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# The circadian clock and diseases of the skin

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## Abstract

Organisms have an evolutionarily conserved internal rhythm that helps them anticipate and adapt to daily changes in the environment. Synchronized to the light-dark cycle with a period of around 24 hours, the timing of the circadian clock is set by light-triggering signals sent from the retina to the suprachiasmatic nucleus. Other inputs, including food intake, exercise, and temperature, also affect clocks in peripheral tissues, including skin. Here, we review the intricate interplay between the core clock network and fundamental physiological processes in skin such as homeostasis, regeneration, immune and stress responses. We illustrate the effect of feeding time on the skin circadian clock and skin function, a previously overlooked area of research. We then discuss works that relate the circadian clock and its disruption to skin diseases, including skin cancer, sunburn, hair loss, aging, infections, inflammatory skin diseases, and wound healing. Finally, we highlight the promise of circadian medicine for skin disease prevention and management.

## Graphical Abstract

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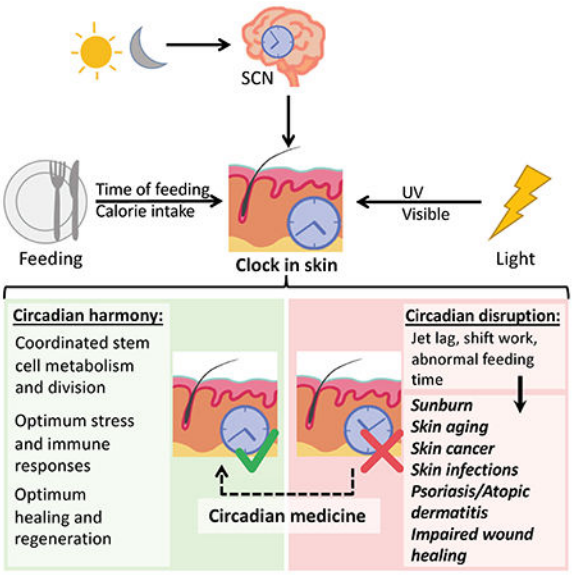
Author contributions

JD, EG and BA designed the review. JD, EG, SK and BA wrote and edited the review. JD and BA drew and edited the figures.




**Keywords**

Circadian clock; skin diseases; stem cells; feeding; cancer; wound healing; aging; psoriasis; stress and immune response; circadian medicine

## Introduction

As the largest organ of the body, skin provides primary defense against the environment, including toxins, microorganisms, and radiation [1,2]. It is also a critical part of organismal homeostasis through fat storage and the production of hormones, including vitamin D. Due to its interactions with external stimuli, the skin must be equipped to adapt to its environment.

The circadian clock, an intrinsic autonomous clock that controls the body's divergent functions during day and night, affects the expression of multiple genes that mediate skin stem cell metabolism and proliferation, DNA repair, stimulus response, and immunity. Circadian clock research has elucidated fundamental regulatory mechanisms that contribute to skin cancer, aging, inflammatory and other skin diseases. Emerging clinical research has begun to employ these findings to refine treatment based on the principles of circadian medicine. While circadian dermatology is a nascent field, studies conducted on the circadian clock continue to unravel the mysteries of the skin and its complex system of regulation.

Understanding the role of the circadian clock in the skin may provide new insights into the pathogenesis of skin diseases and their treatment. To this end, we review the role of the circadian clock in skin, emphasizing the most recent findings in the field, and discuss the role of circadian clock disruption in skin diseases.

## The circadian clock

From bacteria to plants and animals, all living organisms have intrinsic rhythms that are synchronized with the light-dark cycle with a period of around 24 hours. Controlled by internal biochemical oscillators, the circadian clock is autonomous and maintained at both organismal and cellular levels in the absence of outside stimuli [3,4]. In this section, we provide a brief overview of the mammalian circadian clock at the organismal and molecular level.

### The SCN synchronizes peripheral clocks

In mammals, the circadian clock machinery is present in almost all cells. The suprachiasmatic nucleus (SCN), a small region in the hypothalamus of the brain, acts as the central pacemaker that synchronizes the circadian clocks in peripheral tissues through neuronal and hormonal signals [4,5].

The primary entrainment signal for the SCN is light. Upon light reception, intrinsically photosensitive retinal ganglion cells (ipRGCs), pivotal components of the retinohypothalamic tract, send signals to set the circadian time in the SCN. In the SCN, a subpopulation of cells produces neurotransmitters such as vasoactive intestinal peptide (VIP) and arginine vasopressin (AVP) to synchronize and stabilize the cellular circadian clocks within the SCN [4–6]. While the neurons in the SCN are traditionally believed to be the main contributor to circadian rhythm establishment, SCN astrocytes also have the ability to control the circadian rhythm in the SCN [4,5]. As the robustly autonomous SCN sends signals to peripheral tissues, peripheral clocks are synchronized with respect to the SCN and thus the ambient light-dark cycle (Fig. 1).

The SCN clock can synchronize peripheral clocks, but it is not necessary for circadian rhythm maintenance in peripheral organs [7,8]. Although ablation of the SCN in mice disturbs the circadian rhythms in peripheral tissues, the obliteration of BMAL1 in all tissues except the liver does not completely obliterate circadian rhythms in the liver, irrespective of whether light signals are available [7,8]. In addition, other signals such as food intake and temperature can set the clock in peripheral tissues independent of the SCN, causing phase shifts in the peripheral clocks relative to the central clock (Fig. 1) [9].

### The molecular clock is a transcription-translation feedback loop

In each cell, the circadian clock is a biochemical oscillator formed with interlocked transcription-translation feedback loops [10]. The primary feedback loop, which is commonly referred to as the core clock gene network, consists of a positive and a negative arm. In the positive arm, transcription factors Brain and Muscle ARNT-Like 1 (BMAL1) and Circadian Locomotor Output Cycles Kaput (CLOCK) form heterodimers, which bind to the enhancer box (E-box) to activate transcription of Period 1, 2, and 3 (*Per*) and Cryptochrome 1 and 2 (*Cry*). PER and CRY drive the negative arm, inhibiting the expression of BMAL1-CLOCK and thus impeding their own transcription. With the levels of PER and CRY decreasing, the BMAL1-CLOCK dimer regains activation until PER and CRY accumulate

and deactivate BMAL1-CLOCK again, completing the loop with a period of around 24 hours (Fig. 2) [10–12].

In addition to the core clock gene network, a secondary feedback loop reinforces the 24-hour oscillation for *Bmal1* expression. BMAL1-CLOCK activates transcriptions of not only *Per* and *Cry* but also other clock-controlled genes, including RAR-related Orphan Receptors (RORs) and REV-ERBs. While RORs bind to ROR/REV-ERB-response elements (RORE) to activate transcription of *Bmal1*, REV-ERBs bind to the same sites to inhibit *Bmal1* transcription. With the level of BMAL1 decreasing, transcription of REV-ERBs stalls until transcription of *Bmal1* is reestablished (Fig. 2) [10–12].

As simple as the network is, the core clock gene network directly or indirectly modulates the expression of multiple genes and biological processes in all organs, making those processes rhythmic as well. In mice, for example, around 16%, 13%, 12%, and 7% of protein coding genes are rhythmic in the liver, kidney, lung and skin, respectively [13,14]. While the same clock gene network is omnipresent in cells and the SCN acts as a synchronizer for the entire body, the set of genes that are circadian varies greatly between organs. This is because the circadian machinery regulates different biological processes in different organs to modulate their functions. In addition, there is variability in the amplitudes and the peak times of clock gene expressions between organs, as external signals such as temperature and food intake can selectively entrain peripheral clocks (Fig. 1) [9].

## The circadian clock in the skin

Acting as a protective barrier against toxins and other external stressors, skin is a multi-layer organ harboring various cell types organized into several compartments to fulfill a range of tasks such as water loss prevention, sensation, and hormone synthesis [1,2]. In humans and mice, the skin comprises three main layers: the epidermis, the dermis, and the hypodermis; each layer has active circadian clocks. With the outside world changing throughout the 24-hour day, a mechanism has evolved to allow the skin to anticipate the environmental shifts and adjust accordingly. Indeed, diurnal rhythms are observed in multiple cell types across all layers of the skin [15].

As in other organs, the SCN synchronizes circadian clocks throughout the skin. This is supported by SCN ablation studies that abolished skin circadian rhythms [16]. More recent studies have brought to light that epidermal circadian clocks and time-setting mechanisms are independent of the SCN. Selective expression of *Bmal1* only in the epidermis does not disturb the skin diurnal rhythm as long as the mice experience regular light-dark cycles [17], indicating that the epidermal clock does not require the SCN clock or clocks in other tissues. Possible contributors to the maintenance of such diurnality are light sensitive opsin proteins present in various skin cell types (such as melanocytes, keratinocytes, fibroblasts and hair follicle cells) that may be able to entrain the skin circadian clock [18,19].

### The skin is a multi-layer organ with diurnal rhythms in every layer

The outermost layer of the skin is the epidermis (Fig. 3A). Hosting Merkel cells, melanocytes, T-cells, and Langerhans cells, the epidermis itself is organized into four

layers (five on the palm and sole) consisting of keratinocytes at different differentiation stages: *stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum lucidum* (palm and sole specific) and *stratum corneum* (Fig. 3B) [1]. The journey of the keratinocytes in the epidermis starts in the *stratum basale*, where the intrinsic circadian clock is required for the diurnal rhythms in proliferation [14] as well as the coordination between cell division and intermediary metabolism [20] in epidermal stem cells. The keratinocyte progeny of stem cells is continuously pushed upwards until arriving in the *stratum corneum* at the top, where the dead keratin-filled cells slough off [1].

