CONFLICT OF INTEREST
ASP, equity holder in Tycho Therapeutics, focused on targeted therapy of autoimmunity; and is inventor on patent licensed to Novartis for cellular immunotherapy of antibody-mediated disease. CJK states no conflict of interest.

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UV-B-Induced Erythema in Human Skin: The Circadian Clock Is Ticking

Soumyadeep Sarkar[1] and Shobhan Gaddameedhi[1,2]

Acute exposure of skin to UV-B causes DNA damage and sunburn erythema in both mice and humans. Previous studies documented time-of-day-related differences in sunburn responses after UV-B exposure in mice. Because humans are diurnal and mice are nocturnal, the circadian rhythm in human skin was hypothesized to be in opposite phase to the rhythm in mice. A study by Nikkola et al. demonstrates that humans are more prone to sunburn erythema after evening exposure to solar UV-B radiation as compared with morning exposure.

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The majority of living organisms on the Earth are photosensitive and are influenced by the solar day and night cycle created by the axial rotation of the Earth. Consequently, cells have developed an amazing time-keeping mechanism known as the circadian clock that is self-sustained and oscillates with a periodic cycle of approximately 24 hours. In mammals, the molecular clock is entrained daily by the morning sunlight entering the eyes to reset the master clock located in the suprachiasmatic nucleus of the brain. The suprachiasmatic nucleus, via various hormonal and neuronal signals, synchronizes peripheral tissues including the heart, lung, liver, and skin. At the molecular level, the circadian clock is made up of primary as well as secondary feedback loops that are primarily responsible for approximately 24-hour rhythmicity of clock-controlled genes. Proteins including brain and muscle ARNT-like protein 1 (BMAL1), circadian locomotor output kaput (CLOCK), cryptochromes (CRY 1, 2), and periods (PER 1, 2, 3) make up a transcriptional-translational feedback loop that is initiated by dimerization of BMAL1 and CLOCK and the subsequent binding of this complex to the E-Box sequence (CACGTG or CACGTT) found in the promoter region of target clock-controlled genes (Lowrey and Takahashi, 2011). Tissue-specific circadian expression patterns have been observed in as many as 10% of mammalian genes, and many of these genes regulate cell cycle, metabolism, cell death, and DNA repair processes (Sancar et al., 2015).

Apart from its life-giving quality, the Sun emits harmful UVR that induces DNA damage in skin. Exposure to UVR causes a plethora of skin-related issues including photoaging, sunburn erythema, and skin cancers including melanomas, the deadliest skin cancers. In recent years, the skin's circadian clock has been shown to regulate various cellular responses after UVR exposure, including nucleotide excision repair, cell cycle checkpoints, oxidative stress, and apoptosis (Dakup and Gaddameedhi, 2017; Gaddameedhi et al., 2011; Geyfman et al., 2012; Plikus et al., 2015; Sancar et al., 2015; Wang et al., 2017). These robust protective mechanisms attenuate unwanted consequences of UVR-mediated DNA damage. Skin clock regulation of nucleotide excision repair has been well characterized using the SKH-1 hairless mouse model, where it was observed that an elevated rate of

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Because mice are nocturnal and humans are diurnal, their circadian clock outputs are out of phase. Based on this argument, and previous data relating time-of-day UV exposure to severity of UV effects in mouse skin, it was proposed that humans would be more prone to sunburn after late afternoon UV exposure rather than morning exposure.

UV photoproduct removal correlated with higher expression of the core nucleotide excision repair factor Xpa in the evening as compared with the morning (Gaddameedhi et al., 2011). However, acute exposure to UVR also causes a UV-induced inflammatory reaction that is characterized by vasodilation and increased blood flow to the dermis, resulting in sunburn erythema in the affected skin. Using SKH-1 hairless mice, we have shown that more sunburn erythema occurs in morning solar UV-B-treated mice than in evening-treated animals (Gaddameedhi et al., 2015). We determined that during morning time, when DNA repair is lowest and DNA synthesis is highest, the DNA damage caused by UVR leads to stronger activation of the Atr protein kinase, which is known to be activated in response to replication stress and to regulate the stability of the tumor suppressor p53. This results in increased numbers of sunburn apoptotic cells and enhanced inflammation in mouse skin. Because mice are nocturnal and humans are diurnal, their circadian clock outputs are out of phase. Based on this argument, and previous data relating time-of-day UV exposure to severity of UV effects in mouse skin, it was proposed that humans would be more prone to sunburn after late afternoon UV exposure rather than morning exposure.

