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Endocrinology: Advances through omics and related technologies

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ABSTRACT

The rapid development of new omics technologies to measure changes at genetic, transcriptomic, proteomic, and metabolomics levels together with the evolution of methods to analyze and integrate the data at a systems level are revolutionizing the study of biological processes. Here we discuss how new approaches using omics technologies have expanded our knowledge especially in nontraditional models. Our increasing knowledge of these interactions and evolutionary pathway conservation facilitates the use of nontraditional species, both invertebrate and vertebrate, as new model species for biological and endocrinology research. The increasing availability of technology to create organisms overexpressing key genes in endocrine function allows manipulation of complex regulatory networks such as growth hormone (GH) in transgenic fish where disregulation of GH production to produce larger fish has also permitted exploration of the role that GH plays in testis development, suggesting that it does so through interactions with insulin-like growth factors. The availability of omics tools to monitor changes at nearly any level in any organism, manipulate gene expression and behavior, and integrate data across biological levels, provides novel opportunities to explore endocrine function across many species and understand the complex roles that key genes play in different aspects of the endocrine function.

1. Omics and vertebrate non-model species

Omic technologies measure changes in genomes (genomics, epigenomics), global gene expression (transcriptomics), global protein levels (proteomics), and global biochemical molecules involved in metabolism (metabolomics). These measurements have evolved from a focus on a single gene, protein or metabolite endpoint 30 years ago to a focus on rapidly measuring changes in thousands to millions of endpoints. Much of this development was initially driven by the human genome project, which began in 1991 (Collins and Galas, 1993).

An essential step for the development of omics applications for endocrine research was refining their use in model species used in understanding both the highly conserved and species-specific aspects of the endocrine system. A very well characterized model, the zebrafish (*Danio rerio*), has almost 1,800,000 expressed sequence tags deposited in Genbank (http://www.ncbi.nlm.nih.gov/nucest) and >20,000 publications in PubMed (http://www.ncbi.nlm.nih.gov/pubmed). Its genome-sequencing project started in 2001 at the Wellcome Trust Institute and a recent, high-quality assembly of its genome, was able to match 70% of its genes to human orthologs, further supporting its already recognized value as a model species for biomedical research (Howe et al., 2013; Lieschke and Currie, 2007).

The classical cDNA microarray technologies, while simple to construct and permitting easy gene annotation, often presented problems of specificity such as an inability to distinguish close paralogs. Due to deep genomic understanding of this species, oligo-based microarrays were developed (often ≈60-mers mainly

Abbreviations: DEGs, differentially expressed genes; DHP, 17α,20β-dihydroxy-4-pregnane-3-one; ERα, estrogen receptor α; FSH, follicle-stimulating hormone; GVBD, germinal vesicle breakdown; GH, growth hormone; GHR, growth hormone receptor; HPG, hypothalamus–pituitary–gonadal; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; Lmo4, Lim-domain only 4; MIH, maturation-inducing hormone; PBPK, physiologically based computational model; Q-PCR, quantitative polymerase chain reaction; SAGE, Serial Analysis of Gene expression; StAR, Steroidogenic Acute Regulatory protein; SF1, steroidogenic factor 1.

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targeting the untranslated region sequences of transcripts) with commercial arrays available for more than 10 years. A less widely used transcriptomics approach, Serial Analysis of Gene expression (SAGE), is based on cloning 3′-end specific tags of 14–16 nucleotides that are often in the 3′-UTR and then correlates the number of tags to the degree of gene expression. This tool is dependent upon the extent to which the animal model has been sequenced since each single tag must be identified by matching to a corresponding gene. A recent study (Xu et al., 2013) mapped more than 12 million tags and identified nearly 1500 disregulated genes in zebrafish liver in response to arsenic exposure using this approach. However, despite the deep genomic characterization of these model species, interest is growing for "alternative model species" in the fields ranging from evolution and adaptation to extreme environments to toxicology and endocrine research (Perkins et al., 2013). Due to the enormous improvement of the sequencing yield with next-generation sequencing technologies, the ability to produce data for genomic and transcriptomic analysis is becoming increasingly affordable (Davey et al., 2011). As a result, omics techniques can now be applied to "unusual" species to rapidly generate information that can be used to understand novel biological characteristics.

The extent of animal diversity is very large, with nearly 25,000 teleost species, and more than 1,000,000 arthropod species. Evolutionary concerns have driven this extensive interest for the genomic characterization of new species. In May 2010, the release 54 at http://www.ensembl.org/index.html involved 49 species with fully sequenced genome including 5 fish. In September 2013, Release 73 included 63 species with fully sequenced genome, 20 were ongoing and among them 10 were fish. Commercial interests (e.g. aquaculture, oyster-farming) have also driven the development of genome sequencing programs of species of economic interest. Indeed, several species have ongoing genome projects such as the European sea bass (Kuhl et al., 2010), the Atlantic salmon (Ng et al., 2005); the rainbow trout (Palti et al., 2009), the Atlantic cod (Star et al., 2011), eel (Henkel et al., 2012), and the pacific oyster (Zhang et al., 2012).