Just below the epidermis, separated from it by a basement membrane, is the dermis (Fig. 3A). The dermis primarily consists of extracellular matrix with collagen fibers synthesized by fibroblasts. It is much thicker than the epidermis and contributes strength and elasticity to the skin [1]. The diverse cellular populations in the dermis include fibroblasts, multiple immune cells such as T cells, mast cells, macrophages, and dendritic cells [1], as well as dermal white adipocytes (Fig. 3A). All of these cell types participate in immune responses and wound healing [21]. Despite their spatial proximity, the dermis and the epidermis have distinct circadian behaviors. Computational analysis of data collected from skin biopsies from human forearm, buttock, and cheek revealed that oscillation of clock genes is more robust in the epidermis than in the dermis across all three sites [22]. This may be because the cell types in the dermis are more diverse than in the epidermis, leading to less uniform circadian gene expressions in the dermis.

The hypodermis is the layer just below the dermis (Fig. 3A); it mainly consists of adipocytes that form the subcutaneous fat, but also contains fibroblasts and macrophages [1,21]. As an energy reservoir, the adipose tissue has long been studied for its role in metabolic diseases, which are intertwined with the whole-body circadian network. The relationship between the adipose tissue and the circadian clock is reviewed in detail in recent articles [23–25].

## The circadian clock in hair follicles

The hair, one of the defining characteristics of mammals, works together with the epidermis to sense and protect the body from environmental changes. Hair shafts are rooted in hair follicles, which are miniature organs consisting of concentric layers of keratinocytes. They are embedded in the skin, penetrating into the dermis (Fig. 3A). The infundibulum and isthmus are the two most superficial compartments of hair follicles. They are continuous with the epidermis and their circadian activities are similar to that of the epidermis (Fig. 3C, D) [15,26]. The remaining lower compartments of hair follicles, including the anagen-specific bulb, the stem cell-containing bulge, and the mesenchymal-derived dermal papilla, are more dynamic (Fig. 3C, D). In particular, the dermal papilla and the anagen-specific bulb undergo structural changes as the hair follicles move through the three main stages of the hair cycle: the growth stage anagen (Fig. 3C), the regression stage catagen, and the relatively quiescent stage telogen (Fig. 3D) [15,26]. Diurnal genes in telogen and anagen skin only partially overlap [14], suggesting that the circadian clock differentially regulates hair follicles at those two stages.

Anagen marks the beginning of hair renewal. During anagen, quiescent bulge stem cells migrate out of the bulge to become proliferating matrix keratinocytes within the anagen

bulb. The dermal papilla directs the differentiation of those matrix keratinocytes to form the inner layers of the hair follicle and the hair shaft (Fig. 3C) [26,27]. In both the bulge and the anagen bulb of mouse hair follicles, the circadian clock is robustly rhythmic and gates mitotic rhythms by synchronizing the G2/M checkpoint. Due to this synchronization of mitosis, the extension of the hair shafts shows a diurnal pattern, with more growth in the morning than in the evening [28]. *In vivo* studies of the human hair cycle are difficult, but studies indicate that circadian genes are expressed rhythmically in human hair follicles as well [29,30]. Furthermore, *ex vivo* studies suggest that the circadian clock regulates the human hair cycle, as silencing *BMAL1*, *CLOCK* and *PER1* extends anagen [30]. In addition to hair growth, the circadian clock also controls the pigmentation of the hair during anagen, as melanin production by melanocytes in the human anagen hair matrix increases when the core clock genes *BMAL1* and *PER1* are silenced [31].

Hair follicles cease to grow during catagen and eventually enter the resting stage telogen. During telogen, the hair follicles prepare for the next anagen by forming the secondary hair germ just above the dermal papilla (Fig. 3D) [27,32]. Interestingly, in mice, while the hair is not growing during this stage, the amplitude of clock gene expression is significantly higher than during anagen, with the strongest expression in the hair germ. This robust circadian clock in the hair germ regulates stem cell activation as well as anagen initiation [33]. Consistent with this finding, germline *Bmal1* mutation in mice leads to a delay in anagen initiations. Knocking out *Bmal1* specifically in keratinocytes, however, does not significantly delay anagen initiation [14,33]. This suggests that *Bmal1* regulates anagen initiation through the central SCN clock or other non-epidermal cell types. In nature, hair cycles for most mammals are seasonal. Given the role of the clock in seasonal cycles, it is tempting to speculate that the circadian clock has a role in seasonal hair growth [34].

Among the bulge stem cells, circadian rhythms are heterogeneous during telogen, with cells expressing higher levels of *Bmal1* being more prone to activation signals. As anagen begins, circadian heterogeneity gradually decreases with most of the stem cells expressing high levels of *Bmal1* [15,35]. Consistently, overexpression of *Bmal1* by knocking out its repressors *Per1* and *Per2* stimulates proliferation and reduces the number of dormant bulge stem cells [35]. These findings are consistent with a regulatory role of the circadian machinery in the hair cycle and indicate that robust clock output correlates with hair follicle stem cell activation and anagen initiation.

## The skin immune system is circadian clock-regulated

In order to fulfill its infection control function, the skin harbors resident innate immune cells distributed throughout the epidermis and dermis (Fig. 3A) as well as in organized tertiary lymphoid structures [36–39]. The circadian clock modulates the activation of these cells, possibly to match the likelihood of infections and insults throughout the 24-hour day. By regulating the skin immune response in this way, the circadian clock may decrease the tendency for autoinflammatory and autoimmune skin diseases [40–42].

Epidermal-resident immune cells include specialized dendritic cells called Langerhans cells, as well as specialized T cells referred to as $\gamma/\delta^{+}$ T cells. $\gamma/\delta^{+}$ T cells are under clock regulation in that the CLOCK protein directly binds to the promoter of the interleukin 23

receptor (IL-23R) in $\gamma/\delta^+$ T cells, thereby playing a regulatory role in the development the inflammatory skin disease psoriasis [41]. Langerhans cells appear to be regulated by the circadian clock as well. For example, when exposed to a viral mimic, imiquimod (IMQ), Langerhans cells highly upregulate anti-viral genes, the expression of which is affected by *Bmal1* deletion [40].

Other immune cells such as macrophages, mast cells, T cells, and dendritic cells reside in the dermis (Fig. 3A). Macrophages constantly monitor the skin microenvironment and innately respond to pattern-associated molecular patterns and damage-associated molecular patterns produced through infections and inflammation. Other key functions of macrophages, including cytokine production and phagocytosis, are diurnally gated [43,44]. Mast cells cause allergic responses by releasing histamine, which activates other immune cells to cause allergy-like symptoms such as itching, erythema, and edema. Clock deletion in murine mast cells specifically ablates temporal variations in *IgE*-mediated degranulation and thus passive cutaneous anaphylactic reactions [45,46], indicating direct regulation by the circadian clock.

Apart from its resident immune cells, the skin recruits adaptive immune cells through chemokine and cytokine release during infections and inflammation. Circadian expression of chemoattractants, and the subsequent rhythmic infiltration of neutrophils and macrophages into the skin, results in diurnal severity of parasitic infection in murine footpad models [47]. Moreover, all rhythms in infection are abolished in clock-deficient macrophages and mice lacking the circadian clock in immune cells [47]. Additionally, the diurnal pattern of T cell recruitment and function parallels nocturnal itching and exaggeration in atopic dermatitis (AD) [48].

### Skin microbiome

A diverse community of microorganisms, termed the microbiome, colonizes the human body. Appropriate microbial composition and function is essential for proper host immune and metabolic activities [49,50]. Most human studies regarding potential diurnal rhythms in the microbiome have been conducted in the oral cavity [51] or the gut [49,50,52,53], showing that rhythms in the microbiome are primarily driven by food intake. On the skin, taxa abundance, mainly of the phylum *Actinobacteria*, and especially the families *Propionibacteriaceae*, *Micrococcaceae*, *Gordoniaceae* and *Dermacoccaceae*) varies diurnally. This pattern primarily correlates with human activity [54].

## The effect of feeding on the skin circadian clock and skin functions

Although light entrains the SCN clock, additional inputs such as temperature, exercise, and food intake affect the clocks in peripheral tissues [9]. The effects of food intake on circadian clocks in metabolic organs such as liver and fat are extensively studied. In these organs, food intake has major roles in timing of the clock, which is unsurprising given the divergent types of whole-body metabolism required during the day (food intake and activity) and night (fast and rest). In contrast to extensive studies focusing on the liver, the connection between feeding and circadian rhythms in the skin remained unexplored until recently. Below we

review some of the findings showing that feeding regulates the skin circadian clock and biology of the skin.

## Feeding-induced gene expression changes in skin

In experiments with multiple time-restricted feeding schedules, it is possible to rearrange the transcriptome data into feeding time series covering the beginning of feeding to 8 hours after the beginning of feeding. This allows the study of the effect of feeding on skin independent of time of day. In total, the expression of around 2000 genes changes in response to food intake, indicating powerful effects of feeding on gene expression in the skin (Fig. 4, left). The feeding-affected upregulated genes are involved in *lipid biosynthesis* and *protein synthesis*, whereas the downregulated genes are involved in *response to starvation*, *autophagy*, *negative regulation of cell proliferation*, and *response to oxidative stress*. This suggests that the metabolism and cell cycle regulators in the skin respond to feeding. Additionally, the fact that half of the feeding-affected transcripts are diurnal in at least one of the feeding schedules suggests that feeding is a regulator of diurnal gene expression in the skin [55].

## Daytime restricted feeding shifts the phase of the circadian clock in the skin

Daytime restricted feeding, as opposed to normal nighttime feeding schedules for mice, shifts the phase and amplitude of the circadian clock in skin (Fig. 4, middle) [55]. Compared to nighttime feeding groups, daytime-restricted feeding shifts the phase of the core circadian machinery in skin as indicated by *Per2*, *Dbp* and *Per1* mRNA expression patterns. The magnitude and the direction of the shifts depend on the exact feeding time during the day. Additionally, the amplitude of *Per2* mRNA expression decreases with daytime restricted feeding [55]. These changes in the skin circadian clock are different from the changes in the liver, where the phase of the clock is tightly coupled with feeding time, with phase advancements in all daytime-restricted feeding groups. Also, daytime-restricted feeding does not affect the amplitude of the liver clock [55]. These findings demonstrate a strong regulatory effect of feeding time over the skin circadian clock that is different from the effect on the liver clock.

