RESEARCH ARTICLE

Circadian rhythms: influence on skin cancer and exposure paradigms

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Abstract

Approximately 10% of genes oscillate according to a circadian clock. Even though cells are capable of independent oscillation there is a master controller in the brain, the suprachiasmatic nucleus (SCN), that provides a coordinated response throughout the body, influenced by daily and seasonal patterns of light and heat. These genes have widely varied functions but are significantly influential in DNA damage repair, the cell cycle, cellular proliferation and apoptosis, as well as metabolic function. Normal circadian rhythms are essential for the body's natural defence against disease and cancer. Deregulation may enhance the capacity for carcinogenesis in the skin and the influence of the circadian clock helps explain two of the anomalies of melanoma exposure patterns: A higher incidence amongst indoor as opposed to outdoor workers and on intermittently as opposed chronically exposed skin.

1. Introduction

"The primal light the whole illuminates,
And is received therein as many ways
As there are splendours wherewithin it mates."

The Divine Comedy Dante

Solar radiation, which includes ultraviolet radiation (UVR), amongst a range of other wavelengths of light, plays a vital role in a variety of biological functions within living systems. Under natural conditions, we are exposed to solar radiation in a regular 24-hour cycle of day and night with variation according to seasonal changes. This radiation has beneficial as well as potentially harmful effects on the environment and life on earth, and particularly to humans in terms of skin cancer[1,2,3,4]. Light is the regulatory stimulus of circadian rhythms with physical, mental, and behavioural changes that follow this 24-hour cycle. Evolution has provided a range of protective mechanisms in response to this oscillation especially in relation to skin cancer. Approximately 10% of genes oscillate according to a circadian clock. Even though cells are capable of independent oscillation there is a master controller in the brain, the suprachiasmatic nucleus (SCN) that provides a coordinated response throughout the body, influenced by daily and seasonal patterns of light and heat. These genes have widely varied functions but are significantly influential in DNA damage repair, the cell cycle, cellular proliferation and apoptosis, as well as metabolic function[5]. However, lifestyle factors of modern living can influence these biological processes. Widespread use of artificial light overrides the restrictions in activity of natural darkness. Late nights result in missing the entrainment of light at dawn. We experience intermittent and often extended periods of recreational, and less significantly occupational exposure to sunlight. Emigration and air travel have relocated whole populations of individuals to more tropical climates. This deregulation of circadian rhythms within natural exposure patterns may be a factor in the development and progression in a range of skin cancers, including Melanoma[6].

2. Control and regulation of the circadian system

Molecular and gene expression patterns that control the circadian system are now relatively well understood. Light, activating intrinsically photosensitive ganglion cells in the retina, through the photoreceptor pigment melanopsin, transmits the information centrally in the retino-hypothalamic tract running in conjunction with the optic nerve to the hypothalamic suprachiasmatic nucleus (SCN), entraining the central controller. Figure 1.

Other organs and tissues have their own clocks, the skin also harbors a robust intrinsic clock[7,8]. The circadian clock mechanism is an autoregulatory gene expression feedback loop. CLOCK/ BMAL1 transcription factors induce expression of their own inhibitors, PERIOD (PER) and CRYPTOCHROME (CRY), creating a self-sustaining 24-hour rhythm in gene expression[9]. The nuclear receptors Rors and RevErbs constitute an auxiliary transcriptional loop that regulates expression of BMAL1. By acting at their genomic regulatory sequences, the circadian clock transcription factors generate rhythmic oscillations in the expression of a large number of output genes, largely tissue and cell type specific, but particularly influencing skin physiology and adaptive cellular responses to environmental stressors.

The clock mechanism anticipates the vastly different environments of day and night to maintain the homeostasis of the skin in relation to toxins, pathogens, injury, UVR, temperature and water loss. It is an

evolutionary ancient system adjusting physiology to the anticipated diurnal changes.

