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Human and animal cells under influence of different lighting and stimulus

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Abstract

Circadian rhythms, are the basis of homeostasis of organisms like human and other mammals. Violation of circadian rhythms leads to the development of pathological conditions and severe course of preexisting pathologies. For example some work with B16-F10 cells (B16) has shown that molecules like opsins, *Circadian Locomotor Output Cycles Kaput* (CLOCK) and clock genes are changed after a white light pulse (WLP). Like this, melanopsin (OPN4) and rhodopsin (OPN2) through UVA irradiation induced B16 pigmentation. Thus, heat shock reduces secreted rhodopsin expression in normal Melan-a melanocytes, while the opposite effect is found in malignant B16 cells. In both cell lines UVA radiation increases the expression of melanopsin and melanin, interfering with several clock genes, and also increasing the DNA repair enzyme xeroderma pigmentosum, complementation group A (XPA). Furthermore, B16 are more responsive to UVA radiation when compared to normal cells. Thereby, opsins are involved in animal camouflage. And their functions in humans involve different wavelengths, for example in skin the keratinocyte differentiation by (410 nm) involved cone opsin (OPN1) and rhodopsin (OPN2), like this in epidermal keratinocytes irradiation by (447 nm) accelerates closure in wound-healing and violet light (415 nm) induced hyperpigmentation. Furthermore, in B16 cell culture certain wavelengths induce proliferation or inhibition like signs of apoptosis and necrosis. Finally understanding the response of opsins and clock genes to different wavelengths in the skin, we could attribute a therapeutic of photobiomodulation (PBM) to approach various dermatological conditions, such as psoriasis, atopic dermatitis, hair growth, wound healing and tissue regeneration.

Keywords: Circadian rhythms; Melanopsin; Photobiomodulation; Malignant melanocytes.

1. Introduction

All electromagnetic radiation is quantized into photons. The visible or optical spectrum, is the portion of the electromagnetic field whose radiation is composed of photons capable of sensitizing the human eye, which corresponds a smallest piece of electromagnetic radiation. Photons can also be emitted by an unstable nucleus when it decays through some kind of nuclear decay furthermore, photons are produced whenever charged particles are accelerated. Thus, visible photons are produced by atoms when a valence electron moves from one orbital to another orbital with less energy [1]. The proteins involved in vision are rhodopsin's which belong to a group of G protein-coupled receptors (GPCRs) [2]. The opsin is the protein part of rhodopsin, this group of proteins is sensitive to light, via the retinal chromophore mainly found in the photoreceptor cells of the retina. For example, humans possess nine different opsins with some isoforms, see (table 1) [3]. In this way, there are five classical groups of opsins involved in vision three opsins are expressed in cone photoreceptor cells see (figure 1 A), mediating the conversion of a photon of light into an electrochemical signal, which determine the three colours in vision: green medium wave (OPN1MW) red long wave sensitive opsin, (OPN1LW), blue short wave opsin (OPN1SW), and also has the rhodopsin, which work under penumbra conditions expressed in rod photoreceptor cells (figure 1 A). Therefore, melanopsin (OPN4) is the opsin that functions in pupil constriction of the eyes and in circadian regulatory system. In addition to these, we have encephalopsin,

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neuropsin, Retinal Pigment Epithelium-Retinal G Protein Receptor-Opsin (RPE-retinal) also known as (RGR opsin) and peropsin (RRH) [4, 5, 6]. Thus, finally we also know that opsins play an important role in taste and any form of chemical sensitivity [7] for example to detect not only light, but also thermal energy in *Drosophila* [8].

Table 1 (HGNC) Human Genome Organisation Gene Nomenclature Committee.

HGNC ID (gene)	Approved symbol	Approved name	Previous symbols	Aliases	Chromosomal location	Number of introns
HGNC 9936	OPN1LW	opsin 1, long wave sensitive	CBBM, RCP, CBP	COD5	Xq28	5
HGNC 4206	OPN1MW	opsin 1, medium wave sensitive	GCP, CBBM, CBD	OPN1MW1, COD5	Xq28	5
HGNC 26952	OPN1MW2	opsin 1, medium wave sensitive 2			Xq28	5
HGNC 51831	OPN1MW3	opsin 1, medium wave sensitive 3			Xq28	5
HGNC 1012	OPN1SW	opsin 1, short wave sensitive	BCP	BOP, CBT	7q32.1	4
HGNC 10012	RHO	rhodopsin	RP4	OPN2, CSNBAD1	3q22.1	4
HGNC 14007	OPN3	opsin 3	ECPN	ERO, NMO-1, encephalopsin, PPP1R116	1q43	3
HGNC 14449	OPN4	opsin 4		MOP, melanopsin	10q23.2	9
HGNC 19992	OPN5	opsin 5	TMEM13	neuropsin, dJ402H5.1	6p12.3	6
HGNC 9990	RGR	retinal G protein coupled receptor		RP44	10q23.1	6
HGNC 10450	RRH	retinal pigment epithelium-derived rhodopsin homolog		peropsin	4q25	6

For vision formation, in the retina an opsin molecule absorbs a photon of light, that causes a change in retinal cofactor from 11-cis-retinal isomer to all-trans-retinal isomer [9] (Figure 1 B). This change in conformation of retinal pushes against the outer opsin protein to begin a signal cascade, which result in chemical signaling sent to the brain like an electrical signal which is translated in visual perception (Figure 1 C). The retinal is cyclically re-loaded and the biochemical signal is relayed. Like this, light photons are captured by two specialized and morphologically distinct photoreceptor cells from modified neurons: nominated cones and rods that have the same molecular mechanism, then the light signal is transmitted through different cells, and finally reaching ganglion which form the optic nerve which sends signals to synapses in the region responsible for vision in the brain (Figure 1 A) [5].

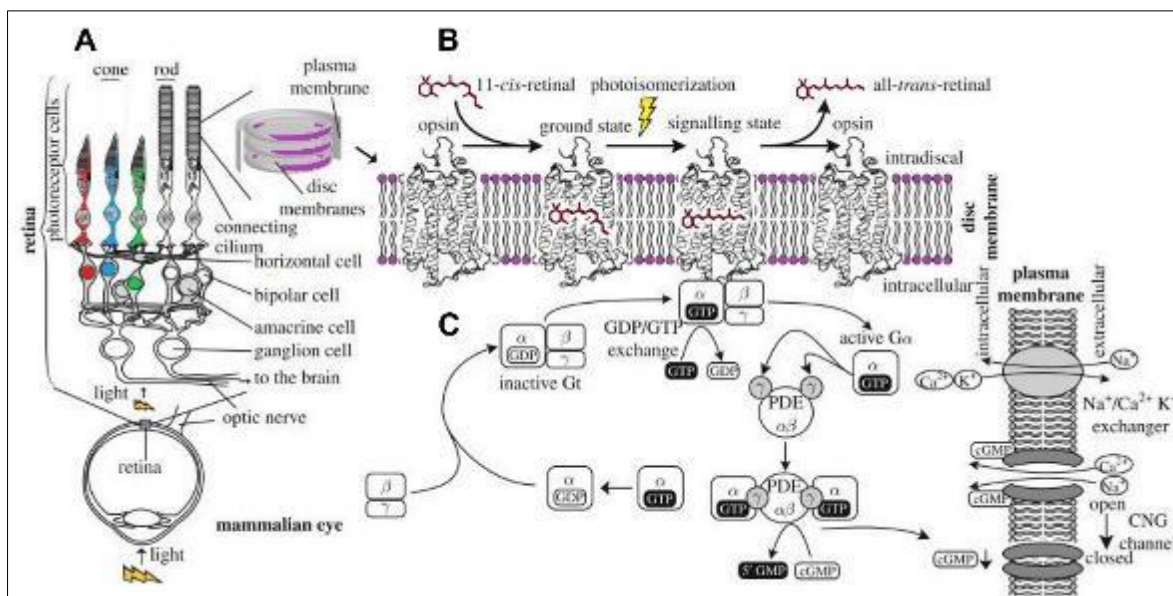


Figure 1 (A) Schematic drawing of phototransduction system in mammalian eyes. (B) Photon is absorbed by opsins in disc membranes of two types of photoreceptor: rods and cones responsible for capturing the three basic colors. (B) In disc membranes opsins absorb photons by change in retinal cofactor. (C) Change in retinal form a signalling state, which activate the G protein GDP to GTP by catalytic change.

To expose molecule active site GTP-bound $G\alpha$ dissociates from $G\beta\gamma$. Thus activated $G\alpha$ binds to its effector, PDE (cyclic nucleotide phosphodiesterase), and activates it. PDE breaks the phosphodiester bond of cGMP producing 5'GMP, and the decrease in the concentration of cGMP causes CNG (cyclic nucleotide gated) channels to close, which creates a signal transmission by hyperpolarization of plasma membrane in rods and cones cells. The photon-activated rhodopsin is unstable so the all-trans-retinal leave the molecule. The hyperpolarization of the membrane potential of the rods and cones cell releases a neurotransmitter-mediated biochemical signal to downstream cells. The Photon signal is transmitted through different cells, and reaching ganglion which form the optic nerve to image synapses in the central nervous system [5].

While humans have three opsins for color, mice have only two opsins, known as S which detect blue/UV and M which detect green light. Thus, mouse cones produce either S-opsins, M-opsins or both. Like this, fewer than 10 percent of cone cells in mice produce just S-opsin, and these cells are essential for color vision [10].