The mechanisms whereby time of feeding shifts the phase and amplitude of the skin circadian clock are unknown. Since feeding regulates the expression of many systemic hormones including insulin, these hormones are potential mediators of the clock phase-shifting effects. Indeed, application of insulin to mouse whisker hair follicles induces activation of *Per2*, leading to circadian synchronization of hair follicles [56]. Interestingly, application of insulin before the peak of PER2 expression leads to phase advances, while application of insulin after the peak does not [56]. Similar patterns are observed in cultured human hair follicles [57], suggesting that the timing of insulin shifts; therefore, food intake, may disturb the circadian rhythm in human hair follicles. While insulin may be one mediator, the exact mechanisms connecting feeding time to circadian rhythms in the skin remain to be investigated.

### Transcriptomic changes in skin are specific to the altered feeding schedules

RNA-seq of the exons, introns, and antisense RNA from the skin of mice under three different restricted feeding schedules shows that while around 2500 to 3000 exons oscillate in each of the schedules, only 147 transcripts are shared in all three groups, including several members of the core clock gene network [55]. Despite the transcriptomic diversity, gene ontology analysis of the diurnal genes indicates *cell death*, *redox regulation*, and *circadian clock* as enriched biological processes for all feeding groups. Significant correlations between the peak expression time of a gene's intron and exon indicated that transcriptional regulation is responsible for the circadian phase shifts induced by restricted feeding. Some cytokinesis genes appear to be the exception to this rule and are regulated post-transcriptionally. Additionally, exons altered by food intake, regardless of the feeding schedule, are mostly related to metabolism, an indicator that feeding plays a role in skin metabolism status [55].

### Time-restricted feeding does not affect diurnal rhythms in epidermal stem cell division but influences the sensitivity to UVB-induced DNA damage

Overall, daytime restricted feeding does not alter the structure of the skin with the exception that the dermis is thinner for the daytime-restricted feeding groups compared to the control [55]. Interestingly, although the cell cycle in epidermal stem cells shows a diurnal rhythm that depends on *Bmal1*, the phase of the circadian clock in skin does not control the cell cycle phase: the peak of S phase does not shift in the restricted feeding groups despite the shift in the phase of the clock (Fig. 4, middle). These experiments suggest that daytime restricted feeding desynchronizes intermediary metabolism and cell division in epidermal stem cells [55].

On the other hand, skin's sensitivity to ultraviolet B (UVB) radiation-induced DNA damage is affected by daytime restricted feeding (Fig. 4, middle) [55]. More damage is caused by UVB applied during the night than during the day in the skin from the nighttime-feeding group, while more damage is caused during the day for the daytime-restricted feeding group. Furthermore, oscillation of xeroderma pigmentosum group A (*Xpa*), a gene responsible for UVB-induced DNA repair, is dampened and less robust in the restricted feeding groups, suggesting that feeding time affects the diurnal rhythms of genes responsible for UVB protection and DNA damage repair in skin [55].

### Time-restricted feeding influences the skin immune response

Expression of interferon-sensitive genes in the skin is also shifted by time-restricted feeding (Fig. 4, middle) [55], suggesting that feeding time influences the skin immune response. Consistent with this idea, the expression pattern of interferon-sensitive genes in response to IMQ in the daytime-restricted feeding group is different from the one in the nighttime feeding group. In the daytime-restricted feeding group, interferon-sensitive genes are expressed at higher levels after IMQ application during the night than during the day, which is the opposite of the pattern shown in the nighttime feeding group (Fig. 4, middle). These findings suggest that time restricted feeding-induced circadian clock alterations affect the skin immune response [40].

### The effect of calorie intake on diurnal gene expression and skin function

Besides food intake as such and time of feeding, the amount of calorie intake affects gene expression and function of the skin. Calorie restriction changes the structure of mouse skin by inducing faster hair regrowth after shaving, thicker epidermis, less dermal white adipose tissue, and greater vascular network [58]. Consistently, calorie-restricted mice have more interfollicular and bulge hair follicle stem cells that contribute to epidermal and hair growth. Additionally, calorie restriction alters the metabolism in mouse skin by decreasing oxidative metabolism in the epidermis but increasing it in the dermis [58]. Interestingly, calorie restriction can impede the aging-related reprogramming of clock-controlled genes, suggesting the connections between calorie restriction, circadian controlled genes and skin function (Fig. 4, right) [59].

On the other hand, high fat diet induces hair thinning and dermal adipose tissue expansion without significantly affecting epidermal thickness [60]. A closer look at the hair follicles in mice taking high fat diet reveals a decrease in hair follicle stem cells in the bulge as well as a fate shift of these stem cells from hair shaft to upper hair follicle components such as sebaceous gland and the epidermis-hair follicle junction zone [60]. On a transcriptomic level, high fat diet induces the expression of around 3000 rhythmic genes enriched for gene ontology terms such as *fatty acid oxidation*, *responses to oxidative stress* and *mitochondrion organization* [59]. This finding suggests a rewiring of the rhythmic genes to adjust skin metabolism in response to high fat diet (Fig. 4, right).

Taken together, food intake itself, the time of food intake, and the amount of calorie intake affect the expression of rhythmic genes in the skin, as well as the cellular composition and function of the skin. These findings suggest that feeding has powerful effects on the skin, in part through modulation of the rhythmic transcriptome (Fig. 4).

## Circadian rhythms and skin diseases

Since the circadian machinery modulates many skin processes, including immunity, cell proliferation, metabolism, and DNA damage repair, it is plausible that circadian dysregulation can contribute to the development and progression of skin diseases.

The connection between circadian dysfunction and skin diseases is being studied both in the laboratory and in clinical settings. In the laboratory, chronic jet lag, prolonged light exposure, and flashlight treatment at night have successfully induced circadian disruption in mice. Additionally, mutation of core clock genes in mice and cultured tissues and cells provide insights into the role of the clock in disease. Human studies mostly focus on epidemiological data collected from shift workers, aiming to delineate the association between shift work-induced circadian disruption and diseases.

In this context, we focus on the role of the circadian clock in various skin diseases, including skin cancer, sunburn, aging, hair loss, inflammatory diseases, infections, and wound healing.

## Skin cancer

The two main forms of skin cancers are carcinoma and melanoma. Carcinomas, such as squamous cell and basal cell carcinomas, are derived from keratinocytes. Melanomas, on the other hand, are derived from melanocytes [61]. Although carcinomas are more frequent, melanomas are more deadly by metastasizing through blood [62].

Ultraviolet (UV) radiation, either from the sun or tanning beds, is the major cause of melanomas and carcinomas. UV radiation from the sun can be classified into three types based on wavelength: UVA, UVB, and UVC. UVC is the most damaging of the three, but fortunately the majority of it is absorbed by the ozone layer. On the other hand, most of UVA and a small percentage of UVB still reach the earth. Those two wavelengths are absorbed by skin cells and cause DNA mutations through different mechanisms. UVB directly causes nucleotide changes while UVA raises the level of reactive oxygen species (ROS) that can lead to DNA damage. Additionally, while UVB is a thousand times more carcinogenic than UVA, UVA can penetrate deeper to cause DNA mutations [63,64].

Epidemiological studies linking work-related circadian disruption to cancer risk have highlighted the role of the circadian clock in several types of cancer, including melanoma [65].

**The skin's susceptibility to UV radiation-induced DNA damage is diurnal—** Circadian rhythms are robustly present in the skin, an organ that directly experiences variations in UV radiation throughout the day. Indeed, the susceptibility of the skin to UV radiation-induced DNA damage is time-of-day dependent, and this diurnality is controlled by the circadian clock. There are at least two possible mechanisms contributing to the diurnal pattern of DNA damage. The first one relates to the susceptibility of the S phase of the cell cycle to UV-induced DNA damage [14] and the other one to the diurnality in DNA repair [66,67].

Exposing the dorsal skin of mice to UVB radiation at different times throughout the day revealed that more DNA damage occurs when the exposure takes place at ZT20 than at ZT8 [14]. Nucleotide excision repair (NER) is ordinarily capable of resolving UV damage, but rate-limiting subunits of the process such as XPA proteins are expressed in a rhythmic pattern due to circadian clock regulation. Because of this rhythmicity, mice that are exposed to UV radiation during dark hours experienced more severe and abundant squamous cell carcinomas compared to mice exposed to UV radiation during light hours [66]. Such time-dependent variation in DNA damage is replaced with a constant high level in *Bmal1*$^{-/-}$ mice, suggesting that the susceptibility to UVB radiation is controlled by the clock [14]. The cell cycle is also diurnal in mouse epidermal cells and controlled by the clock. More cells are in the S phase at night (ZT21) than during the day (ZT9). Similar to the DNA damage, the diurnal variation in the proportion of S phase cells disappeared in single knockout *Bmal1*$^{-/-}$ mice and double knockout *Cry1*$^{-/-}$*Cry2*$^{-/-}$ mice [14,68]. It is noteworthy that the S phase is more prone to DNA damage, which may contribute to increased susceptibility to UVB radiation at night (ZT20) compared to the day (ZT8) [14].

XPA protein is expressed at high levels during the day and is at low levels at night [66,67]. P53 protein also accumulates to a higher level in mouse skin exposed to UV radiation at night (ZT21) than at during the day (ZT9). This variation could be explained by changes in the level of MDM2 protein, which stimulates the degradation of P53 protein and shows a diurnal expression pattern. During the night, the levels of both MDM2 and P53 proteins are low, resulting in less degradation of P53. Thus, when mouse skin is exposed to UV radiation at night, the P53 levels rise to a high level with minimum degradation, leading to more P53-dependent responses such as apoptosis. On the other hand, since the expression level of both P53 and MDM2 proteins are already high during the day, UV radiation does not cause much increase in the P53 level because the level of MDM2 is high enough to degrade most of the P53 proteins. Hence, the P53-dependent responses in mouse skin are less prominent when the radiation occurs during the day instead of at night [69].

