Comparative Biochemistry and Physiology, Part C xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Exposure to 4100 K fluorescent light elicits sex specific transcriptional responses in *Xiphophorus maculatus* skin

William T. Boswell[a], Mikki Boswell[a], Dylan J. Walter[a], Kaela L. Navarro[a], Jordan Chang[a], Yuan Lu[a], Markita G. Savage[a], Jianjun Shen[b], Ronald B. Walter[a,*]

[a] Department of Chemistry and Biochemistry, Xiphophorus Genetic Stock Center, Texas State University, 601 University Drive, San Marcos, TX 78666, USA
[b] Department of Epigenetics and Molecular Carcinogenesis, The University of Texas MD Anderson Cancer Center, Science Park, Smithville, TX 78957, USA

ARTICLE INFO

Keywords:
Xiphophorus
Fluorescent light
RNA-Seq
Differential gene expression
Skin
Transcriptional regulation

ABSTRACT

It has been reported that exposure to artificial light may affect oxygen intake, heart rate, absorption of vitamins and minerals, and behavioral responses in humans. We have reported specific gene expression responses in the skin of *Xiphophorus* fish after exposure to ultraviolet light (UV), as well as, both broad spectrum and narrow waveband visible light. In regard to fluorescent light (FL), we have shown that male *X. maculatus* exposed to 4100 K FL (i.e. "cool white") rapidly suppress transcription of many genes involved with DNA replication and repair, chromosomal segregation, and cell cycle progression in skin. We have also detailed sex specific transcriptional responses of *Xiphophorus* skin after exposure to UVB. However, investigation of gender differences in global gene expression response after exposure to 4100 K FL has not been reported, despite common use of this FL source for residential, commercial, and animal facility illumination.

Here, we compare RNA-Seq results analyzed to assess changes in the global transcription profiles of female and male *X. maculatus* skin in response to 4100 K FL exposure. Our results suggest 4100 K FL exposure incites a sex-biased genetic response including up-modulation of inflammation in females and down modulation of DNA repair/replication in males. In addition, we identify clusters of genes that become oppositely modulated in males and females after FL exposure that are principally involved in cell death and cell proliferation.

1. Introduction

Light is an important exogenous stimulus that has been shown to regulate physiological and behavioral functions in vertebrates (Dijk and Archer, 2009; Kayumov et al., 2007; Kolmos and Davis, 2007). Life evolved over approximately 3 billion years exclusively under full-spectrum sunlight. However, for the past six decades animals, including humans, have transitioned to indoor environments principally illuminated with artificial lighting. In addition, modern indoor spaces are often illuminated by inexpensive energy efficient "cool white" FL designed to maximize apparent brightness. FL is now ubiquitous in homes, schools, and both private and public places of business due to low manufacturing costs, low energy usage, and long bulb life (energy.gov).

Exposure to FL has been documented to increase heart rate, elevate blood pressure, promote inappropriate secretion of hormones, and is associated with increased incidence of cancer, increased stress, and cytoskeletal rearrangement (Heeke et al., 1999; Hollwich and Dieckhues, 1980; Kayumov et al., 2007; Keller et al., 2009; Savvidis and Koutsilieris, 2012; Sripathi et al., 2012). Beral et al. (1982) found that exposure to FL led to an increase in trunk lesions in both men and women, while Mitani and Shima (1995) suggest FL exposure may be involved with oxygen stress and the ability to induce DNA repair proteins (i.e. CPD cyclobutane pyrimidine dimer photolyase). Additionally, we have reported both 6–4 and CPD photolyase induction in varied tissues of *X. maculatus* after FL light exposure (Walter et al., 2014). Surprisingly, the highest post-FL exposure transcriptional induction of these two photolyases was observed in liver, not generally considered an organ involved in primary light photoreception. This led to speculation that light types currently utilized in animal husbandry may alter the genetic state of both light receiving (i.e. skin) and non-light receiving (i.e. liver) organs.

