Abstract Research over the past decade has provided overwhelming evidence that photoreception in the vertebrate eye is not confined to the rod and cone photoreceptors. It appears that photoreceptor cells within the inner retina provide irradiance information to a wide variety of different photosensory tasks including photoentrainment, pupillary constriction and masking behaviour. Action spectra in mice lacking all rod and cone photoreceptors (rd/rd cl) have demonstrated the existence of a previously uncharacterised, opsin/vitamin-A-based photopigment with peak sensitivity at 479 nm (opsin photopigment/OP479). The review addresses the question: has the gene encoding OP479 already been isolated, and if not, what type of gene should we be seeking and where in the eye might this gene be expressed? On the basis of available data, the gene that encodes OP479 remains unidentified, and two broad possibilities exist. On the assumption that OP479 will be like all of the other vertebrate photopigments (ocular and extraocular) and share a close phylogenetic relationship based upon amino acid identity and a conserved genomic structure, then the gene encoding OP479 has yet to be isolated. Alternatively, there may have been a separate line of photopigment evolution in the vertebrates that has given rise to the melanopsin family. If true then the mammalian melanopsin gene may encode OP479. Only when melanopsin and other candidates for OP479 have been functionally expressed, and shown to encode a photopigment that matches the action spectrum of OP479, can firm conclusions about the identity of the non-rod, non-cone ocular photoreceptor of mammals be made.

Keywords Evolution · Melanopsin · Opsin · Photopigment · Circadian

Light, circadian rhythms and novel ocular photoreceptors

The primary role of the circadian system is to set the phases at which physiological and behavioural events occur over a 24-h period so that activity is “fine-tuned” to the varying demands of night and day. To bring about this adjustment, the circadian clock must remain synchronised or entrained to the solar day, and the systematic daily change in the gross amount of light (irradiance) at dawn or dusk seems to provide the most important zeitgeber (time giver) adjusting circadian phase to local time, a process that has been termed photoentrainment (Roenneberg and Foster 1997). In mammals and other vertebrates, the irradiance thresholds are higher and the integration times much longer for photoentrainment than for visual image formation (Foster and Provencio 1999; Nelson and Takahashi 1991). Thus, the point has been made that photic regulation of the circadian system is markedly different from the processes by which a visual representation of the environment is generated (Foster and Helfrich-Forster 2001).

In mammals, light information reaches the master circadian clock in the suprachiasmatic nuclei (SCN) through a dedicated pathway originating in the retina (retinohypothalamic tract or RHT). Eye loss confirms that photoentrainment originates within ocular photoreceptors of mammals (Foster 1998; Lockley et al. 1997; Yamazaki et al. 1999). However, reports have appeared from time to time proposing that mammals possess extraocular photoreceptors which mediate photoentrainment. The most recent of these was by researchers from Cornell University, who suggested that bright light applied to the area behind the human knee would shift circadian rhythms of body temperature and melatonin (Campbell and Murphy 1998). But like earlier reports, these studies have not withstood independent verifica-
tion (Koorengevel et al. 2001; Lindblom et al. 2000a, 2000b; Lockley et al. 1998), suggesting that the original observations resulted from some unrecognised artefact of the experimental procedures (Foster 1998; Yamazaki et al. 1999).

Although clearly ocular, identifying the retinal cells that mediate photoentrainment has been difficult. Until recently the only components of the mammalian CNS thought to be directly light sensitive were the rods and cones of the retina. Thus all light responses were attributed to one or both of these photoreceptor classes. However, work on mice homozygous for the retinal degeneration (rd/rd) mutation challenged this assumption. Despite the massive (but not complete) loss of rods and cones in rd/rd mice these animals showed circadian responses to light that were indistinguishable from non-degenerate congenic control mice (rd/+ , +/+). Removal of the eyes abolished all circadian responses to light in rd/rd mice, confirming that these photoreceptors reside within the eye (Foster et al. 1991; Provencio et al. 1994). However, because small numbers of cones remain in the retina of rd/rd mice, these initial experiments could not establish whether the circadian system is able to retain normal photosensitivity using small numbers of cones, or whether there is another uncharacterised photoreceptor within the eye (Foster et al. 1993). To resolve this issue, lines of transgenic mice were generated that lacked all rod and cone photoreceptors. This was achieved by introducing into mice that lacked rods a synthetic transgene (cl) constructed from the attenuated diphtheria toxin coding sequence driven by a portion of the human red cone opsin promoter (Soucy et al. 1998). These mice lacked rods because they were either homozygous for the rd (retinal degeneration) mutation (Bowes et al. 1990; Carter-Dawson et al. 1978) or because they carried a separate diphtheria toxin-based transgene (rdta) (McCall et al. 1996). Despite rod and cone photoreceptor ablation, rd/rd cl (Fig. 1) and rdta cl mice retain normal cir-

Fig. 1A–D Immunocytochemical analysis of retinas from rd/rd cl mice. Introduction of the cl transgene into mice homozygous for the rd mutation induced a retinal phenotype in mice 80 days of age that lacked both rod and cone cells. Tissue from rd/rd cl (A and B) and wild type (C and D) was fixed with Bouin’s fluid for 24 h, paraffin embedded and 8-µm sections treated with antisera recognising rod (A and C), rod/LWS cone (B and D) photoreceptors. Visualisation was accomplished using ABC methods (Vectastain Elite, Vector Labs.) (GCL ganglion cell layer, IPL inner plexiform layer, INL inner nuclear layer, ONL outer nuclear layer, IS inner segments, OS outer segments, RPE retinal pigment epithelium). For full details, see Lucas et al. (1999)