Marine Genomics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Marine Genomics

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

The Cryptochrome/Photolyase Family in aquatic organisms

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ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form 5 February 2014
Accepted 10 February 2014
Available online xxxx

Keywords:
Light
Aquatic environments
Marine
Cryptochrome
Photolyase
Diatoms
Annelids
Sea urchins
Teleosts
Rhythms

ABSTRACT

The Cryptochrome/Photolyase Family (CPF) represents an ancient group of widely distributed UV-A/blue-light sensitive proteins sharing common structures and chromophores. During the course of evolution, different CPFs acquired distinct functions in DNA repair, light perception and circadian clock regulation. Previous phylogenetic analyses of the CPF have allowed reconstruction of the evolution and distribution of the different CPF super-classes in the tree of life. However, so far only limited information is available from the CPF orthologs in aquatic organisms that evolved in environments harboring great diversity of life forms and showing peculiar light distribution and rhythms. To gain new insights into the evolutionary and functional relationships within the CPF family, we performed a detailed study of CPF members from marine (diatoms, sea urchin and annelid) and freshwater organisms (teleost) that populate diverse habitats and exhibit different life strategies. In particular, we first extended the CPF family phylogeny by including genes from aquatic organisms representative of several branches of the tree of life. Our analysis identifies four major super-classes of CPF proteins and importantly singles out the presence of a plant-like CRY in diatoms and in metazoans. Moreover, we show a dynamic evolution of *Cpf* genes in eukaryotes with various events of gene duplication coupled to functional diversification and gene loss, which have shaped the complex array *of Cpf* genes in extant aquatic organisms. Second, we uncover clear rhythmic diurnal expression patterns and light-dependent regulation for the majority of the analyzed *Cpf* genes in our reference species.
Our analyses reconstruct the molecular evolution of the CPF family in eukaryotes and provide a solid foundation for a systematic characterization of novel light activated proteins in aquatic environments.

1. Introduction

Light is a key environmental signal for life in terrestrial and aquatic habitats. Required as energy source for photosynthesis, light is also an important source of information about the surrounding environment. Rhythmic light changes were characteristic to earth's environment long before the dawn of life and now provide vital input to synchronize basic biological phenomena, allowing species to optimize their growth, propagation and survival (Roenneberg and Merrow, 2005; Dodd et al., 2005). However, light can also be harmful to life. The ultraviolet (UV) component of sunlight can induce several types of DNA damage, which can result in mutagenesis and cell death (Sancar, 2003). Therefore, the capacity to perceive and properly respond to light has been an essential factor affecting growth, development and evolution of all organisms on Earth (Kami et al., 2010; Moglich et al., 2010; Rodriguez-Romero et al., 2010; Chaves et al., 2011a; Gomelsky and Hoff, 2011).

The Cryptochrome/Photolyase Family (CPF) constitutes a large group of UV-A/blue-light activated proteins widely distributed throughout all organisms (Chaves et al., 2011a). They share a common

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http://dx.doi.org/10.1016/j.margen.2014.02.001
1874-7787/

structural organization, with a conserved photosensory domain to which two chromophore cofactors can be bound (Sancar, 2003; Chaves et al., 2011a). The chromophores serve as the primary site of photon absorption and confer to the CPFs their specific photochemical and photophysical properties (Moglich et al., 2010). In all CPFs, a catalytic Flavin Adenine Dinucleotide (FAD) chromophore is located at the C-terminus part of the photosensory domain and differences in the FAD redox states affect both spectral and functional properties of the different CPF members (Chaves et al., 2011a). In some CPFs, another chromophore, a Pterin or an 8-hydroxy-5-deazaflavin, can be bound to the N-terminus part of the photosensory domain acting as a light harvesting cofactor (Chaves et al., 2011a).