Most attempts to quantify the impact of FL have been restricted to examining behavior and/or physiological parameters. Unfortunately, a comprehensive understanding of the genetic pathways and biological functions involved in response to varied light exposures has not been addressed. However, our recent reports using genome-wide gene

* Corresponding author at: The Xiphophorus Genetic Stock Center, Department of Chemistry and Biochemistry, Texas State University, 419 Centennial Hall, 601 University Drive, San Marcos, TX 78666, USA.
E-mail addresses: wb1016@txstate.edu (W.T. Boswell), mboswell@txstate.edu (M. Boswell), juc355@psu.edu (J. Chang), y_l54@txstate.edu (Y. Lu), markita@txstate.edu (M.G. Savage), jianshen@mdanderson.org (J. Shen), RW12@txstate.edu (R.B. Walter).

http://dx.doi.org/10.1016/j.cbpc.2017.09.008
Received 27 July 2017; Received in revised form 14 September 2017; Accepted 20 September 2017

expression profiling to assess light induced changes in transcriptional patterns indicate FL exposure may be capable of repressing cell cycle progression in the skin of male *X. maculatus* (Walter et al., 2015).

Sexually dimorphic responses to external insult have been reported (Mu et al., 2015; Papale et al., 2016; Rousseau et al., 2016), suggesting gender plays an important role in differentiating physiological responses to stimuli. We have reported sex specific genetic responses to UVB (Boswell et al., 2015), showing gender may play a role in the genetic response to light. A recent review by Boyce (2010) suggests FL may depreciate human health, yet detailed studies of the genetic effects of FL light have not been performed. Given quantifiable and sex-biased genetic responses to UVB exposure, a better understanding of the sex specific genetic responses to FL exposure is warranted. Herein, we report analysis of global transcriptional responses in female and male *X. maculatus* skin after exposure to 4100 K FL. We observe female *X. maculatus* skin up-modulates genes associated with inflammation and cell proliferation responses with concomitant down modulation of genes associated with cell death. This is in stark contrast to male *X. maculatus*, that show down modulation of cell proliferation, DNA repair/replication, and up-modulation of gene clusters associated with cell death.

2. Methods

2.1. Fish utilized

The *Xiphophorus maculatus* Jp 163 B used in this study were supplied by the *Xiphophorus* Genetic Stock Center, Texas State University, San Marcos, TX 78666 (http://www.xiphophorus.txstate.edu). All *X. maculatus* Jp 163 B were mature 9-month-old female or male siblings derived from the same brood of fish that were taken from a line-bred stock in its 104th inbred generation.

2.2. 4100 K fluorescent light exposure

Two fish were exposed to either 5 kJ/m^2, 10 kJ/m^2, or 15 kJ/m^2 (20, 40, or 60 min) of 4100 K FL as previously described (Walter et al., 2014, 2015). Briefly, fish were placed in 125 mL flasks containing 100 mL of filtered aquaria water and kept in the dark 12 h prior to FL exposure. Fish were removed from the dark and transferred to UV transparent cuvettes (9 cm × 7.5 cm × 1.5 cm) containing 70 mL of filtered aquaria water. The exposure cuvettes were placed in a 77 cm (length) × 41 cm (height) × 36 cm (depth) wooden box. Air was circulated through the box with 15.5 cm high speed fans mounted at the bottom on each end of the box to maintain an interior temperature of about 24 °C. The fish in the exposure cuvettes were suspended between two banks (total of 4 lights) of unfiltered 24-in. 4100 K lamps (Philips F20 T12/CW Alto “cool white”) that were mounted horizontally on each interior side of the exposure box. After exposure, fish were returned to 125 mL flasks containing 100 mL of filtered aquaria water and placed in the dark for 6 h to allow time for transcriptional remodeling before being euthanized and dissected for RNA isolation.

2.3. RNA isolation and RNA-Seq

Fish were anesthetized by placing them on ice and sacrificed by cranial resection. Skin samples were immediately placed in 1.5 mL microcentrifuge tubes containing 300 μL TRI Reagent (Sigma Inc., St Louis, MO, USA) and flash frozen in an ethanol dry ice bath. Remaining tissues were placed in individual 1.5 mL microcentrifuge tubes with 300 μL RNAlater (Life Technologies, Grand Island, NY, USA).

