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Exposure to Fluorescent Light Triggers Down Regulation of Genes Involved with Mitotic Progression in *Xiphophorus* Skin

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Abstract

We report RNA-Seq results from skin of *X. maculatus* Jp 163 B after exposure to various doses of “cool white” fluorescent light (FL). We show that FL exposure incites a genetic transcriptional response in skin nearly as great as observed for UVB exposure; however, the gene sets modulated due to exposure to the two light sources are quite different. Known light responsive genes involved in maintaining circadian cycling (e.g., *clock*, *cry2a*, *cry1b*, *per1b*, *per2*, *per3*, *arntl1a*, etc.) exhibited expected shifts in transcriptional expression upon FL exposure. Exposure to FL also resulted in down-regulated transcription of many genes involved with cell cycle progression (e.g., *cdc20*, *cdc45*, *cdca7b*, *plk1*, *cdk1*, *ccnb-3*, *cdca7a*, etc.) and chromosome segregation (e.g., *cenpe*, *cenpf*, *cenpi*, *cenpk*, *cenpo*, *cenpp*, and *cenpu*; *cep70*; *knstrm*, *kntc*, *mcm2*, *mcm5*; *smc2*, etc.). In addition, several DNA replication and recombination repair genes (e.g., *pola1*, *pole*, *rec52*, *rad54l*, *rpa1*, *parpbp*, etc.) exhibit reduced expression in FL exposed *X. maculatus* skin. Some genes down modulated by FL are known to be associated with DNA repair and human diseases (e.g., *atm2*, *brip1*, *fancl*, *fancl*, *xrcc4*, etc.). The overall suppression of genes involved with mitotic progression in the skin of adult fish is consistent with entry into the light phase of the circadian cycle. Current efforts are aimed at determining specific wavelengths that may lead to differential expression among the many genes affected by fluorescent light exposure.

Keywords

Fluorescent light; cool white light; RNA-Seq; Differential gene expression; Cell cycle progression; Circadian; DNA damage response

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Introduction

Over the past decade physiological and psychological effects of artificial fluorescent lighting on humans have been shown to be significant and quantifiable (McColl and Veitch, 2001). Both the amount of light and composition of lighting are important parameters associated with human health. Differences in the inflammatory response to specific light wavelengths have been shown between human males and females (Hurlbert and Ling, 2007; Vandewalle, et al, 2010). Other published reports suggest human physiological response to full spectrum vs. “cool white” lamps include differences in oxygen intake, heart rate, absorption of vitamins and minerals, etc (Wurtman, 1975a,b; Holick, 1996; McColl and Veitch, 2001). However, despite behavioral and physiological studies indicating artificial light sources are important to animal health, there exists a paucity of data regarding specific molecular genetic responses that occur in the intact animal upon exposure to varying types of artificial lighting.

Tropical fishes, such as *Xiphophorus*, may be expected to be both very sensitive and responsive to varying light conditions. In the wild, fishes must utilize light conditions for warmth, predation, predator avoidance, and to coordinate breeding cycles. Given the increasing set of genomic tools currently available, *Xiphophorus* represent a tractable vertebrate model to investigate the molecular genetic responses to light composition and varied light wavelengths.

Among the many documented *Xiphophorus* interspecies hybrid melanoma tumor models are a few known to exhibit melanoma induction only after exposure to ultraviolet light (UVB) (Setlow et al., 1989; Setlow et al., 1993; Nairn et al., 1996; Mitchell et al, 2010). Tumor induction studies using the UVB-inducible *Xiphophorus* hybrid tumor models have shown that post-UVB exposure to fluorescent light (FL) greatly diminishes or completely negates melanoma induction (Setlow *et al*, 1989, Setlow et al, 1993). This loss of UVB tumorigenic effect by exposure to FL is widely considered to be due to rapid repair of UVB-induced DNA damage by lesion specific DNA photolyases that are active in fish skin (Ahmed and Setlow, 1993; Meador et al, 2000; Mitchell et al, 2001). Studies from various fish species have shown fish photolyase gene expression is induced by exposure to visible light (Hart et al, 1977; Uchida et al, 1997; Armstrong et al, 2002). To assess this in *Xiphophorus*, we used quantitative real time PCR (qRT-PCR) to follow the expression levels of two photolyase genes (i.e., cyclobutane pyrimidine dimer and 6-4 photoproduct photolyases) in *Xiphophorus* skin upon exposure to FL (Walter et al, 2014). As expected, skin from parental fish (*X. maculatus* or *X. couchianus*) showed visible light induction of photolyase gene expression ($\approx 2-3$ fold). However, skin from interspecies hybrid fish (*X. maculatus* $\times$ *X. couchianus* F₁ interspecies hybrids) appeared to have lost the ability to upregulate transcription of these two photorepair proteins upon FL exposure (Walter et al, 2014). Thus, it becomes difficult to explain loss of UVB-induced melanoma by post-UVB FL exposure solely based upon removal of CPD or 6-4 lesions in hybrid fish skin.

Here, we report RNA-Seq results from skin of *X. maculatus* after exposure to various doses of FL. We show that FL exposures incite a transcriptional response nearly as great in amplitude as that observed after UVB exposure (Yang et al, 2014) and is consistent with

suppression of cell cycle progression as has been observed upon entry into the light phase of the circadian cycle. Although we identified a set of genes in fish skin that are similarly affected by both UVB and FL exposure, only FL serves to down-regulate expression of genes in critical pathways expected to curtail mitotic progression.

Materials and Methods

Fish Utilized and Fluorescent Light Exposure

Fish utilized were mature male *X. maculatus* Jp 163 B, 10 months of age, from the 101st generation (pedigree 101A and D) of sibling inbreeding were used. Skin from 4 individual fish treated as experimental fish but without FL exposure, were utilized for RNA isolation and Illumina sequencing (controls). For each FL time point (20, 40, and 60 min) three fish were individually exposed and RNA from the skin of 2 of these exposed fish was subjected to Illumina sequencing and utilized used for RNA-Seq analysis (Table 1). Power output of the FL light source was determined using a Newport 1918-R power meter (Newport Corporation, Irvine, CA, USA) with a 40 min. FL dose determined to be 10 kJ/m². This is close to the UVB dose of 8kJ/m² utilized in previous analyses of differential gene expression of *X. maculatus* Jp 163 B skin (Yang et al, 2014 and Figure 1). Prior to light exposure, each fish was placed into an individual 125 mL flask filled with 100 mL of filtered water from their home aquaria and kept in the dark 14 hrs. Fish from these individual flasks were randomly chosen for FL light exposure or to remain unexposed and serve as controls.

Light exposures were carried out essentially as previously detailed (Walter et al, 2014; Yang et al, 2014) except that in these studies fluorescent lights (FL) were employed. Briefly, FL exposure occurred in a specially designed wooden box (77 cm in length, 41 cm in height, and 36 cm in depth), with a hinged wooden lid capable of sealing the interior of the box from external light. On the bottom of each of the two sides (41 cm × 36 cm) were 15.5 cm diameter high-speed fans that maintained interior temperatures of the closed box at less than 24°C. For FL exposures single fish were placed into UV transparent (UVT) plastic cuvettes (9 cm × 7.5 cm × 1.5 cm) in about 95 ml water and the cuvettes were suspended in a rack centered between and about 10 cm from the FL bulbs inside the exposure chamber. All animals were kept in the dark 12 hrs prior to exposure. A bank of “cool white” fluorescent lamps (Philips F20T-12/D, 4100K “cool white” lamps) provided FL exposures (20, 40 or 60 min).

