Indian J Med Res 149, March 2019, pp 322-325
DOI: 10.4103/ijmr.IJMR_2192_18

Quick Response Code:

Viewpoint

Medical implications of protein moonlighting

Specific molecular recognition by proteins is the basis of their roles in biocatalysis, signal transduction, metabolism and pathogenesis[1]. Almost all drugs essentially act by interaction with proteins by modulating their activities. Till recently, it was believed that this specificity was critical for all the biological processes and rested on the integrity of the structure of proteins. Lately, both of these premises have been challenged. Protein disorder has been found to be as important as ordered structure[1-3]. The catalytic promiscuity and moonlighting shown by a large number of proteins indicate that not only protein specificity has been overrated but also the non-specificity manifested in these two phenomena is biologically relevant[4-7]. Here we provide an update on the roles of protein moonlighting that are relevant to medical sciences.

What is protein moonlighting?

In the beginning, as living organisms shifted from the RNA world to the DNA world (and central dogma started dictating protein synthesis), the number of enzymes actually was not large and enzymes had broad specificity (also referred to in the literature as substrate promiscuity)[8]. Evolution created more complex organisms, creating needs for a large number of enzymes/proteins and regulation of their biological activities. This led to more efficient and specific enzymes. In fact, the two traits at the molecular design level are not unrelated. Enzyme specificity is quantified by k_{cat}/K_m and thus involves the catalytic rates[9]. Hence, while our appreciation of the importance of protein non-specificity may be rather recent, these molecules inherently were designed to be non-specific; it was the evolutionary need which led to some becoming highly specific.

The protein diversification involves multiple mechanisms: mutation, gene duplication and horizontal gene transfer. In 1989, Piatigorsky and Wistow[6] described their observations on crystallins also behaving as lactate dehydrogenase and enolase and called the phenomenon as gene sharing. This is not to be confused with horizontal gene transfer. Jeffery[7] used a phrase of moonlighting proteins; and this is also called protein multitasking. It is worth noting that moonlighting demolishes the classical boundary between catalytic proteins (enzymes) and other non-catalytic proteins such as structural proteins, signal transduction proteins and other regulatory proteins such as chaperones or repressors. Thus, multiple tasks carried out by proteins could straddle a variety of biological functions. These different functions originate in various non-exclusive modes. A protein in different locations within or outside the cell may have different kinds of biological activities. A protein may have totally different kinds of activities in different cell types. State of oligomerisation (monomer or oligomer) and the concentrations of the substrate/ligand can also dictate the nature of the biological activity of some proteins. An interesting example is that of protein resistin (which has link with diabetes) which forms large oligomers with possible functional relevance[10-12]. The same has been shown as determinant for treatment endpoint for the pulmonary tuberculosis[13]. Moonlighting, in majority of cases, involves different binding sites on a protein. The glycolytic enzyme glucoisomerase is known to act as a cytokine, nerve growth factor and promoter of cell differentiation factor[6,7]. Some other examples of moonlighting proteins are crystallins, lactate dehydrogenase, enolase and quinine oxidoreductase.

Disordered proteins are induced to acquire the desired conformation; so, while preformed binding site is not always required, a macromolecular nature may facilitate the formation of inducible binding site[3-8]. Both catalytic promiscuity and moonlighting reflect that biological specificity is not an essential virtue of proteins/enzymes. The level of protein

expression and metabolic flux (of substrates and ligands) are more important than believed so far. In the former case, the focus has been on the formation of inclusion bodies. For latter, importance seems to go beyond regulation through feedback inhibition/ allosteric interactions. The shift in our view of proteins from a static to inducible conformation (more flexible) happened long ago. The current view of many similar conformations in equilibria seems to be in line with the roles of substrates/ligands as the latter will shift these equilibria. Furthermore, the sanctity of active site turns out to be an invalid concept - it is the combinations of weak interactions which initiate the binding and chemistry of the local amino acid residues which define/dictate the biological activity. The moonlighting requires proteins/enzymes to be macromolecular.

Promiscuity, moonlighting and disorder

The above three terms refer to the emerging paradigm shifts in our understanding of the protein structure and function. It is necessary to clarify how promiscuity and moonlighting differ and how these are related to disorder, at least in some cases.

Jeffery[7,14] has clarified the concept of moonlighting by listing what all do not constitute moonlighting activity; '...the multiple functions are not due to RNA splice variants, gene fusions, or promiscuous enzyme activity'[14]. The list also includes post-translational modifications and proteins which catalyze multisteps in a metabolic pathway[15]. One key difference between promiscuity and moonlighting is that the former involves same active site/region (as for the main activity) whereas moonlighting activities reside at different sites on the protein. It was because these activities were originally coded by different genes which fused into a single one during evolution.

