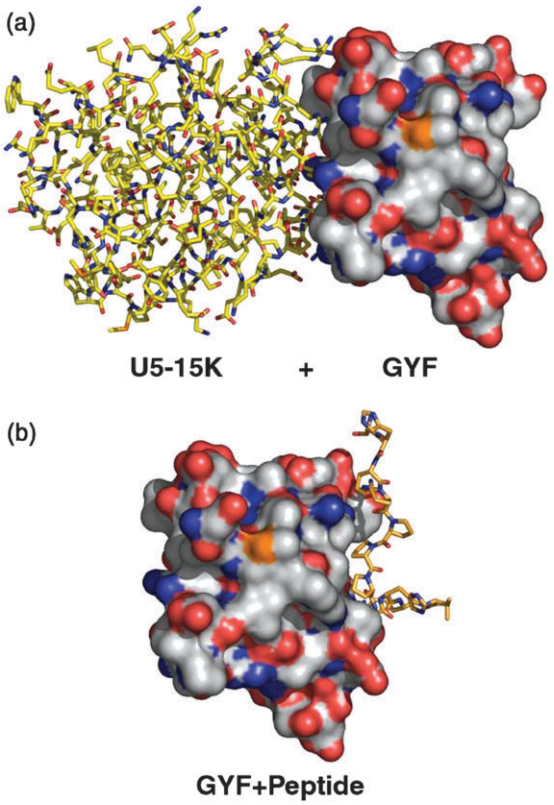

Fig. 1 The GYF domain has two separate binding sites for the U5-15K protein and the polyproline motif of the CD2 receptor. Crystal structures are shown as a surface representation of the GYF domain in complex with (a) the UF-15K protein or (b) a proline-rich peptide (reproduced with permission from ref. 20).

cluster, aconitase/IRP1 is an mRNA binding protein with roles in translation and mRNA degradation. It binds iron-responsive elements (IREs), specific sequences containing a stem-loop in 5′ or 3′ untranslated regions of mRNAs, and controls the translation of proteins involved in uptake, storage, and utilization of iron by affecting the binding of ribosomes or nucleases. For example, when there is a need for more iron uptake aconitase/IRP1 binding blocks translation of ferritin mRNA and stabilizes mRNA for the transferrin receptor.

The X-ray crystal structure of cytosolic aconitase as an enzyme was solved by Dupuy and coworkers.[22] Unfortunately, clear electron density for a bound substrate (or other ligand) was not seen. Walden and coworkers solved an X-ray crystal structure of aconitase in complex with the ferritin H IRE,[23] at 2.8 Å resolution. In the structure with RNA the protein is in a much more open conformation than in the structure containing the iron–sulfur complex. The protein interacts with the IRE stem–loop on two surfaces located 30 Å apart. The protein undergoes large conformational changes to switch between functions (Fig. 2). The conformational changes consist mainly of rigid body motions relative to a core. Domain interfaces become surfaces that interact with the mRNA, and there is some movement of amino acids in the core. The regions of the enzyme that form the active site and the mRNA binding regions overlap, with many amino acid residues playing key roles in both functions. In fact, the authors noted that many of the amino acids that bind RNA

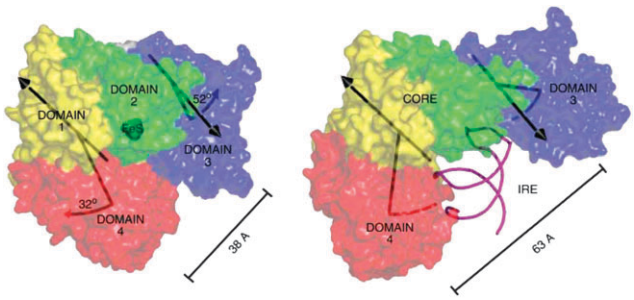

Fig. 2 A large conformational change occurs in c-aconitase/IRP1 when it switches from its enzymatic function to its RNA binding function. Domains 1 and 2 form a core containing the [4e–4S] cluster. After loss of the [4e–4S] cluster, domains 3 and 4 move outward to accommodate the much larger IRE-RNA. In this figure, arcs are included to show the direction of movement of the centers of mass of domains 3 and 4 (reprinted with permission from ref. 23).

are conserved even among c-aconitases that do not bind mRNA and suggested that the amino acids that enable the protein to be so flexible may be a key determinant of the ability of the protein to bind RNA.