The skin is protected from UV radiation by the pigment melanin produced by melanocytes [70]. The core clock component BMAL1 influences melanin production by associating with microphthalmia-associated transcription factor (MITF), the master regulator of melanogenesis. MITF has rhythmic expression levels alongside BMAL1. By overexpressing BMAL1, the resultant melanin from the melanogenesis pathway was correspondingly higher, conferring greater protection against UV radiation for skin cells [71].

For non-UV radiation induced carcinoma, which accounts for about 10% of carcinomas, the loss of *Bmal1* results in decreased development of squamous tumors in mice with an oncogenic background [35]. Compared to the control, mice with *Bmal1* knockout have lower percentages of tumor-initiating cells and increased expressions of tumor suppressors, thus decreasing the severity and abundance of carcinomas.

In sum, the clock affects UV-induced skin cancer initiation by regulating the cell cycle and DNA repair mechanisms in keratinocytes and through regulation of UV-protectant pigment from melanocytes.

**The circadian clock regulates skin cancer progression—**In addition to controlling susceptibility to UV-induced DNA damage and cancer initiation, the clock is involved in skin cancer progression in at least two ways. On the one hand, cancerous cells possess aberrantly expressed clock genes, resulting in impaired clock function [72]. On the other hand, mutations of core clock genes are associated with melanoma progression [73]. As a result, recent studies have targeted components of the circadian clock to restore tumor cell circadian rhythms in cancer treatment [72].

The clock network is dampened in cancer cells, demonstrating that an oncogenic landscape facilitates clock dysfunction [72]. A comparison of gene expression data from human melanoma and healthy skin samples reveals downregulation of clock genes in melanoma [74]. The upregulation of oncogenes such as *Ras* extends the period of the clock, demonstrating mechanisms in cancer progression that disturb the circadian machinery [75]. Consistently, mice injected with B16-F10 melanoma cells possess severely impaired clock gene regulation within and around the tumor site [76]. Core clock components can be indirectly regulated by proteins of the Melanoma Antigen (MAGE) family that

bind to RORs or modify ubiquitination of clock proteins [77,78]. Similarly, circadian machinery is disrupted in oncogenic keratinocytes; ablation of the tumor suppressor gene *Pten* increases BMAL1 in hair follicle stem cells, highlighting the regulatory influence of tumor suppressors on core clock components [79].

In addition to disrupted circadian clock function in melanoma cells, circadian disruption contributes to other oncogenic mechanisms. Myeloid-specific *Bmal1* knockouts lead to metabolic dysregulation in macrophages, thus promoting immune suppression and melanoma tumor burden [80]. In addition, chronically shifting light schedules alters the immune microenvironment in melanomas by inverting the ratio of M1 and M2 macrophages [81]. Thus, clock dysfunction can lead to tumor-promoting changes in the melanoma microenvironment.

Circadian disruption also promotes cell cycle transitions by upregulating cell cycle genes such as *Ccna2* [81]. Clock proteins like PER can couple with nuclear proteins involved in the cell cycle, enabling circadian control of cell cycle checkpoint genes [82]. Normally, specific components of the clock, such as neuronal PAS domain 2 (NPAS2), also directly limit cellular proliferation by gatekeeping portions of the cell cycle, but mutations in these genes promote cell cycle transitions [83]. Overall, since clock proteins act as critical regulators of cell cycle checkpoint control, disruption of the clock leads to unregulated division and tumor proliferation [84].

Given that clock dysfunction enables tumor proliferation, targeting the clock to improve its function could facilitate cancer management. Inducing circadian rhythmicity in tumor cells by dexamethasone decreases *in vivo* melanoma growth in mice [85]. This response is clock-dependent, as knocking down *Bmal1* in tumors prevents the growth-dampening effect from dexamethasone. Pharmacological studies also targeted components of the circadian clock, nuclear hormone receptors NR1D1 and NR1D2, to stimulate apoptosis in malignant cancer cells [86]. By applying agonists of these receptors, senescent cells like those in melanocytic nevi would undergo cell death, demonstrating that treatments targeting core clock machinery impair melanoma proliferation. In addition to targeting the circadian clock machinery, current cancer treatments can utilize the clock by modulating the time of treatment application. The toxicity of the chemotherapeutic cisplatin towards melanoma cells varies throughout the day, indicating that melanoma treatment is more beneficial in the morning (ZT0) for patients [87].

In sum, the circadian clock plays a role in the initiation and progression of skin cancer. The rhythmicity of the clock regulates DNA-damage repair, thus modulating the susceptibility to UV-induced DNA damage and cancer initiation. Carcinomas and melanomas often possess diminished clock function, promoting cell cycle transitions and cell proliferation. Also, dysregulation of the clock can alter the tumor microenvironment to make it more immunosuppressive. Targeting core clock genes therapeutically to restore clock function may be a promising approach to cancer treatment.

## Sunburn

Short-term exposure to excessive UV radiation leads to sunburn. UV radiation-induced DNA damage leads to an inflammatory response characterized by skin redness, swelling, and pain [88]. Consistent with the diurnality in UV-induced DNA damage, the severity of sunburn symptoms also exhibits a diurnal pattern. While erythema severity saturates at high levels of UV exposure on the dorsal skin of mice, moderate levels of radiation applied during the night (ZT21) caused higher levels of inflammatory cytokines, apoptosis, and worse erythema than during the day (ZT9) [69].

Due to the opposite chronotypes of humans and mice, the diurnal pattern found in mice is speculated to be reversed in humans [14,63,69]. Indeed, radiation in the evening (between 7pm to 9pm) caused more serious erythema than in the morning (between 7am to 9am) on human skin, indicating that the human skin is more sensitive to radiation in the evening [89]. Interestingly, P53 protein accumulated more in skin samples collected 24 hours after morning irradiation, which is the same pattern in mice [89]. This could be explained by previous findings wherein UVB-induced accumulation of P53 is not correlated with severity of erythema in human skin [90].

In summary, UV-induced DNA damage directly links to skin carcinogenesis as well as acute inflammatory responses. Furthermore, the effect of UV radiation on skin is time-of-day dependent due to the diurnality of cell cycle and DNA damage repair.

## Hair loss

Hair loss, also known as alopecia, is one of the most common skin concerns. While it usually occurs on the scalp, hair loss can affect any hair bearing part of the body. The most common form of alopecia is androgenetic alopecia, which affects both males and females, albeit with a different pattern of hair loss. There is also an aging-associated hair loss that may be independent of androgenetic alopecia. Telogen effluvium is another hair loss disease; it may be triggered by a number of stimuli, including stress, hormones, medications, and illness [91]. In this condition, hair follicles enter the shedding phase in a synchronized manner [92] whereas normally human hair follicles have desynchronized hair cycle stages. Anagen effluvium on the other hand is caused by cell death in the matrix of growing hair follicles in response to genotoxic stimuli such as ionizing radiation and chemotherapy [93]. Alopecia areata is an autoimmune disease characterized by hair loss. Finally, scarring alopecias are inflammatory conditions that lead to destruction of the hair follicle bulge stem cells and permanent loss of hair follicles [92].

Given the regulatory role of the circadian clock in hair follicles, it stands to reason that clock disruptions could contribute to hair loss, especially aging-related hair loss. Evidence for this, however, is missing from epidemiological studies. The clearest evidence for a role of the circadian clock in hair loss comes from mouse studies showing that the clock gates cell division in growing hair follicles. Thus, $\gamma$ radiation causes more hair loss when applied to anagen hair follicles at the mitosis peak in the morning than in the evening when mitosis levels drop [28]. Based on this finding in mice, we speculate that radiation in the evening would causes more hair loss than radiation in the morning in humans. Hence, arranging

radiotherapy in the morning may be better for managing hair follicle damage during the treatment.

## Aging

At an organismal level, aging correlates with circadian rhythm alterations as indicated by features including advanced sleep pattern, flattened body temperature fluctuation, and decreased activity levels during the wake period [94]. Additionally, aged mice cannot adapt to light-dark schedule alterations as well as young adult mice [95]. One explanation for this is related to the impaired functions of retinal cells and the SCN. Since aging reduces the number of ipRGCs, the amount of SCN neurons expressing VIP, and neuron coupling, the circadian rhythm in aged individuals is not as robust as in young adults at the organismal level [96]. While the SCN function is compromised at a tissue level, the clock machinery evaluated by *Per2* expression in the SCN is as robust as in young adult mice [95]. Similar to the case in the SCN, the molecular clock in mouse peripheral tissues such as kidney, liver, submandibular gland, and epidermis is robust despite the decrease in locomotor activity [59,97].

In the skin, features of aging include wrinkles and lines, dryness, a thin epidermis, hair loss, loss of elasticity, skin fragility with easy breaking, and slow wound healing. The causes of skin aging are on the one hand intrinsic, strongly correlated with chronological age, and on the other hand extrinsic. Intrinsic aging mechanisms include oxidative damage to DNA and proteins from metabolism-generated ROS, accumulating genetic mutations, stem cell dysfunction, and alterations in hormones. Extrinsic skin aging mechanisms involve primarily UV radiation, but also other insults such as cigarette smoking, and pollution [98].

While the exact mechanism is unknown, clock disruption contributes to intrinsic skin aging as mutations of the core clock genes *Bmal1* and *Clock* in mice lead to premature aging in multiple organs, including the skin [99,100]. Conversely, aging influences the circadian function, as aging rewires rhythmic genes in the skin [59]. While the circadian machinery is robust in aged epidermal stem cells, other rhythmic genes in the young and aged groups are quite distinct [59]. Thousands of genes are diurnal in young and aged mouse epidermal stem cells, but only 750 genes overlap the two ages, including some genes from the core clock machinery. Additionally, the rhythmic genes present only in the young adult group are mainly related to homeostasis maintenance, while the genes that oscillate only in the aged group are enriched for stress response. This suggests a reprogramming of rhythmic genes in mouse epidermal stem cells as they age while maintaining the core circadian function [59]. Despite premature aging in core clock gene knockout mice [99,100], the failure to reproduce the transcriptional traits found in physiologically aged epidermal stem cells in *Bmal1* knockout and *Per1/Per2* double knockout mice suggests that the reprogramming of rhythmic genes is due to aging instead of loss of circadian function [59].