After FL exposure, fish were maintained in the dark for 6 hrs to allow for gene expression changes prior to sacrifice and tissue dissection. At dissection fish were anesthetized in an ice bath and upon loss of gill movement were sacrificed by cranial resection. Skin were dissected directly into Tri-Reagent (Sigma Inc. St. Louis) placed in a dry ice-ethanol bath if the RNA was isolated at the time of dissection, or into RNALater (Ambion Inc. Austin, TX) and kept at -80°C for RNA isolation at a later time.

RNA isolation and RNA sequencing

Total RNA was isolated as previously detailed (Walter et al., 2014; Yang et al., 2014) using Tri-Reagent (Sigma Inc., St. Louis, MO, USA). Skin from each individual fish was homogenized in 600 µl Tri-Reagent followed by addition of 120 µl chloroform and the samples vigorously shaken and subjected to centrifugation at 12,000 g for 15min at 4°C. Total RNA was further purified using RNeasy mini RNA isolation kit (Qiagen, Valencia, CA, USA). Residual DNA was eliminated by DNase digestion on the column at 25°C for 15 min. RNA integrity was assessed by gel electrophoresis (2% agarose in TAE running buffer) and total RNA concentration was determined using a spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). RNA quality was verified on a 2100 Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) to confirm that RIN scores were above 8 prior to sequencing.

RNA sequencing was performed on libraries constructed using the Illumina TrueSeq library preparation system that employs a polyA selection. RNA libraries were sequenced as 75 bp paired-end fragments using an Illumina Hi-Seq 2000 system (Illumina, Inc., San Diego, CA, USA). 70–80 million raw reads were generated for each fish skin RNA sample. All raw reads were subsequently truncated by similarity to library adaptor sequences using a custom Perl script (Table 1). Short reads were then trimmed and filtered based on quality scores by using a custom filtration algorithm that removed low-scoring sections of each read and preserved the longest remaining fragment (Garcia et al., 2012). Our read depth was no less than 40x (O-D sample) for any sample and averaged about 58.5x (Table 1).

Computational Analyses

Filtered reads were mapped to the *X. maculatus* reference transcriptome (Ensembl v71) using Bowtie2 (<http://bowtie-bio.sourceforge.net/bowtie2/index.shtml>), (Langmead et al, 2009; Langmead and Salzberg, 2013) and a custom Perl script to quantify gene expression. The percentage of reads mapped and unmapped as well as the nucleotide coverage was identified using SAMtools flagstat and depth respectively (Li et al., 2009). Differentially expressed (DE) genes were determined using the R-Bioconductor (www.bioconductor.org) package DESeq (Anders and Huber, 2010) with a fold-change cutoff of ± 2 and a *p*-adj 0.05. Briefly, a gene expression count table containing raw counts, that represent how many short reads align to each gene was calculated by eXpress (Roberts, 2013) and used as the input file of DESeq. The raw count of each gene was then normalized by the size of each sample. The test for differential expression is based on a model using the negative binomial distribution. We defined differentially modulated genes as genes that have $\log_2$ Fold Change ($\log_2FC$) -1 or 1 , with a False Discovery Rate Adjusted *p*-value (*p*-adj) 0.05 between light-exposed skin samples and unexposed skin samples.

Determination of Basal Gene Expression Determination

To normalize gene expression levels on both transcript length and RNA-Seq library size, the eXpress generated gene expression raw count values were used to calculate Reads Per Kilobase per Million reads (RPKM) values, where $RPKM = [(raw\ counts/total\ reads\ count\ per\ library) \times 1\ million]/(transcript\ length/1000)$. We defined a gene as expressed if its RPKM value was > 0.3 (Ramskold et al, 2009).

Biological Functional Analysis

Interacting genes may be expected to show nonrandom patterns in cell localization, molecular function, and/or biological process. Bioinformatics analysis of exposure data was performed using DAVID (<http://david.abcc.ncifcrf.gov>). DAVID allowed significant DE genes in each gene set to be clustered into functional gene ontologies. GeneMania (www.genemania.org, Warde-Farley et al., 2010) was used to visualize interactions and associations of gene networks. Nodes are differentially colored to designate between queried (black) and predicted (grey) genes.

NanoString

RNA isolated from *X. maculatus* skin was subjected to NanoString nCounter analysis (<http://www.nanostring.com/applications/technology>) to assess gene expression by direct counting (NanoString technologies, Seattle, WA). Hybridization protocols were strictly followed according to manufacturer's instructions (Geiss *et al.*, 2008) at the Baylor College of Medicine Microarray Core Facility (Houston, TX). Hybridized samples (500 ng RNA in 7 μ L of solution) were incubated overnight at 65°C with a custom set of probes in the NanoString Prep Station and immediately evaluated with the NanoString nCounter based on color code signals. Data analysis was performed by lane normalization using a set of standard NanoString probes followed by sample normalization using a set of 10 housekeeping genes. Counts were generated by nCounter Digital Analyzer. Fold changes were calculated on normalized counts and plotted using Microsoft Excel. Two biological samples previously used in RNA-Seq analysis were selected for NanoString. Supplemental Table S1 presents the NanoString custom designed probe set for 18 test targets and 10 housekeeping controls.

Quantitative real time PCR

Quantitative real time PCR (qRT-PCR) was performed as previously described (Yang et al., 2014; Walter et al., 2014). Seven transcripts that exhibited differential modulation in response to FL by RNA-Seq analyses were confirmed using qRT-PCR. Briefly, cDNA synthesis was performed with 100 mM dNTPs and 10X RT random primers, RNase inhibitor, and Multiscribe Reverse Transcriptase mixed with 1.5 μ g of RNA in a 20 μ L reaction. Thermocycler conditions for cDNA synthesis were 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min.

Each cDNA reaction was diluted to a final volume of 500 μ L and quantified using a Qubit 2.0 fluorometer (Life Technologies, Grand Island, NY). Each sample was analyzed with qRT-PCR on Applied Biosystems 7500 Fast Real Time PCR System (Applied Biosystems, Carlsbad, CA, USA). Primers used for qRT-PCR were designed using Geneious (Biomatters Ltd., Auckland, New Zealand) software version 6.1. Primer design included melting temperatures (T_m) between 60 and 62°C (50 mM Na, 3 mM K, and 0.8 mM dNTPs), lengths of 18–25 nt with 55–60% GC content, and not more than 1°C between T_m s of each primer in a set. Target amplicons were limited to 100–150 bps in length, and were designed to amplify across at least one intron-exon junction. Primers specific for the *Xiphophorus* transcripts (Table S2) were tested for efficiency and only primers with efficiency values between 70 and 120% were utilized in downstream analyses. Each primer set was used to

test the relative expression of each gene from cDNAs (15 ng) produced from *X. maculatus* skin in four qRT-PCR technical replicates. All plates were run using the SYBR Green protocol as previously described (Walter et al., 2014; Yang et al., 2014) wherein amplification for each target is normalized against the 18S rRNA transcript to determine CT values. UV modulated fold changes are relative to 0 point control (CT) values.