The evolution of these two different kinds of multifunctional proteins in archaea has been reviewed by Jia *et al*[15]. The promiscuity in both Embden-Meyerhof-Parnas (EMP) and Entner-Doudoroff pathway found in archaea indicated that these organisms used promiscuity to utilize available nutrients with limited set of enzymes. The above review also points out that the proteins of archaea are rich in disorder[15]. It seems likely that disorder plays a more important role in promiscuity as it requires same binding site accommodating very different kinds of substrates. The disorder also plays an important role in moonlighting as well[16].

How moonlighting impacts medical sciences?

Sriram *et al*[17] have pointed out the various factors which can complicate prediction of phenotype from genotype in cases of single gene disorders. If the metabolic enzyme responsible for the disorder has (unknown) moonlighting activities, these complicate the clinical phenotype picture. Phosphoglucoisomerase deficiency is responsible for haemolytic anaemia, but its neuroleukin activity may lead to neurological defects as well. Glycerol kinase has many moonlighting activities. The mutation(s) in its gene can result in a variety of phenotypes which have been difficult to predict[17]. There is enough evidence that moonlighting activities of glyceraldehydes-3-phosphate dehydrogenase play a role in many neurodegenerative diseases including Huntington, Alzheimer's and Parkinson's diseases. A clear indication is that several promising drugs for Alzheimer's disease decrease the expression of these enzymes. Further, ceruloplasmin has been extensively studied as a protein with several intriguing moonlighting activities[18]. Aceruloplasminaemia and haemochromatosis lead to systemic haemosiderosis and diabetes while the former alone results in neural and retinal degeneration[18]. Elevated levels of this protein are associated with several inflammatory processes and metastatic cancers. While it acts as a protection against oxygen reactivity, when localized in the vessel walls, the available copper ions switch its activity to cause oxidative damage to the invading pathogen[18].

Moonlighting proteins as virulence factors

While it has been suggested that many glycolytic enzymes are found on the cell surface of Gram-positive organisms, the work from Götz's laboratory[19] shows that in methicillin-resistant *Staphylococcus aureus*, two cytoplasmic enzymes of EMP, fructose-1,6-bisphosphate aldolase and glyceraldehyde phosphate dehydrogenase moonlight in their secretory forms. These enzymes were shown not only to enhance the binding of the bacterium to host cells but also binding to some host matrix proteins. In fact, moonlighting activity has emerged as a mechanism of virulence in several cases[20]. Furthermore, during evolutionary phase, *Mycobacterium tuberculosis* assigned newer roles to proteins, many of these have virulence attributes[21]. Isocitrate dehydrogenase and aconitase of *M. tuberculosis* are examples of moonlighting proteins[22,23].

Moonlighting, tuberculosis and antibiotic resistance

Chaperonin 60.2 (hsp65) of *M. tuberculosis* is also secreted and believed to facilitate the entry of

the bacterium in the macrophages[24]. *M. tuberculosis* peptidyl-prolyl isomerases (PPIases) show immunological and chaperone-like activity though these do not carry the crystallin motif[25,26]. There is another interesting aspect of moonlighting activity in the case of *M. tuberculosis* with respect to its developing antibiotic resistance towards ciprofloxacin[27]. Glutamate racemase is an enzyme important for the cell wall synthesis by producing D-glutamate. The enzyme also showed DNA gyrase inhibition activity thereby creating resistance towards the antibiotic. It is likely that this may turn out to be a more general phenomenon. There are many more examples of moonlighting where a protein displays many other functions[28-31].

Neomorphic moonlighting functions in disease

Jeffery[14] has defined 'neomorphic moonlighting function' as a 'specific biochemical function (catalytic activity, binding activity *etc*.) of a protein' because of mutations in its coding region or a deleterious change in the conformation of the polypeptide chain. Several examples have been provided where such moonlighting functions have led to diseases. β-2-microglobulin is a major histocompatibility complex (MHC) Class I protein on the surface of B-lymphocytes. Diminished kidney function is associated with its formation of amyloid fibres. Altered forms of triose phosphate isomerase form disordered aggregates and may lead to neurological disorders and other severe diseases. Some other important examples of neomorphic moonlighting proteins are glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase and dihydrolipoamide dehydrogenase[14]. Thus, understanding moonlighting is critical to understand complete clinical picture.

