Published in final edited form as:

J Invest Dermatol. 2015 April ; 135(4): 943–945. doi:10.1038/jid.2014.553.

When the Circadian Clock Meets the Melanin Pigmentary System

Andrzej T. Slominski[1,2], **Rüdiger Hardeland**[3], and **Russel J. Reiter**[4]

[1]Department of Pathology and Laboratory Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, USA

[2]Division of Connective Tissue Diseases, Department of Medicine, University of Tennessee, Memphis, Tennessee, USA

[3]Johann Friedrich Blumenbach Institute of Zoology and Anthropology, University of Göttingen, Göttingen, Germany

[4]Department of Cellular and Structural Biology, UT Health Science Center, San Antonio, Texas, USA

Abstract

Silencing of BMAL1 and PER1 stimulates melanogenic activity of follicular and epidermal melanocytes, indicating a novel role for peripheral circadian clock processes in the regulation of melanin pigmentation. Linking the expression levels of BMAL1/PER1 with changes in melanogenesis opens exciting opportunities to study the role of the local molecular clock in modulation of melanocyte functions in the hair follicle and the epidermis with attendant effects on epidermal barrier functions in general.

Silencing of the BMAL1 and PER1 stimulates melanogenic apparatus in the skin

In this issue, Hardman *et al.* (2015) provide evidence for the involvement of a peripheral circadian oscillator in the control of human pigmentation. The experiments were conducted using micro-dissected hair follicles and cultured primary skin melanocytes. Although melanogenesis in the hair follicle is anagen-associated (Slominski and Paus, 1993), the role of the peripheral clock was notably demonstrated under the conditions independent of the hair cycle (Hardman *et al.*, 2015). Conclusions were convincingly drawn from silencing of either BMAL1 (brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1) or PER1 (period 1), using respective small interfering RNAs. These two proteins are components of the circadian core oscillator, in which a heterodimer formed from BMAL1 and its interaction partner CLOCK (circadian locomotor output cycles kaput) stimulates the expression of PER (period) and CRY (cryptochrome) variants. These factors jointly block BMAL1/CLOCK actions, a fundamental basis for the cyclicity of circadian oscillators. The

Correspondence: Andrzej T. Slominski, Department of Pathology and Laboratory Medicine, University of Tennessee Health Science Center, 930 Madison Avenue, Memphis, Tennessee 38163, USA. aslominski@uthsc.edu.

CONFLICT OF INTEREST

The authors state no conflict of interest.

BMAL1/ CLOCK heterodimer activates PER and CRY expression by binding to an E-box within the respective promoters. In addition, BMAL1/CLOCK stimulates the expression of various circadian-driven genes carrying an E-box. Concerning melanogenesis, E-boxes are found in the genes of tyrosinase, tyrosinase-related proteins 1 and 2, in which they also act as binding sites of the melanogenic master regulator MITF (microphthalmia-associated transcription factor; Slominski *et al.*, 2004). With regard to the circadian oscillator mechanism, silencing of BMAL1 can be expected to substantially decrease PER1 expression. In a cycling oscillator, PER/CRY heterodimers are the key players of the negative feedback limb downregulating BMAL1/CLOCK activity. However, silencing of PER1 may not necessarily lead to BMAL1 overexpression, as in knockdown experiments the oscillator is more or less fixed in a non-cycling phase of the artificially deregulated oscillator.

Under these conditions, silencing of either BMAL1 or PER1 led to remarkably clear effects: increased pigmentation, expression of tyrosinase, tyrosinase-related proteins 1 and 2, and phosphorylation of the melanogenic master regulator MITF. Silencing of PER1 caused mostly stronger effects than that of BMAL1, findings that may indicate a greater importance of the PER protein in stimulating melanogenesis, perhaps also under cycling conditions. In addition to the molecular data, increased numbers of melanosomes, higher melanocyte dendricity, and stimulation of melanosome transfer to keratinocytes were shown to result from PER1 silencing, which likely indicates involvement of extramelanocytic cutaneous signaling systems (Slominski *et al.*, 2012). Comparison of hair follicle explants with melanocyte cultures allowed a dissection between cell-autonomous actions and other effects requiring cell–cell interactions. In the isolated melanocytes, silencing of the core oscillator components increased melanogenesis, whereas gp100 expression and melanocyte dendricity were not substantially changed and may thus require melanocyte–keratinocyte interactions, as reviewed by Slominski *et al.* (2004).