RNA was isolated from skin using a TRI Reagent (Sigma Inc., St Louis, MO, USA) chloroform extraction followed by the Qiagen RNeasy (Qiagen, Valencia, CA, USA) isolation protocol. Skin was homogenized in 300 μL TRI Reagent using a handheld tissue disruptor, and an additional 300 μL of TRI Reagent was added to the homogenate, followed by 120 μL of chloroform. Samples were vigorously shaken for 15 s. and then phases partitioned by centrifugation (12,000 x*g* for 5 min at 4 °C). The aqueous phase containing nucleic acids was transferred to a new microcentrifuge tube and extracted a second time (300 μL TRI Reagent and 60 μL chloroform). After extraction, the nucleic acids were precipitated with 500 μL 70% EtOH. RNA was then purified using a Qiagen RNeasy mini RNA kit following the manufacturer's protocol. Residual DNA was eliminated with an on-column DNase treatment at 25 °C for 15 min. RNA quality was assessed by determining the RNA Integrity Number (RIN) with an Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA), and quantified with a Qubit 2.0 fluorometer (Life Technologies, Grand Island, NY, USA). RNA samples that were subject to sequencing had RIN scores ≥ 8.0.

Individual sequencing libraries were constructed using the Illumina TruSeq Stranded mRNA Library Prep Kit with polyA selection. Libraries were sequenced (75 bp, paired-end [PE] reads) on a HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA) at the M.D. Anderson Cancer Center Science Park Next-Generation Sequencing Facility (Smithville, TX). Sequencing adaptors were trimmed from raw sequencing reads, and processed sequencing reads were subsequently filtered using a custom Perl script (Garcia et al., 2012) that removed low-scoring sections of each read while preserving the longest remaining fragments.

2.4. Computational and pathway analyses

GSNAP (Wu and Nacu, 2010) was used to map filtered sequencing reads to the *X. maculatus* reference transcriptome (Ensembl v71). The percentage of reads mapped and the nucleotide coverage was identified using samtools flagstat and samtools depth respectively (Li et al., 2009). Gene expression was assessed by eXpress (http://bio.math.berkeley.edu/eXpress/), and differentially expressed genes (DEGs) were determined using the R-Bioconductor (www.bioconductor.org) package edgeR (Robinson et al., 2010) with a fold change (FC) ≥ |2.0| and False Discovery Rate (FDR) < 0.05. Two experimental replicates were used for DEG analysis. DEGs that were based solely on gender prior to FL exposure (i.e. comparing female and male 0 min controls) were removed from the datasets prior to FL DEG analysis.

After removing sex specific basal DEGs, single gene lists for each sex were constructed of genes differentially modulated in at least 2 of the 3 exposures (5 kJ/m^2, 10 kJ/m^2, and 15 kJ/m^2); all genes that exhibited differential expression in 2 of the 3 exposures are listed in Table S1. The shared and unique genetic response for *X. maculatus* female and male skin were quantified with http://www.venndiagrams.net.

Ingenuity Pathway Analysis (IPA, Qiagen, Redwood City, CA) was used for clustering, assessing effected genetic pathways and the biological function (i.e. functional class) behind gene expression data, and for suggesting up-stream regulators to aid in mechanistic interpretation. Herein, the term “pathways” is short for canonical pathways assigned by IPA based on the light exposure input DEG data. Known pathways are drawn as pictures with input DEGs overlaid onto them that are identified by symbols and colors indicating known functions and direction of modulation. A z-score algorithm is used to determine if a pathway is up or down regulated based on the genes that fall into that particular pathway. IPA assignment of DEGs into “functions” or “functional classes” relates the input DEGs to known disease states and biological functions as published in the scientific literature. Functional classes are visualizations of the biological trends in the light effected DEG dataset and may be used to predict the effect of gene expression changes of the entire dataset on biological processes and known cellular functions. Function assignment uses an algorithm to assess the dataset as a whole and predict what is collectively occurring on a larger downstream scale.

In this dataset, genetic pathways, functional classes, and/or biological processes of the shared and unique gene sets identified with venndiagrams.net were analyzed with IPA. ConsenuspathDB (http://consensuspathDB.org) with *p*-values < 0.01 was also used to visualize genetic pathways due to its ability to query multiple (12) online