Results and Discussion

Genes up- and down-modulated in *Xiphophorus* skin by FL exposure

Data on the basal transcription activity of tissues from human and mouse studies have shown the number of genes expressed in most tissues varied from 11,000 to 13,000 applying a threshold of 0.3 RPKM. This corresponds to roughly 60–70% of RefSeq protein-coding genes (Ramskold et al, 2009). As many as 7,897 genes (42%) are observed to be ubiquitously expressed in all tissues and cell lines. Further, compared to the other tissues, testis was a clear outlier, expressing more than 15,000 different genes (84% of RefSeq genes) (Ramskold et al, 2009). Applying the same threshold constraints to RNA-Seq data generated for 10 individual skin samples derived from control *X. maculatus* Jp 163 B (i.e. dissected after 14hrs dark incubation) we observe 16,505 $\pm$ 147 genes to be transcriptionally active in the fish skin. This is about 80.5% of the total gene models queried in the transcriptome (20,498 gene models; [ftp.ensembl.org/pub/release-80/fasta/xiphophorus_maculatus/cdna/Xiphophorus_maculatus.Xipmac4.4.2.cdna.all.fa.gz](ftp://ftp.ensembl.org/pub/release-80/fasta/xiphophorus_maculatus/cdna/Xiphophorus_maculatus.Xipmac4.4.2.cdna.all.fa.gz)). The large fraction of active genes observed, approaching the fraction observed in mammalian testes, highlights the dynamic nature and complexity of fish skin compared to the skin of mammals (Rakers et al, 2010; Xu et al, 2013).

Our conservative application of DEseq to the RNA-Seq data derived from FL exposed skin samples identified 522 genes to be differentially expressed (DE). Thus, we observe significant up- or down-transcriptional modulation for about 2.5% of the gene models, or about 3.0% of the number of genes expected to have been active in the fish skin before exposure to one of the three FL doses (20, 40 or 60 min.; Table S3).

The total number of DE genes (up or down modulated) in skin due to UVB (8 kJ/m²; 322) or FL light exposures of 20 min (271), 40 min (282), and 60 min (274) are presented in Figures 1 and 2. Overall, 522 differentially expressed (DE) genes from the transcriptome queried (20,218 gene models; [ftp.ensembl.org/pub/release-80/fasta/xiphophorus_maculatus/cdna/Xiphophorus_maculatus.Xipmac4.4.2.cdna.all.fa.gz](ftp://ftp.ensembl.org/pub/release-80/fasta/xiphophorus_maculatus/cdna/Xiphophorus_maculatus.Xipmac4.4.2.cdna.all.fa.gz)) showed significant up- or down-transcriptional modulation for at least one FL exposure (presented in Table S3). Of these genes, 11 DE genes are currently “uncharacterized” while 511 could be assigned gene names with presumed encoded protein functions.

The data show FL exposure resulted in a transcriptional response nearly as great as observed previously for exposure to 8 kJ/m² UVB exposure (311 nm; see Yang et al, 2014). For example, by magnitude of the DE response, the 40 min FL exposure (282 genes) represents 87.5% of the UVB response (322 genes). However, UVB exposure results in a much higher fraction of up-regulated genes (54%) compared to FL exposure at 20 (38%), 40 (32%), or 60 min (48%) (Figure 1).

All genes that exhibited significant DE in two or more exposure time points are listed in Table S4 (216 genes). Of the 216 DE genes in Table S4, 6 were “uncharacterized” and could not be assigned a gene name after extensive homology comparisons to Ensembl and NCBI databases. Of the remaining 210 genes in this set, 115 (55%) were clustered into five overrepresented functional groups (discussed below) while the remaining 101 named genes did not segregate into clearly defined functional clusters, but appeared to represent a large and varied group of pathways.

Shared genes modulated by FL or UVB exposure

Comparing the sets of differentially expressed genes from each FL exposure (20, 40, and 60 min), we identified 91 genes that are DE similarly across all three FL doses (Figure 2). Additionally, nearly half of the responsive genes are common between subsequent time points with over 40% of the DE genes shared between the shortest and longest exposures (Figure 2).

A set of 32 genes were identified as similarly affected by UVB or FL exposure (Figure 3). This set of 32 genes represents genes known to be responsive to light exposure, associated with mitosis, or involved in intercellular signaling. Interestingly, the light-responsive (*ybx2*, *sox3*) and circadian cycle genes (*clock*, *per2*) are up-regulated, while many of the down-regulated genes play a role in mitosis (*aspm*, *cenpe*, *cenpf*, *FAM64a*, *kif14*, *kif20a*, *kif22*, *knstrn*, *kpn2*, *tacc3*, etc.), cell cycle control (*ccnb1*, *cdc20*), or intercellular signaling (*raver1*, *arhgap19*, *depdc1a*). Outside of this small set of 32 genes, FL exposure modulates a fundamentally different set of genes in *Xiphophorus* skin than does UVB (Yang et al, 2014).

Clustering of the FL Results

DAVID gene ontology was employed to identify biological associations among the 210 named genes modulated in *X. maculatus* skin by exposure to FL and shared between two or more exposures (Table S4). Statistical significance was restricted for each category to p-values < 0.005. This analysis showed very strong clustering of 115 DE genes modulated in two or more exposures into five functional categories; Light Responsive (17 genes, p-value 6.60e-06), Oxidation/Reduction (14 genes, p-value 3.10e-04), Mitosis and Cell Cycle (38 genes, p-value 1.00e-39), Chromosome Structure (24 genes, p-value 1.10e-04), and DNA Replication and Repair (22 genes, p-value 4.60e-11).

Of the 210 named genes showing DE in two or more FL exposures, 69 (33%) were up-modulated. However, after segregation of the 115 DE genes into five functional clusters, we observe only 23 (20%) up-modulated while 92 (80%) exhibited suppressed transcription after FL exposure. Among these up-regulated genes, 19 of the 23 genes are disproportionately present in only 2 of the 5 functional classes; Light Responsive and Oxidation/Reduction. The 4 genes that exhibited up-modulation in the other 3 functional classes (*ybx2*, Chromosome structure; *dusp1* and *pim1*, Mitosis and Cell Cycle; and *nupriL*, DNA Replication and Repair) each has shared functions that could have also resulted in their being assigned to the Light Responsive or Oxidation/Reduction classes. For example, *ybx2* was up-regulated in both UVB and FL light exposures (Figure 3), while *dusp1* is

known to be responsive to oxidative stress and may also function within the Oxidation/Reduction functional class.

Inspection of the 115 genes DAVID segregated into the five functional classes (Table S4) shows many DE genes that could have been assigned to two or more classes (e.g., Mitosis/Cell Cycle and DNA Replication/Repair, or Mitosis/Cell Cycle and Chromosome Structure, etc.) as one might expect from these overlapping functional categories. Even so, the majority of genes within each functional category are clearly related by both functional and genetic interaction (Figure 4).