Moonlighting also complicates the drug discovery approaches. A drug is a targeted molecule designed to inhibit a particular protein function. It is often difficult to predict how it will impact the moonlighting activities. It is likely that in several cases, the side effects of a drug may originate in the affected moonlighting activities. The current methods (gene knockouts, antisense RNA or RNA interference) which have proved invaluable in establishing the genotype-phenotype correlations now need to be relooked in light of moonlighting activities.

Concluding remarks

The overview by James and Tawfik[32] provides a broader perspective on the functional diversity which originates from the protein flexibility; the most extreme case of that being proteins with varying extent of disorder. Not only that facilitates catalysis and signal transduction (especially through post-translational modifications) but also seems to be a prerequisite for the evolution of new and diverse kinds of proteins. Thus, moonlighting is a part of the overall evolutionary design[33]. Just as understanding the phenomenon of isoenzymes paved the way for valuable diagnostic applications several decades ago, appreciation of these 'new views' about protein structure-function correlation will be useful for developing future contours of the practice of medicine.

***Financial support & sponsorship*:** The last two authors (NZE and SEH) thank the Department of Biotechnology, Ministry of Science and Technology, New Delhi, for research grants. SEH is a JC Bose National Fellow and Robert Koch Fellow of the Robert Koch Institute, Berlin, Germany.

***Conflicts of Interest*:** None.

M.N. Gupta[1], Saurabh Pandey[3], Nasreen Z. Ehtesham[5] & Seyed E. Hasnain[2,4,6,*]

[1]Former Professor, Department of Chemistry & [2]Molecular Infection and Functional Biology Laboratory, Kusuma School of Biological Sciences, Indian Institute of Technology Delhi, New Delhi 110 016, [3]Department of Biochemistry & [4]JH Institute of Molecular Medicine, Jamia Hamdard, New Delhi 110 062, [5]National Institute of Pathology, New Delhi 110 029 & [6]Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad 500 007, Telangana, India

[*]*For correspondence*: seyedhasnain@gmail.com

Received December 4, 2018

References

1. Petsko GA, Ringe D. *Protein structure and function*. London: New Science Press Ltd.; 2004 p. 46-7.
2. Gupta MN, Mukherjee J. Enzymology: Some paradigm shifts over the years. *Curr Sci* 2013; *104* : 1178-86.
3. Mittag T, Kay LE, Forman-Kay JD. Protein dynamics and conformational disorder in molecular recognition. *J Mol Recognit* 2010; *23* : 105-16.
4. Hult K, Berglund P. Enzyme promiscuity: Mechanism and applications. *Trends Biotechnol* 2007; *25* : 231-8.
5. Kapoor M, Gupta MN. Lipase promiscuity and its biochemical applications. *Process Biochem* 2012; *47* : 555-69.
6. Piatigorsky J, Wistow GJ. Enzyme/crystallins: Gene sharing as an evolutionary strategy. *Cell* 1989; *57* : 197-9.
7. Jeffery CJ. Moonlighting proteins. *Trends Biochem Sci* 1999; *24* : 8-11.