2. Discovery of the opsins in the skin

For a long time, opsins were associated with the detection of photon radiation involving the eyes, or in specific tissues such as the pineal gland and brain of non-mammals [11, 12]. However later a new opsin was found in a skin tissue that produces melanin called melanophores of frog species *Xenopus laevis* and because of this the new opsin was called melanopsin [13]. Another relevant finding was the discovery of melanopsin mRNA in the human retinal ganglion cell layer [14] because we know that axons of this subpopulation cells project into the suprachiasmatic nuclei (SCN), where a gene encoding a basic helix-loop-helix-PAS transcription factor known as *Circadian Locomotor Output Cycles Kaput* (CLOCK) (Figure 3), handle circadian oscillation by the light/dark cycle information of day and night light and dark, controlling (Figure 4) other proteins and transcription of other genes known as clock genes (Figures 3 and 4) [15]. One of the first findings of opsins in the skin was from Miyashita group [16] and Castrucci's laboratory [17], reported the expression of rhodopsin in the immortalized murine melanocytes Melan-a, and in mouse skin, simultaneously thus confirming the findings of opsin expression and photoreception in tissues not linked to organs of vision [18, 19]. Since when, opsins were discovered in human skin (Figure 5) [20] its functions have been investigated, like this immunolabeling of rhodopsin-OPN2 as well as S and L/M cone opsins OPN1 were found in epidermis of human (Figure 5) [20]. Therefore, Wicks and colleagues [21] were able to confirm in human melanocytes only expression of rhodopsin, in contrast to the findings in mice [22] and human skin [23], where the OPN5 gene and protein expression were detected. However in another job, of Oncea's laboratory [24, 21], demonstrated the presence of short wave sensitive named blue-sensitive opsin (OPN1SW), encephalopsin-OPN3 and neuropsin-OPN5 in human melanocytes and keratinocytes (Figure 5). Confirming the conspicuousness of rhodopsin transcripts in human skin, keratinocytes [25]

and hair follicles [26]. The Castrucci's laboratory group show the levels of mRNA by RNASeq and protein levels of melanopsin *Opn4* in murine Melan-a and B16-F10 cells [27, 23], as well as confirmed the levels of mRNA of rhodopsin-*Opn2*, (*OPN1MW*) and (*OPN1LW*). Castrucci's group also showed in human sun-exposed and unexposed skin the variations in levels of opsin-encoding genes rhodopsin-*OPN2*, encephalopsin-*OPN3*, melanopsin *OPN4*, and neuropsin-*OPN5* [28, 29] (Figure 5). Thereafter, Toh and colleagues [30] found the peropsin mRNA and protein in keratinocytes of human skin (. Concurrently in 2019, detected the mRNA and protein levels of *OPN1*, *OPN2*, *OPN3*, *OPN4*, and *OPN5* in dermal human fibroblasts [31] (Figure 5). Then, was also demonstrated *OPN4* expression in human melanocytes and keratinocytes [32]. Surprisingly, the isomerase RPE65, responsible in regeneration of 11-cis retinal from all-trans retinal has found in human keratinocytes [33].

3. Suprachiasmatic nucleus (SCN)

The gene responsible for making the link between light variation and the circadian cycle is melanopsin *OPN4*, this gene is expressed by Optic Chiasm cells, in a brain structure close to Hypothalamus (Figure 2) [4, 5, 6]. Circadian genes are expressed in specific groups of neurons in *Drosophila* (Figure 2 A and Figure 3 A) or located in the hypothalamus of vertebrates in a structure known as suprachiasmatic nucleus (SCN) (Figure 2 B and Figure 3 B). The circadian cycle is mainly controlled by transcription factors and proteins known as clock genes associated with chronotype such as period (*PER*), cryptochrome (*CRY*), brain muscle Arnt-like protein (*BMAL*) and the *CLOCK* gene which encoding a protein with domain of basic helix-loop-helix-Per-Arnt-Sim (*PAS*) a type of transcription factor which affects both the persistence and period of circadian rhythms [34]. In *Drosophila* *CLOCK* neural system make up a network of approximately 150 *CLOCK* neurons, in several magnitude orders fewer of mammals, which still shares a similar function to vertebrate suprachiasmatic nucleus (SCN) located inside mammals, hypothalamus (Figure 2 A) [35, 36 37]. Thus in mammals the (SCN) is the central oscillator (Figure 2 B) responsible for controlling the temporal expression of clock genes and *CLOCK*-controlled genes (*CCG*) in peripheral tissues, and ultimately align the entire organism under a single timing [38, 39, 40]. The cycle of light and dark (LD) is the most important environmental stopwatch timer, of cycle known as zeitgeber synchronizer, in mammals. So the data generated is interpreted by a subset of retinal ganglion cells, that express melanopsin *OPN4* into electrical stimuli which reach the SCN through the retinohypothalamic synapses, and entrain in SCN [15, 41]. The *CLOCK* genes was first reported in isolated mammalian fibroblasts in 1998 [42], and two years later in human skin [43]. In 1997 the *CLOCK* gene was first identified by Joseph Takahashi group. Takahashi used mice treated with N-ethyl-N-nitrosourea as a mutagen to create and identify mutations in key genes that largely affect circadian activity [44, 45]. The mice mutants *CLOCK* sequence discovered through the screen displayed an abnormally long period of daily activity. Thus, this trait proved to be heritable and heterozygous mice showed longer periods of 24.4 hours compared to the control 23.3 hour period. Withal, mice homozygous for the mutation showed a long period of 27.3 hours, and lost all circadian rhythmicity after several days in constant darkness. So, this discovery showed that "CLOCK genes" are necessary for normal mammalian circadian rhythm [46]. To obtain the data of light and dark, the SCN located in hypothalamus receives afferent neural synapses from the retina through the retinohypothalamic tract (Figure 2 B) [47]. Thus, the neural synapses of the SCN send efferent projections to other parts of the brain, such as the paraventricular nucleus (PVN), medial preoptic area, dorsomedial nucleus in region of the hypothalamus and the pineal gland, to transfer timing information and to regulate secretion of pituitary hormones and melatonin, as well as to control sleep, food intake and body temperature [44, 48].

CLOCK protein has been found to play a central role as a transcription factor in the circadian cycle mechanism. [49, 50] In *Drosophila*, before entering the nucleus the newly synthesized *CLOCK* is hypophosphorylated in the cytoplasm (Figure 3 A). Once in the nuclei, *CLOCK* is localized in nuclear foci and is later homogeneously redistributed. Another transcription factor that dimerizes with *CLOCK* is called *CYCLE* (*CYC*) in *Drosophila* (Figure 3 A) and in mammals also known as brain-muscle-arnt-like protein 1 (*BMAL1*), which belong to the basic helix-loop-helix (bHLH)-*PER*-*ARNT*-*SIM* (*PAS*) superfamily of transcription factors ortholog (Figure 3 B) thus *BMAL1* dimerizes with *CYCLE* via their respective *PAS* domains (Figure 3). Then this dimer recruits co-activator CREB-binding protein (*CBP*) and is further phosphorylated [51]. In *Drosophila*, this *CLOCK*-*CYC* complex once phosphorylated binds to the E-box elements of the promoters of period (*PER*) and timeless (*TIM*) via its basic helix-loop-helix (bHLH) domain, causing the stimulation of gene expression of *PER* and *TIM*. A large molar excess of period (*PER*) and timeless (*TIM*) proteins causes a negative feedback by formation of the *PER*-*TIM* heterodimer which prevents the *CLOCK* -*CYC* heterodimer by blocking the primary transcript to binding in the E-boxes of *PER* and *TIM* (Figure 3 A) [52].

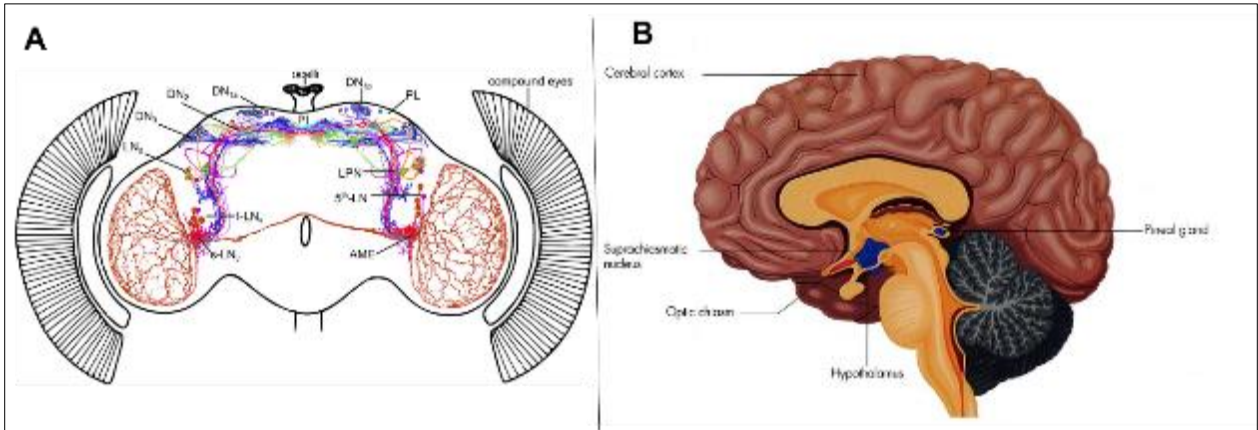


Figure 2 (A) The circadian cluster neurons in the Lobes of *Drosophila melanogaster*. The neural synapse system with the CLOCK genes consists of Lateral Neurons (s-LNVs, 5th-LN, l-LNVs, LNds, and LPNs) which are highly connected with the neurites of Dorsal Neurons (DN1as, DN1ps, DN2s, DN3s). Many of them project axons into the dorsal protocerebrum including the neurosecretory centers in the pars intercerebralis (PI) and pars lateralis (PL) as well as into the accessory medulla (AME) of both hemispheres [49]. (B) Illustration of the human brain showing the Cerebral Cortex, the Suprachiasmatic Nucleus, the Optic Chiasm, the Hypothalamus and the Pineal Gland.

