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Mini Review

New Approaches to Comparative and Animal Stress Biology Research in the Post-genomic Era: A Contextual Overview

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ABSTRACT

Although much is known about the physiological responses of many environmental stresses in tolerant animals, studies evaluating the regulation of stress-induced mechanisms that regulate the transitions to and from this state are beginning to explore new and fascinating areas of molecular research. Current findings have developed a general, but refined, view of the important molecular pathways contributing to stress-survival. However, studies utilizing newly developed technologies that broadly focus on genomic and proteomic screening are beginning to identify many new targets for future study. This minireview will provide a contextual overview on the use of DNA/RNA sequencing, microRNA annotation and prediction software, protein structure and function prediction tools, as well as methods of high-throughput protein expression analysis. We will also use select examples to highlight the existing use of these technologies in stress biology research. Such tools can be used in comparative stress biology in the characterization of animal responses to environmental challenges. Although there are many areas of study left to be explored, research in comparative stress biology will always be continuing as new technologies allow the further analysis of cell function, and new paradigms in gene regulation and regulatory molecules (such as microRNAs) are continuing to be discovered. Building upon the findings of past research, while utilizing new technologies in the appropriate manner, future studies can be carried out in new and exciting areas still unexplored. Proper use of rapidly developing technologies will help to create a complete understanding of the animal stress response and survival mechanisms utilized by many diverse organisms.

Contents

1. Introduction

Currently, the field of comparative animal biology is at the beginning of a large expansion of experimental knowledge as studies start to

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utilize new high-throughput technologies. To date, research has discovered much about the physiological responses of many tolerant animals to environmental stress [1–7], however new studies are broadly focused on genomic and proteomic screening and have been identifying many new targets for future study [8–10]. Many of these technologies should be intriguing to the comparative stress biologist, who now has available technology to assess the global expression of nearly all genes and proteins that contribute to survival in stress-tolerant animals [11]. Although there are many areas of study left to be explored, research in comparative and animal stress biology will always be continuing with the advancement of technologies that allow new insight into cell function, and new paradigms in gene regulation and regulatory molecules (such as microRNAs) are continuing to be discovered. Proper knowledge and use of genomic and proteomic-based technology will help to create a complete understanding of the stress response and survival mechanisms that are utilized by many diverse organisms.

Within the next few years the entire genome of many organisms, including those that display tolerances to extreme environmental conditions, will most likely be sequenced. For example, the genome of the anoxia/freeze-tolerant Western painted turtle (*Chrysemys picta bellii*) was only sequenced in 2013 and the hibernating thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) has been sequenced since 2008 [12]. With the current and future availability of genomic information, the prepared comparative biologist will be provided with a blue-print for protein structure, control domains and sites of post-translational modifications that are either conserved or perhaps unique in those organisms. The overall goal for global modeling of the cell is to better predict the behavior of biological systems. This type of research will have profound implications for the understanding of basic biology and improving future stress-tolerance of human systems.

As previously mentioned, being able to successfully utilize newly developed technologies and resources, researchers will be able to build upon previously explored areas of study. The end result will most likely be a deeper and extensive understanding of the biological processes that underlie natural mechanisms of animal stress tolerance. It should also be noted that the ability to analyze the stress response at a global level is not limited to the availability of a genome [13]. It is one of the goals of this minireview to provide a contextual overview of technologies and tools that can provide omic-level analysis, without the absolute need for an annotated genome. Several technologies have emerged in the recent years that allow researchers to quantitatively analyze the cellular response in a relatively short period of time and at low cost. These technologies include (1) the use of microarrays to examine the responses of mRNA [14], protein and microRNAs, (2) the use of RNA sequencing (RNASeq) to evaluate the state of transcription among all expressed genes [15], (3) multiplexed assays that have the ability to assess the expression of multiple analytes (mRNA, protein and enzyme activity) [16], and (4) the prediction of protein structure and function [17]. Below are brief overviews of each technology and its application to the field of comparative molecular biology.

2. Microarray analysis of gene and protein expression

Microarrays are widely available in the research marketplace, functioning as a solid-support for thousands of different sequences that are fixed at specific locations [18]. To date, there are a variety of microarray types and formats. Essentially, microarrays can be received as an advancement on end-point RT-PCR or immunoblotting as they have the ability to measure the expression of a very large number of genes (cDNA/oligo-based capture) or proteins (antibody-based capture) at the same time and within a single sample [19]. As a result, they are typically a chosen technology for experiments that require a large number of genes to be measured quickly or when sample amount is extremely limited for study. These arrays are also useful when discovery or initial characterization of a new model organism is necessary because they allow either the generation of project "leads" (heterologous screening) or a quantitative assessment of gene/protein expression when a homologous array is used (Box. 1) [20,21]. As microarrays can be used to examine the expression of hundreds (protein) or thousands (gene) of targets at once, it holds the promise to complete multiple years of RT-PCR or immunoblotting expression research (target-based) within days. However it is critical to note that the results obtained by microarray experiment need to be validated through other methods of expression analysis (ie. Immunoblotting (protein) or qRT-PCR (gene)). One must also realize that this technology is steadily changing and improving as new advances are being made to increase both array reproducibility and specificity. Nevertheless, at its current state this technology provides an excellent research tool to the comparative biologist to obtain complete expression data or a simple generation of project leads, using either homologous or heterologous arrays, that can be used to identify possible areas of future study. Ultimately, microarray-based studies promise to expand the knowledge of the cellular stress response, revealing patterns of coordinated gene expression and perhaps even uncovering entirely new stress-responsive cellular pathways. When combined with appropriate bioinformatic tools, microarray technology also aids in integrating target expression data with function at the cellular level, revealing hypotheses of how multiple targets may work together to produce a particular stress response to match a particular cellular need (such as metabolic adjustments, cytoskeletal reorganization, etc.) [22]. Outlined below is specific information regarding both DNA and protein microarrays.

2.1. Gene expression

Microarrays can be used to detect mRNA expression patterns comparatively within different stresses, organisms, tissues or time-points. The previous research from the Storey lab, using heterologous cDNA microarrays, has indicated that there may not be a large variety of genes involved in regulating the typical animal stress response [20]. This makes it critical to be able to detect "all-of-the-few" genes that play important roles, no matter how seemingly obscure. For a comparative biologist, an expression microarray experiment could be designed where gene expression data are generated over multiple stress points in multiple arrays and referenced to control conditions. Unfortunately, it must be noted that data obtained from cDNA microarray experiments do not yield sequence information and do not provide an indication of organism-specific novel genes or organism-specific "oddities" within the gene (such as mutation of splicing events that alter protein function). It is also important to note that two main types of DNA microarrays exist in today's marketplace, (1) oligomeric microarrays, and (2) cDNA microarrays. Oligomeric microarrays are spotted with synthesized oligos anywhere between 30 and 60 bp in length. Typically, these oligos are designed to have complementarity to the 3′ UTR (some companies differ, so you must check with your company of interest) and are often used because of their high stringency. By contrast, microarrays spotted with cDNA contain the complete transcript sequence. Classically, heterologous cDNA microarrays allow the highest degree of hybridization for use with new/unsequenced animals, because they are "forgiving" enough that small, poorly conserved regions of sequence do not dictate overall binding to the array. Overall, it is critical to

Box 1

Heterologous array: Using an array with sample from an animal that is different from the animal that the array was designed for (ex. testing for squirrel gene expression using a mouse cDNA microarray).
Homologous array: Using an array with samples from the same species that the array was designed for (ex. Using mouse samples on a mouse cDNA microarray for which it was designed).