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Characterization and differential expression of CPD and 6–4 DNA photolyases in *Xiphophorus* species and interspecies hybrids ☆

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ABSTRACT

Among the many *Xiphophorus* interspecies hybrid tumor models are those that exhibit ultraviolet light (UVB) induced melanoma. In previous studies, assessment of UVB induced DNA damage and nucleotide excision DNA repair has been performed in parental lines and interspecies hybrids. Species and hybrid specific differences in the levels of DNA damage induced and the dark repair rates for cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine pyrimidine photoproducts (6–4PPs) have been reported. However, UVB induced DNA lesions in *Xiphophorus* fishes are thought to primarily be repaired via light dependent CPD and 6–4PP specific photolyases. Photolyases are of evolutionary interest since they are ancient and presumably function solely to ameliorate the deleterious effects of UVB exposure. Herein, we report results from detailed studies of CPD and 6-4PP photolyase gene expression within several *Xiphophorus* tissues. We determined photolyase gene expression patterns before and after exposure to fluorescent light in *X. maculatus*, *X. couchianus*, and for F_1 interspecies hybrids produced from crossing these two parental lines (*X. maculatus* Jp 163 B × *X. couchianus*). We present novel results showing these two photolyase genes exhibit species, tissue, and hybrid-specific differences in basal and light induced gene expression.

1. Introduction

The various cellular effects of ultraviolet light (UVB) exposure on vertebrate organisms have been well studied and continue to be of scientific interest (McKenzie et al., 2003). In humans, UV exposure has been linked with increased risk of skin cancers including cutaneous malignant melanoma (Armstrong et al., 1997; Jemal et al., 2006). Despite the inherent interest in induced biological effects of UVB (290–320 nm), animal models that lend themselves to experimentally controlled UVB exposure and melanoma induction are few (Walter and Kazianis, 2001). *Xiphophorus* fish interspecies hybrids have emerged as a tractable experimental model to study the genetics underlying UV induced melanoma (Nairn et al., 2001; Walter and Kazianis, 2001; Mitchell et al., 2010). Efforts to better understand UVB induced melanoma have led to many reports detailing UVB induced DNA damage and relative rates of repair of UVB induced DNA photoproducts. In particular, UVB induction and repair of cyclobutane pyrimidine dimers (CPDs) and 6–4 pyrimidine pyrimidine photoproducts (6–4PPs) has been assessed in several *Xiphophorus* species and interspecies hybrids (Meador et al., 2000; Mitchell et al., 2001; Fernandez et al., 2012).

Penetration of UVB through the epidermis may cause a litany of DNA photoproducts (Rastogi et al., 2010), the most common of which are CPDs and 6–4PPs. UVB induced DNA damage may ultimately lead to activation of growth factors and is associated with an increasing incidence of human melanoma worldwide (Marks, 2000). In previous studies it has been shown that UVB induced DNA damage (CPDs and 6–4PPs) may lead to an increase in transcription of many DNA repair genes (Funayama et al., 1996), including two distinct photolyase proteins that possess specificity to repair CPDs and 6–4PPs upon exposure to visible light (Kim et al., 1996; Sancar, 2003). The repair mechanism for CPD photolyase has been defined in much greater detail than the more complex 6–4 photolyase mechanism. CPD photolyase repair occurs via photon absorption, triggering electron release into a tunneling pathway that serves to split the CPD cyclobutane ring, while the 6–4 photolyase is thought to perform repair using a mechanism involving cyclic proton transfer between the photolyase and 6–4PP substrate (Li et al., 2010).

Genes encoding CPD and 6–4 photolyases have been shown experimentally to be inducible by exposure to fluorescent light (Hirayama et al., 2009; Rastogi et al., 2010). In vertebrates these two photolyase genes are members of the cryptochrome superfamily and have only been studied in a few select organisms (opossum, amphibians, and

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several fishes). Research into photolyase gene level effects is confounded by an undercurrent of confusing gene nomenclature exacerbated by recent sequencing of many new genomes having disparate annotation of cryptochrome gene family members that may or may not include CPD and 6–4 photolyases. The cryptochrome gene family is comprised of a large number of genes that are known to function in maintenance of circadian rhythm, contain similar cofactors, often exhibit light induced transcription, and with the exception of the photolyases do not possess DNA repair capability. This lack of repair capability in most cryptochrome superfamily members is largely due to C-terminal extensions that may be used to distinguish cryptochromes from photolyases (Eker et al., 2009).

Here we present identification of putative *Xiphophorus* CPD and 6–4 photolyase genes based on sequence similarity to *Xenopus*, zebrafish (*Danio rerio*), and medaka (*Oryzias latipes*, Japanese rice fish) photolyases that have been functionally charaterized. We show results of both phylogenetic and syntenic analyses of photolyase genes in *Xiphophorus* and other fishes that ensure identification of true platyfish CPD and 6–4 gene orthologs. We present novel quantitative real time PCR (qRT-PCR) data showing basal expression profiles for both CPD and 6–4 photolyase genes in several organs and induced expression levels after exposure to fluorescent light. We report these data for two *Xiphophorus* species (*X. maculatus* Jp 163 B and *X. couchianus*) and for F_1 interspecies hybrids produced from crossing these two species.