A similar observation was made in rat abdominal skin explants. There is no significant overall change in the average value of phase, amplitude and period lengths in rat skin explants as they age, although the phases are significantly more variable in the aged group [101]. However, when looking at the circadian rhythm in fibroblasts derived from these explants collected at different ages, *Per1* expression amplitude attenuates uniformly as the

age increases [101]. Along the same line, a microRNA *miR-31* that is upregulated in aged mouse hair follicle stem cells targets at the core clock gene *Clock*, suggesting the potential effects of aging on the clocks in hair follicle stem cells [102]. While the differences in results could be caused by different experimental methods, the question about whether aging affects circadian clocks differently in different cell types warrants further investigation.

As discussed before, the rhythms in the skin regulate cell proliferation in a way to avoid exposing possible genotoxic and radiation stress to the vulnerable S phase [14,20]. Intriguingly, without affecting the core clock gene network, aging affects the cell cycle timing in mouse epidermal stem cells, which could be an explanation for the transcriptional enrichment of stress response and DNA damage repair in aged cells. While the S phase takes place mostly during the night in young adult epidermal stem cells [14,20,59], the peak DNA replication time is shifted to the day for the cells from the aged group [59]. Despite this delay, mitosis is rhythmic and still takes place at ZT20 in the aged group, which is the same as in the young adult group. Such delay may lead to the possible exposure of unwound DNA to oxidative stresses and increase the possibility of cells entering the mitosis with DNA damage [59]. This observation is consistent with some of the skin aging symptoms, as oxidative stress is one of the contributors to interfollicular epidermis stem cell senescence, which could lead to skin wound healing delay, thinning of the epidermis in aged skin, and age-related hair loss [15].

One way to lessen the effect of aging in mouse epidermal stem cells is through calorie restriction. For mice under calorie restriction (70% of the amount taken by the control group), both physiological and transcriptional traits found in young mice are maintained with rhythmic expressions preserved in genes related to homeostasis maintenance and lower level of oxidized DNA. Also, S phase peaks at ZT12, which is closer to the peaking time in young adult epidermis. Despite a 4-hour phase advancement for the calorie restriction group due to feeding anticipation, other circadian features such as the amplitude and the period length of some of the core clock genes are conserved in the calorie restriction group [59].

Since circadian rhythms in the skin may protect cells from UV-induced DNA damage by regulating cell proliferation [14], circadian reprogramming and cell cycle shifts caused by intrinsic aging may contribute to extrinsic skin aging. Furthermore, clock-related hormone function impairment caused by intrinsic aging can affect skin function and promote extrinsic skin aging. Melatonin is one of the hormones that regulates peripheral clocks and its receptor is present in the skin [103]. Interestingly, melatonin receptor MT1 plays a key role in DNA repair as it activates the P53-dependent DNA damage response [104]. In human skin, MT1 levels drop as we age, leading to increased sensitivity to UV radiation [105]. The receptor MT1 level is drastically lower in cultured dermal fibroblasts from a 67-year old donor when compared with cells from a 19-year donor. Furthermore, after UV radiation, the amount of UV-induced DNA damage and ROS generation is much higher in MT-1 knockdown human fibroblasts, suggesting that aged human dermal fibroblast are more susceptible to UV damage [105].

Much of the connections between skin aging and circadian rhythms remain to be explored. Questions such as whether aging affects the circadian rhythm differently in different cell

types, what mechanisms cause the shifts in cell cycle while not affecting the core circadian clock in epidermal stem cells, and what kind of mechanisms contribute to the rescuing effect of calorie restriction on epidermal stem cell aging remain to be answered.

## Skin infection

As a first line of protection against outside insults, the skin harbors various defensive strategies, including a physical barrier formed by corneocytes (terminally differentiated keratinocytes), resident innate immune cells, and antimicrobial peptides (AMPs), which are secreted to induce direct antimicrobial effects [37,38,106]. The immune system is circadian regulated, possibly to anticipate and prepare for outside insults when they are most likely to occur [15,107]. The circadian clock regulates immune responses to infections in the respiratory system [108,109], the liver [110], the digestive system [111], and other body sites [112]. Consistent with these findings, a growing body of animal research supports the hypothesis that immune responses and clinical outcomes of skin infections are influenced by the host's circadian rhythms and time of infection [14,15,47,113].

**Bacterial and parasite skin infections—**The survival of bacteria on the skin depends on the time of infection. *Staphylococcus aureus* shows maximum survival on mouse skin when applied at ZT22 and minimum survival when applied at ZT16. This may be due to the diurnal expression of certain AMPs in the skin. The expression levels of *Rarres2* (chemerin), *Camp* (cathelicidin CRAMP), and *Defb1* (β-defensins) are diurnal, with the highest expression during the early night when mice are under 12:12 light-dark cycle. However, the rhythms for those genes in mouse skin are lost in constant darkness, although they are maintained in the liver. In contrast, *Defb3* and *Defb14* (β-defensins) aare circadian under both 12:12 light-dark cycles and in constant darkness, with expression peaking during the day [113].

The severity of parasite infections in skin is diurnal as well [47]. Leishmania parasites are transmitted through sandfly bites and use neutrophils and macrophages as host cells. Injection of *Leishmania major* parasite into mice at different times of the day revealed that inflammation is the mildest when the injection takes place at ZT3, with minimum macrophage recruitment six hours after injection. *In vitro* experiments confirm that the attachment of the parasite to mouse macrophages is circadian and dependent on the circadian machinery, which also regulates the time-dependent release of chemokines and cytokines [47].

**Viral skin infections—**Skin also has the important task of defending against many viruses, which are biological entities that can only thrive and multiply in a living cell. While there are few studies on circadian regulation of defenses against viral infections of the skin, studies in other organs suggest a role for the circadian clock in regulating this activity. Mice intranasally infected with herpes or influenza viruses during the daytime have greater infection loads and mortality than mice infected at nighttime. This time-of-day dependent difference may be clock controlled as deletion of *Bmal1* exacerbates the infection [114]. In addition, disruption of circadian rhythms by *Bmal1* deletion or altered sleep/wake patterns enhanced acute viral bronchiolitis after Sendai virus and influenza A viral infections [115].

Topical application of the viral mimic IMQ during the day results in greater expression of anti-viral defense response genes compared to application at night. Absence of *Bmal1* results in heightened levels of those same genes [40]. This result suggests that the diurnal gating of sensitivity to viral infection is modulated by circadian clock control of anti-viral factors expressed in the skin. These results have implications for anti-cancer defenses as anti-viral mechanisms limit cancers as well. For example, IMQ is prescribed as an anti-tumor treatment for basal cell carcinomas, as it activates the immune system through type I interferon secretion.

## Inflammatory skin diseases

Skin inflammation, which includes redness, heat, itching, sensitivity, and swelling serves to defend against invading pathogens and microbes. While controlled skin inflammation is necessary to regain homeostasis after infection, there are cases in which skin inflammation is triggered by self-peptides or persists following an infection, leading to skin diseases. Inflammatory responses are linked to circadian rhythms [40] and circadian disruption can lead to inflammatory diseases. In mice, for example, chronic jet lag induces ulcerative dermatitis [116]. For humans, psoriasis and AD are two of the most common autoimmune skin diseases. Both of these diseases, which can significantly impair patients' life quality, are linked to circadian rhythms.

Psoriasis is caused in part by the immune system attacking healthy skin cells, resulting in intense inflammation. In humans, disruption of the circadian clock through night shift work is associated with psoriasis [117,118]. The symptoms of psoriasis also show a diurnal pattern, with more than 70% of patients reporting more severe itch occurring in the evening or at night [119]. With most of the itch occurring at night, AD and psoriasis symptoms compromise sleep quality [120], which in turn disrupts circadian rhythms and increases severity of the disease. This circadian disruption also contributes to the development of metabolic diseases such as obesity [121], which may be causally linked to psoriasis [122].

At a mechanistic level, the inflammatory response in psoriasis is controlled by the circadian machinery [40,41]. Mutation of *Clock* relieves the induced psoriasis-like symptoms significantly by controlling the transcription of IL-23R in $\gamma/\delta^{+}$ T cells while mutation of *Per2*, the inhibitor of *Clock*, promotes IMQ-induced symptoms [41]. Additionally, deletion of *Bmal1* promotes IMQ-induced psoriasis [40]. Conversely, psoriasis inflammation disturbs the circadian clock locally in the skin lesions. In IMQ-induced psoriasis mouse model, the rhythmicity of keratinocyte proliferation is lost, and the expression of core clock genes is dampened [40]. Similar to the case in mice, bulk RNAseq studies using human patients skin samples show that circadian genes such as *CRY2*, *PER3*, *NR1D1* and *RORC* are downregulated in the psoriasis lesion as well as the adjacent normal skin when compared to skin from non-psoriatic individuals [40,123]. These findings support the intricate connection between psoriasis and the circadian machinery and suggest that the circadian clock is a potential target in psoriasis treatment.

AD is another common inflammatory skin disease characterized by impaired barrier function, eczematous dermatitis, and chronic itching. The causes of AD are not fully understood, but may relate to barrier impairment, dysregulated immune system, and/or

dysregulated commensal microbiota. AD symptoms tend to be greater at night and impair sleep quality [124,125]. There are several mouse models of AD. These typically apply an inflammatory insult (a chemical or hapten) to the skin to activate macrophage cytokine secretion, which in turn provokes release of histamines by mast cells that causes itch; the insult is then re-applied later to induce an adaptive immune response driven by T cells. Disruption in circadian rhythms by forcing mice to run during the day and rest during the night causes loss of rhythmicity in circadian gene expressions and more severe AD symptoms with increased expression of histamine and cytokines, including TNF-$\alpha$ and interleukins (IL-4, IL-10, IL-13). Additionally, more dendritic cells, mast cells and T helper cells are recruited to the skin in mice with the reversed chronotype [126]. Also, hapten induces more inflammation in the skin of mice living in constant light than in the skin of mice living in regular 12:12 light-dark cycle [127]. Implicating the circadian clock on the molecular level, deletion of *Clock* causes more severe delayed-type skin allergic reactions [128]. As the circadian clock regulates the immune responses that contribute to AD symptoms, studying the relationship between the circadian rhythm and AD will yield insights into symptom management.