Light responsive genes modulated by FL exposure

Seventeen genes are known to be light responsive and are largely up-regulated after FL exposure (Table 2). Many members of this cluster are circadian associated genes (*arntl1a*, *bhlhe40*, *clock*, *cry2a*, *npas2*, *per1b*, *per2*, *per3*, *timeless*) and are well-documented to exhibit light-affected transcriptional regulation (for reviews see Somade, 2014; Borgs, et al, 2009). These genes behaved exactly as one might predict from previously published studies. In addition, other genes previously shown to exhibit light dependent regulation and associated with DNA repair (*cipc*, *il12b*, *junb*, *nfil3-2*) or genes we have observed in many independent experiments to be light inducible (*dnah7*, *fggy*, *klhl38b*, *opn1*) (Caballero, 2015; Chang et al, 2015) appear in this category.

Overall the behavior of these known light responsive genes, showing similar values in all three exposures (11 of 17), with the overwhelming up-regulation (10 of the 17 genes) and the down regulation of specific circadian genes (i.e., *bhlhe40*, *per1b*, *per 3*, and *timeless*) upon FL light exposure is in agreement with previous published reports (Somade, 2014; Johnson, 2010).

Oxidation/reduction

Fourteen genes associated with Oxidation/Reduction states are observed modulated in skin upon exposure to FL light (Table 3). Of these, 9 are up-regulated. The spectrum of the “cool white” FL light (Supplemental Figure 1) used for these exposures shows some emission in the lower blue-violet wavelengths with two peaks of emission between 400–450 nm. This region may be expected to result in absorption by many different cellular photosensitizers (i.e. melanin, flavins, heme, etc.) and photoactive proteins (Horner and Weber. 2012). Proteins that require light in this region often use flavin or modified rhodopsin as a photoreceptor to become active (e.g., photolyases and flavin, Li et al., 1991; Mitani et al., 1996). For example, the cryptochrome family proteins, involved in circadian phasing, have many members that undergo photoactivation (Bouly et al., 2007). It seems likely the intense FL light encountered by the fish skin in these exposures led to excited electron states in cellular photosensitizers that may have resulted in production of reactive oxygen species (ROS). In addition, the regulation of heavy metals within the cell becomes important in the presence of light energy. Consistent with this, several genes up-regulated in the Oxidation/Reduction category are expected to encode proteins that bind metals (e.g., *cyp24a1*, *ferritin*, *smox*, etc.). Others in this cluster are involved with peroxide metabolism (*gpx6*, *ehhadh*), aromatic amino acid or sterol metabolism (*akr1d1*, *hgd*, *pah*, *tm7sf2*), associated with the

control of mitochondria-derived reactive oxygen species (*ucp2*, *chdh*), or involved with general metabolic activities (*grhph*, *hpdb*). Overall, these 14 genes exhibit a very strong cluster p-value (3.10×10^{-4}) as an Oxidation/Reduction functional class. However, as with most proteins, overlapping functions of the encoded proteins in several cellular functional classes is very likely.

Mitosis, cell cycle and chromosome structure

One of the most striking co-regulatory shifts in the transcription of skin RNA after FL exposure is a generalized suppression of 62 genes (i.e., 38 in the Mitosis and Cell Cycle, Table 4; and 24 in Chromosome Structure, Table 5) that are collectively associated with cell cycle progression and mitosis. Genes encoding protein components of the centromeres, kinetochore, and involved with spindle attachment (*cenpe*, *cenpf*, *cenpi*, *cenpk*, *cenpo*, *cenpp*, *cenpu*, *cep70*, *haus5*, *kif14*, *kif15*, *kif20a*, *kif20b*, *kif22*, *kif18a*, *kif23*, *kifc*, *kifc1*, *knstm*, *kntc12*, *ndc80*, *nuf2*, *ttk*), chromosome condensation and chromatin structure (*aspm*, *ckap2l*, *chtfl8*, *esp1*, *ncapg*, *smc2*, *pik1*), as well as cell cycle regulators and kinases (*aurka*, *bub1*, *ccb1*, *ccb2*, *ccb3*, *cdc7a*, *cdc20*, *cdkn1d*, *cit*, *ect2*, *pask*, *pim1*, *prc1a*, *prc1b*, *ybx2*) all appear suppressed in *Xiphophorus* skin after exposure to FL light. Other cell cycle associated genes are similarly affected and many in this group also share function, not only between Mitosis/Cell Cycle and Chromosome Structure categories, but also with the DNA Replication and Repair cluster of genes (*gins4*, *mcm2*, *cmc5*, *pttg1*, *wdhd1*, etc.). Overall, this list of suppressed genes suggests cessation of mitotic progression in cells that prior to exposure were actively dividing or preparing to do so. GeneMania analysis of genes in these functional classes show genetic association and strong pathway association among the encoded gene products (Figure 4). This level of interaction may be expected in a coordinated response to light as has been reported for cells entering the light phase of the circadian cycle (Borgs et al., 2009; Johnson, 2012; Somade, 2014). Circadian control of the cell cycle has been demonstrated in most organisms including mammalian cultured cells, rodent models (Reppert and Weaver, 2002; Destici et al, 2011) and circadian-entrained mitotic activity has been shown active in human skin (Bjarnason and Jordan, 2002). Zebrafish has been shown to possess light regulated cell cycling in larvae and evidence of peripheral circadian clock oscillators by direct light absorption has been suggested (Whitmore et. al, 1998; Whitmore et. al, 2000; Dekens et al, 2003; Peyric et al, 2013). The results shown here extend this work into live-bearing fish and imply the cell cycle stalls in the skin of adult male *Xiphophorus* upon FL exposure. Given the time of exposure and dissection it is likely this occurs via direct light absorption by the skin and thus implicates peripheral circadian oscillators that gate cell cycle progression upon FL light exposure.

DNA replication and repair

A large number of FL DE genes (20) segregated into the functional class of DNA Replication and Repair (Table 6). Several of these genes are involved with replication as one may expect during S phase of the cell cycle (i.e., *cdc45*, *orc1*, *rpa1*, *rnaseh2a*) and these share functional similarity with several genes that are segregated into both the Mitosis and Cell Cycle, and Chromosome Structure classes (*gins4*, *mcm2*, *chtfl8*, *cmc5*, *pttg1*, and *wdhd*). This repression of transcription in genes encoding products that function in S phase may suggest that cells actively in mitosis, at the time of FL exposure, cease their mitotic

progression. This would be in addition to the stalling of cells at the G₁-S transition by reduction in many mitotic and cell cycle regulators (see above).

Quite a few of the DNA repair genes suppressed in light-exposed skin are thought to be involved with recombination (*rad52*, *rad54l*) and are noteworthy in being associated with human disorders when mutated (i.e., *atm*, ataxia telegentasia; *brip1* and *hmmr*, BRCA1 complex; *fanci* and *fancl*, Fanconi's anemia; *xrcc4* and *rpa1*, xeroderma pigmentosum class D). Other genes that become suppressed upon FL exposure are also associated with DNA repair processes (*cpd-phr*, *cit*, *neil3*, *pif1*) as well as DNA repair polymerases (*polα1* and *pole*).