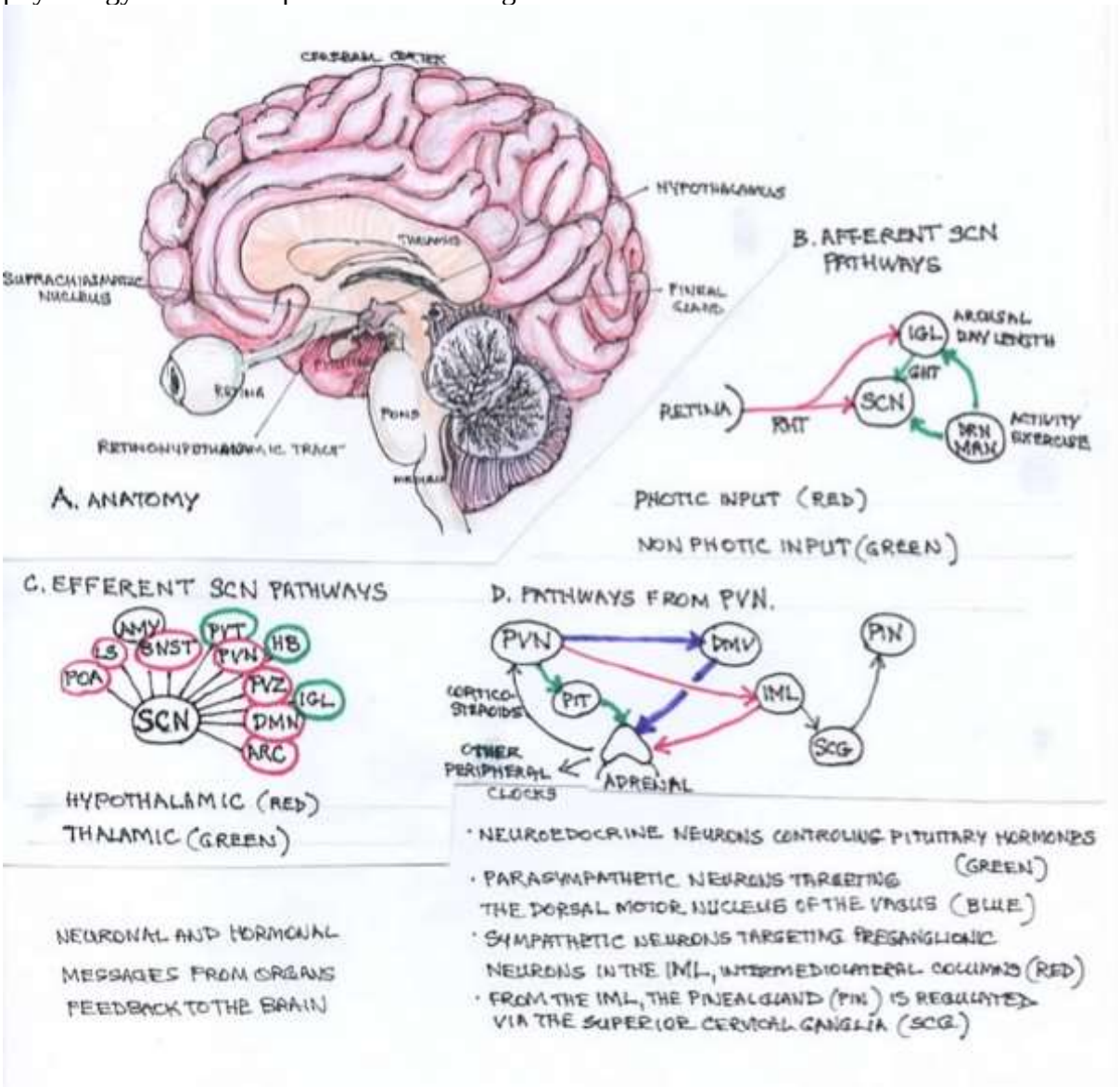

Figure 1. The suprachiasmatic nucleus: A relay to synchronise physiology to environmental change. AMY, amygdala; ARC, arcuate; BNST, bed nucleus of stria terminalis; DMH, dorsomedial hypothalamus; DRN, dorsal raphe nucleus; GHT, geniculate-hypothalamic tract; HB, Habenular; IGL, intergeniculate leaflet; LS, lateral septum; MRN, medial raphe nucleus; POA, preoptic area; PVN, paraventricular nucleus of hypothalamus, PVT, paraventricular nucleus of thalamus; RHT, retino-hypothalamic tract; SC, suprachiasmatic nucleus; SPVZ, sub-paraventricular zone. (Adapted from Dibner et al, 2010[10])

3. Circadian involvement in DNA damage repair response

There are several types of DNA repair in mammals. Direct repair by alkyl transferases, base excision repair (BER) by glycosylases and AP endonucleases, double strand break/crosslink repair and nucleotide excision repair (NER). NER is strongly clock controlled and is carried out by 6 factors: Replication protein A (RPA), Xeroderma

Pigmentosum Complementation Groups A, C, G and F (XPA/C/G/F) and Transcription factor II Human (TFIIH). Figure 2.