Both a new function and a new X-ray crystal structure were determined for an intron-encoded homing endonuclease, *I-TevI endonuclease*. This protein is a homing endonuclease that is encoded within the group 1 *td* intron of phage T4 and binds to and cleaves a specific DNA sequence in a target allele. Edgell and coworkers found that the enzyme also binds to a different DNA sequence as a transcriptional autorepressor.[24] It binds an operator site upstream of its own coding sequence within the intron so that it can repress transcription of RNA and thereby prevent making more copies of the protein. Edgell and co-workers demonstrated that the enzyme binds the homing site and the operator with equal affinity, but they observed 100-fold less cleavage of the operator DNA sequence. They also solved a 2.5 Å resolution X-ray crystal structure of I-TevI in complex with 20-base pairs of operator site duplex DNA from the T4 phage late promoter. Comparison of the new structure with a previously reported structure of the protein in complex with an oligonucleotide containing the endonuclease site showed that the protein's interactions with the two DNA sites are similar, but use different hydrogen bonding patterns.[25] Their model involves multiple domains of the protein, and the flexibility of function of the catalytic site is dependent on which other domains in the protein interact with DNA (Fig. 3). One end of the protein binds the same DNA sequence in both functions, but if the catalytic domain does not find the preferred cleavage sequence at the preferred distance, the catalytic domain binds to a closer site and the middle domain changes its interaction with DNA. In this conformation the protein has less catalytic ability. This ability to bind multiple sequences enables the endonuclease to have multiple functions, and the conformation of the overall protein helps determine which function is used.

Some moonlighting proteins are intrinsically unstructured proteins (IUPs)

Tompa and coworkers have noted that some other moonlighting proteins might be proteins that lack a well-defined

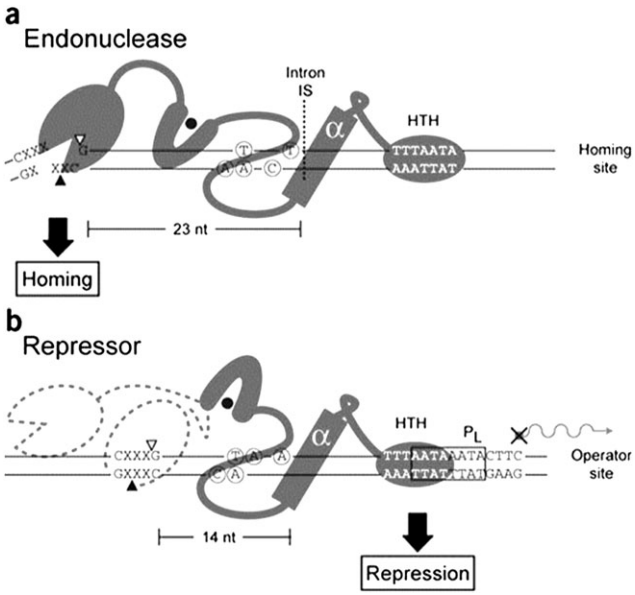

Fig. 3 I-TevI binds to DNA in different conformations as an endonuclease or as a repressor. In both functions, one end of I-TevI binds to a TTTAATA sequence, but the interactions between the central extended domain and DNA vary depending on whether the catalytic domain locates homing site sequence or not. When I-TevI binds to its homing site DNA (a), the catalytic domain finds the CXXXG sequence at the optimal distance of 23 and 25 nucleotides and is able to cleave it. When I-TevI binds to operator site DNA (b) the catalytic domain finds the CXXXG sequence 9 bp closer, which results in a different interaction of the central domain with the DNA and an inactive conformation of the catalytic domain (reprinted with permission from ref. 24).