The observed suppression on DNA repair gene transcription is consistent with dramatically reduced cell division in the skin, and is likely due to circadian control upon light exposure.

NanoString and qRT-PCR Results

Validation of a subset of RNA-Seq gene expression values was performed by analysis of 18 individual transcripts using a custom NanoString nCounter panel. Each exposure (20, 40 and 60 min FL) was subject to nCounter analysis; ten transcripts were differentially modulated following 20 min FL, four transcripts were differentially modulated following 40 min FL and 13 transcripts were differentially modulated following 60 min FL. Generally, the direction and magnitude of gene expression change was confirmed for all 18 genes, with an R² value of 0.86 (Figure 5 and Table 7).

In addition to the NanoString nCounter analysis, an additional confirmation of 7 DE gene targets was performed using qRT-PCR. Like the nCounter analysis, all reported fold changes from the RNA-Seq analysis agreed with the qRT-PCR in direction (Figure 6).

Conclusions

Fluorescent light exposure, from commonly utilized “cool white” lights, induced a robust genetic response (~ 87% of the response induced by UVB) in the skin of male *X. maculatus* across all examined FL exposures. The FL response is significantly different than that observed from UVB exposure (Figure 2). The FL genetic response appears to saturate at the earliest exposure (20 min.) since the genes modulated remain relatively constant in number, type, and direction of effect though 60 min.

One-third of the genes that demonstrated significant DE after FL which includes over half of the genes modulated in at least two of the three FL doses, cluster into 5 functional classes (Table S4): (1) Light Responsive, (2) Oxidation/Reduction, (3) Mitosis and Cell Cycle, (4) Chromosome Structure, and (4) DNA Replication and Repair (Tables 2–6).

Exposure to FL results in depression of transcription levels for of a large set of genes involved in mitosis and cell cycle progression, as well as genes involved in the repair (recombination) and replication of DNA. Repression of this gene set in the Mitosis/Cell Cycle and Chromosome Structure categories shows genetic interaction and encoded gene products that are functionally related (Figure 4).

Tumor induction studies using the UVB inducible *Xiphophorus* interspecies hybrid models aimed at determination of the wavelengths responsible for melanomagenesis have been controversial. While it is established that UVB exposure can induce melanoma in susceptible *Xiphophorus* hybrids (Nairn *et al*, 1996; Patton *et al*, 2010; Mitchell *et al*, 2010) there are also reports showing UVA may play a major role in tumor induction (Setlow *et al*, 1993; Wood *et al*, 2006). We have shown that exposure to as little as 20 min of fluorescent light may substantially decrease the transcription levels of genes involved in cell cycle regulation, mitosis, chromosome structure, and DNA repair. While fluorescent light does not induce melanoma the molecular genetic effects of FL exposure in fish skin appear to be substantial. Thus, the light conditions prior to a UV tumor induction experiment have the potential to play a role in the outcome. It remains to be seen if *Xiphophorus* hybrid skin behaves genetically as the parental *X. maculatus* Jp 163 B utilized herein. In previous studies of DNA repair, *Xiphophorus* hybrid fish have been shown to lose light induced regulation or have reduced DNA repair capability compared to their parental lines (Mitchell *et al*, 2004; David *et al*, 2004; Walter *et al*, 2014).

The rather rapid and sustained (20 to 60 min) reduction on transcription of genes involved in cell cycle progression suggests peripheral circadian oscillators may exist in *Xiphophorus* skin capable of gating the cell cycle. It remains to be determined how different light sources, with substantially varied spectral emission, may affect such mechanisms. Having evolved in a full spectrum environment, the genes of tropical fishes such as *Xiphophorus*, are likely to be fine tuned to a full spectrum of wavelengths that fluorescent lighting does not mimic. As a result, one cannot say the genetic effect shown herein is “normal”, but only that it is observed upon “cool white” fluorescent light exposure. At the minimum, given the cost associated with maintaining aquatic research animals (*Axolotl*, *Xiphophorus*, zebrafish, *Fundulus*, medaka, *Aplysia*, sea urchins, etc.) more investigations are warranted to ensure lighting conditions utilized are most appropriate. Given the amount of fluorescent light that humans and animals are subjected to on a daily basis, further investigation of these effects would be prudent.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The total number of up- and down-modulated genes in *X. maculatus* Jp 163 B skin due to UVB (8 kJ) or FL light exposures (20, 40, or 60 min). Within each bar are numbers of genes with increased transcription levels (top bar), or genes exhibiting depressed transcription levels (bottom bar) compared to the skin of unexposed sibling animals. The total number of genes showing modulated transcription (DE genes) are 322 for UVB, 271 for 20 min FL, 282 for 40 min FL, and 274 at 60 min FL.

Figure 2.

Venn diagram comparison of DE genes from each FL exposure (20, 40, and 60 min). There are 134 genes in common between the 20 min (49%) and 40 min (48%) time points, 147 genes in common between the 20 min (54%) and 60 min (54%) time points, and 117 genes in common between the 40 min (41%) and 60 min (43%) time points. Among all three exposures, 91 DE genes are shared and expressed similarly (see Table S4).

Figure 3.

Relative transcription levels of a set of genes identified as similarly affected by UV or FL exposure. This set of 32 genes include known light responsive genes (which are upregulated) and genes involved in mitosis and intercellular signaling (which are downregulated). For instance, light responsive (*ybx2*, *sox3*) and circadian cycle genes (*clock*, *per2*) are up regulated while many of down regulated genes play a role in mitosis (*aspm*, *cenp-e*, *-f*, *fam64a*, *kif-14* and *-20a*, *knstrn*, *kpna2*, *tacc3*, etc.), cell cycling (*ccnb1*, *cdc20*), or intercellular signaling (*raver1*, *arhgap19*, *depdc1a*).

Figure 4.

Genetic associations of chromosome structure and mitosis genes differentially modulated in at least 2 time points (20, 40, and 60 min FL exposure) in *X. maculatus* JP 163 B skin. Biological associations are identified through genetic interaction (green lines) and pathways (blue lines). Grey circles are genes predicted through the GeneMania algorithm to be associated with query genes (black circles).

Figure 5.

NanoString analysis confirmed the RNA-Seq data for 18 genes in both direction and magnitude ($R^2 = 0.86$) following 20 (red), 40 (green) and 60 (blue) min FL exposure. Fold changes are represented as the average change of 2 biological replicates determined independently for each target plotted and at each exposure.

Figure 6.

RNA-Seq validation (20 min FL- light grey; 40 min FL- dark grey) was performed on seven targets using qRT-PCR following 20 (red) and 40 (green) min FL exposure. Mean fold change relative to an unexposed sample was calculated using the $\Delta\Delta C_T$ method with an 18S rRNA internal standard for 3 technical replicates.

Table 1

RNA-Seq and read mapping statistics.