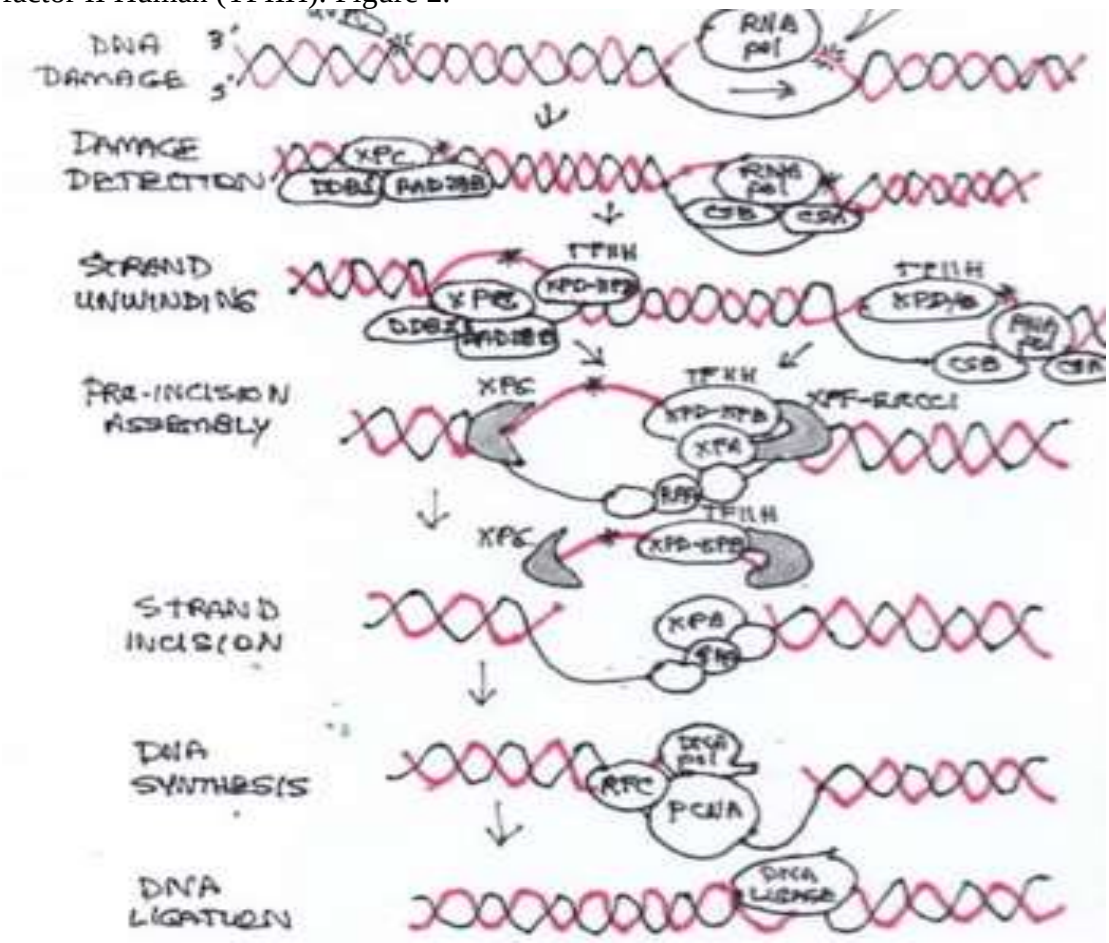

Figure 2. NER- Global genomic or Transcription-coupled Nucleotide incision repair.
Sensors detect damage. GG-NER; XPC, RAD23, DDB2: TC-NER; RNA pol, CSA, CSB.
Sensors initiate unwinding and stabilisation. RPA, TFIIH and XPA.
Helicase enzymes XPB and XPD (part of TFIIH) unwind DNA, XPA stabilises DNA.
Binding of specific 5' and 3'strand endonucleases; XPF, ERCC1 and XPG to repair fork,
Cleaving a 25-30 nucleotide strand (containing the lesion).
DNA synthesis. RPA, PCNA, RF-C, DNA polymerases (Pol) **δ** and ε.
New DNA is ligated at 5' by DNA ligases. (Adapted from Sarkar & Gaddameedhi 2020)[11]