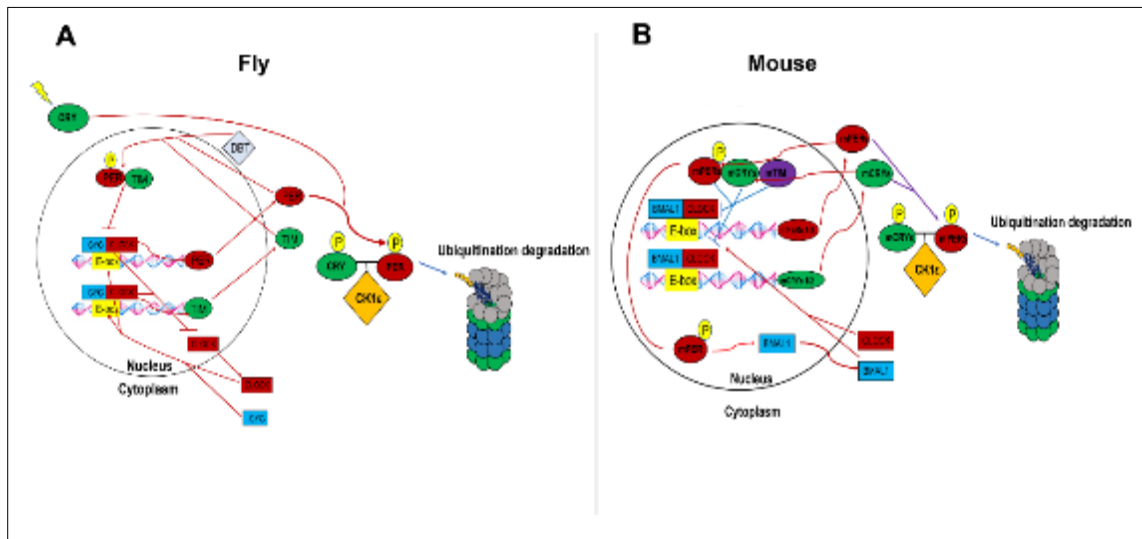


Figure 3 The circadian oscillator mechanisms from flies and mice. (A-B) Models depicting the regulatory interactions within the (A) *Drosophila* and (B) mouse feedback loops. Arrows indicate positive regulation and lines ending in bars denote negative regulation. Gene symbols are as described in the text. The 'P' on Per and mPer represents phosphorylation due to Dbt/CK1ε. The lightning bolt represents light acting on *Drosophila* Cry mouse per orthologs (mPer). An E-box (enhancer box) is a DNA response element [50].

When double time (DBT) kinase interacts with complex CLOCK-CYC acts phosphorylating CLOCK through a PER-dependent interaction, and both are phosphorylated destabilizing CLOCK and PER, what causes degradation of both proteins in the ubiquitin proteasome pathway (Figure 3 A) [52, 53]. Thus, after Hypophosphorylated the levels of CLOCK increase, allowing CLOCK to enter in cell nucleus to act as transcription factor in E-boxes of PER and TIM and activates their transcription once again [52]. This cycle of post-translational phosphorylation imply that temporal phosphorylation of CLOCK acts in the circadian timing mechanism of CLOCK [51]. In mice there is a similar cycle which BMAL1 dimerizes with CLOCK to activate PER and cryptochrome (CRY) transcription (Figure 3 B) [51,52]. Thereby, PER and CRY proteins form a heterodimer which acts on the CLOCK-BMAL heterodimer as negative feedback in transcription of PER and CRY (Figure 3). The heterodimer CLOCK:BMAL1 functions similarly to other transcriptional activator complexes; CLOCK:BMAL1 interacts with the E-box regulatory elements[34]. During the day PER is phosphorylated by CRY, which allows it interacting with Double-Time (DBT)/Casein Kinase 1 ε (CK1 ε), a protein kinase that destabilizes cytoplasmic PER while TIM increases (Figures 3 and 4). Thus CK1 ε are essential components of one feedback loop in the *Drosophila* circadian CLOCK, PER and TIM by physically interact. Co-expression of PER and TIM promotes their

nuclear accumulation and influences the activity of DBT: although DBT phosphorylates and destabilizes PER, this is suppressed by TIM (Figure 3) [54]. Through the analysis of crystallography has been confirmed that PER and CRY proteins accumulate and dimerize during subjective night, and translocate into the nucleus to inhibiting by directly interact with CLOCK:BMAL1 complex, by negative feedback their own expression (Figure 4) [55].

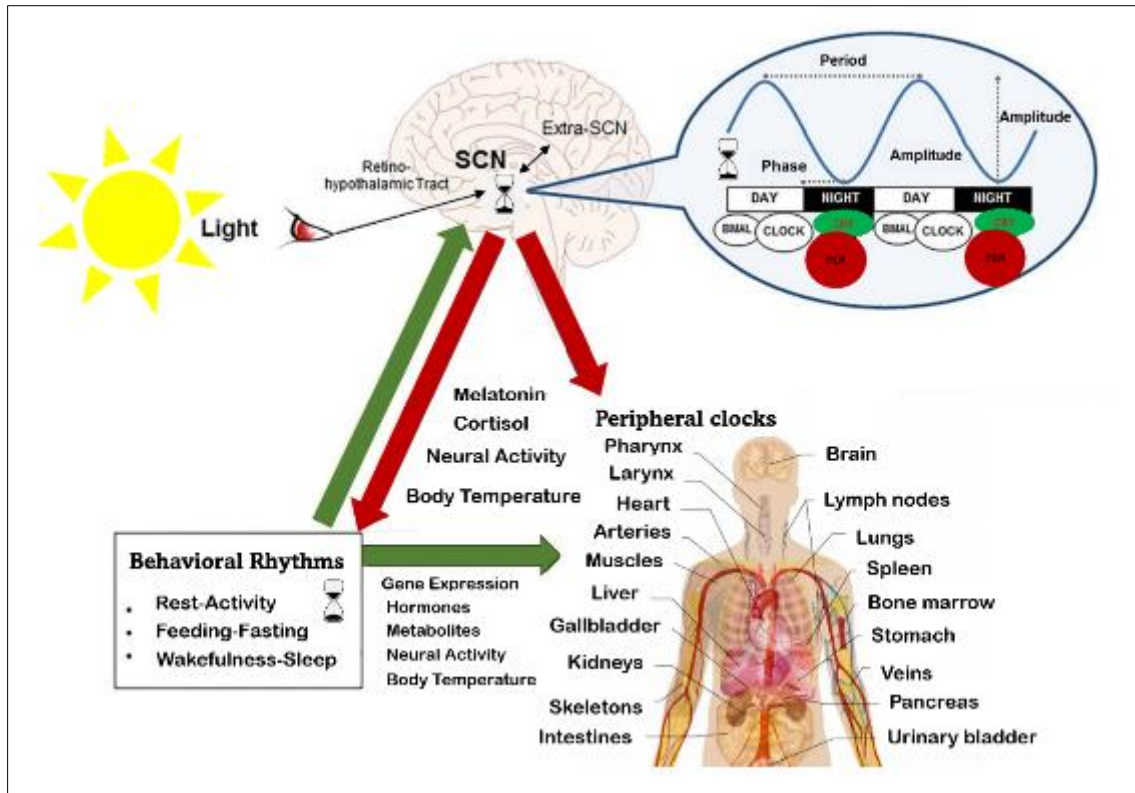


Figure 4 Molecular control by suprachiasmatic nucleus of the zeitgeber timing system. During the light phase BMAL1 and CLOCK are active as transcription factors linked to their respective E-BOX and during the night their products CRY and PER act, and finally degraded by ubiquitin during the new light phase. Legend: (BMAL) brain and muscle Arnt-like protein; (CLOCK), circadian locomotor output cycles kaput; (CRY), cryptochrome; (PER), period; and SCN, suprachiasmatic nucleus [56].

4. CLOCK genes in skin

Experimental findings have shown that clock genes are not expressed exclusively in the SCN of the brain but also in epithelial tissue and in other organs constituting the peripheral clocks. The human clock genes hCLOCK, hTIM, hPER, hCRY, and hBMAL are expressed in skin, with a circadian profile consistent with that found in the suprachiasmatic nuclei and the peripheral tissues of rodents. For example hPER, hCRY, hCLOCK and hBMAL have a rhythmic expression, peaking early in the morning, in late afternoon, and at night (Figure 4) [56, 57]. Thus, there are studies that investigate the effect of different types of stimulus on the regulation of clock genes expression in murine and human skin, like for example the white light stimulus (Table 2) [58, 59, 60]. The important role of clock genes has been reported in pigmentation regulation of skin [61], likewise has been proposed that the skin cells, for example keratinocytes, fibroblasts, and melanocytes display distinct clock machineries which comprise a local circadian system in epithelial tissue [58, 61].