Exposure	Filtered Reads	Reads Mapped	% Mapped	Coverage
0 Control A	44,846,553	41,104,701	73.4	67.1X
0 Control B	44,699,687	40,949,014	73.4	66.8X
0 Control C	32,175,519	30,439,113	73.4	49.7X
0 Control D	24,988,902	24,178,684	73.4	39.5X
FL –20 min A	43,612,238	39,827,303	73.6	65.0X
FL –20 min B	47,343,728	42,550,103	75.7	69.5X
FL –40 min A	48,980,752	43,639,847	76.8	71.2X
FL –40 min B	56,608,706	45,536,298	74.8	74.3X
FL –60 min A	57,118,191	52,357,400	73.8	85.5X
FL –60 min B	48,035,072	43,179,638	78.2	70.5X

Light responsive genes showing DE upon FL exposure (20, 40 and 60 min). Shaded boxes are up-regulated while down-regulated are unshaded. np = not significant for that exposure.

Table 2

Ensembl ID	Gene	Fold Change			Description
		20	40	60	
ENSXMAT000000015691	<i>arntl1a</i>	2.75	2.33	2.35	aryl hydrocarbon receptor nuclear translocator-like 1a
ENSXMAT00000002257	<i>bhlhe40</i>	-3.74	-2.41	-2.44	basic helix-loop-helix family, member e40
ENSXMAT000000017010	<i>clock</i>	3.62	3.51	3.45	clock
ENSXMAT000000008440	<i>cipc</i>	-2.07	np	-2.07	clock interacting pacemaker
ENSXMAT000000009327	<i>cry2a</i>	3.32	np	3.13	cryptochrome 2a
ENSXMAT000000007381	<i>dnah7</i>	3.72	2.74	3.62	dynein, axonemal, heavy chain 7
ENSXMAT000000018361	<i>fggy</i>	-3.34	-3.47	-3.47	FGGY carbohydrate kinase domain containing
ENSXMAT00000000472	<i>il12bb</i>	6.61	3.83	3.58	interleukin 12B, with IL23 activates Jak/Stat pathway
ENSXMAT000000019606	<i>junbb</i>	2.50	np	3.55	jun B proto-oncogene b
ENSXMAT000000008690	<i>klh138b</i>	14.81	19.37	17.14	kelch-like 38b (Drosophila)
ENSXMAT000000019605	<i>nfil3-2</i>	2.54	2.56	2.34	nuclear factor, interleukin 3 regulated, member 2
ENSXMAT000000012307	<i>npas2</i>	3.47	np	3.71	neuronal PAS domain protein 2, SAD associated
ENSXMAT000000013132	<i>opn1sw1</i>	-2.45	np	-4.04	opsin 1 (cone pigments), short-wave-sensitive 1
ENSXMAT000000015376	<i>perl1b</i>	-2.49	-2.92	-2.45	period homolog 1b (Drosophila)
ENSXMAT000000016248	<i>per2</i>	3.54	2.84	4.42	period homolog 2 (Drosophila)
ENSXMAT000000004015	<i>per3</i>	-3.11	-2.63	-2.01	period homolog 3, p53 degradation
ENSXMAT000000003313	<i>timeless</i>	-2.00	-2.29	np	timeless homolog (Drosophila), stress response, pole

Table 3

Oxidation/reduction responsive genes showing DE upon FL exposure (20, 40, and 60 min). Shaded boxes are up-regulated while down-regulated are unshaded. np = not significant for that exposure.

Ensembl ID	Gene	Fold Change			Description
		20	40	60	
ENSXMA.T000000004428	<i>akr1d1</i>	2.27	2.76	2.21	aldo-keto reductase family 1, member D1
ENSXMA.T00000019354	<i>chdh</i>	-3.39	-3.80	-2.96	choline dehydrogenase
ENSXMA.T00000010260	<i>cyp24a1</i>	4.49	np	3.27	cytochrome P450, family 24, subfamily A, polypeptide 1
ENSXMA.T00000015599	<i>ehhadh</i>	-2.94	-3.46	np	Enoyl-CoA hydratase/3-hydroxyacyl Co A dehydrogenase
ENSXMA.T00000008621	LOC102238129	2.46	np	2.53	ferritin, middle subunit-like; 100% identity
GPX6.CDS	<i>gpx6</i>	-2.66	np	-4.42	Glutathione Peroxidase 6 (Olfactory)
ENSXMA.T000000008475	<i>grhpr</i>	-2.53	np	-2.60	glyoxylate reductase/hydroxypyruvate reductase a
ENSXMA.T00000019083	<i>hgd</i>	2.58	3.72	2.69	homogentisate 1,2-dioxygenase
ENSXMA.T00000015288	<i>hpdh</i>	2.27	2.75	2.24	4-hydroxyphenylpyruvate dioxygenase b
ENSXMA.T00000017348	<i>pah</i>	2.28	3.08	2.50	phenylalanine hydroxylase
ENSXMA.T00000005538	<i>smox</i>	3.12	2.22	np	spermine oxidase
ENSXMA.T00000014172	<i>snca</i>	4.18	4.02	np	synuclein, alpha (non A4 component of amyloid precursor)
ENSXMA.T00000005176	<i>tm7sf2</i>	-5.94	-3.91	-5.22	transmembrane 7 superfamily member 2
ENSXMA.T00000011982	<i>ucp2</i>	2.59	2.76	2.61	uncoupling protein 2, mitochondrial oxidation/reduction

Mitosis and cell cycle progression genes showing DE upon FL exposure. Shaded boxes are up-regulated while down-regulated are unshaded. np = not significant for that exposure.