In a study by Gaddameedhi et al they were able to show that circadian rhythms have an impact on the skin's ability to cope with UVB damage. Xeroderma pigmentosum group A (XPA) is critical to the body's DNA damage repair mechanism, being one of several proteins which carry out DNA nucleotide excision repair (NER). This is the major repair mechanism that deals with bulky UV-induced cyclobutene pyrimidine dimers (CPDs) and 6-4 photo-products (6-4 PP)[12,13]. They showed that XPA oscillates in the skin with circadian rhythmicity, peaking in mice in the early evening and reaching its lowest point of expression in the early morning. They then when on to show that mice subjected to UVB radiation in the early morning demonstrated 5 times the frequency and a faster growth rate of skin cancer than mice subjected to the same level of radiation in the early evening.

The study showed that normal circadian oscillation is important in the body's natural defence against UV-induced skin cancer and should be considered when studying UV exposure and skin cancer. They suggested that circadian rhythm could be exploited to reduce skin cancer incidence in humans. As the core circadian clock and their outputs exhibit opposite phases in mice (nocturnal) versus humans (diurnal) they predict that humans will have a higher rate of repair in the morning and would be less prone to the carcinogenic effects of UV radiation early in the day. They further suggest advising patients to restrict occupational, therapeutic, recreational, and cosmetic UV radiation exposure to the morning hours[14].

In line with Gaddameedhi's work, Sancar's group showed that NER activity is rhythmic in the brain and liver. In the skin, XPA was found to be the only NER related protein to demonstrate oscillation, in antiphase to CRY1. UVB-induced damage was found to be highest in the afternoon (4-5 pm) and lowest in the morning (4-5am)[15].

More recent studies in humans have again supported this concept with milder UVB-induced erythema in the morning as compared to the afternoon[16].

BER may also be rhythmic in humans as the expression of 8 oxo guanine DNA glycosylase (OGG1) is rhythmic. Oxidative DNA repair in lymphocytes is higher in the morning as compared to the afternoon and Bmal1 knockdown leads to loss of rhythmic expression of OGG1[17].

4. DNA damage response in melanocytes

The significance of NER in the protection of melanocytes is evident in Xeroderma Pigmentosum patients who have compromised NER genes. These patients are 2000 times more likely to develop melanoma as compared to normal individuals[18].

Inter- and intracellular signalling cross-talk maintains skin homeostasis against environmental stressors, such as UVR, with strong interaction between the various cell types. In one of the best known examples UV-induced damage in keratinocytes triggering p53-mediated αMSH synthesis. This acts as a ligand binding to the melanocortin 1receptor (MC1R) on the melanocyte, initiating cAMP-mediated melanogenesis. Other hormones have been shown to influence pigmentary regulation in melanocytes. Fibroblast and hepatocyte growth factors and stem cell factor[19]. A complex of paracrine and autocrine networks influences melanogenesis and DNA defence response (DDR) signalling mechanisms, including NER and BER. Stimulation of MC1R enhances NER efficacy and genomic stability. MC1R stimulation resulting in PKA-mediated ATR phosphorylation, stabilising XPA and allowing co-localisation of DNA photolesions[20]. MC1R genotype influencing DDR in melanocytes with multiple single nucleotide polymorphism in MC1R affecting function. This is most strongly expressed in the red hair phenotype with its extreme sensitivity to UV exposure[21]. It has been shown that in the Australia approximately 50% of the population carry at least one of these dysfunctional MC1R genes making sun exposure pattern choices and protective measures vital to maintaining good health[22].

Both MC1R and ET-1 influence localisation of XPA, in nuclear and chromatin fractions, on UV exposure. These distinct signalling pathways regulated common targets in DDR signalling. Their backup providing more effective clearance of DNA mutations. Figure 3.

