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Characterization of basal gene expression trends over a diurnal cycle in *Xiphophorus maculatus* skin, brain and liver

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ABSTRACT

Evolutionarily conserved diurnal circadian mechanisms maintain oscillating patterns of gene expression based on the day-night cycle. *Xiphophorus* fish have been used to evaluate transcriptional responses after exposure to various light sources and it was determined that each source incites distinct genetic responses in skin tissue. However, basal expression levels of genes that show oscillating expression patterns in day-night cycle, may affect the outcomes of such experiments, since basal gene expression levels at each point in the circadian path may influence the profile of identified light responsive genes. Lack of knowledge regarding diurnal fluctuations in basal gene expression patterns may confound the understanding of genetic responses to external stimuli (e.g., light) since the dynamic nature of gene expression implies animals subjected to stimuli at different times may be at very different stages within the continuum of genetic homeostasis. We assessed basal gene expression changes over a 24-hour period in 200 select *Xiphophorus* gene targets known to transcriptionally respond to various types of light exposure. We identified 22 genes in skin, 36 genes in brain and 28 genes in liver that exhibit basal oscillation of expression patterns. These genes, including known circadian regulators, produced the expected expression patterns over a 24-hour cycle when compared to circadian regulatory genes identified in other species, especially human and other vertebrate animal models. Our results suggest the regulatory network governing diurnal oscillating gene expression is similar between *Xiphophorus* and other vertebrates for the three *Xiphophorus* organs tested. In addition, we were able to categorize light responsive gene sets in *Xiphophorus* that do, and do not, exhibit circadian based oscillating expression patterns.

1. Introduction

The diurnal or circadian cycle has been shown to affect the physiology of all organisms where it has been studied (Panda et al., 2002a). These physiological changes are coincide with oscillating gene expression patterns that are coordinated to adapt cellular activity to periods of activity and inactivity. The core mechanisms that lead to endogenous circadian oscillation of gene expression involve a transcription-translation feedback loop controlled by transcription factors CLOCK and brain and muscle ARNT-like protein 1 (BMAL), and their antagonistic transcriptional targets Cryptochrome (CRY) and Period (PER). CRY and PER serve as repressors of CLOCK and BMAL transactivation activity (Mohawk et al., 2012). Oscillating expression patterns of these circadian regulators can be entrained by external environmental cues, such as alteration in light periods, feeding patterns, or changes in temperature (Raible et al., 2017; Migaud et al., 2010; Carr et al., 2006; Rensing and Ruoff, 2002).

Teleost fish represent useful vertebrate model systems to investigate circadian gene expression. The diversity of physiological adaptations to extremely varied environments allows exploration of the plasticity in patterns of basal gene expression. Circadian cycle regulator genes have been characterized in several teleost fish species, including zebrafish, medaka, flounder, amberjack, sea bass and blind cavefish (Beale et al., 2013; Cavallari et al., 2011; Cuesta et al., 2014; Sanchez et al., 2010; Toyama et al., 2009; Wang, 2008; Watanabe et al., 2012). Comparative genetic analyses among fish models have identified different regulatory mechanisms associated with basal gene expression. For example, cave-dwelling populations of blind cavefish *Astyanax mexicanus*, compared to non-cave living populations, both exhibit robust circadian cycling of the *per1* gene, but the cave-dwelling blind cavefish show elevated levels of light-induced genes (e.g., *per2*; Beale et al., 2013). The understanding of differences in regulation of basal gene expression, as a result of

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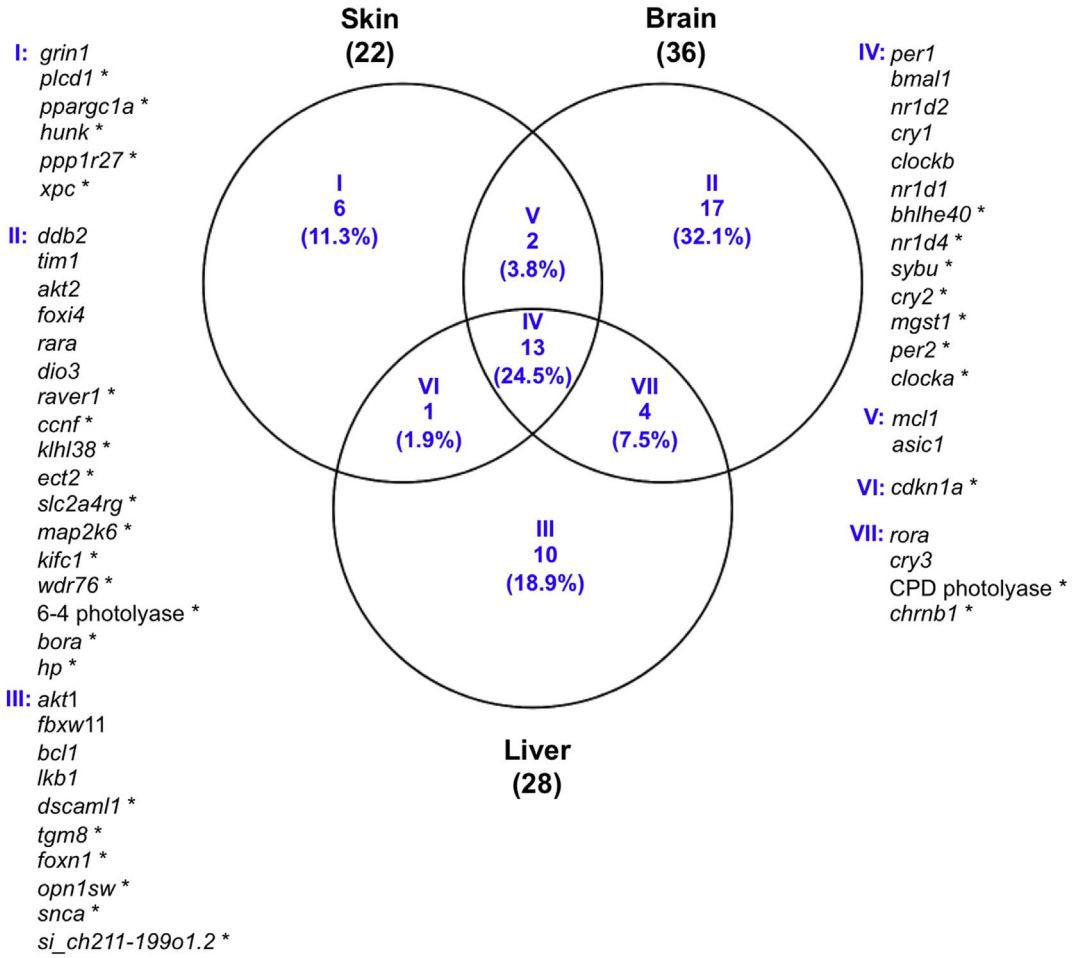