Table 4

Ensembl ID	Gene	Fold Change			Description
		20	40	60	
ENSXMAT00000000723	<i>aspm</i>	-3.15	-5.80	-4.14	asp (abnormal spindle)-like, coordination of mitotic processes.
ENSXMAT00000004556	<i>bub1</i>	-3.41	-3.47	np	BUB1 mitotic checkpoint ser/threo kinase, Mitotic complex activating spindle checkpoint
ENSXMAT000000008618	<i>cnb1</i>	-4.09	-4.78	-5.01	cyclin B1
ENSXMAT00000014334	<i>cnb2</i>	-4.92	-4.78	-5.71	cyclin B2 role in the regulation of cytokinesis.
ENSXMAT00000015109	<i>cnb3</i>	-3.47	-3.68	-3.27	cyclin B3
ENSXMAT00000016054	<i>cde20</i>	-4.71	-4.84	-6.77	cell division cycle 20 homolog
ENSXMAT00000013526	<i>cenpi</i>	-2.08	-2.25	np	centromere protein I, Mitotic porgression, Kinetochore assembly
ENSXMAT00000000137	<i>cenpk</i>	-3.25	np	-2.93	centromere protein K
ENSXMAT00000015198	<i>cenpo</i>	-2.39	-2.86	-2.61	centromere protein O, Centromere, spindle assembly
ENSXMAT00000010514	<i>cenpp</i>	-2.14	np	-2.18	centromere protein P, Centromere-kinetochore
ENSXMAT00000004074	<i>cenpu</i>	-2.63	-2.78	-2.36	centromere protein U, Kinetochore assembly, replaces histone H3
ENSXMAT00000018911	<i>cep70</i>	-3.05	-3.25	np	centrosomal protein
ENSXMAT00000018396	<i>cit</i>	-2.40	-4.71	-2.40	citron rho-interacting ser/threo kinase
ENSXMAT00000016865	<i>ckap2l</i>	-2.47	-2.67	-2.30	cytoskeleton associated protein 2-like, Mitotic spindle formation and cell cycle progression
ENSXMAT00000005117	<i>diaph3</i>	-3.18	-4.03	-3.18	diaphanous homolog 3 (Drosophila), Required for cytokinesis
ENSXMAT00000009693	<i>dusp1</i>	2.25	np	2.69	dual specificity phosphatase 1, Skin fibroblast oxidative stress, inactivates MSAP kinase
ENSXMAT00000014150	<i>ect2</i>	-4.97	-5.23	-4.88	epithelial cell transforming sequence 2 oncogene
ENSXMAT00000004776	<i>g2e3</i>	np	-5.03	-4.85	G2/M-phase specific E3 ubiquitin ligase
ENSXMAT00000001883	<i>gins4</i>	-2.35	-2.48	np	GIN5 complex sub4 (Sld5 homolog), DNA replication progression, S phase
ENSXMAT00000006058	<i>kif14</i>	np	-4.44	-3.79	kinesin family member 14, Important role in cytokinesis, spindle
ENSXMAT00000013566	<i>kif15</i>	-2.16	-3.61	np	kinesin family member 15, Spindle assembly
ENSXMAT00000012995	<i>kif20a</i>	-3.64	-4.65	-3.71	kinesin family member 20A, Mitotic segregation
ENSXMAT00000004879	<i>kif20b</i>	-2.71	-4.29	-2.40	kinesin family member 20Ba, Chromosome movement, G2 phase, Cytokinesis
ENSXMAT00000018645	<i>kif22</i>	-2.75	-2.98	-2.81	kinesin family member 22
ENSXMAT00000015773	<i>kif2c</i>	np	-3.92	-3.96	kinesin family member 2C, Important role in cytokinesis, spindle
ENSXMAT00000007687	<i>knstrn</i>	-3.64	-4.01	-3.42	kinetochore-localized astrin/SPAG5 binding protein, Mitotic segregation
ENSXMAT00000017710	<i>kntc1</i>	-2.87	-3.72	-2.43	kinetochore associated 1

Ensembl ID	Gene	Fold Change				Description
		20	40	60		
ENSXMAT000000008371	<i>kpan2</i>	-3.99	-4.52	-4.88		karyopherin alpha 2 (RAG cohort 1, importin alpha 1), Essential for cell cycle control G2/M
ENSXMAT000000008384	<i>masl</i>	np	-3.68	-2.48		microtubule associated serine/threonine kinase-like, entry into M phase
ENSXMAT000000000306	<i>mcm2</i>	-2.29	-3.26	-2.08		MCM2 minichromosome maintenance deficient 2, MCM complex, S phase entry
ENSXMAT000000004575	<i>mcm5</i>	-2.03	-2.42	np		MCM5 minichromosome maintenance deficient 5, MCM complex. Go-G1/S phase replication
ENSXMAT000000011043	<i>MTBP</i>	-3.13	-3.65	-2.24		MDM2 binding protein
ENSXMAT000000006891	<i>nuf2</i>	-2.06	-2.32	np		NUF2, NDC80 kinetochore complex component, Centromere Prophase
ENSXMAT000000011193	<i>numa1</i>	-3.09	-3.97	-4.43		nuclear mitotic apparatus protein 1, Circadian clock gene
ENSXMAT000000008117	<i>phf19</i>	-2.69	-2.96	-2.23		PHD finger protein 19, binds histone H3-traHC3, transition from active to repressed state.
ENSXMAT000000016450	<i>pim1</i>	2.21	np	2.66		pim-1 oncogene, Cell cycle progression, regulates MYC
ENSXMAT000000012882	<i>plk1</i>	-3.47	-4.78	-4.40		polo-like kinase 1, Master regulator of M phase, centrosome/spindle many other functions
ENSXMAT000000009875	<i>wldh1</i>	-2.14	-2.91	np		WD repeat and HMG-box DNA binding protein, Replication initiation

Chromosome structure genes showing DE upon FL exposure. Shaded boxes are up-regulated while down-regulated are unshaded. np = not significant for that exposure.

Table 5

Ensembl ID	Gene	Fold Change			Description
		20	40	60	
ENSXMAT00000006097	<i>aurka</i>	np	-3.56	-3.87	aurora kinase A, ser/thr kinase important for cell cycle progression and p53
ENSXMAT00000000581	<i>cdca7b</i>	-2.77	-2.42	-2.04	cell division cycle associated 7b
ENSXMAT000000004312	<i>cdkl1d</i>	2.23	2.18	2.14	cyclin-dependent kinase inhibitor 1D, Circadian regulator
ENSXMAT000000010065	<i>cenpe</i>	-2.71	-4.45	-2.87	centromere protein E, Kinetochore, chromosome alignment and movement
ENSXMAT000000015178	<i>cenpf</i>	-2.76	-4.41	-3.27	centromere protein F, (mitotin), Centromere at G2, localizes to spindle in anaphase & telophase
ENSXMAT000000007642	<i>chtf18</i>	-2.09	-2.55	np	CTF18, chromosome transmission fidelity factor 1, Chromosome cohesion, S phase
ENSXMAT000000000722	<i>depdc1a</i>	-3.04	-4.58	-4.26	DEP domain containing 1a, represses A20 leading to transport of NF- κ B
ENSXMAT000000002159	<i>espl1</i>	-3.00	-4.42	-3.27	extra spindle poles like 1, Mitotic spindle function
ENSXMAT000000018919	<i>haus5</i>	-2.92	-3.16	np	HAUS augmin-like complex, subunit 5, Involved in mitotic spindle, vital to spindle assembly
ENSXMAT000000003800	<i>kif18a</i>	-2.48	-3.05	np	kinesin family member 18A, Kinetochore function, regulates CENPE
ENSXMAT000000003706	<i>kif23</i>	-2.58	-3.70	-2.71	kinesin family member 23, Chromosome movement, spindle, cytokinesis
ENSXMAT000000016886	<i>kifc1</i>	np	-3.96	-3.26	kinesin family member 2C, Important role in cytokinesis, spindle
ENSXMAT000000009865	<i>ncapg</i>	np	-3.54	-3.11	non-SMC condensin II complex, subunit D3, condensation of chromosomes during mitosis
ENSXMAT000000017114	<i>ndc80</i>	-2.29	-2.40	-2.10	NDC80 homolog, kinetochore complex component, Kinetochore, chromosome segregation
ENSXMAT000000015146	<i>pass</i>	-2.53	-2.78	-2.21	PAS domain ser/threo kinase, Regulates changing environmental conditions, energy homeostasis
ENSXMAT000000006913	<i>prc1a</i>	np	-3.44	-3.49	protein regulator of cytokinesis 1a
ENSXMAT000000018132	<i>prc1b</i>	-2.90	-4.04	-3.91	protein regulator of cytokinesis 1b
ENSXMAT000000013737	<i>pttg1</i>	-2.20	np	-2.34	pituitary tumor-transforming 1, Chromosome stability, DNA repair, negatively regulates p53
ENSXMAT000000004597	<i>smc2</i>	-2.58	-3.11	-2.38	structural maintenance of chromosomes 2, Critical for mitotic condensation and DNA repair
ENSXMAT000000019592	<i>sox3</i>	2.96	2.33	3.38	SRY-box containing gene 3
ENSXMAT000000010119	<i>spag5</i>	-2.81	-3.57	-3.20	sperm associated antigen 5, Functions at spindle, chrom. segregation, anaphase progression
ENSXMAT000000013574	<i>tacc3</i>	-2.51	-2.92	-3.02	transforming, acidic coiled-coil containing protein 3, Stabilization of the mitotic spindle
ENSXMAT000000007097	<i>tkk</i>	np	-3.34	-2.98	tkk protein kinase, centrosome alignment, mitotic segregation
ENSXMAT000000015815	<i>ybx2</i>	3.59	2.91	3.81	Y box binding protein 2, transcription factor and RNA binding

Table 6

DNA replication and repair genes showing DE upon FL exposure. Shaded boxes are up-regulated while down-regulated are unshaded. np = not significant for that exposure.