## Wound healing

Skin injuries invoke complex repair mechanisms that require regulation and coordination among cell types throughout the skin. These mechanisms go awry in chronic wounds, a major health problem sometimes caused by metabolic diseases like diabetes and obesity [129]. Several elements of skin wound healing have diurnal features and interact with the core clock machinery.

Wound healing progresses through five steps: hemostasis, inflammation, growth, re-epithelialization, and remodeling. Hemostasis stops the bleeding by forming a hemostatic plug at the injury site [130]. This very first step of wound healing displays diurnal rhythms in humans, with hemostasis being upregulated in the morning and fibrinolysis activation peaking in the afternoon [131]. As discussed earlier, elements of the inflammation step are under circadian control.

The growth stage of skin wound healing displays circadian rhythms. The actin polymeric state, as measured by the ratio of F:G actin produced by fibroblasts, is rhythmic and such rhythm is dependent on the cells' internal circadian machinery [132]. Additionally, the circadian clock affects senescence of myofibroblasts through NONO, a binding partner of PER that regulates cell cycle inhibitors [82]. These findings support the idea that the circadian clock regulates both proliferation and migration of fibroblasts, key functions required for wound healing.

The time-of-day of wounding may affect the length of recovery [132]. Synchronized murine skin fibroblast monolayers scratch-wounded at the peak time of PER2 expression healed almost completely after 60 hours while those wounded at the PER2 trough time were far from closing. This can be explained by the significantly faster initial migration, stronger initial cell adhesion, and greater wound-oriented polarization of the most motile fibroblasts in monolayers wounded at the peak time of PER2 expression. *Ex vivo* wounding experiments done on 5-day-old murine skin explants harvested during the active phase and

the resting phase show similar results, with twice as many fibroblasts invading the wound in the explants collected during the active phase when compared with the sample collected during the resting phase. This observation is reproduced in *in vivo* wounding experiments on adult mouse back skin, suggesting that the invasion of fibroblasts into the wounded area, and thus possibly the recovery of the wound, are dependent on the wounding time in mice [132].

A *post hoc* analysis of the datasets from the Burn Injury Database reveals that burns happening at night took around 60% more time to heal than the burns that took place during the day [132]. While wounds incurred during the active phase of humans and mice take less time to heal [132], the opposite pattern is true in nocturnal hamsters [133]. According to the wound sizes recorded daily, wounds incurred at ZT18 (night) take longer to recover by 50% than wounds incurred at ZT3 (day), even though wounds incurred at both times take the same number of days to heal completely [133].

Re-epithelization may also display circadian rhythms. While there is no *ex vivo* or *in vivo* wounding experiments done so far on human skin, scratch-wounds incurred on human keratinocyte monolayers at the peak time of fibroblast motility healed faster than the monolayers scratch-wounded at the trough time [132]. On the other hand, while the proliferation of mouse epidermal keratinocytes is circadian, with more cells at the S phase at night than during the day [14], the proliferation elevates and loses its rhythmicity when there is inflammation [40].

Circadian disruption delays skin wound healing. Cutaneous wounds in disruptive light-treated hamsters took longer to heal compared to hamsters with normal circadian rhythm, no matter the time of wound occurrence [133]. Similarly, dim light exposure to mice during dark hours before wounding slows down the healing process significantly [134].

Manipulation of the circadian machinery does not always impede wound healing. Partial or complete knock out of *Npas2*, whose protein is a paralog of CLOCK, significantly shortens the duration of dorsal skin wound healing in mice *in vivo*. *In vitro* experiments using the fibroblasts isolated from $Npas2^{-/-}$, $Npas2^{+/-}$ and wild type mice uncovered accelerated proliferation of $Npas2^{-/-}$, $Npas2^{+/-}$ fibroblasts without alternation in the rhythmic expression of most of the core clock genes except *Per2*. Additionally, $Npas2^{-/-}$ fibroblasts contract faster than wildtype and $Npas2^{+/-}$ fibroblasts. Intriguingly, the expression level of *Acta2* and *Actb*, along with other integrin genes do not differ among the genotypes. Some collagen genes (*Col12a1* and *Col14a1*) are upregulated in cultured $Npas2^{-/-}$ and $Npas2^{+/-}$ fibroblasts, in agreement with promoted collagen fiber formation observed in those knockout cells [135].

While more extensive studies are necessary to delineate the connection between the circadian clock and skin wound healing, current findings indicate that the time of wounding has an effect on the healing duration [132,133] and that the circadian machinery plays a role in many wound healing processes [133–135]. The field remains to be explored, especially the possibility of promoting wound healing via circadian clock manipulation [135].

## The future of circadian medicine for skin health

Circadian medicine and chronotherapy are receiving increasing attention as important components of preventive medicine and patient care [136–138]. At its core, circadian medicine considers circadian rhythms in pre-clinical research and when developing disease management plans to improve clinical outcomes while minimizing side effects. For example, vaccination in the morning results in greater trained immunity and antibody responses than vaccination in the evening in humans [139–141]. Additionally, perioperative myocardial injury is more common after on-pump cardiac surgeries in the morning than in the afternoon [142]. In mice, non-steroidal anti-inflammatory drugs better manage pain and promote recovery with less adverse effects on healing after tibia fracture surgery when taken during the active phase than during the resting phase [143]. Such diurnal variation in treatment efficiency is also observed in other tissues and diseases [136–138], including cancer [144,145].

Circadian medicine uses three main approaches: lifestyle adjustments to regulate the internal clock, drugs targeting the clock machinery at a molecular level, and administration of drugs at the time of day that maximizes effectiveness and minimizes side effects (chronotherapy) [68,137]. Although the skin harbors a robust circadian clock, circadian medicine for skin diseases is a fledging field. Here, we discuss how circadian medicine can benefit skin health.

### Managing the internal clock via lifestyle adjustment

Research in model animals and cultured human cells shows how disruptions of circadian rhythms via clock gene manipulations can contribute to the development and progression of various kinds of skin diseases including cancer, sunburns, radiation-induced hair loss, aging, infections, inflammatory diseases, and wounds. Consistent with these experimental studies, epidemiological studies on shift workers show that circadian disruption is associated with increased incidence of metabolic disease [146], cancer [65], and several skin diseases [68,117,118]. Therefore, it stands to reason that a healthy lifestyle aimed at maintaining normal circadian rhythms may improve skin health.

Currently, shift work, social jet lag, sleep disorder and excessive light exposure at night are common causes of circadian rhythm disruption in modern society. Shift work is extensively studied as it leads to disruption of the core clock machinery, including the skin [147,148]. Compared to normal daytime workers, shift workers possess altered circadian rhythms in the skin as indicated by significantly attenuated peak expression level of *Per1* [147]] and altered expression patterns and levels of *Per3*, *Nr1d1* and *Nr1d2* [148] in hair follicles. Shift workers are more prone to skin diseases, including psoriasis, dry skin, itching, dandruff/seborrheic dermatitis, acne, and melanoma [65,68,117,118].

Conversely, skin diseases such as psoriasis and AD can affect the patients' organismal circadian rhythm by disturbing sleep [120,125,149], which then adversely feeds back to disease progression. Besides being predisposed to greater disease risk, shift workers may experience worse side effects. In mice, circadian disruption via jet lag or clock gene mutations leads to more severe radiation-induced dermatitis [150]. Because of this,

maintaining a normal sleeping pattern and avoiding shift work would be beneficial for minimizing the risk and severity of skin diseases.

As feeding can influence skin circadian rhythms in mice, and thus the immune response, adjusting feeding behavior in terms of both of the feeding time and the caloric intake may be a way to support circadian function in the skin [138,151]. In mice, time of feeding affects the skin immune response [40]. Additionally, calorie restriction delays the age-associated reprogramming of circadian controlled genes from homeostasis maintenance to stress response [59]. In contrast, high fat diet induces hair loss [60] and rewires the expression of rhythmic genes to favor oxidative metabolism [59]. While well-controlled experimental research in humans on the effect of feeding on skin health is lacking, a recent clinical study focusing on psoriasis patients before and after Ramadan fasting, which happens during daylight time for an entire month, showed alleviation of psoriasis after the fasting [152,153].

## Targeting the clock machinery at a molecular level

Circadian rhythms are often disrupted in diseased tissues. Most often, this takes the form of dampened rhythms due to a loss of synchronization or suppressed circadian gene expression [40,85,144,154]. For example, expression of *Bmal1* is dampened in the skin of IMQ-treated mice [40]. Therefore, it is possible that restoring or enhancing the circadian rhythm can control disease progression. Dexamethasone, for example, activates the circadian clock in B16 melanoma tumors, which decreases cell proliferation and tumor growth [85]. Indeed, the circadian machinery is a reservoir that may host promising targets for developing skin disease treatments.

For example, without harming normal cells, REV-ERBs agonists SR9009 and SR9011 adversely affect cancer cells derived from tumors, including melanoma [86]. In mice, mutation of *Clock* relieves psoriasis-like symptoms [41] and mutation of CLOCK protein paralog shortens wound healing duration [135]. Additionally, topical application of ROR inverse agonist SR1001 quenches inflammatory response in AD mouse models. For humans, melatonin supplement alleviates AD symptoms [155]. Future studies should explore drugs that target the circadian machinery in skin diseases, including inflammatory and hyperproliferative skin diseases.

## Chronotherapy

Circadian rhythms regulate many metabolic enzymes that participate in drug metabolism in the skin [14]. Hence, more clinical studies should be conducted to assess how time of application impacts treatment efficacy and side effects. Experiments done in mice show that maxacalcitol, a vitamin D3 analogue for psoriasis symptom relief, is more effective when applied during the early to middle active phase of mice than during the early to middle resting phase [48]. For AD, application of topical treatment in the evening is proposed to improve symptoms [156].