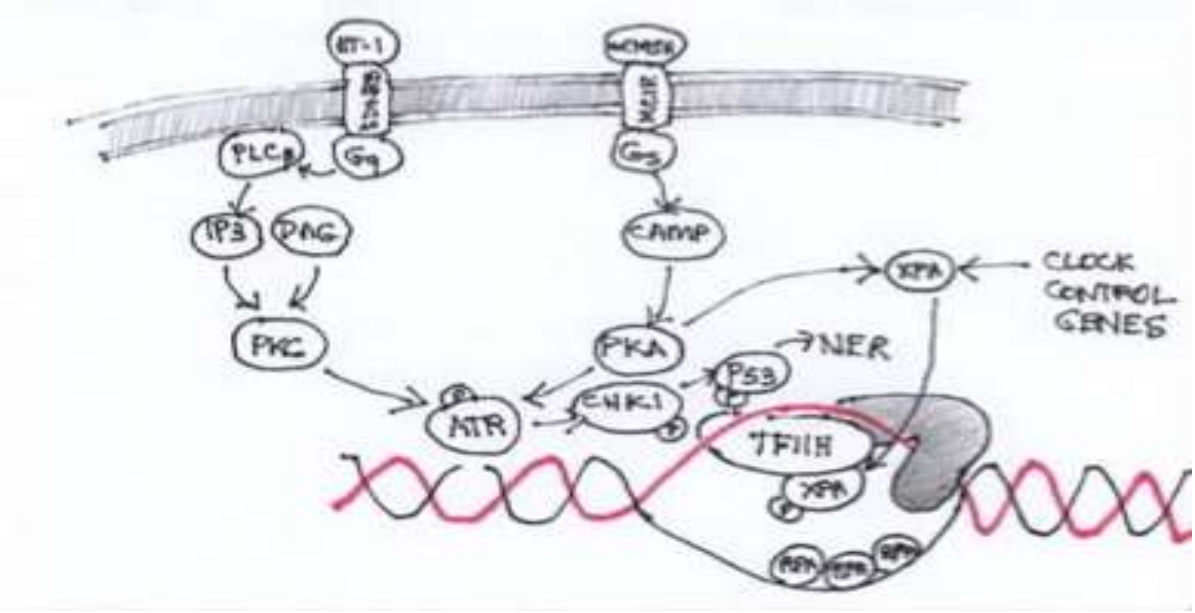

Figure 3. Regulators of NER in Melanocytes.
NER is a major DNA repair pathway in mammals which removes UV-induced photolesions from genomic DNA. NER is a self-driven process initiated with detection by XPC (sensor proteins), but it takes input from cellular processes to function efficiently. In melanocytes, apart from endogenous regulators it is influenced by exogenous paracrine factors αMSH and ET-1. These factors influence the damage sensor protein ATR, which in turn influences NER, either directly through a p53-mediated pathway or by stabilisation of XPA, an essential rate-limiting NER protein. In parallel, the circadian clock influences NER through transcriptional regulation of XPA. Clock regulation of NER anticipates the requirements of NER according to the time of day. (Adapted from Sarkar & Gaddameedhi 2020)[11]

5. Oxidative effects

Control of reactive oxygen and nitrogen species (ROS/RNS) is important for cellular signalling and homeostasis as these molecules act as secondary messengers used to control a variety of cellular processes. ROS are a by-product of normal cellular metabolism, mainly mitochondria. Their production is balanced by protective enzymes and small-molecule antioxidants that also express a circadian rhythmicity. Exposure to environmental stresses, and in the case of melanomagenesis, UVR, can disrupt this control with increased levels of these molecules creating a state of oxidative stress (OS), macromolecular damage, such as DNA, and eventually apoptosis or oncogenesis[23]. Circadian regulation of protein expression plays a significant role in cellular response to OS.

Base excision repair (BER) is mainly responsible for removal of oxidative DNA damage such as 8 oxo guanine (8 oxo G) via 8 oxo-guanine DNA glycosylate (OGG1). Manzella et al investigated the circadian modulation of 8 oxo G DNA damage repair in samples from human subjects. They found that *Ogg1* expression demonstrated a daily variation with higher levels in the morning as compared to the evening, and lymphocytes exposed to oxidative damage to DNA at 8.00am displayed a lower accumulation of 8 oxo G than at 8.00 pm. Altered levels of *Ogg1* expression were also observed in a group of shift workers experiencing deregulation of circadian clock genes as compared to controls. BMAL1 knockdown fibroblasts with deregulated molecular clock showed abolishment of circadian variation of *Ogg1* expression and an increase in OGG1 activity. From their results they suggested that circadian modulation of 8 oxo G DNA repair, according to variation of *Ogg1* expression,

could render humans less susceptible to accumulated 8 oxo G DNA damage with exposure in the morning hours[17].