three-dimensional structure in the native state, known as intrinsically unstructured proteins (IUPs).[31] Examples of IUPs have been found in signal transduction, gene expression, and chaperones. IUPs utilize a template-induced folding process that in many cases can result in a single IUP with the ability to adopt different conformations when it binds to different proteins. Some IUPs can even cause different responses, for example activation or inactivation, from different protein partners. An IUP's interaction with different proteins might involve the same protein surface or overlapping surfaces. In some cases different combinations of short interaction motifs are used. An IUP could even bind the same partner in two different conformations or at different binding sites.

Summary

The list of known moonlighting proteins and our knowledge of their three-dimensional structures and molecular mechanisms is continuing to grow. Some of the types of functions and methods of switching between functions that are seen in the proteins described above have been observed in previously identified moonlighting proteins, but in many proteins there is a novel combination of functions. A wide variety of proteins have developed a second function during evolution, and there does not appear to be any common structural features among the moonlighting proteins. In many of the cases above, biochemical characterization of a specific protein under study

led to identification of a second function for that protein, but one example was found through a microarray DNA binding survey of hundreds of proteins. In the future, similar proteomics studies have the potential of identifying more moonlighting proteins relatively quickly because they tend to use high throughput methods. In addition, the observation that many proteins with intrinsically unstructured regions moonlight suggests there might be many more moonlighting proteins that have not yet been identified.

Acknowledgements

Funding for research on moonlighting proteins and other projects in the Jeffery lab has been through grants from the UIC Cancer Center, American Cancer Society, the American Heart Association, the National Science Foundation, and the Society for Biomolecular Sciences.