For cancer, studies from the 1990’s describe more effective melanoma tumor shrinkage in mice when interferon was infused during the day as opposed to night [157]. Additionally, radiation chronotherapy in the morning for cancer treatment reduces its damaging effect on skin [158].

It may also be important to consider the time of diagnostic measures. For example, the time of diagnostic measures is critical for accurate diagnosis in endocrinology, as a number of hormones oscillate in a time-of-day dependent manner [159]. Similarly, it is possible that the time of measurement is important in dermatology, since features such as mitosis [14,28] trans-epidermal water loss [160], and immune components display diurnal rhythms [40,55,113].

### Challenges

As promising as circadian medicine is, we face challenges. Most studies are done *in vitro* or *in vivo* using model organisms such as mice, rats and hamsters. Those animals are nocturnal while humans are diurnal, which can lead to difficulty in translating from mice to humans. Also, most pre-clinical intervention studies in mice are carried out during the day, the resting phase of mice. One possible step to reduce the gap between human and model nocturnal animals is to use inverted-light housing, so researchers can still make the measurements during the day while it will be the night for the nocturnal model animals.

Additionally, since circadian medicine works the best when it is correlated with the patient's internal circadian rhythm, health professionals need a feasible method to accurately determine each patient's circadian rhythm before establishing a treatment plan [161]. The most traditional and established way of measuring circadian phase of a patient includes a tedious process of collecting multiple saliva samples throughout the day in a dim room for the dim-light melatonin-onset assay [162–164]. For clinical applications, several approaches have been proposed to determine a patient's clock phase [165], including body temperature [166], transcript biomarkers in blood [164,167,168], cortisol concentration in sweat [169], as well as volatile organic compounds and nitric oxide fraction in breath [170]. The accessibility of skin makes this organ a source to measure rhythms: indeed, transcripts from hair follicles [171], skin biopsies [163] and tape-stripped epidermis [172] can potentially be used for circadian time estimation [161]. However, the question as to which method is the best in terms of accuracy and accessibility remains unanswered. Another challenge to utilizing circadian medicine for skin diseases lies in the complexity of skin. The circadian rhythm in skin may not be as uniform as in other organs [15]. Additionally, two other clocks, hair cycle and natural aging, influence the circadian controlled gene expression, raising questions about whether these variables need to be considered [15,59].

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## Abbreviations:

| | |
|---|---|
| **SCN** | suprachiasmatic nucleus |
| **ipRGCs** | intrinsically photosensitive retinal ganglion cells |
| **VIP** | Vasoactive Intestinal Peptide |
| **AVP** | Arginine Vasopressin |

| | |
|---|---|
| **BMAL1** | Brain and Muscle ARNT-Like 1 |
| **CLOCK** | Circadian Locomotor Output Cycles Kaput |
| **E-box** | Enhancer Box |
| **PER** | Period |
| **CRY** | Cryptochrome |
| **RORs** | RAR-related Orphan Receptors |
| **RORE** | ROR/REV-ERB-response element |
| **IL** | Interleukin |
| **AD** | Atopic Dermatitis |
| **IMQ** | Imiquimod |
| **UV** | Ultraviolet |
| **NER** | Nucleotide Excision Repair |
| **XPA** | DNA Damage Recognition and Repair Factor |
| **NPAS2** | Neuronal PAS Domain Protein 2 |
| **P53** | Tumor Protein 53 |
| **ROS** | Reactive Oxygen Species |
| **MITF** | Microphthalmia-associated Transcription Factor |
| **AMPs** | Antimicrobial Peptides |

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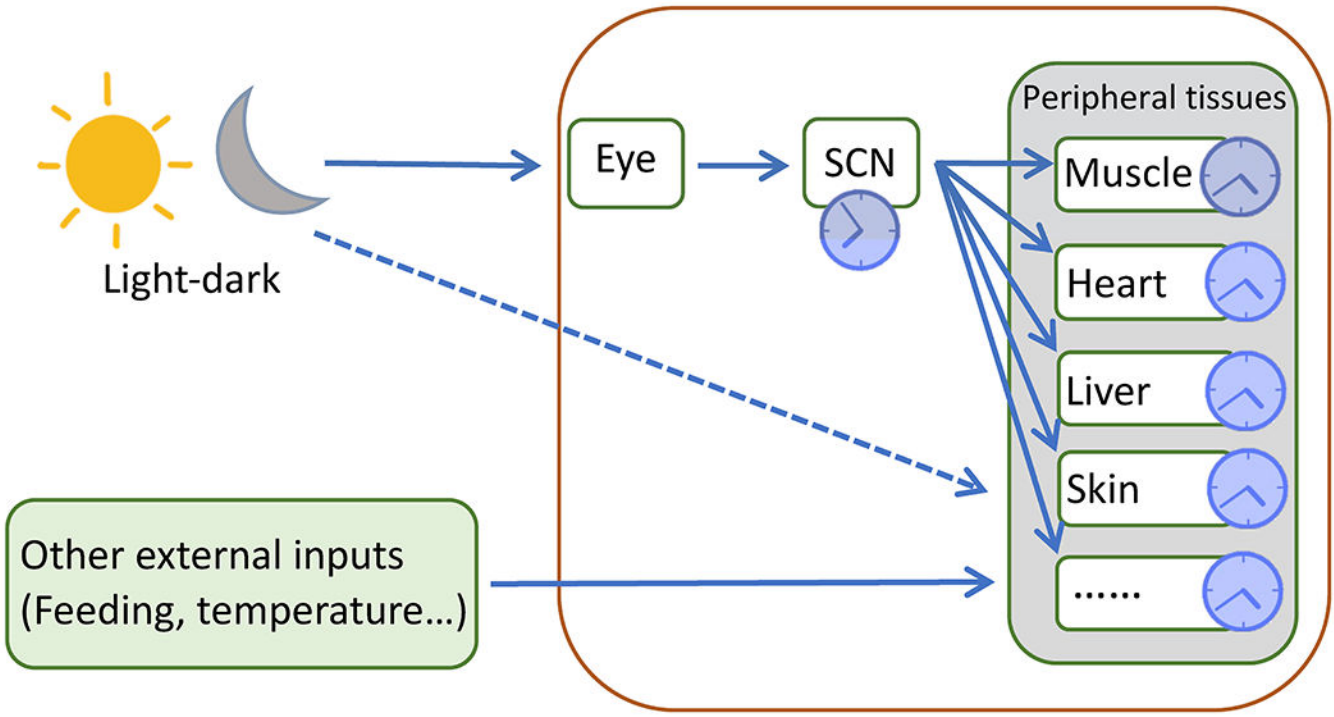


**Figure 1: The SCN synchronizes peripheral clocks.**

Light sends signals through the retina to set the clock in the SCN, which synchronizes peripheral clocks via neuronal and hormonal signals. As indicated, the phase of peripheral clocks is delayed by a few hours compared to the SCN clock. The SCN, however, is not required for the maintenance of peripheral clocks. It is also possible that light-dark cycles directly regulate clocks in the skin. Additionally, other external inputs such as feeding and temperature entrain peripheral clocks, including in the skin.

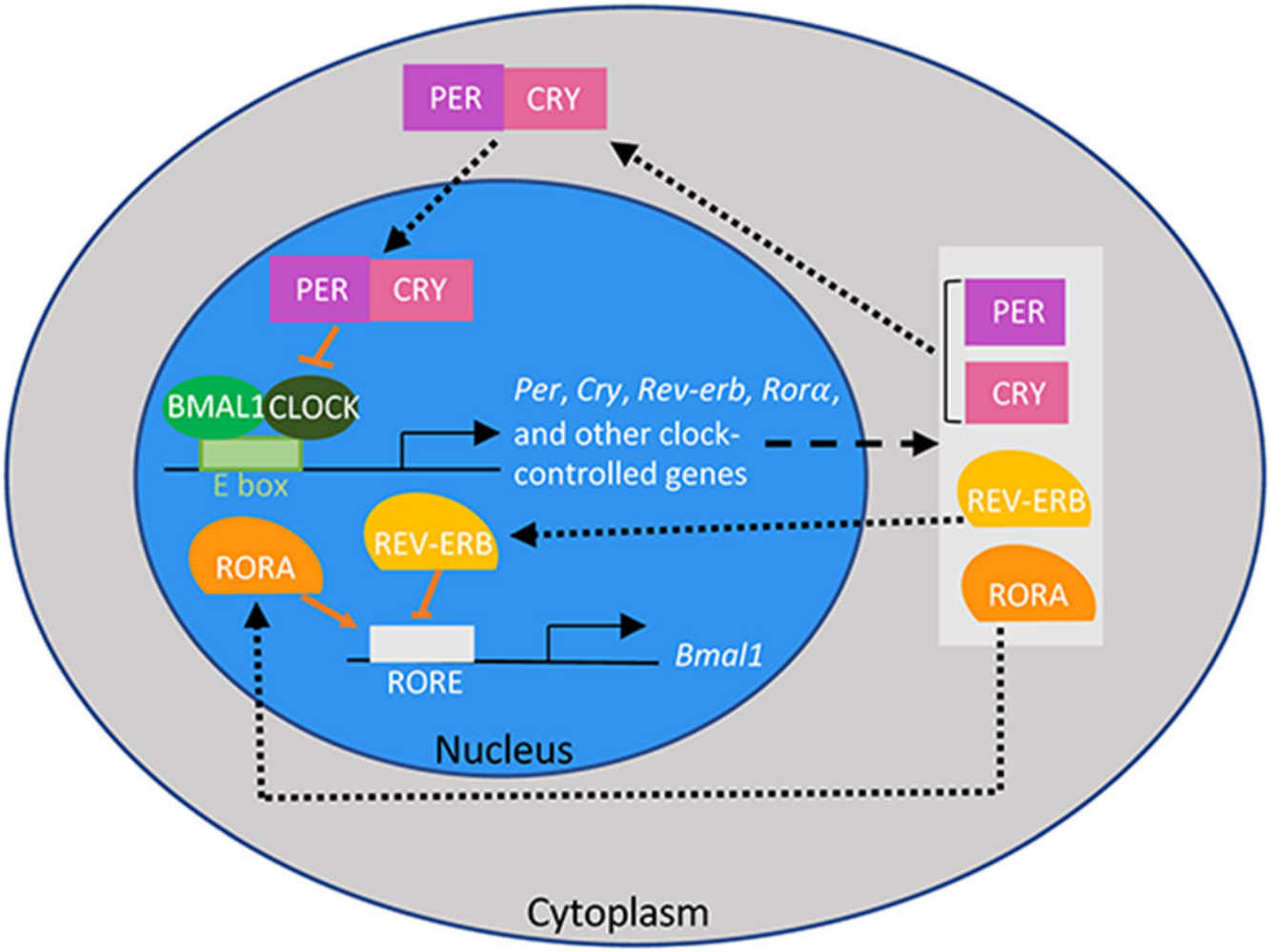


**Figure 2: A transcription-translation feedback loop generates circadian rhythms.**

The core clock has a positive arm, consisting of BMAL1 and CLOCK, and a negative arm, consisting of PER and CRY, which inhibits the BMAL1-CLOCK heterodimer. RORs, REV-ERBS forms a secondary feedback loop to reinforce the oscillatory expression of *Bmal1*. The clock network directly and indirectly regulates the expression of about 10–20% of genes in each peripheral tissue.