There is clear evidence, when considering both NER and BER, that exposure is less damaging in the early morning hours as compared to any other time of day. Obviously, it is not going to be possible for most individuals to restrict their sun exposure to this time frame but it is worth trying to modify exposure patterns with this in mind.

6. Inter-relationship of the circadian and cell cycles

Skin homeostasis is imperative to its protective function for the whole organism, highlighting the importance of DNA damage repair and apoptotic systems. However, considering the high proliferative rate of most skin cells, regulation of the cell cycle and it's check-point control would be assumed to also have an important role. The clock oscillates independently of the cell cycle in single cells. In contrast, in mammalian tissues, the circadian clock controls the expression of cell cycle-related genes. Thus, the intracellular clockwork can control the cell division cycle in proliferating cells[24].

A relatively early study, investigating the circadian expression of clock genes in human mucosa and skin found *Clock, Tim, Per1* and *Bmal1* expression in these tissues consistent with the circadian profile of the SCN. *Per1, Cry1* and *Bmal1* peaking at early morning, late afternoon and night, respectively. In concurrent oral mucosal biopsies, thymidylate synthetase, a marker for DNA synthesis, also had a circadian variation peaking in the early afternoon coinciding with S phase from a previous study. The *Per1* peak expression occurring at the same time as peak G1 phase. They identified the anti-phasic relationship between repair and proliferation, correctly suggesting the intimate interrelationship between circadian and cell cycles[25].

Similar to the circadian cycle, the cell cycle is a closely regulated system with check-points placed at key transitions in the cycle to regulate fidelity of DNA replication and mitosis. In addition to transcriptional control the circadian clock is regulated at the level of translation and post-translational modifications that fine-tune the system. Circadian gating of mitosis has been reported for many years in some peripheral tissues but the molecular mechanisms were not well understood. However, it has more recently been shown in plants, cyanobacteria, zebrafish[26] and mice[27] that the circadian clock specifically regulates key phases of the cell cycle. Circadian clock-dependent regulation of S-phase is important for vulnerability to UV-induced DNA damage in proliferating skin cells[28].

Circadian gating of the cell cycle could have developed as an adaptive mechanism to minimise the overlap between sensitive cell cycle phases and endogenous factors such as reactive by-products of oxidative metabolism. Daily oscillations in epidermal stem cell metabolism is consistent with this model. In humans, the highest proportion of keratinocytes are in S phase during the day, as opposed to nocturnal mice where the situation is reversed. Cell cycle kinetics suggests this regulation is mediated through S phase duration.

- *p20* and *p21*, significant regulators of the G1/S transition, are clock controlled genes, temporarily expressed in a tissue specific manner [26,29].
- WEE 1 kinase is highly circadian and is responsible for regulation of cyclin dependent kinase 1 (CDK1) complex and subsequent entry into mitosis[27]. The *Wee1* promoter contains sites of CLOCK/BMAL1 binding and is subject to chromatin remodelling in response to light. Figure 4.
- *Myc, Cyclin D1, Cyclin B1* and *Cdc2* are other cell cycle genes subject to circadian

oscillation in expression. Chromatin remodelling permitting circadian influence but the extent to which chromatin modification plays a role in light-dependent remodelling at cell cycle promoters is unclear.