Like this, the skin contains local circadian systems that regulate manifold processes in a mold dependent of time [58, 62] for example, skin barrier protection temperature, water loss, and pH [63, 64]. Another important protein of the skin that displays a circadian feature is DNA repair: xeroderma pigmentosum, complementation group A (XPA), [65] activation of the DNA repair pathway enzyme, oscillates in nice skin [66]. Is also known that oxidative phosphorylation in S-phase of the cellular cycle is another important CLOCK regulated event in the skin. Interestingly, when (BMAL) is specifically deleted in keratinocytes, the time dependent cell division is lost, a fact that, cause a constitutively elevated cell proliferation [67, 68]; Thus, we can associate the importance of clock genes with the regulation of the cell cycle.

5. Characteristic of B16 and Melan-a cells under influence of different lights and stimulus

For example, the effects of wavelengths belonging to visible spectrum on B16 cells depend of intensity and exposure time; in addition factors such chemical composition of the culture medium and characteristics of the cell lineage also be taken into account. Thus, scientific findings have already demonstrated that B16 under low dosage of light regime preserve characteristic of this tumor like intact tissue safe junctions, functionally active cells with highly irregular nuclei, developed organelles and a relatively low content of melanin. But in contrast the B16 melanoma tissue structure and the ultrastructure under the action of high constant lighting exhibits accelerated growth, increased melanin levels, as significant number of cells in the state of apoptosis and necrosis, like signs of embryonization with background of adaptation to oxygen deficiency [69]. For example, the mice genes, tyrosinase (tyr) [28, 70], solute carrier family 45 member 2 (SLC45A2) [28, 71] solute carrier family 24 member 5 (SLC24A5) [28, 72] melanopsin, (OPN4) and , rhodopsin, (OPN2) have been identified withal important role in the production of melanin in B16-F10 and Melan-a cells (Table 2) [27, 28, 73]. As soon, experimental probes in B16-F10 cells have shown that twenty-four hours after the white light pulse (WLP) the OPN4 protein was found in the cell membrane. So, in cells that were kept in constant dark (DD) the OPN4 protein was found in a small area capping the cell nuclei [27]. When compared to Melan-a the B16-F10 cells expressed less mRNA of OPN2 and OPN4 genes, however data imply that B16-F10 are more photoresponsiveness. Was also found that in B16-F10 cells CLOCK gene is severely downregulated when compared to Melan-a cells, and the expression levels of mRNA PER1, PER2, and BMAL1 are increased in B16-F10 cells in response to WLP (Table 3). Although no response in CLOCK gene expression to WLP was observed in Melan-a cells, (Table 3); therefore this data suggest a minor gene correlational by effect of WLP [27]. Thus, in contrast to opsins and clock genes, melanogenesis is significantly upregulated in malignant melanocytes in comparison to Melan-a cells. Like this tyrosinase expression increased after WLP only in B16-F10 cells; however no statistical increase was observed in melanin content after WLP in either cell line (Table 3) [27]. Like this Melan-a and B16-F10 cells express Opn2, Opn4, and S-opsin (Table 3) but not M-opsin and Opn5 [27]. For example, thermal shock is also a factor that influences in genetic expression, thus was demonstrate that a heat shock increases the levels of OPN2 mRNA in B16-F10 and has been a reduction in levels of OPN2 gene in normal Melan-a cells (Table 3). Like this, in both cell lines, the UVA radiation increases the expression of Opn4 mRNA (Table 3) and melanin content, (table 2). We also know that mRNA expression of clock genes and DNA repair XPA, in malignant melanocytes are more responsive by UVA radiation when in comparison Melan-a cells (Table 3). But an interesting fact is that most effects of UVA are antagonized by heat shock, (table 2 and Table 3) [68].

Has also been demonstrated that UVA in a dose of (4.4 kJ/m²) leads to a phenomenon called immediate pigment darkening (IPD) in B16-F10 and Melan-a cells (table 2). Thus the OPN2 and OPN4 work like a sensor of UVA, because the ability of UVA induce IPD was fully abolished when OPN4 was pharmacologically inhibited by AA92593 or when OPN2 and OPN4 were knocked down by siRNA or knockout in OPN4 in both cell lines (table 2) [74]. The data, however, demonstrate that UVA-induced IPD is not dependent of a classical OPN4 pathway phospholipase C/protein kinase C pathway, in either cell line [74]. Despite what, in both cell types the intracellular calcium signal is necessary for IPD induced by UVA; as the involvement of calmodulin-dependent protein kinase II (CaMK II), whose inhibition, abolished the UVA-induced IPD (table 2). Thereby an inhibition of either nitric oxide synthase NOS or soluble guanylyl cyclase sGC abolished the UVA-induced IPD by the way that involves the CAMK II, (NOS), (sGC) and Cyclic guanosine monophosphate (cGMP) (table 2) [28]. Like this, has been demonstrated that OPN2 and OPN4 participate together in the induction of IPD by UVA in B16-F10 and Melan-a cells through a conserved common pathway. Another important verification is the fact that after knockdown of OPN2 or OPN4, the IPD induced by UVA is completely lost, which suggests that both opsins are required and cooperatively in IPD signal in both B16-F10 and Melan-a mice cell (table 2) [28].

Thus, was investigated the effects of the tumor in a non-metastatic melanoma model observing the characteristics of tumor in micro-environment (TME) and macro-environments (TMaE). Therefore, in C57BL/6J mice inoculated with B16 cells, was found that presence of localized tumor depress the biological clock of tumor-adjacent skin and affected the oscillatory expression of genes involved in light, thermo-reception, proliferation, melanogenesis, and DNA repair. In this way, was found what the expression of tumor molecular clock was significantly reduced but still displayed an oscillatory profile compared to healthy skin [29]. Another important finding is fact that molecular clocks of lungs and liver (common sites of metastasis), and the suprachiasmatic nucleus (SCN) were significantly affected by tumor presence, which entaile a chronodisruption in each organ. Thereby, generally the presence of non-metastatic melanoma significantly debilitates the biological clocks of organism [29]. In skin the melanopsin (OPN4) has the function of detects temperature and UVA radiation. Thus to evaluate the interaction between OPN4 and UVA radiation in B16-F10 as well in Melan-a. Knockout cells (OPN4KO) as with as intact gene (OPN4WT) has been carried out, an experiment where the melanocytes was exposed to three daily doses (total 13.2 kJ/m²) of UVA radiation. The UVA radiation led to a reduction of proliferation in both OPN4WT cell lines; however, only in melanoma cells this effect was associated with increased cell death by apoptosis. Like this, was verified that the daily stimulus by UVA induced a persistent pigment darkening (PPD) in both OPN4WT cell lines (Table 2). However in the lineages which the gene OPN4 was knocked out, all UVA-

induced effects were lost. Was also verified in OPN4WT cells that PER1 gene was reduced after radiations by UVA (Table 3).

Table 2 Factors that influence melanin production

Factors that influence in melanin production			
Cells		B16-F10 stimulus	Melan-a stimulus
Chemical agents	AA92593, melanopsin antagonist	-	-
	adenylyl cyclase activator Forskolin	+	+
	adenylyl cyclase inhibitor, SQ22536	-	-
	calcium/calmodulin kinase II inhibitor, KN-93	-	-
	calcium chelator, BAPTA-AM	-	-
	guanylyl cyclase inhibitor, ODQ	-	-
	L-tyrosine	+	+
	nitric oxide synthase inhibitor, L-NAME	-	-
	phospholipase inhibitor, U-73122	-	-
	protein kinase C inhibitor, RO 31-8220	-	-
Gene Knockout	melanopsin, OPN4	-	-
	rhodopsin, OPN2	-	-
	solute carrier family 45 member 2, SLC45A2	-	-
	solute carrier family 24 member 5, SLC24A5	-	-
	tyrosinase, Tyr	-	-
Light environment and	constant dark	Neutral	Neutral
	heat shock	-	-
	UVA radiation	+	+
	white light pulse (WLP)	Neutral	Increase tendency

Table 3 Rhodopsins and clock genes in B16 and Melan-a cells under influence of different lighting.

Rhodopsins and clock genes in B16 and Melan-a cells under influence of different lighting									
B16-F10 genes	Ligths mRNA stimulus				Melan-a genes	Ligths mRNA stimulus			
	Dark	White light	UVA	Heat shock		Dark	White light	UVA	Heat shock
BMAL1	down	raise	raise	raise	BMAL1	no variations	no variations	raise	down
CLOC	down	raise	raise	raise	CLOC	no variations	no variations	raise	down
KCRY1	down	no variations	no data	no data	KCRY1	no variations	no variations	no data	no data
PER1	down	raise	down	no variations	PER1	no variations	no variations	down	down
PER2	down	raise	no data	no data	PER2	no variations	no variations	no data	no data
Mice OPN2	no variations	down	raise	raise	Mice OPN2	no variations	no variations	raise	down
Mice IPN4	no variations	raise	raise	raise	Mice IPN4	no variations	no variations	raise	no variations
Mice S-Opisin	no variations	no variations	no variations	no variations	Mice S-Opisin	no variations	no variations	no variations	no variations
MITF	no variations	no data	no data	no data	MITF	no variations	no data	no data	no data
Tyrosinase	no variations	raise	no variations	no data	Tyrosinase	no variations	no variations	no variations	no data
XPA	no variations	no data	raise	down	XPA	no variations	no data	no variations	no variations

But in the opposite way OPN4KO melanocytes and melanoma cells, showed an acute increase of PER1 expression immediately after UVA stimulus (Table 4). Also was found that OPN4 expression is downregulated in human melanoma compared to normal skin. Thus interestingly, metastatic melanomas with low expression of OPN4 present increased expression of BMAL1 and longer overall survival [75]. The skin melanocytes possess a complex photosensitive system comprised of opsins, which display light and thermo-independent responsive functions. Like this, has been investigated whether melanopsin, OPN4, displays such function in normal melanocytes, consequently was found that murine OPN4KO melanocytes displayed a faster proliferation rate compared to OPN4WT melanocytes, thus the cell cycle population analysis demonstrated that OPN4KO melanocytes exhibited a faster cell cycle progression with reduced G0-G1, and highly increased S and slightly increased G2/M cell populations compared to the OPN4WT counterparts. The alterations exhibited by OPN4KO in expression of specific cell cycle-related genes promotes in these cells a faster cell cycle progression. Was also found a significant modification in gene and protein expression levels of important regulators of Melan-a cell physiology. For example, the protein level of PER1 was higher while BMAL1 decreased in OPN4KO Melan-a compared to OPN4WT cells (Table 4).