The CPF family is classically divided in two types of proteins: the Photolyases and the Cryptochromes (Sancar, 2003; Chaves et al., 2011a; Todo, 1999). Photolyases are enzymes that can catalyse light-dependent DNA repair. These enzymes are divided into two major groups based on the capacity to fix different types of UV-induced DNA damages: the cyclobutane pyrimidine dimer (CPD), or the 6–4 pyrimidine–pyrimidone photoproducts (6–4 PP) (Sancar, 2003; Todo, 1999; Hitomi et al., 2009; Sancar, 2004; Sancar, 2008). Despite the structural similarity and common evolutionary origin with the photolyases, cryptochromes (Cry) have generally lost the capacity to repair damaged DNA but have conversely acquired novel specialized functions in light perception and transcriptional regulation (Chaves et al., 2011a; Gegear et al., 2010; Ozturk et al., 2007; Liu et al., 2011).

In several organisms Crys play important roles in circadian clocks, which are endogenous timekeeping mechanisms that synchronize biological processes to the length of the environmental day–night cycle (Roenneberg and Merrow, 2005; Harmer, 2009). The underling common mechanism of the circadian clock is based on a central oscillator that measures time *via* molecular feedback loop(s) cycling over about 24 h (Dodd et al., 2005; Doherty and Kay, 2010; Dunlap, 1999). In plants, Crys act as photoperiodic photoreceptors. Following blue light activation, they transduce the light input into the clock *via* the activation of a still largely uncharacterized signalling cascade (Chaves et al., 2011a; Liu et al., 2011; Yu et al., 2008; Yanovsky and Kay, 2002). In animals, Crys can impact on circadian rhythms by two very different molecular pathways. The vertebrate Crys (also called type 2 Crys, or transcriptional Crys, here referred as vCrys) act as light-independent core clock components, by interacting with the positive transcriptional regulators CLOCK and BMAL1 (CLOCK and CYCLE in insects) and repressing their transcriptional activity (Sancar, 2004; Ko and Takahashi, 2006). A second animal Cry class is typified by the only *Cry* gene known in *Drosophila melanogaster* (Drosophila-type Cry, also called type 1 Cry, or light Cry, here referred as dCry). Where tested, dCrys encode photoreceptor molecules that are light-activated and transduce the input into the clock mechanism *via* the modulation of the degradation of the core clock gene *timeless* (Tim) (Kobayashi et al., 2000; Vinayak et al., 2013; Collins et al., 2006; Emery et al., 2000; Yuan et al., 2007).

The discovery of the Cry DASH family has provided novel information about the evolution and functional diversification of the CPFs (Brudler et al., 2003). CRY DASH proteins are widely distributed throughout the tree of life and both crystallographic (Huang et al., 2006) and biochemical characterizations have revealed that these proteins bind both single and double-stranded DNA (Pokorny et al., 2008; Selby and Sancar, 2006); Even though it has been shown that CRY DASHs have single-stranded DNA repair activity (Pokorny et al., 2008) and function in the light-dependent regulation of metabolism in fungi (Castrillo et al., 2013), a more comprehensive understanding of their function in the context of light signaling is still missing.

Members of the CPF family have been extensively characterized in bacteria, terrestrial animals and plants. In contrast, little information is available about their orthologs in aquatic organisms. The recent characterization of members of the CPF family in aquatic microalgae revealed new functional and photochemical aspects of this family. In particular, novel animal-like Crys showing both 6–4 photolyase and photoreceptor activities have been identified in the marine diatom *Phaeodactylum tricornutum* and in the green alga *Ostreococcus taurii* (Coesel et al., 2009; Heijde et al., 2010). Additionally, a novel flavin binding Cry photoreceptor, responding to both blue and red light, has been identified in *Chlamydomonas reinhardtii* (Beel et al., 2012).