Nikkola et al. evaluated the circadian time effect of narrow band-UV-B exposure on sunburn erythema in human skin. They selected 19 subjects (16 females and 3 males) having skin types II and III as defined by Fitzpatrick sun-reactive skin type scale (Fitzpatrick, 1988). Subjects were exposed to increasing doses of narrow band-UV-B up to 4 suberythemal dose, once in the morning between 7 am and 9 am on one buttock and once in the evening between 7 pm and 9 pm on the other buttock of the same subject. Twenty-four hours after UV-B treatment, the erythema of the skin patches was quantified and skin biopsies were irradiated with the highest dose of UV-B (i.e., 4 suberythemal dose = 40 mJ/cm^2 CIE (Commission Internationale de l'Eclairage, Vienna, Austria)) were collected. Protein levels of the tumor suppressor proteins p53 and the clock proteins CRY1 and CRY2 were measured in biopsies. Interestingly, the authors found that human subjects treated with UV-B in the evening had a significantly higher erythema index scores in comparison with the subjects exposed in the morning. In addition, different levels of CRY1 and CRY2 were detected in irradiated skin using immunohistochemistry, and human subjects with a negligible amount of CRY2 showed more erythema relative to individuals with high levels of CRY2. This is a novel observation.

The authors speculated that CRY2 protects against UV-B-mediated skin damage. In a different study, the Lamia group reported that CRY2 regulated c-MYC proteasomal degradation by acting as a cofactor for the SCF substrate adaptor FBXL3 (an E3 ligase protein) (Huber et al., 2016). Because c-MYC plays important roles in cell proliferation, cell growth, and apoptosis, high levels of CRY2 could enhance c-MYC degradation, which in turn might reduce sunburn-induced apoptosis and other characteristic features of sunburn erythema. It would be interesting to evaluate levels of core clock proteins including CRY1, 2, PER1, 2, 3, BMAL1, and CLOCK in a larger sample set. Also, knowing the chronotype (behavioral manifestation of the underlying biological clock) of the patients with negligible amount of CRY2 levels would facilitate understanding the effects of circadian clock disruption on sunburn erythema. Collectively, the above additional information would define mechanistic similarities and circadian phase output differences between human and mouse models as shown in Figure 1. In addition to CRY protein levels, Nikkola et al. also measured p53 concentrations in skin biopsies and observed increased p53 levels in the morning samples compared with the evening group. This observation appears to contradict the predicted human skin sunburn model, but the authors suggested that the possible reasons for this observation were either delayed (24 hours after UV-B irradiation) biopsy collection in comparison with the mouse study previously conducted by us or a dissociation of human p53 elevation and erythema as previously suggested (Healy et al., 1994). However, the paper by Healy et al. did not consider the circadian aspect of p53 and erythema, nor were subjects selected according to chronotype. Thus, the observations by Healy et al. may not be directly relatable to the current study. In contrast, strong evidence of p53 involvement in sunburn erythema was shown by Brash's group using p53 knockout mouse models, where they demonstrated that deleting p53 gene in mouse skin decreased the number of sunburn-mediated apoptotic cells after exposure to UV-B (Ziegler et al., 1994).

In another recent paper (Guan et al., 2016) in which erythema induction was examined as a function of time-of-day of UV exposure, the authors reported enhanced erythema in human subjects after morning treatment in comparison with evening exposure. However, this study used solar simulated radiation, which is primarily composed of UV-A radiation that is much less efficient at inducing canonical UV bipyrimidine dimers in DNA, rather than narrow band-UV-B as in the Nikkola et al. study. Guan et al. also did not evaluate core clock gene expression levels in skin biopsies of the subjects, which is important to understand the circadian phase of the observations. In future studies, it will be interesting to determine how different experimental parameters and variables in the Guan