Another interesting fact is the gene expression of microphthalmia-associated transcription factor (MITF) was upregulated in OPN4KO melanocytes, which is in line with a greater proliferative capability. Like this, was demonstrated that OPN4 regulates cell proliferation, cell cycle, and affects the expression of several important factors of the melanocyte physiology [74]. In this way was also shown that OPN4KO Melan-a cell display independent roles like light, thermal response and faster proliferation by interference in cell cycle progression of murine melanocytes[74, 75].

Table 4 Rhodopsins and clock genes in B16 OPN4KO and Melan-a cells OPN4KO under different lights.

Rhodopsins and clock genes in B16 OPN4KO and Melan-a cells OPN4KO under different lights									
B16-OPN4KO	Ligths mRNA stimulus				Melan-a OPN4KO	Ligths mRNA stimulus			
Genes	Dark	White light	UVA	Heat shock	Genes	Dark	White light	UVA	Heat shock
BMAL1	raise	no variations	raise	raise	BMAL1	down	no variations	raise	no variations
CLOC	no variations	no data	raise	raise	CLOC	no variations	no variations	raise	no variations
KCRY1	no variations	no variations	no data	no data	KCRY1	no variations	no variations	no data	no data
PER1	no variations	raise	raise	no variations	PER1	no variations	no variations	raise	no variations
PER2	no variations	raise	no data	no data	PER2	no variations	no variations	no data	no data
Mice OPN2	no variations	down	raise	raise	Mice OPN2	no variations	no variations	raise	raise
Mice S-Op sin	no variations	no variations	no variations	no variations	Mice S-Op sin	no variations	no variations	no variations	no variations
MITF	down	no data	no data	no data	MITF	raise	no data	no data	no data
Tyrosinase	no variations	no data	no variations	no data	Tyrosinase	no variations	no data	no variations	no data
XPA	no variations	no data	raise	down	XPA	no variations	no data	no variations	no variations

By use of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) to knock out the OPN4 gene in B16-F10 cells was evaluated the putative role of OPN4 in the carcinogenic process of melanoma investigating how the absence of OPN4 in tumor cells would affect the development and progression of melanoma in a murine model using OPN4KO cells [74]. Thus, was verified that in B16-F10 cells the absence of OPN4 results in a slower tumor growth and increased immune cell infiltration in the tumor microenvironment (TME) and consequently was demonstrated that OPN4 can act as an oncogene in murine melanoma B16-F10 cells [76].

6. The role of opsins in animal and human skin

For instance, humans do not have the ability to camouflage for survival (Figure 5) as frogs (Figure 6). Like this, the main functions of opsins in human and animal skin are response to light, temperature variation and sensitivity. Nevertheless, a wide range of opsins is expressed throughout different cell types in skin for example: keratinocytes, melanocytes, dermal fibroblasts, and hair follicle cells (Figure 5). Thus, expression of visual opsins, cone opsin (OPN1) and rhodopsin (OPN2), in human culture of facial skin and normal human epidermal keratinocytes (NHEKs) suggested that OPN2 could be involved in the regulation of keratinocyte differentiation by verification that (410 nm) irradiation of light violet decreases keratinocyte differentiation markers with an increase in OPN2 expression [20, 77]. In addition, two other opsins the OPN3 and OPN5 were also identified in keratinocytes derived from neonatal foreskin [24]. Also was identified in human skin and in NHEKs cultures a type of tetraopsin known by the name of peropsin (RRH) (Table 1), despite RRH had previously identified in cells of the eye on retinal pigmented epithelial (RPE) [30]. Like this was reported that irradiation of cultured keratinocytes with violet light (380 nm) increase Ca²⁺ transients dependent of the all-trans-retinal ligand, suggesting that RRH may contribute to the phototransduction of violet light in keratinocytes. The spectral sensitivity of RRH is unknown, but neuropsin and retinal G protein-coupled receptor opsins (RGR), have peak absorption at 380 and 470 nm, respectively [78]. The expression of OPN1-SW, OPN3, and OPN5 was confirmed in epidermal keratinocytes of human facial and abdominal skin (Figure 5) and showed that irradiation with blue light (447 nm) accelerates closure in wound-healing model in ex vivo experiment [79].

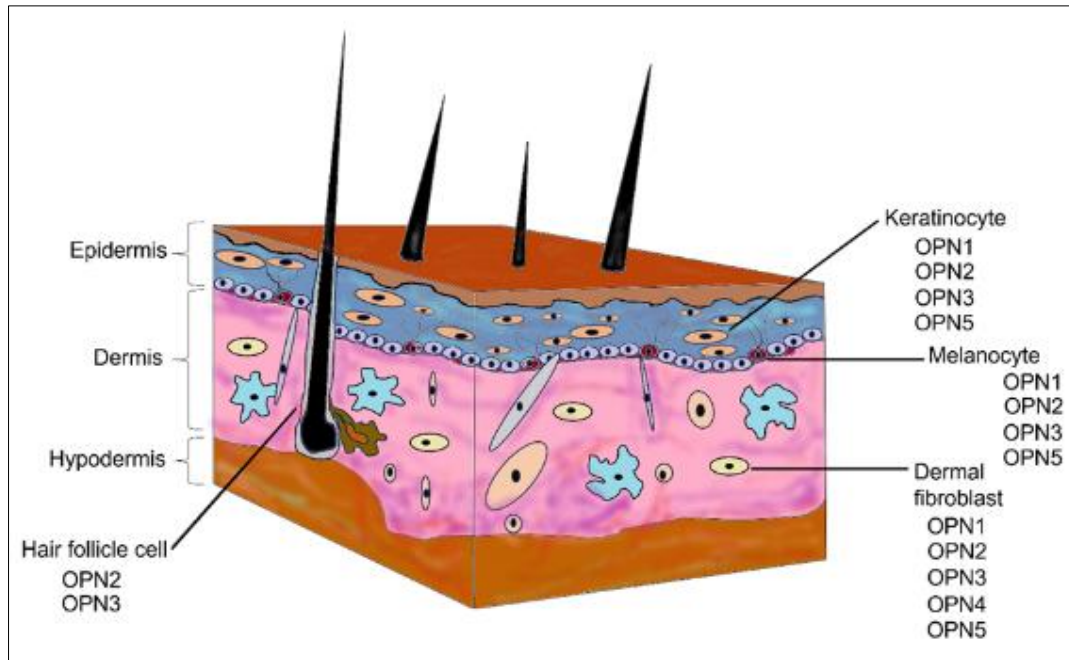


Figure 5 Opsins in human skin

In cultured human epidermal melanocytes (HEMs) has been identified the expression of OPN2 in addition to reported that UV irradiation induces calcium influx and melanin production [21]. In this way it was proposed that melanin synthesis occurs in same pathway, mechanism of visual phototransduction in retina via calcium-mediated $G_{\alpha q}/11$ [80]. Consequently was detected three other opsins OPN1-SW, OPN3, and OPN5 in cultured normal human melanocytes (NHMs), although OPN2 and OPN3 were expressed most abundantly (Figure 5)[24]. Scientific findings demonstrated that opsins, principally encephalopsin (OPN3), have regulatory functions in cellular biology in addition to light or thermal sensors. As soon, OPN3 acts as a negative regulator of melanogenesis through an interaction with melanocortin 1 receptor (MC1R) [81] and participates in apoptotic processes of human melanocytes [82]. Therefore, has been proposed that OPN3 is a key receptor responsible for visible light- because violet light irradiation (415 nm) induced hyperpigmentation in cultured NHMs by pathway of calcium-dependent microphthalmia-associated transcription factor (MITF). Whereas the silencing OPN3 annulled the violet light effect [83]. In this way, has been suggested that OPN3 might play a key role in the regulation of melanogenesis, [81]. Thus has been proposed different mechanism of OPN3 action like for example as a negative modulator via coupling to $G_{\alpha i}$ pathway of melanin production, which inhibits melanocortin 1 receptor (MC1R)-mediated Cyclic adenosine monophosphate (cAMP) response.

Another find reported that OPN3 is a key receptor responsible for survival of human epidermal melanocytes [82]. Thus, the downregulation of OPN3 reduces the intracellular calcium level and triggers the conventional mitochondria apoptosis pathway. The evidence supports the expression of several opsin types in human melanocytes with OPN3 being most abundantly expressed [77].

Like this, many reports of positive effects on hair growth by photobiomodulation is attributed to presence of opsins in the hair follicle stem cells (PBM) [77, 84]. For example, OPN2 and OPN3 was detected in anagen hair follicles (Figure 5) and demonstrated that blue light (453 nm), which corresponds to the absorption spectra of OPN3, prolongs anagen hair growth phase. In contrast, red light did not affect hair growth and silencing OPN3 cancels the stimulatory effects of blue light [26]. Thus, there is few evidence on the functional role of opsin in hair follicle cells. And for example elucidating this mechanism will open new door for utilization of light therapy in alopecia.