The function and photophysical properties of CPFs have been studied in detail in only a few aquatic animal species such as in teleosts (Kobayashi et al., 2000; Tamai et al., 2007; Daiyasu et al., 2004). Moreover, the presence of different classes of CPF (mostly Crys) has been described in sponges (Rivera et al., 2012), cnidarians (Reitzel et al., 2010), protostomes (Zhang et al., 2013; Teschke et al., 2011; Zantke et al., 2013) and deuterostomes. Just to name a few, it has been reported that UV light exposure induces the expression of the repair enzyme CPD photolyases in Antarctic sea urchin larvae (Isely et al., 2009), and also that the levels of *Cpf* transcripts and proteins are influenced by the moonlight in corals (Levy et al., 2007). In *Platynereis dumerilii*, *tr-Cry* (*transcriptional-Cry*, ortholog of *vCry*) transcripts show a robust circadian cycling and are influenced by nocturnal light, which entrains the worm's circalunar clock (Zantke et al., 2013). Moreover, the vCrys of *P. dumerilii* and of the marine crustacean *Eurydice pulchra* can repress BMAL/CLOCK-mediated transcription (Zhang et al., 2013; Zantke et al., 2013). Together, these findings strongly suggest fundamental roles for Crys in biological rhythms also in marine organisms that possess more than one type of endogenous clock.

The functional diversification of CPFs has likely been shaped by the distinct light properties found in aquatic environments when compared to the terrestrial environment (Depauw et al., 2012). Indeed, underwater light is highly absorbed at wavelengths below 250 nm and above 700 nm, resulting in a progressive enrichment of the blue-green (400–500 nm) light components with depth, due to the absorptive and scattering properties of water and the presence of colored dissolved organic matter. Not surprisingly, the recent availability of sequenced genomes from representative organisms has revealed an expansion of the CPF family and novel blue light receptors in aquatic organisms (*e.g.* (Kobayashi et al., 2000; Depauw et al., 2012; Suetsugu and Wada, 2013)).

In addition to being complex light environments, marine habitats are governed by a multitude of rhythms, including the daily (circadian) rhythm, but also rhythms with shorter (ultradian) or longer (infradian) periods, such as tides, the lunar cycle, or seasons. Changes in light spectrum and intensity carry much information about these different types of rhythms. Consistently, the synchronization of many marine biotic processes, ranging from growth and cell division to sexual reproduction, uses light as a key signal (Bentley et al., 2001; Coppard and Campbell, 2005; Dickman et al., 2006; Fabioux et al., 2005; Iliffe and Pearse, 1982; Hastings, 2007; Ragni and Ribera d'Alcala, 2007). Despite their fundamental significance, the molecular players underlying

Fig. 1. Phylogenetic analysis of the CPF family.Phylogenetic reconstruction of the CPF family evolution in opistokonta, archeaplastidia, heterokonta, cryptophyta, haptophyta and bacteria. The four major groups, here referred as super-classes, of CPF proteins are highlighted with different letters (A–D) and are consistent with what reported in previous studies (*e.g.*, (Lucas-Lledo and Lynch, 2009; Ozturk et al., 2008)). In particular: (A) the animal CRYs/6–4 photolyases, (B) the CRY DASH, (C) the Plant CRYs and (D) the Class II CPD Photolyases groups. For each super-class, colored bars highlight different classes. Black arrows indicate the position of CPF members from our representative model species *D. rerio* (Dre), *O. latipes* (Ola), *P. tricornutum* (Ptr), *P. dumerilii* (Pdu) and *S. purpuratus* (Spu). The shown tree is midpoint rooted and was obtained using the ML method. For clarity, bootstrap supports are shown only for the basal nodes and were obtained using both the NJ and ML methods. Both approaches referred to 100 pseudoreplicates, with the first value corresponding to the NJ and the second to the ML method, respectively.

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Please cite this article as: Oliveri, P., et al., The Cryptochrome/Photolyase Family in aquatic organisms, Mar. Genomics (2014), http://dx.doi.org/10.1016/j.margen.2014.02.001