BMAL1 and PER1 affect melanin pigmentation.

Central and peripheral regulators of circadian rhythm

During recent years, abundant evidence has accumulated regarding the importance of peripheral circadian oscillators (Fu and Kettner, 2013; Hardeland, 2013; Reiter *et al.*, 2014). Some of these cellular clocks are to a variable extent autonomous and do not receive a direct input from the hypothalamic pacemaker, the suprachiasmatic nucleus (SCN) (Hardeland, 2013). However, they may be indirectly influenced by SCN-dependent cycles, such as the rhythms of glucocorticoids, ACTH, or melatonin. It must be noted that melatonin (Slominski *et al.*, 2008; Hardeland, 2013; Reiter *et al.*, 2014), ACTH, and glucocorticoids are also produced in peripheral organs including the skin (Slominski *et al.*, 2013). The partial autonomy of peripheral cellular clocks must also be considered in the context of the cell cycle, which is gated by the respective circadian oscillator via controlling G1-, intra-S-, and G2/M checkpoints and modulating mitogenic signals (Fu and Kettner, 2013). The circadian influence on cell division, as controlled by different clocks, is usually cell type–specific.

Skin and local circadian clock

In the skin, the gating of cell cycle progression by a peripheral clock has been recently addressed in the context of regenerative hair cycling, uncovering diurnal mitotic gating as an essential mechanism protecting anagen hair follicle (Plikus *et al.*, 2013). The study by Hardman *et al.*, (2015) explored an additional role of a peripheral circadian oscillator on melanin formation in hair follicles and epidermal melanocytes. In this context, it is important to mention that different signaling molecules including melatonin, ACTH, and cortisol, whose systemic fluctuation is regulated by the central pacemaker, are also produced in the skin and are involved in the regulation of hair and epidermal functions (Slominski *et al.*, 2012, 2013, 2014). As these factors are also produced in melanocytes, the challenge for future research is to determine whether their local production as well as their receptors are regulated by a peripheral circadian clock, and how these cycling cascades affect final skin phenotype.

Dermatological implications

The study by Hardman *et al.*, (2015), although limited to the pigmentary system, suggests a multitude of possibilities in experimental and clinical dermatology. Knowing that the clock influences melanin formation and melanosome transfer, the anagen-associated processes of pigmentation can be analyzed in-depth from a new perspective. One may consider other aspects of cell cycle control and the roles of accessory oscillator components that are associated with or driven by core oscillator proteins. This may be the case for the PER1-associated factor NONO (non-POU domain-containing octamer binding protein), which has been reported to couple the circadian oscillator to the cell cycle (Kowalska *et al.*, 2013).

Another area that can be explored in the pigmentary system is the role of the aging suppressor sirtuin 1 (SIRT1), which has been shown to associate with BMAL1/CLOCK dimers (Sahar and Sassone-Corsi, 2013). In various peripheral circadian oscillators, the surprising observation has been made that neither CLOCK nor SIRT1 expression is rhythmic. However, contrary to protein levels, the activities of SIRT1 and, thus, of the BMAL1/CLOCK/SIRT1 complex do exhibit rhythms, because the stability of the ternary complex depends on the SIRT1 substrate NAD^+, which exhibits a rhythm due to the E-box-controlled expression of nicotinamide phosphoribosyltransferase, which regulates NAD^+ formation (Sahar and Sassone-Corsi, 2013). This would represent an unexpected but exciting area of research in skin biology in general and interaction between melanogenesis and cellular metabolism (Slominski *et al.*, 2014).