Was identified the expression of OPN1-SW, OPN2, and OPN3 in culture of dermal fibroblasts from human facial and abdominal skin tissue [79] (Figure 5). Although the function of these options is still not fully elucidated, it is believed that they are involved in cell proliferation by the antiproliferative effect of blue light (450-490 nm) on fibroblasts in vitro, thus that opsins might be involved in the regulation of cell proliferation [85, 86, 87]. The modulation of dermal fibroblasts by blue light has the potential for the treatment of hypertrophic scarring, such as keloids and other fibrotic skin diseases, and the role of opsins in fibroblasts is worthy of further investigations. [88].

Furthermore, there is evidence that OPN3 is the key sensor responsible for upregulating matrix metalloproteases (MMPs), and in dermal fibroblasts exposed to (UVA) the upregulate of matrix contributes to the skin aging. It has been well known that chronic exposure to UVA radiation induces an increase in MMPs, which leads to the degradation of fibrous connective tissue and skin photoaging. Like this was found five opsins, OPN1- OPN5, in normal human dermal fibroblasts (NHDFs) by qPCR and Western blot analysis, as well as was verified that exposure to UVA particularly increased the expression level of OPN3 and triggered the phototransduction and the expression of MMPs [77].

Biological evidence of dermal opsins found in nonhuman animals support that several dermal opsins mediate dispersed photoreception across the body surface and allow the animals to instantly respond to changes in irradiation of local surrounding [77, 89]. In animals that face a constant threat of predators, dermal photoreception allows them to move away from potential danger, by camouflage or shadow reflex, and control circadian rhythms (Figure 6) [13, 90].

For example, the (OPN4) in dermal melanophores of frogs (*Xenopus laevis*, Chordata) [13, 90, 91] probably works like a molecular mechanism of light-detecting responsible for melanosome migration, and synthesis of various pigments which enables frogs to change their skin colors and avoid of predators (Figure 6) [13,91,92,93]. In this way, the animal's skin cells detect the wavelengths present in the environment and respond by producing pigments which mixed in skin reflect the environmental colors around the animal (Figure 6). However, which opsins are involved in this process requires further investigation [93].

Opsins have been involved in different proposed models, to explain the mechanisms of pigments from organelles called melanosomes in melanocytes to superficial skin or integument (colored) (Figure 6). Figure 6 shows the cytophagocytosis of melanocyte dendrites; fusion of melanocyte in integument cells membranes; shedding of melanosome-laden globules by melanocytes; and exocytosis/phagocytosis of the melanin core. These are the main mechanisms of production of colours in feathers and skin (fish, reptile and amphibian) (Figure 6). produced predominantly by red or yellow pigments in epidermis (Figure 6). Like this, the OPN4 is a light-sensing receptor which mediates melanosome migration, showed by molecular components of OPN4 signaling pathway that were found in cultured dermal melanophores of *Xenopus*. And light increases the intracellular level of inositol trisphosphate and activity of phospholipase C in dermal melanophores during the melanosome migration, suggesting that OPN4 is the key sensor mediating melanosome dispersion in *Xenopus* [92,93].

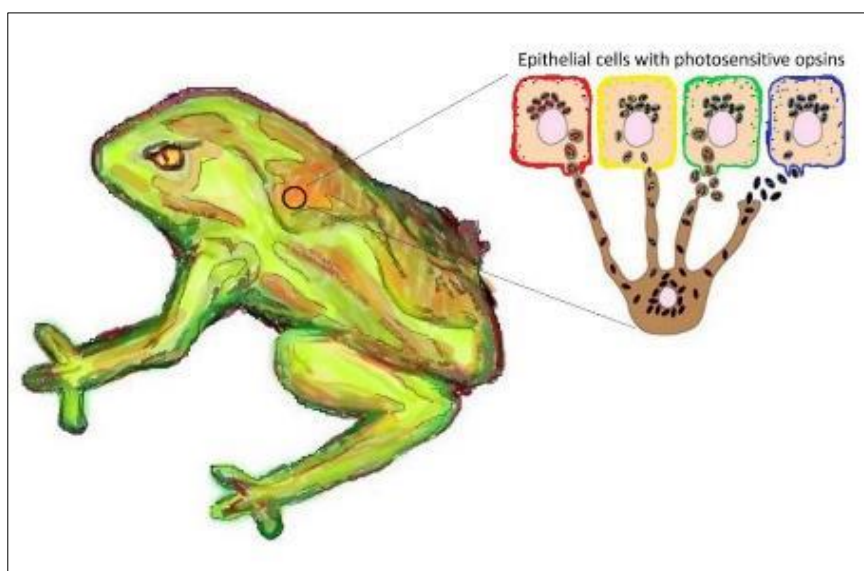


Figure 6 Mechanisms of pigments from melanosomes in melanocytes to superficial skin or integument. The colors show the cytophagocytosis of melanocyte dendrites; fusion of melanocyte in integument cells membranes; shedding of melanosome-laden globules by melanocytes; and exocytosis/phagocytosis of the melanin core. In feathers and skin (fish, reptile and amphibian). produced predominantly by red or yellow pigments in epidermis.

7. Discussion

Thus, by understanding the function of opsins in the skin, we could attribute a therapeutic of photobiomodulation (PBM) to approach various dermatological conditions, such as psoriasis, atopic dermatitis, hair growth, wound healing and

tissue regeneration [94, 95, 96]. For example, each opsin has distinct absorption spectra and signaling transduction, thus optimization of light therapy to each opsin is important for adapt and maximize the benefit of PBM for medical treatments [77]. The opsins are important for the camouflage of animals that mimic the environment by changing the color of their skin. Like this, the OPN4 in dermal melanophores of frog identify the light, promoting the migration of melanosome, to enables the amphibian modify skin color [13].

Thus, many studies have supported the existence of skin photosensors across different species suggesting the influence of opsins on behavior especially on the circadian cycle in addition to electrochemical communication and maintain the body temperature. Thereby, most studies supported the existence of dermal photosensitivity through animal behavior and physiology. For example studies demonstrated that opsins are involved in various physiological processes of the skin, including wound healing, melanogenesis, photoaging, and hair growth [79, 81].

Thereby, many studies have been performed on cultured skin cells, which might not display the exact morphological and physiological properties of native tissue *in vivo*. For example cultured cells like B16 and Melan-a after multiple passages alter their specific phenotypes. Therefore, it's hard to correlate the data with animal tissue. As soon research in dermal opsins has great potentials for advancing the clinical applications of PBM. The application of light therapy to clinical cases owing to the advantages of a cost-effective and noninvasive approach. Thus, to maximize the benefit of light therapy, is important to understand the underlying mechanisms of opsins in physiological processes in skin to define the light parameters that elicit such responses. For example studies, has demonstrated the stimulatory effect on hair follicle stem cells and melanocytes with specific wavelengths of light irradiation, like this PBM could be a new promising treatment for hair loss and skin pigmentation disorders [26, 81, 95, 96, 97].

8. Conclusion

Finally, the mechanistic role of opsins in the skin, can be understanding in future investigations using *in vitro* treatments with different cell types and knockout mice as tool to confirm the therapeutic of PBM. As soon, optimization of light exposure time, wavelength, intensity, and treatment interval will meliorate the clinical application of PBM. Thus, a deeper understanding of BPM treatment in different models of PBM still requires a understanding of biochemical processes, as soon current literature which supports that PMB is a promising therapeutic modality in clinical dermatology and skin aging.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

References

- [1] Gatti F. Molecular dynamics simulated by photons. *Nature*. 2018;557(7707):641-2.
- [2] Deupi X. Relevance of rhodopsin studies for GPCR activation. *Biochim Biophys Acta*. 2014;1837(5):674-82.
- [3] Terakita A. The opsins. *Genome Biol*. 2005;6(3):213.
- [4] Nordström K, Larsson TA, Larhammar D. Extensive duplications of phototransduction genes in early vertebrate evolution correlate with block (chromosome) duplications. *Genomics*. 2004;83(5):852-72.
- [5] Kuraku S, Meyer A, Kuratani S. Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? *Mol Biol Evol*. 2009;26(1):47-59.
- [6] Shichida Y, Matsuyama T. Evolution of opsins and phototransduction. *Philos Trans R Soc Lond B Biol Sci*. 2009;364(1531):2881-95.
- [7] Pergner J, Kozmik Z. Amphioxus photoreceptors - insights into the evolution of vertebrate opsins, vision and circadian rhythmicity. *Int J Dev Biol*. 2017;61(10-11-12):665-81.