Often non-model species have poorly characterized, or a complete lack of, genomic/transcriptomic information. As a first step, researchers can use omics to produce large amounts of information that drastically increase their knowledge of a particular species. Non-model species can then be compared to other better-characterized genomes and/or transcriptomes to aid in their characterization. Comparative genomics was first applied between human and chimpanzee 10 years ago (Fujiyama et al., 2002). This approach was based on synteny group search, a tool for evolutionary understanding and genome rearrangement identification such as duplication or reorientation events between species. By comparison and identification of similarity in genes and genomics, one can then infer functional relationships to aid in determining whether or not endocrine pathways are conserved across species (Burgess-Herbert and Euling, 2011). However, despite this advancement, not all genes, proteins, or metabolites can be identified and are therefore not interpreted.

A review of the early use of microarrays for aquatic species can be found in (Denslow et al., 2007). The quality and quantity of sequence data generated by rapidly evolving sequencing techniques revolutionized research with non-model species, allowing researchers to construct high-quality microarrays for relevant species instead of focusing research efforts in model species. Such is the case of the development of a largemouth bass microarray (Garcia-Reyero et al., 2008), used to study estrogenic response in males; or a goldfish cDNA array (Martyniuk et al., 2006), used to study estrogenic effects on neuroendocrine function. The development of microarrays for non-model species though often faces the problem of poorly of annotated probes. For example, 37% of unique transcripts had no annotation in a 19,048 sequence database from European sea bass (Ferraresso et al., 2010). Similarly, a microarray recently built to study embryogenesis of killifish, *Fundulus heteroclitus,* under air-exposed conditions versus water-exposed condition had 65% percent of probes missing annotations (Tingaud-Sequeira et al., 2013). Therefore, investing in further sequencing for better coverage and annotation seems crucial when working with non-model species.

1.1. Use of omics technologies in endocrine research

The hypothalamus–pituitary–gonadal (HPG) axis is a clear example of a system highly conserved across vertebrates, as evidenced by sequence similarities among the estrogen receptor α (ERα) ligand-binding domain from several species (Fig. 1; Norris, 2006). Conservation of these molecules/receptors/signaling pathways involved in reproduction should allow non-mammalian vertebrate models to be used to explore and understand the function of the HPG axis and reproduction both in non-mammalian and mammalian species (Perkins et al., 2013). It is a fact that there are still many unknowns in the processes involved in reproduction. For instance, the molecular pathways involved in oogenesis to produce competent female gametes are still incomplete. Omics technologies are being widely used to provide an overview of the processes underlying the reproductive success through gamete production quality. The mRNA content of an oocyte is shared between the need for oogenesis to be completed and the need for successful oocyte-embryo transition until zygotic genome activation, the maternal inheritance. Comparative transcriptomic analysis suggests that this maternally inherited pool of mRNA is evolutionary conserved whereas those mRNAs necessary for gamete production are divergent between species (Evsikov et al., 2006; Sylvestre et al., 2013).

Although many endocrinology aspects are often conserved across species, several specific aspects exist. In teleosts, reproductive strategies can be extremely diverse. For example, eggs can be benthic or pelagic; spawning can occur in salt or fresh water; fish ovaries can be synchronized or asynchronized; or embryos can develop immersed or aerially exposed. Some species still reproduce poorly in captivity, requiring the capture of young animals in the wild to maintain populations in captivity. Strong efforts are therefore being made to improve the quality of reproduction for species of economic and conservation interest. Transcriptomic patterns are now available to use as reference and track molecular marker of gametogenesis. Zebrafish was among the first species used for that purpose. One comparative transcriptomic approach of ovary versus testis revealed more than 3500 genes differentially expressed between the two organs with nearly 50 presenting a large difference in expression, suggesting gene silencing in one of the two sexes (Santos et al., 2007a). Also, while individual variability between mature males does not appear to influence testis transcriptomic pattern, ovaries have been found to have significant variation between individuals (Santos et al., 2007b). The zebrafish ovary is asynchronous, therefore the relative ratio of oogenesis stages across whole ovary is not stable and impacts transcriptomic patterns.

Omics approaches have also been used to understand processes involved in and affecting the endocrine system. For instance, Martyniuk et al. (2007) used a transcriptomics approach to identify novel genomic responses after exposure to 17α-ethinylestradiol (a potent estrogenic compound) in the zebrafish liver and telencephalon, demonstrating that multi-tissue gene profiling is needed to improve understanding of the effects of human pharmaceuticals on aquatic organisms (Martyniuk et al., 2007). Garcia-Reyero et al. (2009a) used transcriptomics approaches to analyze gene expression profiles in male fathead minnow (*Pimephales promelas*)