Ensembl ID	Gene	Fold Change			Description
		20	40	60	
ENSXMAT00000017007	<i>atm-2</i>	-6.54	-12.28	-3.53	ATM serine/threonine kinase, DNA repair, ATM (Ataxia telangiectasia), recombination
ENSXMAT00000005761	<i>brp1</i>	-2.26	-2.87	-2.15	BRCA1 interacting protein C-terminal helicase 1, BRCA1 Complex, also called FANCI
ENSXMAT00000014828	<i>cdc45</i>	-2.36	-2.29	np	CDC45 cell division cycle 45 homolog, Required for Initiation of DNA replication
ENSXMAT000000004764	<i>cpd-phr</i>	np	2.03	2.35	deoxyribopyrimidine photolyase like
ENSXMAT00000000980	<i>fanci</i>	-2.44	-3.43	np	Fanconi anemia, compl. group I, DS break DNA repair, FANC complexes and BRCA1
ENSXMAT000000007264	<i>fanc1</i>	-3.55	-3.43	np	Fanconi anemia, complementation group L
ENSXMAT00000010557	<i>hmmr</i>	-3.00	-4.08	-2.59	hyaluronan-mediated motility receptor, complex with BRCA1, BRCA2, DNA repair
ENSXMAT00000016798	<i>map2K6</i>	-3.86	-4.09	-2.71	mitogen-activated protein kinase 6
ENSXMAT00000011013	<i>mdc1</i>	-2.24	-3.08	np	mediator of DNA-damage checkpoint1, G2-M phase checkpoint control, BRCA1
ENSXMAT000000004105	<i>mms22l</i>	-2.31	-2.62	np	MMS22-like, DNA repair protein, Genome integrity during replication
ENSXMAT00000002242	<i>nei3</i>	-2.42	-3.32	np	nei endonuclease VIII-like 3 (E. coli), Oxidative damage N-glycosylase
ENSXMAT000000020089	<i>nupr1l</i>	2.22	3.76	2.46	nuclear protein, transcriptional regulator, 1-like
ENSXMAT00000013066	<i>orc1</i>	-2.34	-3.22	np	origin recognition complex, subunit 1, Part of complex for replication initiation, S phase
ENSXMAT00000017412	<i>parbp</i>	-5.24	-7.44	-9.15	PARP1 binding protein
ENSXMAT00000003968	<i>pif1</i>	np	-5.89	-7.52	PIF1 5'-3' DNA helicase homolog (S. cerevisiae) replication and repair
ENSXMAT00000019149	<i>pol1</i>	-2.25	-3.08	np	polymerase (DNA directed), alpha 1, Initiation of Replication, S phase
ENSXMAT00000007844	<i>pole</i>	-2.45	-3.69	np	polymerase (DNA directed), epsilon, DNA repair and replication
ENSXMAT000000009167	<i>rad52</i>	-2.58	-2.61	np	RAD52 homolog (S. cerevisiae), Double strand break repair and recombination
ENSXMAT000000000633	<i>rad54l</i>	-2.21	-2.88	np	RAD54-like (S. cerevisiae), Interaction with BRCA1 complex
ENSXMAT000000004146	<i>rpa1</i>	-2.41	-3.10	np	replication protein A1, Replication and DNA repair, xeroderma pigmentosum
ENSXMAT000000006984	<i>maseh2a</i>	-2.51	-2.56	-2.45	ribonuclease H2, subunit A, recombination, removal of priming RNA
ENSXMAT000000006766	<i>xrcc4</i>	-2.47	np	-2.78	X-ray repair complementing repair in Chinese hamster 4, XPD, DS break repair

Table 7

NanoString targets (18) tested confirmed RNA-Seq determined fold changes following 20, 40 and 60 min of FL exposure.

Exposure	Ensembl ID	Gene Name	NanoString	RNA-Seq
20 min FL	ENSXMAT00000002257	<i>bhlhe40</i>	-2.9	-2.0
	ENSXMAT00000002359	<i>nr1d4b</i>	3.4	2.5
	ENSXMAT00000015815	<i>ybx2</i>	2.6	2.2
	ENSXMAT00000017010	<i>clock</i>	2.7	1.9
	ENSXMAT00000000471	<i>adrb2b</i>	3.0	2.9
	ENSXMAT00000008690	<i>klhl38b</i>	3.6	4.9
	ENSXMAT00000009163	<i>tgm8</i> *	11.8	3.4
	ENSXMAT00000014172	<i>snca</i>	2.9	2.0
	ENSXMAT00000016248	<i>per2</i>	5.8	3.5
	ENSXMAT00000016798	<i>map2k6</i>	-2.5	-2.0
40 min FL	ENSXMAT00000009327	<i>cry2a</i>	2.3	2.0
	ENSXMAT00000015815	<i>ybx2</i>	2.2	2.1
	ENSXMAT00000017010	<i>clock</i>	2.5	2.1
	ENSXMAT00000016248	<i>per2</i>	1.9	2.8
60 min FL	ENSXMAT00000004757	<i>ccnf</i>	-2.0	-2.2
	ENSXMAT00000014334	<i>ccnb2</i>	-2.1	-2.8
	ENSXMAT00000008371	<i>kpna2</i>	-2.1	-2.6
	ENSXMAT00000011193	<i>numa1</i>	-2.0	-2.4
	ENSXMAT00000014517	<i>arhgap19</i>	-1.9	-2.1
	ENSXMAT00000015815	<i>ybx2</i>	2.1	2.3
	ENSXMAT00000017010	<i>clock</i>	2.0	3.9
	ENSXMAT00000003968	<i>pif1</i>	-3.9	-3.3
	ENSXMAT00000008690	<i>klhl38b</i>	3.8	5.2
	ENSXMAT00000009163	<i>tgm8</i>	6.6	3.5
	ENSXMAT00000016248	<i>per2</i>	7.0	4.4
	ENSXMAT00000016798	<i>map2k6</i>	-2.3	-2.0
	ENSXMAT00000019435	<i>xpc</i>	3.6	2.2

* *tgm8* confirmed the RNA-Seq determined fold change in direction but did not correlate in magnitude and was therefore not included Figure 5.