- [8] Moraes MN, de Assis LVM, Provencio I, Castrucci AML. Opsins outside the eye and the skin: a more complex scenario than originally thought for a classical light sensor. *Cell Tissue Res.* 2021;385(3):519-38.
- [9] Navidi M, Yadav S, Struts AV, Brown MF, Nesnas N. Synthesis of 9-CD. *Tetrahedron Lett.* 2018;59(51):4521-4.
- [10] Nadal-Nicolás FM, Kunze VP, Ball JM, Peng BT, Krishnan A, Zhou G, et al. True S-cones are concentrated in the ventral mouse retina and wired for color detection in the upper visual field. *Elife.* 2020;9.
- [11] Max M, McKinnon PJ, Seidenman KJ, Barrett RK, Applebury ML, Takahashi JS, et al. Pineal opsin: a nonvisual opsin expressed in chick pineal. *Science.* 1995;267(5203):1502-6.
- [12] Yokoyama S, Zhang H. Cloning and characterization of the pineal gland-specific opsin gene of marine lamprey (*Petromyzon marinus*). *Gene.* 1997;202(1-2):89-93.
- [13] Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: An opsin in melanophores, brain, and eye. *Proc Natl Acad Sci U S A.* 1998;95(1):340-5.
- [14] Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. *J Neurosci.* 2000;20(2):600-5.
- [15] Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, et al. Melanopsin is required for non-image-forming photic responses in blind mice. *Science.* 2003;301(5632):525-7.
- [16] Miyashita Y, Moriya T, Kubota T, Yamada K, Asami K. Expression of opsin molecule in cultured murine melanocyte. *J Investig Dermatol Symp Proc.* 2001;6(1):54-7.
- [17] Lopes GJ, Góis CC, Lima LH, Castrucci AM. Modulation of rhodopsin gene expression and signaling mechanisms evoked by endothelins in goldfish and murine pigment cell lines. *Braz J Med Biol Res.* 2010;43(9):828-36.
- [18] Bellingham J, Whitmore D, Philp AR, Wells DJ, Foster RG. Zebrafish melanopsin: isolation, tissue localisation and phylogenetic position. *Brain Res Mol Brain Res.* 2002;107(2):128-36.
- [19] Oshima N. Direct reception of light by chromatophores of lower vertebrates. *Pigment Cell Res.* 2001;14(5):312-9.
- [20] Tsutsumi M, Ikeyama K, Denda S, Nakanishi J, Fuziwara S, Aoki H, et al. Expressions of rod and cone photoreceptor-like proteins in human epidermis. *Exp Dermatol.* 2009;18(6):567-70.
- [21] Wicks NL, Chan JW, Najera JA, Ciriello JM, Oancea E. UVA phototransduction drives early melanin synthesis in human melanocytes. *Curr Biol.* 2011;21(22):1906-11.
- [22] Denda M, Fuziwara S. Visible radiation affects epidermal permeability barrier recovery: selective effects of red and blue light. *J Invest Dermatol.* 2008;128(5):1335-6.
- [23] Kojima D, Mori S, Torii M, Wada A, Morishita R, Fukada Y. UV-sensitive photoreceptor protein OPN5 in humans and mice. *PLoS One.* 2011;6(10):e26388.
- [24] Haltaufderhyde K, Ozdeslik RN, Wicks NL, Najera JA, Oancea E. Opsin expression in human epidermal skin. *Photochem Photobiol.* 2015;91(1):117-23.
- [25] Kim HJ, Son ED, Jung JY, Choi H, Lee TR, Shin DW. Violet light down-regulates the expression of specific differentiation markers through Rhodopsin in normal human epidermal keratinocytes. *PLoS One.* 2013;8(9):e73678.
- [26] Buscone S, Mardaryev AN, Raafs B, Bikker JW, Sticht C, Gretz N, et al. A new path in defining light parameters for hair growth: Discovery and modulation of photoreceptors in human hair follicle. *Lasers Surg Med.* 2017;49(7):705-18.
- [27] de Assis LV, Moraes MN, da Silveira Cruz-Machado S, Castrucci AM. The effect of white light on normal and malignant murine melanocytes: A link between opsins, clock genes, and melanogenesis. *Biochim Biophys Acta.* 2016;1863(6 Pt A):1119-33.
- [28] de Assis LVM, Moraes MN, Magalhães-Marques KK, Castrucci AML. Melanopsin and rhodopsin mediate UVA-induced immediate pigment darkening: Unravelling the photosensitive system of the skin. *Eur J Cell Biol.* 2018;97(3):150-62.
- [29] de Assis LVM, Moraes MN, Magalhães-Marques KK, Kinker GS, da Silveira Cruz-Machado S, Castrucci AML. Non-Metastatic Cutaneous Melanoma Induces Chronodisruption in Central and Peripheral Circadian Clocks. *Int J Mol Sci.* 2018;19(4).

- [30] Toh PP, Bigliardi-Qi M, Yap AM, Sriram G, Stelmashenko O, Bigliardi P. Expression of peropsin in human skin is related to phototransduction of violet light in keratinocytes. *Exp Dermatol*. 2016;25(12):1002-5.
- [31] Lan Y, Wang Y, Lu H. Opsin 3 is a key regulator of ultraviolet A-induced photoageing in human dermal fibroblast cells. *Br J Dermatol*. 2020;182(5):1228-44.
- [32] Kusumoto J, Takeo M, Hashikawa K, Komori T, Tsuji T, Terashi H, et al. OPN4 belongs to the photosensitive system of the human skin. *Genes Cells*. 2020;25(3):215-25.
- [33] Hinterhuber G, Cauza K, Brugger K, Dingelmaier-Hovorka R, Horvat R, Wolff K, et al. RPE65 of retinal pigment epithelium, a putative receptor molecule for plasma retinol-binding protein, is expressed in human keratinocytes. *J Invest Dermatol*. 2004;122(2):406-13.
- [34] Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, et al. Role of the CLOCK protein in the mammalian circadian mechanism. *Science*. 1998;280(5369):1564-9.
- [35] Yao Z, Bennett AJ, Clem JL, Shafer OT. The Drosophila Clock Neuron Network Features Diverse Coupling Modes and Requires Network-wide Coherence for Robust Circadian Rhythms. *Cell Rep*. 2016;17(11):2873-81.
- [36] Nitabach MN, Taghert PH. Organization of the Drosophila circadian control circuit. *Curr Biol*. 2008;18(2):R84-93.
- [37] Vansteensel MJ, Michel S, Meijer JH. Organization of cell and tissue circadian pacemakers: a comparison among species. *Brain Res Rev*. 2008;58(1):18-47.
- [38] Ralph MR, Foster RG, Davis FC, Menaker M. Transplanted suprachiasmatic nucleus determines circadian period. *Science*. 1990;247(4945):975-8.
- [39] Brown SA, Azzi A. Peripheral circadian oscillators in mammals. *Handb Exp Pharmacol*. 2013(217):45-66.
- [40] Mohawk JA, Green CB, Takahashi JS. Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci*. 2012;35:445-62.
- [41] Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, et al. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science*. 2002;298(5601):2213-6.
- [42] Balsalobre A, Damiola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell*. 1998;93(6):929-37.
- [43] Zanello SB, Jackson DM, Holick MF. Expression of the circadian clock genes clock and period1 in human skin. *J Invest Dermatol*. 2000;115(4):757-60.
- [44] Takahashi JS, Hong HK, Ko CH, McDearmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet*. 2008;9(10):764-75.
- [45] King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, et al. Positional cloning of the mouse circadian clock gene. *Cell*. 1997;89(4):641-53.
- [46] Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science*. 1994;264(5159):719-25.
- [47] Meijer JH, Schwartz WJ. In search of the pathways for light-induced pacemaker resetting in the suprachiasmatic nucleus. *J Biol Rhythms*. 2003;18(3):235-49.
- [48] Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, et al. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab*. 2006;4(2):163-73.
- [49] Reinhard N, Schubert FK, Bertolini E, Hagedorn N, Manoli G, Sekiguchi M, et al. The Neuronal Circuit of the Dorsal Circadian Clock Neurons in. *Front Physiol*. 2022;13:886432.
- [50] Hardin PE. From biological clock to biological rhythms. *Genome Biol*. 2000;1(4):REVIEWS1023.
- [51] Hung HC, Maurer C, Zorn D, Chang WL, Weber F. Sequential and compartment-specific phosphorylation controls the life cycle of the circadian CLOCK protein. *J Biol Chem*. 2009;284(35):23734-42.
- [52] Yu W, Zheng H, Houl JH, Dauwalder B, Hardin PE. PER-dependent rhythms in CLK phosphorylation and E-box binding regulate circadian transcription. *Genes Dev*. 2006;20(6):723-33.
- [53] Lundgren J, Masson P, Mirzaei Z, Young P. Identification and characterization of a Drosophila proteasome regulatory network. *Mol Cell Biol*. 2005;25(11):4662-75.
- [54] Cyran SA, Yiannoulos G, Buchsbaum AM, Saez L, Young MW, Blau J. The double-time protein kinase regulates the subcellular localization of the Drosophila clock protein period. *J Neurosci*. 2005;25(22):5430-7.