References

1 C. J. Jeffery, *Trends Biochem. Sci.*, 1999, **24**(1), 8–11.
2 J. Piatigorsky, *Ann. N. Y. Acad. Sci.*, 1998, **842**, 7–15.
3 J. Piatigorsky, *J. Biol. Chem.*, 1992, **267**(7), 4277–4280.
4 C. J. Jeffery, *Trends Genet.*, 2003, **19**(8), 415–417.
5 C. J. Jeffery, *Ann. Med.*, 2003, **35**(1), 28–35.
6 C. J. Jeffery, in *Molecular Chaperones and Cell Signalling*, ed. B. Henderson and A. G. Pockley, Cambridge University Press, New York, USA, 2005, pp. 61–77.
7 Y. Qi, X. He, X. J. Wang, O. Kohany, J. Jurka and G. J. Hannon, *Nature*, 2006, **443**(7114), 1008–1012.
8 J. Hanna, N. A. Hathaway, Y. Tone, B. Crosas, S. Elsasser, D. S. Kirkpatrick, D. S. Leggett, S. P. Gygi, R. W. King and D. Finley, *Cell*, 2006, **127**(1), 99–111.
9 A. Kunert, J. Losse, C. Gruszin, M. Huhn, K. Kaendler, S. Mikkat, D. Volke, R. Hoffmann, T. S. Jokiranta, H. Seeberger, U. Moellmann, J. Hellwage and P. F. Zipfel, *J. Immunol.*, 2007, **179**(5), 2979–2988.
10 N. G. Markova, A. Pinkas-Sarafova and M. Simon, *J. Invest. Dermatol.*, 2006, **126**(9), 2019–2031.
11 Y. Huang, M. A. Bayfield, R. V. Intine and R. J. Maraia, *Nat. Struct. Mol. Biol.*, 2006, **13**(7), 611–618.
12 H. H. Jang, K. O. Lee, Y. H. Chi, B. G. Jung, S. K. Park, J. H. Park, J. R. Lee, S. S. Lee, J. C. Moon, J. W. Yun, Y. O. Choi, W. Y. Kim, J. S. Kang, G. W. Cheong, D. J. Yun, S. G. Rhee, M. J. Cho and S. Y. Lee, *Cell*, 2004, **117**(5), 625–635.
13 M. Shimojima, S. Hoffmann-Benning, R. M. Garavito and C. Benning, *Arch. Biochem. Biophys.*, 2005, **436**(1), 206–214.
14 D. Deutsch, A. Haze-Filderman, A. Blumenfeld, L. Dafni, Y. Leiser, B. Shay, Y. Gruenbaum-Cohen, E. Rosenfeld, E. Fermon, B. Zimmermann, S. Haegewald, J. P. Bernimoulin and A. L. Taylor, *Eur. J. Oral Sci.*, 2006, **114**(Suppl 1), 183–189.
15 X. J. Chen, X. Wang, B. A. Kaufman and R. A. Butow, *Science*, 2005, **307**(5710), 714–717.
16 N. Entelis, I. Brandina, P. Kamenski, I. A. Krasheninnikov, R. P. Martin and I. Tarassov, *Genes Dev.*, 2006, **20**(12), 1609–1620.
17 D. A. Hall, H. Zhu, T. Royce, M. Gerstein and M. Snyder, *Science*, 2004, **306**, 482–484.
18 P. Scheerer, A. Borchert, N. Krauss, H. Wessner, C. Gerth, W. Höhne and H. Kuhn, *Biochemistry*, 2007, **46**(31), 9041–9049.
19 C. Freund, R. Kühne, S. Park, K. Thiemke, E. L. Reinherz and G. Wagner, *J. Biomol. NMR*, 2003, **27**(2), 143–149.
20 T. K. Nielsen, S. Liu, R. Luhrmann and R. Ficner, *J. Mol. Biol.*, 2007, **369**(4), 902–908.
21 J. M. De Pereda, W. F. Waas, Y. Jan, E. Ruoslahti, P. Schimmel and J. Pascual, *J. Biol. Chem.*, 2004, **279**(9), 8111–8115.
22 J. Dupuy, A. Volbeda, P. Carpentier, C. Darnault, J. M. Moulis and J. C. Fontecilla-Camps, *Structure*, 2006, **14**(1), 129–139.
23 W. E. Walden, A. I. Selezneva, J. Dupuy, A. Volbeda, J. C. Fontecilla-Camps, E. C. Theil and K. Volz, *Science*, 2006, **314**(5807), 1903–1908.
24 D. R. Edgell, V. Derbyshire, P. Van Roey, S. LaBonne, M. J. Stanger, Z. Li, T. M. Boyd, D. A. Shub and M. Belfort, *Nat. Struct. Mol. Biol.*, 2004, **11**(10), 936–944.
25 P. Van Roey, C. A. Waddling, K. M. Fox, M. Belfort and V. Derbyshire, *EMBO J.*, 2001, **20**(14), 3631–3637.
26 C. J. Jeffery, *Drug Discovery Today*, 2004, **3**, 71–78.
27 C. J. Jeffery, *Mass Spectrom. Rev.*, 2005, **24**(6), 772–782.
28 C. J. Jeffery, *Curr. Opin. Struct. Biol.*, 2004, **14**(6), 663–668.
29 L. M. Sampaleanu, F. Vallee, C. Slingsby and P. L. Howell, *Biochemistry*, 2001, **40**, 2732–2742.
30 J. Piatigorsky, W. E. O'Brien, B. L. Norman, K. Kalumuck, G. J. Wistow, T. Borras, J. M. Nickerson and E. F. Wawrousek, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**(10), 3479–3483.
31 P. Tompa, C. Szász and L. Buday, *Trends Biochem. Sci.*, 2005, **30**(9), 484–489.