Fig. 1. Basal oscillation of gene expression over 24-hour light-dark cycle in *Xiphophorus* skin, brain and liver. Genes exhibiting basal oscillating expression patterns over a 24 h diurnal cycle were identified using the RAIN algorithm ($p < 0.05$). Among the light responsive and reference circadian gene targets on the NanoString panel, 22 genes were identified in skin, 36 genes were identified in brain and 28 genes were identified in liver that showed circadian oscillation. Genes exhibiting oscillating expression patterns identified from different organs were compared to each other. Organ specific oscillating genes and shared oscillating genes among the three organs are categorized into seven groups. Group I, II and III represent genes that were uniquely identified from skin, brain and liver. Group IV represent genes that are shared by all three organs, and Group V, VI and VII represent genes that are shared by two of the three organs. A NanoString nCounter panel was designed to capture gene expression of known light-responsive genes and known circadian rhythm regulator genes. Asterisk (*) highlights previously identified *Xiphophorus* light-responsive genes.

adaptation to an environment niche, may enhance our understanding of chronobiology. Compared to mammals in which the suprachiasmatic nucleus (SCN) serves as a master oscillation regulator, fish appear to utilize a photoreceptive pineal gland as the autonomous clock to drive melatonin synthesis and circadian rhythm (Bailes and Lucas, 2010). Additionally, fish have been shown to possess oscillation centers in peripheral organs (i.e., “peripheral clocks”) that may be directly entrained by light exposure (Vallone et al., 2004; Whitmore et al., 2000). These attributes make fish attractive models to study circadian gene regulation and light-induced genetic effects.

Xiphophorus represent a tractable vertebrate model that has recently been employed to investigate the molecular genetic responses to exposure from varied light sources and select light wavebands (Boswell et al., 2015; Chang et al., 2015; Lu et al., 2015; Walter et al., 2014; Walter et al., 2015; Yang et al., 2014; Boswell et al., 2017a; Boswell et al., 2017b). Genome sequence and assembly for several *Xiphophorus* species indicates they possess compact genomes retaining remarkable syntenic conservation with mammalian genomes (Amores et al., 2014; Schartl et al., 2013; Shen et al., 2016). Previous studies employing gene expression profiling of *Xiphophorus* skin reported that ultraviolet light effects the transcription of genes associated with apoptosis, cell cycle, circadian rhythm, fatty acid/lipid biosynthesis, wound healing, and cell differentiation (Boswell et al., 2015; Lu et al., 2015; Yang et al., 2014). In contrast, exposure to 4100 K (“cool white”) fluorescent light (FL) was shown to incite a genetic response in *Xiphophorus* skin involving transcriptional suppression of gene sets (< 130 genes) associated with cell cycle progression, chromosome segregation, DNA repair and recombination, as well as expected induction of circadian genes (Walter et al., 2015). Our previous reports showing light induced changes in transcriptional profiles were performed at a single time point (i.e., 7 am) in the normal diurnal or circadian cycle. However, due to oscillating expression patterns, light responsive genes are expected to be in different homeostatic activity states over the circadian cycle, perhaps affecting light induced gene expression. To better understand this experimental parameter, we sought to define basal gene expression patterns inherent to genetic homeostasis through the circadian cycle.

Herein, we assessed basal gene expression levels of previously identified light-responsive genes, to determine if they would exhibit oscillating gene expression patterns. We report gene expression patterns for a selected gene set in *Xiphophorus* skin, brain and liver over a 24-hour period. The three target organs represent external (skin), and internal (brain and liver) organs that play central roles in behavior, physiology, and metabolism. We designed a custom NanoString nCounter panel that contains 65 previously identified light-responsive genes, 60 reference circadian genes, and 10 housekeeping genes as internal controls to measure basal gene expression. Additionally, as tumors are known to have disrupted circadian cycle, for future studies involved with melanoma etiology, we have also assessed diurnal gene