Comparative Biochemistry and Physiology, Part A 165 (2013) 417–428

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Review

Stress response and adaptation: A new molecular toolkit for the 21st century☆

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ARTICLE INFO

Article history:
Received 30 November 2012
Received in revised form 15 January 2013
Accepted 17 January 2013
Available online 31 January 2013

Keywords:
Biochemical adaptation
Enzyme regulation
Posttranslational modification
MicroRNA
Posttranscriptional regulation
Multiplex assay systems
Luminex technology

ABSTRACT

Much research in comparative biochemistry is focused on understanding the molecular mechanisms that allow organisms to adapt to and survive diverse environmental challenges. In recent years, genomic and proteomic approaches have been key drivers of advancement in the field, for example, providing knowledge about gene and protein expression, regulation of signal transduction pathways, and functional control of enzymes/proteins by reversible protein phosphorylation. Advances in comparative biochemistry have always drawn upon conceptual and technological advances that arise from "mainline" biochemistry and molecular biology, often from medical models. The present article discusses three such advances that will have major impacts on comparative biochemistry in the 21st century. The first is the crucial role of posttranslational modification in metabolic control, expanding outwards from reversible phosphorylation to explore the individual and interacting effects of protein modification by acetylation, methylation, SUMOylation and O-GlcNAcylation, among others. The second is the newly recognized role of non-coding RNA in the regulation of gene expression, particularly the action of microRNAs. The third is the emergence of powerful multiplex technology that allows rapid, high-throughput detection of analytes and will revolutionize RNA and protein profiling in the comparative biochemistry laboratory. Commercial tools such as Luminex allow researchers to simultaneously quantify up to 100 different analytes in a single sample, thereby creating broad functional analyses of metabolism and cell signaling pathways.

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; FOXO, forkhead box class O; HBP, hexosamine biosynthetic pathway; HNF, hepatocyte nuclear factor; HSP, heat shock protein; G6PDH, glucose-6-phosphate dehydrogenase; IL1-F9, interleukin-1 family member 9; LDH, lactate dehydrogenase; NF-κB, nuclear factor-kappa B; Nrf2, NF-E2 related factor 2; O-GlcNAc, *O*-linked N-acetylglucosamine; PCR, polymerase chain reaction; PDH, polyol dehydrogenase; PKG, cyclic GMP dependent protein kinase; PP1, protein phosphatase 1; PPAR, peroxisome proliferator activated receptor; PTM, posttranslational modification; Q-PCR, quantitative PCR; SUMO, small ubiquitin-like modifier; Tf, transcription factor; ULM, ubiquitin-like modifier; UDP-GlcNAc, uridine diphosphate-N-acetylglucosamine.

☆ This paper was presented at the 'First International Conference on Oxidative Stress in Aquatic Ecosystems' held in San Jose del Cabo, Baja California Sur, Mexico on November 20–24, 2012.

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1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.01.019

1. Introduction

The field of comparative biochemistry explores the diversity of life at the molecular level and the wonderful biochemical adaptations that allow organisms to survive and prosper in virtually every environment that can be found on Earth. Over the past few decades, the increasing availability of molecular tools has allowed researchers to dive ever more deeply into the genomic and proteomic aspects of comparative biochemistry (Storey, 2006). The field has undergone a revolution in both the experimental methodologies available for use and the types of data that can be produced. For example, where earlier studies could often focus only on the responses of small groups of targets (e.g. heat shock proteins, glycolytic enzymes, antifreeze proteins, etc.), the introduction of genome and proteome array screening methods is now allowing exploration of the global changes in gene and protein expression that underlie adaptation to environmental stress. This new holistic view is now allowing a better understanding of integrated responses by cellular metabolism and providing the opportunity to identify the involvement of previously unsuspected genes/proteins in the adaptive process. Together with the ever-expanding list of completed genome sequencing projects for non-mammalian vertebrates and invertebrates, as well as a huge range of bioinformatics prediction tools, the comparative biochemist is now well-armed with tools to better explore how animals work.

However, conceptual and methodological advances continue to be made and these give comparative biochemists even more opportunities and options for exploring biochemical adaptation. In the present article, we review selected advances that offer crucial new ideas and opportunities for exploring organismal adaptation to environmental stress. Three topics are covered: (a) integrated enzyme/protein control by multiple forms of posttranslational modification, (b) posttranscriptional control of gene expression by non-coding RNA, particularly microRNA, and (c) high-throughput multiplex technologies for screening and quantifying gene and protein expression, based on microsphere bead technology.