Finally, the role of BMAL1, PER1, or other circadian regulators should also be explored in the development and progression of melanoma, a tumor of melanocytic origin. This may identify novel therapies for this devastating disease. This possibility is strengthened by the well-known deregulation of circadian oscillators in other tumor cells (Fu and Kettner, 2013; Hardeland, 2013; Reiter *et al.*, 2014).

Concluding remarks

This work (Hardman *et al.*, 2015) from Dr Paus' group opens a "Pandora's box" of possibilities that relate not only to regulation of melanin pigmentation by BMAL1 and PER1 but also to the role of peripheral circadian rhythms in regulation of skin physiology overall, with important health-care implications. The biggest challenge is to decipher how the local neuroendocrine systems are integrated and participate in proper function of the molecular clock that would also include melatonin, glucocorticoids, ACTH, and other signals/messengers now associated with the systemic circadian clock but which also operate on the local level (Hardeland, 2013; Slominski *et al.*, 2013; Reiter *et al.*, 2014; Slominski *et al.*, 2014).

Acknowledgments

This was in part supported by NIH grants 1R01AR056666-01A2, 2R01AR052190, and R21 AR066505-01A1 to AS.

References

Fu L, Kettner NM. The circadian clock in cancer development and therapy. Prog Mol Biol Transl Sci. 2013; 119:221–82. [PubMed: 23899600]

Hardeland R. Chronobiology of melatonin beyond the feedback to the suprachiasmatic nucleus-consequences to melatonin dysfunction. Int J Mol Sci. 2013; 14:5817–41. [PubMed: 23481642]

Hardman JA, Tobin DJ, Haslam IS, et al. The peripheral clock regulates human pigmentation. J Invest Dermatol. 2015; 135:1053–64. [PubMed: 25310406]

Kowalska E, Ripperger JA, Hoegger DC, et al. NONO couples the circadian clock to the cell cycle. Proc Natl Acad Sci USA. 2013; 110:1592–9. [PubMed: 23267082]

Plikus MV, Vollmers C, de la Cruz D, et al. Local circadian clock gates cell cycle progression of transient amplifying cells during regenerative hair cycling. Proc Natl Acad Sci USA. 2013; 110:E2106–15. [PubMed: 23690597]

Reiter RJ, Tan DX, Galano A. Melatonin: exceeding expectations. Physiology (Bethesda). 2014; 29:325–33. [PubMed: 25180262]

Sahar S, Sassone-Corsi P. The epigenetic language of circadian clocks. Handb Exp Pharmacol. 2013; 217:29–44.

Slominski A, Kim TK, Brozyna AA, et al. The role of melanogenesis in regulation of melanoma behavior: Melanogenesis leads to stimulation of HIF-1α expression and HIF-dependent attendant pathways. Arch Biochem Biophys. 2014; 563:79–93. [PubMed: 24997364]

Slominski A, Paus R. Melanogenesis is coupled to murine anagen: toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth. J Invest Dermatol. 1993; 101:90S–7S. [PubMed: 8326158]

Slominski A, Tobin DJ, Shibahara S, et al. Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev. 2004; 84:1155–228. [PubMed: 15383650]

Slominski A, Tobin DJ, Zmijewski MA, et al. Melatonin in the skin: synthesis, metabolism and functions. Trends Endocrinol Metab. 2008; 19:17–24. [PubMed: 18155917]

Slominski AT, Kleszczynski K, Semak I, et al. Local melatoninergic system as the protector of skin integrity. Int J Mol Sci. 2014; 15:17705–32. [PubMed: 25272227]

Slominski AT, Zmijewski MA, Skobowiat C, et al. Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. Adv Anat Embryol Cell Biol. 2012; 212:1–115.

Slominski AT, Zmijewski MA, Zbytek B, et al. Key role of CRF in the skin stress response system. Endocr Rev. 2013; 34:827–84. [PubMed: 23939821]