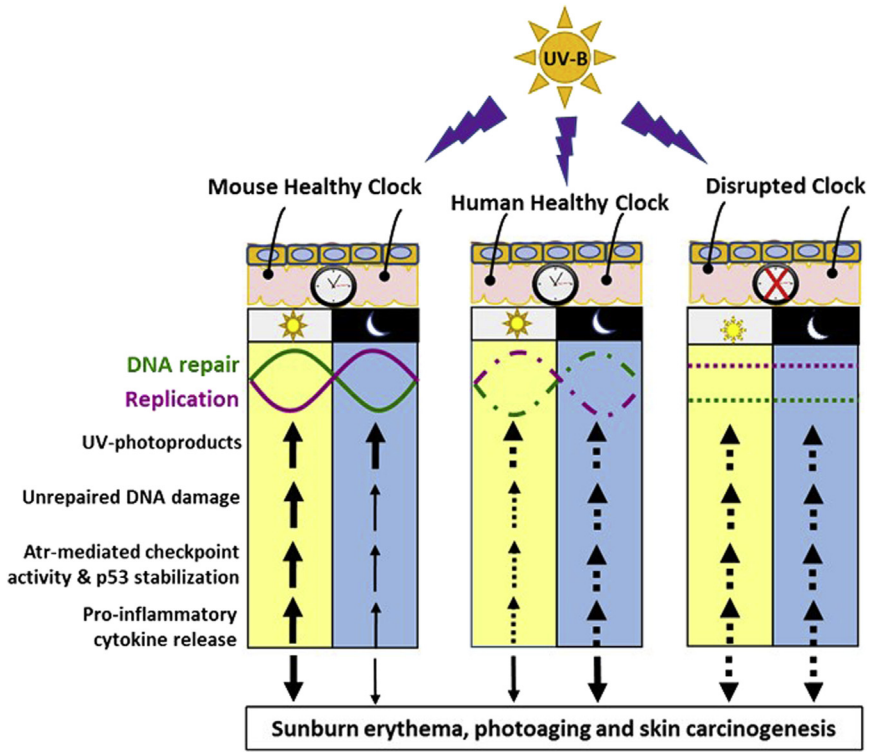

Figure 1. Schematic representation of the role of the circadian clock in sunburn erythema, photoaging, and skin cancer in mice and humans. Mice and humans have similar circadian clocks, but are opposite phase to each other. From the left is a modified figure from Gaddameedhi et al. (2015), which shows that in mice, DNA replication is high and DNA repair is low during the morning, which leads to unrepaired UV photoproducts, causing replication stress, followed by activation of DNA damage checkpoint causing enhanced p53-mediated apoptosis and sunburn erythema compared with the evening time. The middle figure represents a hypothetical human sunburn erythema model based on the mice model, showing that the outcomes of UV damage in humans are in opposite phase to mice. Based on the study by Nikkola et al. (2017), the development of sunburn erythema in humans due to UV-B exposure seems to fit this proposed model, that is, humans are more prone to sunburn erythema during the evening time compared with morning. Finally, the figure on the right shows the effects of a disrupted skin clock in the development of sunburn erythema, where regardless of the time-of-day of UV-B exposure, the skin is hypothesized to be vulnerable and is more prone to develop sunburn erythema.

et al. and Nikkola et al. studies contribute to the unique time-of-day erythemal responses in humans.

It should be noted that there are additional factors that may contribute to circadian effects on UV responses in human skin, such as skin type, sleep cycles, and feeding behaviors of the participating human volunteers. These issues are more difficult to control for in human studies than in mice. In a recent elegant study, the Andersen group showed that restricting feeding to the inactive phase (during day time) in mice shifts the skin's circadian clock and its response to solar UV-B irradiation (Wang et al., 2017). The group concluded that daytime feeding reverses diurnal sensitivity to UVB-induced DNA damage specifically by influencing the oscillatory expression pattern of the *Xpa* gene. Feeding and social behaviors of human subjects may also affect UV responses and should therefore be considered when designing future studies involving humans. A few possible solutions to this problem include evaluating participating human subjects in sleep research labs, where their sleep cycles, physical activity, and feeding patterns can be closely monitored, or formulating detailed questionnaires for the volunteers at the time of enrollment.