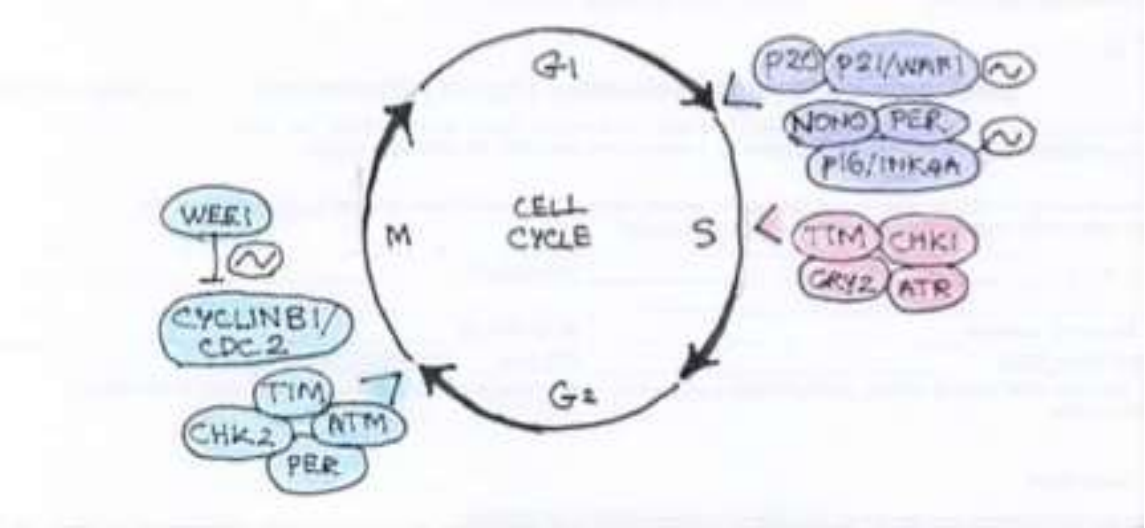

Figure 4. Gating of the cell cycle by the circadian clock.
Expression of genes reported to have circadian oscillation indicated by (〜). Cyclin D1 that controls G1/S checkpoint and c-Myc that regulates proliferation via G0/G1 transition are not shown. (Adapted from Masri et al, 2013)[24].

Geyfman et al investigated circadian control over epidermal cell proliferation and subsequent susceptibility to UVB-induced DNA damage in mice by looking at the circadian transcriptome at two distinct phases in the hair growth cycle, anagen and telogen, the latter dominated by genes of proliferation and metabolism. They found that the expression of many metabolic genes was antiphasic to cell cycle related genes, the metabolic genes peaking during the day and cell cycle genes peaking at night. Accumulation of ROS (as a product of oxidative phosphorylation) and S phase antiphasic in telogen skin.

Keratinocyte-specific deletion of *Bmal 1* obliterated time-of-day-dependent synchronicity of cell division in the epidermis leading to constitutively elevated proliferation. They found that mice were most sensitive to UVB-induced DNA damage at night i.e. S phase. In the human epidermis the maximum number of keratinocytes go through S phase in the late afternoon. They speculated that the circadian clock imposes regulation of epidermal cell proliferation, so that skin is at its most vulnerable stage during times of maximal UV exposure contributing to skin cancer[28].

It has also been shown that the cell cycle has some control over the Circadian clock. Circadian period length of proliferation in N1H3T3 fibroblasts is longer than non-proliferating fibroblasts, suggesting cell cycle can influence the period of the circadian clock[30]. Cell division can alter PER/CRY repressor protein concentrations and subsequently alter circadian rhythms. RNA binding protein p54^{nrb} or NONO partnered with PER protein to activate *p16-Ink4A* cell checkpoint gene in a circadian manner[31]. It is clear that there is bidirectional control between the two circuits, with common regulators, to provide homeostatic and an adaptive response to environmental change or metabolic demands.

7. Circadian system link to apoptotic regulation

Sancar et al linked the circadian system to apoptosis regulation through NF-κB signalling. It had been reported that a CRY mutation in p53 null mice delayed the onset of cancer. It was suggested that the mutation might activate a p53-independent pathway that eliminates pre-malignant cells. They were able to show that the CRY mutation sensitises p53 mutant and oncogenically transformed cells to TNFα. This initiated apoptosis by interfacing with NF-κB signalling pathway through GSK3β kinase, alleviating post-survival NF-κB signalling. They concluded that clock disruption sensitises the transformed cells to apoptosis by inflammatory cytokines (such as TNFα, elevated in CRY mutants) which is the link between the two cellular pathways. The circadian clock, which introduces an oscillatory variability into cellular physiological and cellular function, and the NF-κB pathway which is a key nodal focus in the inflammatory response at a cellular and organisational level[32].

8. The role of melatonin

Light may play a secondary role in the maintenance of circadian rhythms though its regulation of the pineal hormone, melatonin which is known to oscillate in a circadian manner[33]. It plays a role in both circadian regulation with a direct effect on circadian proteins and acting as a signalling molecule to synchronise certain peripheral cells. Melatonin is an inhibitor of SIRT1, a histone deacetylase, the acetylation state of BMAL1 affecting its activity. Melatonin is also a strong antioxidant and can attenuate UVR-mediated oxidative stress in the skin. There is good evidence for melatonin biosynthesis in the skin with the presence of precursor molecules, necessary enzymes and higher local than serum levels[34]. Melatonin is highly lipophilic with the ability to cross cellular membranes allowing protection of intracellular structures such as DNA and mitochondria. It has the ability to modulate UV-induced apoptotic pathways and upregulate other antioxidant enzymes. Fischer et al showed higher viability of keratinocytes, pre-treated with melatonin, after UV exposure[35]. As with all circadian proteins its oscillation is sensitive to light and changes in the normal light/dark cycle disturbs melatonin expression.

