

Cryptochromes: The novel circadian photoreceptors

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The circadian clock maintaining daily light-dark cycles of rhythmicity in physiology and behaviour is present in all species. The circadian photo-response system mainly comprises three components – a photoactive pigment (chromophore) that senses the light and transmits the signal, the clock that oscillates with about 24 h periodicity, and the clock-controlled genes. In the past few years, remarkable progress has been made in discerning the clock components – the *period* (*per*) and *timeless* (*tim*) genes in *Drosophila*, the *frequency* (*frq*) gene in *Neurospora*, and the *clock* gene in mouse¹. To date, genetic approaches have yielded the most success in the study of circadian biology to elucidate the molecular mechanisms governing the circadian clock. In humans and other mammals, the body's 'master clock' consists of paired groups of cells, collectively known as suprachiasmatic nucleus (SCN) that resides at the base of the hypothalamus. These cells have been identified to contain novel genes – *clock*, *per1*, *per2*, *per3* and *Bmal1* that appear to be translated in sequence; the protein helps to keep cellular time by acting in a feedback loop in which the production of other proteins is shut off during certain phases of the day².

Early chronobiological experiments indicated that blind humans have a daily cycle that lasts for 24 h which seemed to stress the importance of light as a zeitgeber. Thus, light acts as a primary entraining agent for circadian clocks. Setting or 'entraining' of the biological clock requires photopigments – molecules that can perceive light³. The eyes communicate with the pineal gland by a neuronal route which includes the retino-hypothalamus tract, the SCN, and the pre- and post-ganglionic fibres of the peripheral sympathetic nervous system. In the pineal gland, the photoperiodic information is translated into a chemical message, the nocturnal secretion of melatonin. The duration of this secretion is proportional to the dark period and in this way photoperiodic information is integrated at the level of the central nervous system.

Although rod and cone cells of the retina are thought to mediate all the light responses of the vertebrate eye, our preliminary experiments have shown that photo-regulated neuroendocrine response of pineal gland, i.e. the melatonin levels in the serum and synaptic ribbon complex (SRC) in the pinealocytes were normal in genetically mutant anophthalmic rats lacking a complete visual system including retina⁴. Furthermore, circadian photoreception in retinally degenerated mouse (*rd/rd*) and retinally degenerated slow (*rds/rds*, an ablation of photoreceptor outer segments) have reported to suppress melatonin secretion by pineal. Thus, neither rod photoreception nor rod and cone outer segments are required for photic regulation of the pineal gland⁵. Reports also indicate that many completely blind persons exhibit normal photic entrainment of the circadian rhythm. Thus, all these examples have led to the involvement of some unidentified non-rod, non-cone, non-ocular-based photopigment (photoreceptor) that allows the photic impulses to reach the pineal gland.

At present, the identity of the putative circadian photoreceptors remains elusive. Recent reports regarding plant blue-light photoreceptors—cryptochromes that have high degree of sequence homology to the light activated DNA repair enzymes called DNA photolyases were found to be essential for phototropism and photoperiodism of flowering period in some plants, raising the possibility of their involvement in circadian rhythms, at least in plants. Recently, cryptochromes have also been reported in animals⁶.

The first indication that cryptochrome photoreceptors existed in humans came with the identification of two genes with sequence homology to the photolyase/plant blue-light photoreceptor gene family. Like the plant blue-light photoreceptors, the human cryptochrome homologs were found to contain FAD and a pterin as chromophore/cofactor but did not exhibit DNA repair activity. Later on, these two proteins were designated as cryptochromes 1 and 2 (CRY1 and CRY2). Although *mcry1* and *mcry2* are

expressed in most of the neural and non-neural tissues, particularly their expression within the inner retina and the suprachiasmatic nucleus of the hypothalamus – the location of the 'master' clock in the mouse suggested their prominent role as circadian photoreceptors. In order to justify this statement, van der Horst *et al.*⁷ generated mutant mice that lacked either functional *cry1* or *cry2* genes or both. The observed arrhythmic behaviour in these mutant mice suggests the involvement of cryptochromes in the central clock mechanism. Thus, the presence of cryptochromes in the SCN with their expression with a strong day-night rhythm, and data from the mice lacking CRY1 and CRY2 are providing sufficient proof for their role as circadian photoreceptors.

In conclusion, the discovery and study of cryptochromes have provided a clue to the precise role of these proteins as circadian photoreceptors in phylogenetically diverse organisms but their role as a 'universal' entraining photopigment remains unproven.

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