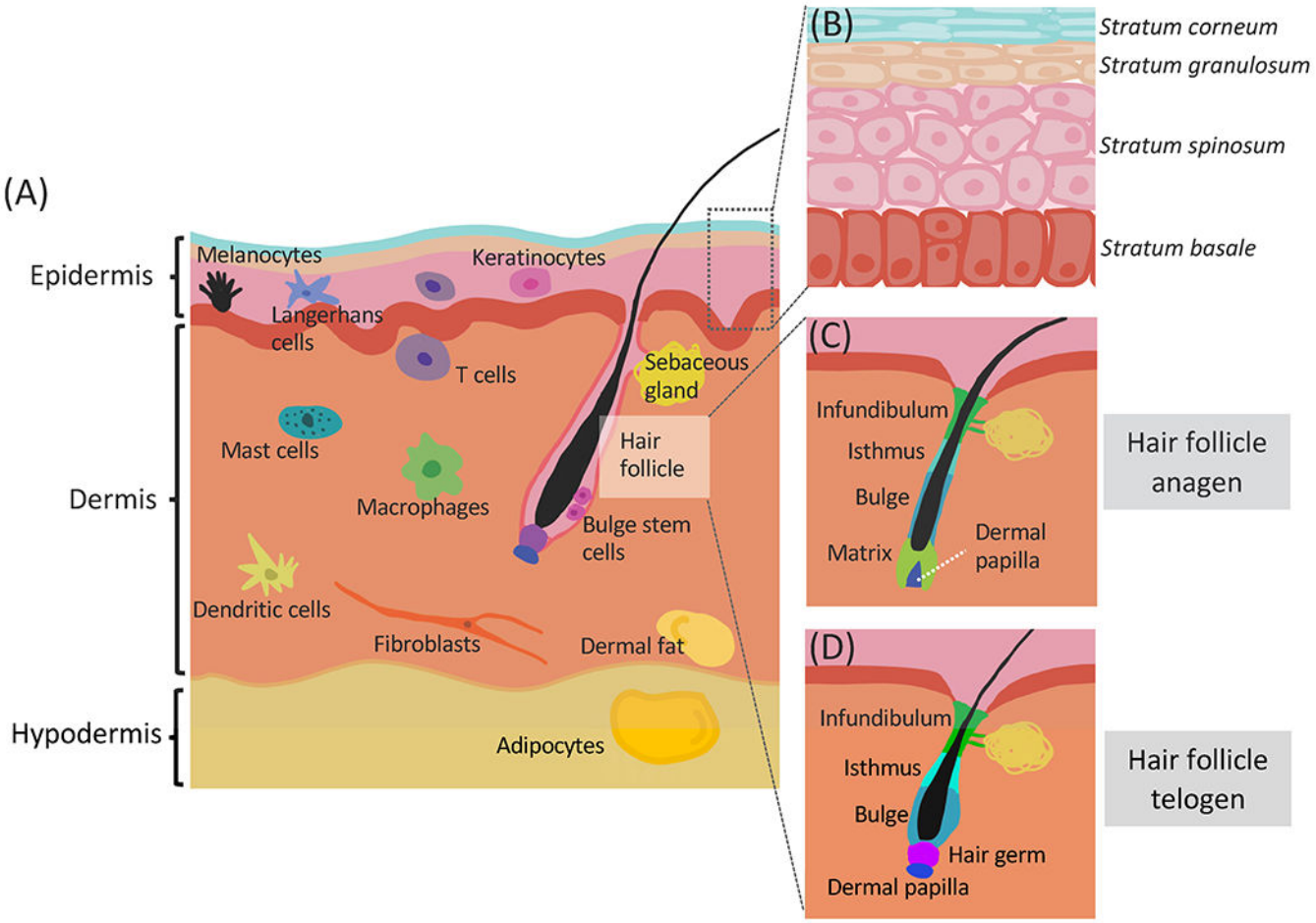


**Figure 3: The skin is a multi-layer, compartmentalized organ.**

(A) There are three main layers in the skin: epidermis, dermis and hypodermis. Each layer contains multiple cell types. (B) The interfollicular epidermis contains mostly keratinocytes organized into four layers based on differentiation status. The epidermal stem cells reside in the *stratum basale*. (C) The anagen hair follicle. The matrix contains proliferating keratinocytes derived from the secondary hair germ, giving rise to the hair shaft. (D) The telogen hair follicle. The hair follicle bulge contains the slow cycling stem cells. The hair germ contains the progenitor cells for the hair. The dermal papilla is a mesenchymal structure that signals to the hair germ and stem cells.

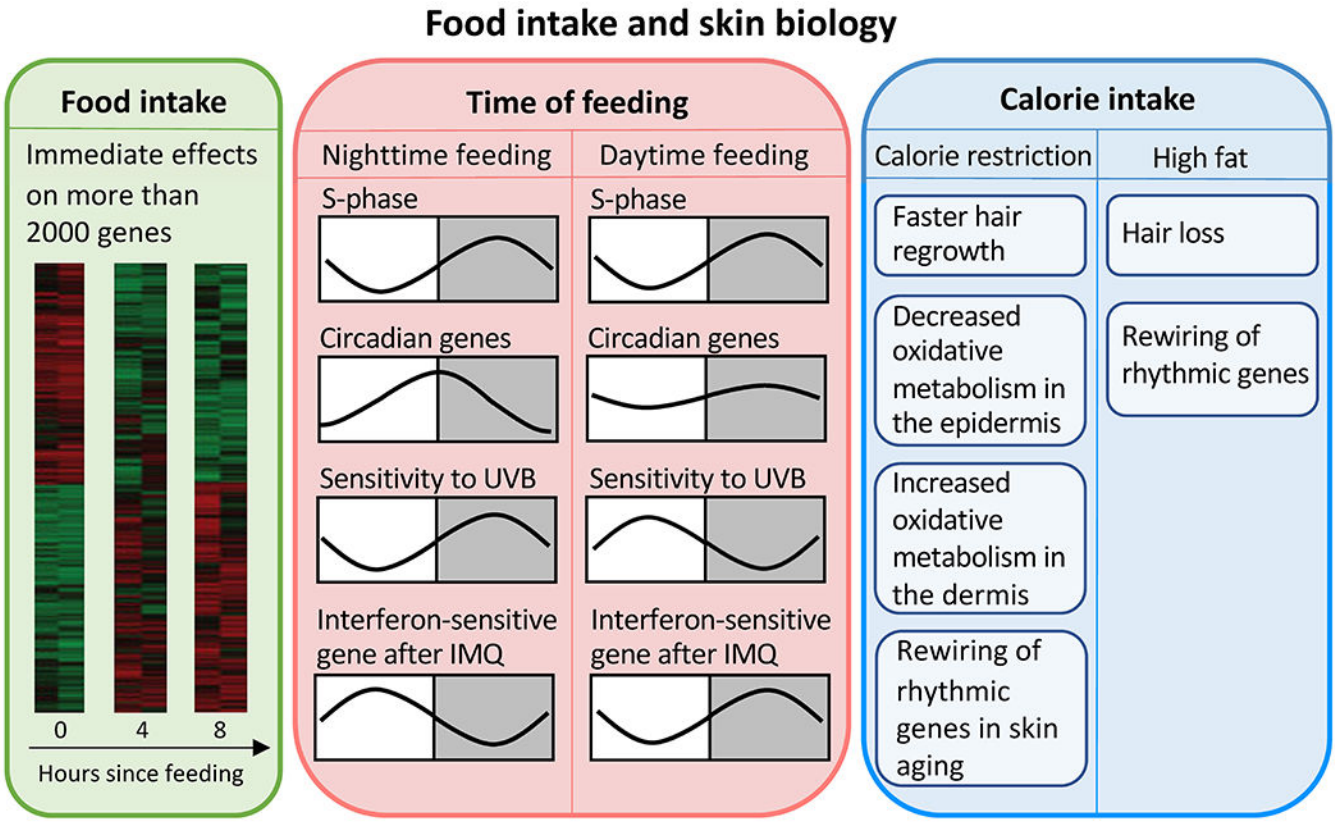


**Figure 4: Food intake regulates skin functions.**

Left: Food intake immediately changes the expression of more than 2000 genes in the skin. Heat maps show gene expression before, four hours, and eight hours after food intake. Middle: Time of feeding does not affect the diurnal pattern of cell proliferation in the skin. However, daytime feeding shifts and dampens the expression of circadian genes. Daytime feeding also changes the diurnal variation in sensitivity to UVB-induced DNA damage and the IMQ-induced interferon response. Right: Calorie restriction and high fat diet affect metabolism and expression of rhythmic genes, hair follicle stem cell function, and skin aging. IMQ, imiquimod.