- [55] Huang N, Chelliah Y, Shan Y, Taylor CA, Yoo SH, Partch C, et al. Crystal structure of the heterodimeric CLOCK:BMAL1 transcriptional activator complex. *Science*. 2012;337(6091):189-94.
- [56] Covassin N, Singh P, Somers VK. Keeping Up With the Clock: Circadian Disruption and Obesity Risk. *Hypertension*. 2016;68(5):1081-90.
- [57] Bjarnason GA, Jordan RC, Wood PA, Li Q, Lincoln DW, Sothorn RB, et al. Circadian expression of clock genes in human oral mucosa and skin: association with specific cell-cycle phases. *Am J Pathol*. 2001;158(5):1793-801.
- [58] Sandu C, Dumas M, Malan A, Sambakhe D, Marteau C, Nizard C, et al. Human skin keratinocytes, melanocytes, and fibroblasts contain distinct circadian clock machineries. *Cell Mol Life Sci*. 2012;69(19):3329-39.
- [59] Spörl F, Schellenberg K, Blatt T, Wenck H, Wittern KP, Schrader A, et al. A circadian clock in HaCaT keratinocytes. *J Invest Dermatol*. 2011;131(2):338-48.
- [60] Tanioka M, Yamada H, Doi M, Bando H, Yamaguchi Y, Nishigori C, et al. Molecular clocks in mouse skin. *J Invest Dermatol*. 2009;129(5):1225-31.
- [61] Hardman JA, Tobin DJ, Haslam IS, Farjo N, Farjo B, Al-Nuaimi Y, et al. The peripheral clock regulates human pigmentation. *J Invest Dermatol*. 2015;135(4):1053-64.
- [62] Gutierrez D, Arbesman J. Circadian Dysrhythmias, Physiological Aberrations, and the Link to Skin Cancer. *Int J Mol Sci*. 2016;17(5).
- [63] Yosipovitch G, Sackett-Lundeen L, Goon A, Yiong Huak C, Leok Goh C, Haus E. Circadian and ultradian (12 h) variations of skin blood flow and barrier function in non-irritated and irritated skin-effect of topical corticosteroids. *J Invest Dermatol*. 2004;122(3):824-9.
- [64] Yosipovitch G, Xiong GL, Haus E, Sackett-Lundeen L, Ashkenazi I, Maibach HI. Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *J Invest Dermatol*. 1998;110(1):20-3.
- [65] van Steeg H, de Vries A, van Oostrom CTh, van Benthem J, Beems RB, van Kreijl CF. DNA repair-deficient Xpa and Xpa/p53+/- knock-out mice: nature of the models. *Toxicol Pathol*. 2001;29 Suppl:109-16.
- [66] Gaddameedhi S, Selby CP, Kaufmann WK, Smart RC, Sancar A. Control of skin cancer by the circadian rhythm. *Proc Natl Acad Sci U S A*. 2011;108(46):18790-5.
- [67] Geyfman M, Kumar V, Liu Q, Ruiz R, Gordon W, Espitia F, et al. Brain and muscle Arnt-like protein-1 (BMAL1) controls circadian cell proliferation and susceptibility to UVB-induced DNA damage in the epidermis. *Proc Natl Acad Sci U S A*. 2012;109(29):11758-63.
- [68] de Assis LVM, Moraes MN, Castrucci AML. Heat shock antagonizes UVA-induced responses in murine melanocytes and melanoma cells: an unexpected interaction. *Photochem Photobiol Sci*. 2017;16(5):633-48.
- [69] Areshidze DA, Kozlova MA, Chernikov VP, Borisov AV, Mischenko DV. Characteristic of Ultrastructure of Mice B16 Melanoma Cells under the Influence of Different Lighting Regimes. *Clocks Sleep*. 2022;4(4):745-60.
- [70] Schubert R, Bae T, Simic B, Smith SN, Park SH, Nagy-Davidescu G, et al. CRISPR-clear imaging of melanin-rich B16-derived solid tumors. *Commun Biol*. 2023;6(1):370.
- [71] Liu Y, Chi W, Tao L, Wang G, Deepak RNVK, Sheng L, et al. Ablation of Proton/Glucose Exporter SLC45A2 Enhances Melanosomal Glycolysis to Inhibit Melanin Biosynthesis and Promote Melanoma Metastasis. *J Invest Dermatol*. 2022;142(10):2744-55.e9.
- [72] Ginger RS, Askew SE, Ogborne RM, Wilson S, Ferdinando D, Dadd T, et al. SLC24A5 encodes a trans-Golgi network protein with potassium-dependent sodium-calcium exchange activity that regulates human epidermal melanogenesis. *J Biol Chem*. 2008;283(9):5486-95.
- [73] Shakil M, Harlalka GV, Ali S, Lin S, D'Atri I, Hussain S, et al. Tyrosinase (TYR) gene sequencing and literature review reveals recurrent mutations and multiple population founder gene mutations as causative of oculocutaneous albinism (OCA) in Pakistani families. *Eye (Lond)*. 2019;33(8):1339-46.
- [74] de Assis LVM, Moraes MN, Mendes D, Silva MM, Menck CFM, Castrucci AML. Loss of Melanopsin (OPN4) Leads to a Faster Cell Cycle Progression and Growth in Murine Melanocytes. *Curr Issues Mol Biol*. 2021;43(3):1436-50.
- [75] de Assis LVM, Mendes D, Silva MM, Kinker GS, Pereira-Lima I, Moraes MN, et al. Melanopsin mediates UVA-dependent modulation of proliferation, pigmentation, apoptosis, and molecular clock in normal and malignant melanocytes. *Biochim Biophys Acta Mol Cell Res*. 2020;1867(10):118789.

- [76] de Assis LVM, Lacerda JT, Moraes MN, Domínguez-Amorocho OA, Kinker GS, Mendes D, et al. Melanopsin (Opn4) is an oncogene in cutaneous melanoma. *Commun Biol.* 2022;5(1):461.
- [77] Suh S, Choi EH, Atanaskova Mesinkovska N. The expression of opsins in the human skin and its implications for photobiomodulation: A Systematic Review. *Photodermatol Photoimmunol Photomed.* 2020;36(5):329-38.
- [78] Kumbalasisiri T, Provencio I. Melanopsin and other novel mammalian opsins. *Exp Eye Res.* 2005;81(4):368.
- [79] Castellano-Pellicena I, Uzunbajakava NE, Mignon C, Raafs B, Botchkarev VA, Thornton MJ. Does blue light restore human epidermal barrier function via activation of Opsin during cutaneous wound healing? *Lasers Surg Med.* 2019;51(4):370-82.
- [80] Bellono NW, Najera JA, Oancea E. UV light activates a $G\alpha_q/11$ -coupled phototransduction pathway in human melanocytes. *J Gen Physiol.* 2014;143(2):203-14.
- [81] Ozdeslik RN, Olinski LE, Trieu MM, Oprian DD, Oancea E. Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with melanocortin 1 receptor. *Proc Natl Acad Sci U S A.* 2019;116(23):11508-17.
- [82] Wang Y, Lan Y, Lu H. Opsin3 Downregulation Induces Apoptosis of Human Epidermal Melanocytes via Mitochondrial Pathway. *Photochem Photobiol.* 2020;96(1):83-93.
- [83] Regazzetti C, Sormani L, Debayle D, Bernerd F, Tulic MK, De Donatis GM, et al. Melanocytes Sense Blue Light and Regulate Pigmentation through Opsin-3. *J Invest Dermatol.* 2018;138(1):171-8.
- [84] Mignon C, Botchkareva NV, Uzunbajakava NE, Tobin DJ. Photobiomodulation devices for hair regrowth and wound healing: a therapy full of promise but a literature full of confusion. *Exp Dermatol.* 2016;25(10):745-9.
- [85] Opländer C, Hidding S, Werners FB, Born M, Pallua N, Suschek CV. Effects of blue light irradiation on human dermal fibroblasts. *J Photochem Photobiol B.* 2011;103(2):118-25.
- [86] Tafliński L, Demir E, Kauczok J, Fuchs PC, Born M, Suschek CV, et al. Blue light inhibits transforming growth factor- $\beta 1$ -induced myofibroblast differentiation of human dermal fibroblasts. *Exp Dermatol.* 2014;23(4):240-6.
- [87] Masson-Meyers DS, Bumah VV, Enwemeka CS. Blue light does not impair wound healing in vitro. *J Photochem Photobiol B.* 2016;160:53-60.
- [88] Mamalis A, Garcha M, Jagdeo J. Light emitting diode-generated blue light modulates fibrosis characteristics: fibroblast proliferation, migration speed, and reactive oxygen species generation. *Lasers Surg Med.* 2015;47(2):210-5.
- [89] Sunada H, Sakaguchi T, Horikoshi T, Lukowiak K, Sakakibara M. The shadow-induced withdrawal response, dermal photoreceptors, and their input to the higher-order interneuron RPeD11 in the pond snail *Lymnaea stagnalis*. *J Exp Biol.* 2010;213(Pt 20):3409-15.
- [90] Leung NY, Montell C. Unconventional Roles of Opsins. *Annu Rev Cell Dev Biol.* 2017;33:241-64.
- [91] Shawkey MD, D'Alba L. Interactions between colour-producing mechanisms and their effects on the integumentary colour palette. *Philos Trans R Soc Lond B Biol Sci.* 2017;372(1724).
- [92] Isoldi MC, Rollag MD, Castrucci AM, Provencio I. Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. *Proc Natl Acad Sci U S A.* 2005;102(4):1217-21.
- [93] chweikert LE, Bagge LE, Naughton LF, Bolin JR, Wheeler BR, Grace MS, et al. Dynamic light filtering over dermal opsin as a sensory feedback system in fish color change. *Nat Commun.* 2023;14(1):4642.
- [94] Serrage H, Heiskanen V, Palin WM, Cooper PR, Milward MR, Hadis M, et al. Under the spotlight: mechanisms of photobiomodulation concentrating on blue and green light. *Photochem Photobiol Sci.* 2019;18(8):1877-909.
- [95] Aragona SE, Grassi FR, Nardi G, Lotti J, Mereghetti G, Canavesi E, et al. Photobiomodulation with polarized light in the treatment of cutaneous and mucosal ulcerative lesions. *J Biol Regul Homeost Agents.* 2017;31(2 Suppl. 2):213-8.
- [96] Hamblin MR. Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. *AIMS Biophys.* 2017;4(3):337-61.
- [97] Eric Almeida Xavier. The macromolecular machinery of the nucleus in the senescence process. *World Journal of Advanced Research and Reviews.* 2022; 15(02), 393–404