2. Materials & methods

2.1. Identification of CPD and 6–4 photolyase orthologs in Xiphophorus

The medaka CPD (Funayama et al., 1996), zebrafish 6–4 (Kobayashi et al., 2000), and both CPD and 6–4 photolyases from *Xenopus* (Kim et al., 1996; Todo et al., 1997; Tanida et al., 2005) have been cloned and their respective proteins functionally verified.

Therefore, we used these gene sequences as queries for BLAST searches of the platyfish genome within Geneious vR6 (http://www.geneious.com/). The platyfish exons were annotated by alignment with the query genes by applying their gene models.

2.2. Syntenic analysis

Genomicus v72.01 (http://www.genomicus.biologie.ens.fr/genomicus-72.01/cgi-bin/search.pl) (Louis et al., 2013) was used to examine positions of the predicted platyfish CPD and 6–4 photolyase genes within conserved syntenic regions of many other fishes (Supplemental Fig. 1; for supplemental materials see http://www.xiphophorus.txstate.edu/publications-data/publishedworks-older/supplement.html).

2.3. Phylogenetic analysis

Xiphophorus and other CPD and 6–4 photolyase sequences were identified using the Gene Tree in Ensembl vs.71 (http://apr2013.archive.ensembl.org/index.html); all genus and species names and Ensmbl IDs for these taxa are provided in Supplemental Table 1. The gar photolyase sequences for these genes were identified by similarity — searching the Pre Ensembl genome and manually annotated the coding sequence based on similarity to other annotated photolyases. In addition to the taxa representing CPD and 6–4 photolyase in Fig. 1, several sequences that were functionally verified representatives of CRY1, and CRY4 were also included to increase the rigor of the analysis (data not shown). Protein sequences ($n = 46$) were aligned using the Opalescent v2.1 (Wheeler and Kececioglu, 2007) module in Mesquite v2.75 (Maddison and Maddison, 2011; http://mesquiteproject.org). The accuracy of the alignment was assessed by ensuring that known conserved regions (e.g., conserved Trp triad residues) were generally aligned for all taxa in the dataset. Phylogenetic analysis was performed using MrBayes v3.2.1 (Ronquist and Huelsenbeck, 2003), with 2 million generations and discarding a burn-in fraction of 25%. The analysis included 2 independent runs using the WAG model for amino acid substitution (Whelan and Goldman, 2001), which had been identified as the best-fitting model in prior model-test runs. Convergence of the two runs was verified visually with Tracer v1.5 (Rambaut and Drummond, 2007; http://tree.bio.ed.ac.uk/software/tracer/) and using the convergence diagnostic from MrBayes, and the trees retained after 25% burnin were used to build a consensus tree. The consensus tree was visualized and midpoint-rooted using Archaeopteryx v0.9813 A1ST (Han and Zmasek, 2009).

2.4. Fishes utilized

All fishes utilized were supplied by the *Xiphophorus* Genetic Stock Center, Texas State University, San Marcos, TX 78666 (http://www.*Xiphophorus*.txstate.edu). All parental and F_1 hybrid fishes were mature males between 9 and 12 months old (Fig. 2). The *X. maculatus* Jp 163 B (pedigree 100B) was in its 100th generation of sibling inbreeding, while the *X. couchianus* (pedigree 77B) was in its 77th generation of inbreeding (Walter et al., 2006; http://www.xiphophorus.txstate.edu/stockcenter/stockcentermanual.html).

Interspecies hybrids were produced by mating the two parental lines (*X. maculatus* Jp 163 B × *X. couchianus*) in their 99th and 75th inbred generations, respectively.

2.5. Fluorescent light exposures

Fluorescent light (FL) exposures were carried out 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 14 h prior to exposure. FL exposures were 2 h with a bank of "cool white" fluorescent lamps (Philips F20T-12/D, 4100K "cool white" lamps) filtered through Mylar 500D to exclude any wavelengths <320 nm.

After FL exposure, fish were maintained in the dark for appropriate times (see Results) to allow for gene expression prior to sacrifice and tissue dissection. At dissection fish were sacrificed using a lethal dose of anesthesia (MS-222) and skin, as well as other organs, dissected directly into RNAlater (Ambion Inc., Austin, TX, USA), frozen, and stored at −80 °C.

2.6. RNA isolation

Total RNA was isolated after maceration of liquid nitrogen-frozen whole tissues using a pestle followed by resuspension in TRizol (Invitrogen, Carlsbad, CA, USA). RNA was further purified using 5Prime phase lock gel tubes (ThermoFisher Scientific, Waltham, MA, USA). Any residual DNA was eliminated by performing column DNase I digestion at 37 °C (30 min). The integrity of RNA was determined by gel electrophoresis (2% agarose in TAE running buffer) and concentration was determined using a spectrophotometer (Nano Drop Technologies, Willmington, DE, USA).

2.7. Quantitative real time PCR

Total RNA isolated from tissues of two independent biological replicates of highly inbred parental and hybrid fishes. Isolated RNA was used