Collectively, the Nikkola et al. and Guan et al. papers show involvement of circadian timing in UVR-induced sunburn erythema in humans, but report different observations relating the intensity of sunburn erythema to time-of-day UVR exposure. Observations of core clock gene expression pattern in the −/+UV-B-treated skin samples bolster the involvement of circadian clock regulation in UV-B-induced sunburn erythema in humans. However, it would have been fascinating to perform additional mechanistic studies to understand the signaling events occurring through the skin's circadian clock on UVR exposure and correlate the results with the observed phenotype in humans.

Our environment is constantly changing, challenging us both socially and biologically, and pushing us to adopt erratic time schedules. Irregular and unhealthy sleep cycles and feeding patterns are disrupting our natural circadian rhythm and making us vulnerable to environmental stresses that vary depending on the time-of-day. Despite the strong evidence that the circadian clock impacts on sunburn erythema in mice, there are still active debate about the vulnerability of human skin to UVR-induced erythema as a function of the time-of-day of exposure. One reason for continued controversy could be huge variations in the natural rhythm among humans, which make such studies much more difficult and time consuming. Hence, special efforts should be made to select appropriate human volunteers. Identification of the optimal time-of-day to minimize sunburn erythema in humans may soon be possible, and may be consistent with the model proposed by Gaddameedhi et al. (2015). Until then, minimization of sun exposure and photoprotection should continue to be recommended.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Propionibacterium acnes Strains Differentially Regulate the Fate of Th17 Responses in the Skin

Emmanuel Contassot[1] and Lars E. French[1]

Agak et al. demonstrate that different strains of *Propionibacterium acnes*, a bacterium colonizing pilosebaceous units in healthy skin and acne, have the ability to induce T helper type 17 cells secreting either IFN-γ or IL-10 and exhibiting either pathogenic or protective properties, respectively. This work contributes to growing evidence indicating that the phenotype of T helper type 17 cells is largely dependent on their microbiological environment.

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Acne is an inflammatory disorder of the pilosebaceous unit that commonly begins at puberty and affects up to 85% of adolescents and young adults. Currently, acne is considered to be a multifactorial disease involving factors such as excess sebum production, disturbed keratinization in hair follicles, colonization of pilosebaceous units by the bacterium *Propionibacterium acnes* (*P. acnes*), and the release of proinflammatory molecules. However, despite being a highly prevalent and extensively studied inflammatory skin disorder, the precise pathophysiology of acne is still not completely understood.

P. acnes is an anaerobic Gram-positive bacterium present in the human skin microbiota. This commensal accumulates preferentially in pilosebaceous units in both patients with acne and healthy individuals. *P. acnes* overgrowth in acne microcomedones is associated with inflammation of pilosebaceous units. However, the role of this bacterium in the pathogenesis of acne has long been debated and how it contributes to acne while being a major component of the normal skin flora remains unclear. Several reports demonstrated that *P. acnes* is able to induce strong inflammatory responses by inducing the release of proinflammatory cytokines and chemokines from cells present in, or in close proximity to, pilosebaceous units. Keratinocytes respond to *P. acnes* exposure by releasing IL-6 and IL-8 (CXCL8), IL-1α, tumor necrosis factor, and GM-CSF (Graham et al., 2004), whereas *P. acnes* stimulates the secretion of IL-1β, tumor necrosis factor, IL-8, and IL-12 by myeloid cells (Kistowska et al., 2014; Qin et al., 2014). Moreover, sebocytes also respond to *P. acnes* exposure by releasing IL-8 (CXCL8), tumor necrosis factor (Nagy et al., 2006), and IL-1β (Li et al., 2014).

Despite the ability of *P. acnes* to induce robust innate immune responses, cells infiltrating early acne lesions consist mainly of $CD4^+$ T cells. IL-17A-secreting $CD4^+$ T cells (T helper type 17 [Th17] cells) bridge innate and adaptive immunity, and their important role in mediating host defense and controlling different microorganisms is well documented. Increasing evidence suggests that *P. acnes* is able to induce Th17 cells that may contribute to inflammation in acne. Our group and the group led by Jenny Kim at UCLA provided experimental evidence showing that the secretion of IL-17A by naïve $CD4^+$ T cells can be induced by *P. acnes* (Agak et al., 2014; Kistowska et al., 2015). We also showed that IL-$17A^+$/IFN-γ^+ Th17 cells—initially called "Th1/Th17" cells and also termed "Th1-like" cells—could be inhibited by the use of a blocking antibody to

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