2. Regulatory control of metabolism by posttranslational modification

The role of reversible protein phosphorylation in the regulation of cellular metabolism is very well known and has been extensively studied for many years. The mechanism is pervasive throughout the Eukaryota and many proteins are known to be phosphorylated on serine, threonine or tyrosine residues by hundreds of protein kinases with reversal by protein phosphatases. For example, the Human Protein Reference Database lists over 95,000 phosphosites mapped to more than 13,000 proteins (Goel et al., 2012). Reversible protein phosphorylation is a central mechanism of metabolic regulation and affects many kinds of cellular proteins (e.g. enzymes, structural proteins, and signaling proteins) to initiate changes in their properties including activity, sensitivity to effectors, protein–protein interactions, and subcellular localization. For example, signal transduction cascades frequently propagate by sequential phosphorylation of protein kinases in a chain, taking a signal received by a cell surface receptor and amplifying and spreading it to create coordinated action by multiple functional proteins (e.g. metabolic enzymes, and transcription factors) (MacDonald, 2004). In comparative biochemistry, reversible phosphorylation of selected proteins frequently mediates adaptive responses to environmental stress and, in particular, our laboratory has documented the broad use of reversible phosphorylation as a primary means of coordinating entry into and arousal from states of hypometabolism including hibernation, estivation, anoxia tolerance and freeze tolerance (Storey and Storey, 2007, 2010).

Much remains to be learned about reversible phosphorylation and its role in metabolic regulation, but our 21st century approach to biochemical adaptation also requires us to broaden our scope and evaluate new directions in posttranslational modification (PTM). Two themes are of current interest: (a) the widespread phosphorylation of non-regulatory enzymes and what this means to intermediary metabolism, and (b) protein/enzyme regulation by PTMs other than phosphorylation.

2.1. Reversible phosphorylation of non-regulatory enzymes

Reversible phosphorylation control is well-known for regulatory enzymes (e.g. those gating pathways or branchpoints in metabolism), functional proteins (e.g. ribosomal initiation and elongation factors, transcription factors, transporters of multiple types, etc.) and components of signal transduction pathways. However, many enzymes in metabolic pathways are considered to be non-regulatory (e.g. most dehydrogenases) with their responses to stress or stimuli dictated by substrate availability (often controlled by regulatory enzymes at other sites in a pathway). Are non-regulatory enzymes also subject to reversible phosphorylation and/or other forms of PTM? The answer is proving to be yes and a key goal for the 21st century enzymologist is to identify and explore the range of PTMs that modify enzymes and determine how these contribute to enzyme/pathway control and to modulating enzyme function in response to external stress.

A recent study of glucose-6-phosphate dehydrogenase (G6PDH) from hepatopancreas of the land snail, *Otala lactea*, triggered our interest in reversible phosphorylation control of a dehydrogenase. G6PDH is the initial enzyme of the pentose phosphate pathway and along with the second enzyme of the pathway (6-phosphogluconate dehydrogenase) is a major source of the NADPH reducing equivalents needed for cellular biosynthesis and antioxidant defense. We found that *O. lactea* G6PDH was a phosphoenzyme and that the enzyme from estivating snails had a much higher phosphate content than the enzyme in active control snails. The low (control) and high (estivating) phosphate forms of G6PDH differed in activity, substrate affinity for glucose-6-phosphate and other parameters (Ramnanan and Storey, 2006). Interconversion of the two forms was linked with cyclic GMP protein kinase (PKG) and protein phosphatase 1 (PP1) and it was proposed that phosphorylation of G6PDH when snails enter estivation may enhance relative carbon flow through the pentose phosphate cycle and contribute to sustained NADPH production for use in antioxidant defense. A parallel study of G6PDH from hepatopancreas of a freshwater crayfish (*Orconectes virilis*) showed comparable anoxia-responsive phosphorylation of the crustacean enzyme, again with an increase in glucose-6-phosphate affinity, and links to PKG and PP1 action (Lant and Storey, 2011). This shows a conserved method of altering G6PDH properties both between diverse invertebrate groups and in response to two different forms of stress-induced hypometabolism, indicating that reversible phosphorylation is a principle of G6PDH control. Another novel instance of phosphorylation modification of a dehydrogenase is the case of polyol dehydrogenase (PDH) from the cold-hardy gall moth, *Epiblema scudderiana* (Holden and Storey, 2011). PDH catalyzes the NADPH-linked conversion of glyceraldehyde to form glycerol, the winter cryoprotectant that builds up to over 2 M in the larvae and helps to push their winter supercooling point down to near −40 °C (Storey and Storey, 1988). The low phosphate form of PDH from 5 °C-acclimated larvae undergoing active glycerol synthesis showed a 2-fold higher affinity for glyceraldehyde as well as differential sensitivity to denaturants (urea, guanidine hydrochloride) as compared with the high phosphate form of the enzyme from −15 °C, winter cold-hardened larvae. Treatments that stimulated the actions of endogenous protein kinases versus protein phosphatases interconverted the enzyme between the two forms and ProQ Diamond phosphoprotein staining as well as immunoblotting with a phosphoserine antibody showed a much higher content of phosphate on the enzyme in −15 °C acclimated larvae. Hence, it is apparent that reversible phosphorylation of PDH makes a significant contribution to controlling the