9. Vitamin D and melanoma

It is accepted that sun exposure is the major environmental factor in melanoma incidence, however sun exposure is critical for Vitamin D synthesis. Sub-optimal levels are associated with reduced bone health and increasing literature linking increased risk of other diseases, so that a balance between exposure and protection is needed. Overall, genetic-epidemiological data suggests intermittent exposure and sun-burn have been responsible for the drastic increase in melanoma in Caucasian populations this century[36]. Excluding the high-risk phenotypes, the epidemiological data is complex but suggests that exposure can be protective in some circumstances, possibly through photoadaptation or higher Vitamin D levels[37]. Vitamin D is anti-proliferative in vitro for some melanoma cell lines[38]. At melanoma diagnosis, lower Vitamin D levels are associated with thicker tumours and poorer prognosis [39,40]. Sun-sensitive people have lower Vitamin D levels[41]and patients with a previous diagnosis of melanoma are likely to practice sun-avoidance behaviours. Should we be measuring levels and suggesting supplementation for our melanoma patients? Shield's English study suggested optimal level of 60nmol/L and supplemented dose of 1000 IU daily for levels < 40 nmol/L and 400 IU for 40-60 and avoiding supplantation >60 nmol/L. This is for a population that generally had a low intake of fatty fish and the evidence is

lacking for supplementation reducing melanoma risk.

10. Discussion

Biology is linked to the astronomical cycles of the rotation of the planet on its axis and its movement around the sun. A clockwork is built into our systems programmed to anticipate these cyclic changes including a complex layered system of protective and adaptive mechanisms that has evolved to provide protection from environmental stressors and change. We have come a long way from early Homo sapiens but still carry a paleolithic genome not necessarily adapted to an indoor sedentary lifestyle under artificial light with a continual availability of calorie rich food. The clock evolved in a vastly different environment from the one we now face and we do not seem to full comprehend the consequences to human health. Our patterns of modern living attempt to disregard the innate knowledge and intelligence of cellular systems replacing them with the products of technological advancement yet we continue to be plagued by disease and cancer. We may be better served by using circadian rhythm biochemistry to our advantage in terms of exposure patterns.

The extremely fair skinned individuals require maximum protection all daylight hours and would probably require Vitamin D supplementation but for the rest of the population with Fitzpatrick type 2 skin and better, early morning exposure is ideal. It has been shown that there is a higher rate of repair in the morning, less prone to carcinogenic effects of UVR as compared to other times of day. This also means separating exposure from cellular proliferation, when the epidermal cells are most vulnerable. By exposure I mean staying below the burn threshold with protection from hats, clothing and sunscreens and limiting the amount of time exposed but this maybe gradually extended once a degree of photoadaptation has been achieved.

The ideal sport and recreational activity for my patients living in coastal SE Queensland is surfing. Arriving at the beach pre-dawn, present outdoors at first light to entrain the circadian system and then exposed in swim-wear for a couple of hours in the water. Exposure at this time of day does not require sunscreen. It is well known that melanoma has a propensity for intermittently exposed skin sites, the most common areas of melanoma incidence the shoulders and back. This exposure pattern allows for these anatomical sites to be exposed under the most ideal time for DNA repair mechanisms to operate. This can be followed by remaining indoors at work or school with the sun at its zenith and avoiding the period of most intense UVR exposure. Afternoon exposure is less advantageous in terms of repair mechanisms but still avoids maximum UV exposure.

Both melatonin and Vitamin D have anti-proliferative effects and are influential in reducing the risk of carcinogenesis. Vitamin D biosynthesis in the skin is dependent on UVB exposure and melatonin is dependent on the normal day/night rhythm. Both are good examples of the potential benefits of sunlight exposure that can be adversely affected by through modern life styles with prolonged indoor living under artificial light causing dysregulation of the circadian cycle. I provide in this paper a summary of skin biochemistry that supports recommendations for sun exposure.

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