8. Gupta MN, Roy I. Rear mirror view of some biological tools and processes. *Proc Indian Natl Sci Acad* 2018; *84* : 611-24.
9. Purich DL. *Contemporary enzyme kinetics and mechanisms,* 3rd ed. Saint Louis: Elsevier/Academic Press; 2009. p. 683.
10. Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L. Disulfide-dependent multimeric assembly of resistin family hormones. *Science* 2004; *304* : 1154-8.
11. Suragani M, Aadinarayana VD, Pinjari AB, Tanneeru K, Guruprasad L, Banerjee S, *et al.* Human resistin, a proinflammatory cytokine, shows chaperone-like activity. *Proc Natl Acad Sci U S A* 2013; *110* : 20467-72.
12. Ghosh S, Singh AK, Aruna B, Mukhopadhyay S, Ehtesham NZ. The genomic organization of mouse resistin reveals major differences from the human resistin: Functional implications. *Gene* 2003; *305* : 27-34.
13. Ehtesham NZ, Nasiruddin M, Alvi A, Kumar BK, Ahmed N, Peri S, *et al.* Treatment end point determinants for pulmonary tuberculosis: Human resistin as a surrogate biomarker. *Tuberculosis (Edinb)* 2011; *91* : 293-9.
14. Jeffery CJ. Proteins with neomorphic moonlighting functions in disease. *IUBMB Life* 2011; *63* : 489-94.
15. Jia B, Cheong GW, Zhang S. Multifunctional enzymes in archaea: Promiscuity and moonlight. *Extremophiles* 2013; *17* : 193-203.
16. Tompa P, Szász C, Buday L. Structural disorder throws new light on moonlighting. *Trends Biochem Sci* 2005; *30* : 484-9.
17. Sriram G, Martinez JA, McCabe ERB, Liao JC, Dipple KM. Single-gene disorders: What role could moonlighting enzymes play? *Am J Hum Genet* 2005; *76* : 911-24.
18. Bielli P, Calabrese L. Structure to function relationships in ceruloplasmin: A "moonlighting" protein. *Cell Mol Life Sci* 2002; *59* : 1413-27.
19. Ebner P, Rinker J, Nguyen MT, Popella P, Nega M, Luqman A, *et al.* Excreted cytoplasmic proteins contribute to pathogenicity in *Staphylococcus aureus*. *Infect Immun* 2016; *84* : 1672-81.
20. Henderson B. *Moonlighting proteins*: *Novel virulence factors in bacterial infections*. New Jersey: John Wiley & Sons, Inc.; 2017.
21. Singh Y, Kohli S, Sowpati DT, Rahman SA, Tyagi AK, Hasnain SE, *et al.* Gene cooption in mycobacteria and search for virulence attributes: Comparative proteomic analyses of *Mycobacterium tuberculosis*, *Mycobacterium indicus* pranii and other mycobacteria. *Int J Med Microbiol* 2014; *304* : 742-8.
22. Banerjee S, Nandyala A, Podili R, Katoch VM, Murthy KJR, Hasnain SE, *et al. Mycobacterium tuberculosis* (Mtb) isocitrate dehydrogenases show strong B cell response and distinguish vaccinated controls from TB patients. *Proc Natl Acad Sci U S A* 2004; *101* : 12652-7.
23. Banerjee S, Nandyala AK, Raviprasad P, Ahmed N, Hasnain SE. Iron-dependent RNA-binding activity of *Mycobacterium tuberculosis* aconitase. *J Bacteriol* 2007; *189* : 4046-52.
24. Henderson B, Lund PA, Coates ARM. Multiple moonlighting functions of mycobacterial molecular chaperones. *Tuberculosis (Edinb)* 2010; *90* : 119-24.
25. Pandey S, Sharma A, Tripathi D, Kumar A, Khubaib M, Bhuwan M, *et al. Mycobacterium tuberculosis* peptidyl-prolyl isomerases also exhibit chaperone like activity *in vitro* and *in vivo*. *PLoS One* 2016; *11* : e0150288.
26. Pandey S, Tripathi D, Khubaib M, Kumar A, Sheikh JA, Sumanlatha G, *et al. Mycobacterium tuberculosis* peptidyl-prolyl isomerases are immunogenic, alter cytokine profile and aid in intracellular survival. *Front Cell Infect Microbiol* 2017; *7* : 38.
27. Sengupta S, Ghosh S, Nagaraja V. Moonlighting function of glutamate racemase from *Mycobacterium tuberculosis*: Racemization and DNA gyrase inhibition are two independent activities of the enzyme. *Microbiology* 2008; *154* : 2796-803.
28. Tundup S, Mohareer K, Hasnain SE. Mycobacterium tuberculosis PE25/PPE41 protein complex induces necrosis in macrophages: Role in virulence and disease reactivation? *FEBS Open Bio* 2014; *4* : 822-8.
29. Kumar A, Lewin A, Rani PS, Qureshi IA, Devi S, Majid M, *et al*. Dormancy associated translation inhibitor (DATIN/Rv0079) of *Mycobacterium tuberculosis* interacts with TLR2 and induces proinflammatory cytokine expression. *Cytokine* 2013; *64* : 258-64.
30. Akhter Y, Ehebauer MT, Mukhopadhyay S, Hasnain SE. The PE/PPE multigene family codes for virulence factors and is a possible source of mycobacterial antigenic variation: perhaps more? *Biochimie* 2012; *94* : 110-6.
31. Ahmad J, Farhana A, Pancsa R, Arora SK, Srinivasan A, Tyagi AK, *et al*. Contrasting function of structured N-terminal and unstructured C-terminal segments of *Mycobacterium tuberculosis* PPE37 protein. *MBio* 2018; *9*. pii: e01712-17.
32. James LC, Tawfik DS. Conformational diversity and protein evolution - A 60-year-old hypothesis revisited. *Trends Biochem Sci* 2003; *28* : 361-8.
33. Nam H, Lewis NE, Lerman JA, Lee DH, Chang RL, Kim D, *et al.* Network context and selection in the evolution to enzyme specificity. *Science* 2012; *337* : 1101-4.