

How Senescent Vascular Cells Lose Their *Clock* Age-Dependent Impairment of Circadian Rhythmicity in Smooth Muscle Cells

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“Oh dear! Oh dear! I shall be too late!”

—*Alice’s Adventures in Wonderland*, Lewis Carroll

In mammals, as well as in flies and even in prokaryotes, circadian rhythmicity is allowed by a genetic clockwork that tightly regulates organisms’ adaptation to daily variations of light, temperature, and other living conditions.¹ The master pacemaker of the hierarchically organized cell-autonomous circadian clocks² is localized in the densely packed 2×10^4 neurons of the hypothalamic suprachiasmatic nuclei (SCN), which is reset mainly by light signals captured by the retina. Although clocks resident in peripheral tissues, the so-called peripheral clocks, are mainly controlled by the SCN, an abrupt change in feeding time synchronizes these clocks independently of the central clock.³ In fact exogenous (eg, light) or endogenous rhythms can temporally adjust the cellular clocks, whose main characteristics are (1) feedback regulation and (2) 24 hours oscillatory period.

At molecular level, circadian rhythms are maintained by the intracellular feedback loop of the clock genes.⁴ The principal members of this family are the *mPer 1* and *2* genes,⁵ *Brain and Muscle RNA-t-like protein (BMAL-1)*,⁶ *Clock*,⁷ the *2 Cryptochrome* genes (*mCry1* and *2*),⁸ *Casein Kinase 1 ϵ (CK1 ϵ)*,⁹ and the orphan receptor *Rev-erba*.¹⁰ These genes constitute a well-conserved transcription/translation-based negative feedback loop. In mammals, *Clock* and *BMAL-1* proteins are in the positive limb of the loop, whereas *mPERs* and *Cryptochromes* are in the negative.^{2,11} *Clock/BMAL-1* heterodimeric complex, associated to the histone acetyltransferase *p300*,¹² bind to an E-box motif in the promoters of *Per* and *Cry* genes, allowing their transcription. *Per* and *Cry* protein products translocate from the cytoplasm to the nucleus, acting as negative regulators of *Clock/BMAL-1* complex, repressing their own transcription. Further, *BMAL-1* gene transcription is negatively regulated by *REV-erba*, whose transcription is activated by *Clock/BMAL-1*.¹³ This mechanism accounts for the circadian oscillation of *Bmal-1*

expression which is in antiphase with *Per* expression. Besides this transcription/translation-based oscillatory mechanism, which is common to central and peripheral clocks,¹⁴ post-translational modifications of clock gene products, such as phosphorylation and ubiquitination, modulate their stability and/or function.^{15,16}

Aging alters mammalian circadian rhythms by a dysregulation of clock genes expression¹⁷ and the work of Kunieda et al¹⁸ in this issue of *Circulation Research*, gives new insights about the molecular mechanisms underlying the loss of circadian rhythmicity in senescent cells, with particular emphasis on vascular human smooth muscle cells (HSMCs). Although it has been demonstrated that the peripheral clock is responsive to a variety of stimuli and some molecular mechanisms have been already elucidated,^{19,20} no information were available about the modification of the oscillatory rhythms in aged vascular cells. The authors clearly demonstrate an attenuation of clock genes expression in senescent HSMCs compared with the young counterpart, which is related, at least in part, to telomeres shortening and impaired CREB activation. In fact, either prevention of telomere shortening or the restoration of CREB activity overcome the impairment of circadian rhythmicity in senescent HSMCs. Moreover, as already demonstrated in other cellular systems,²¹ the authors describe a correlation between CREB activation and clock genes expression, providing novel data regarding a direct involvement of CREB in the maintenance of *BMAL-1* circadian oscillations.

SCN rhythmic transcription of *Per1* and *2* gene depends on histone acetylation/deacetylation waves occurring in their promoter regions²² (see Figure, panel A). Chromatin acetylation is required for vascular specific gene expression^{23,24} and histone acetyltransferases for the chromatin remodelling of vascular clock genes promoter in a circadian time-dependent manner.²⁵ Is the impairment of circadian expression of clock genes in senescent HSMCs paralleled by the loss of acetylation/deacetylation waves in their promoter regions? And if so, is this phenomenon dependent on an inappropriate recruitment of transcriptional complexes onto the chromatin? Is the chromatin of senescent vascular cells less accessible to the circadian transcriptional machinery? The answer to these fundamental questions could indicate, perhaps, temporally modified histones as a reasonable therapeutic target for age-associated pathologies characterized by an impairment of circadian rhythms.

In the attempt to dissect the signal transduction pathway responsible for the decreased circadian response in senescent HSMCs, Kunieda et al ascribed a pivotal role to extracellular

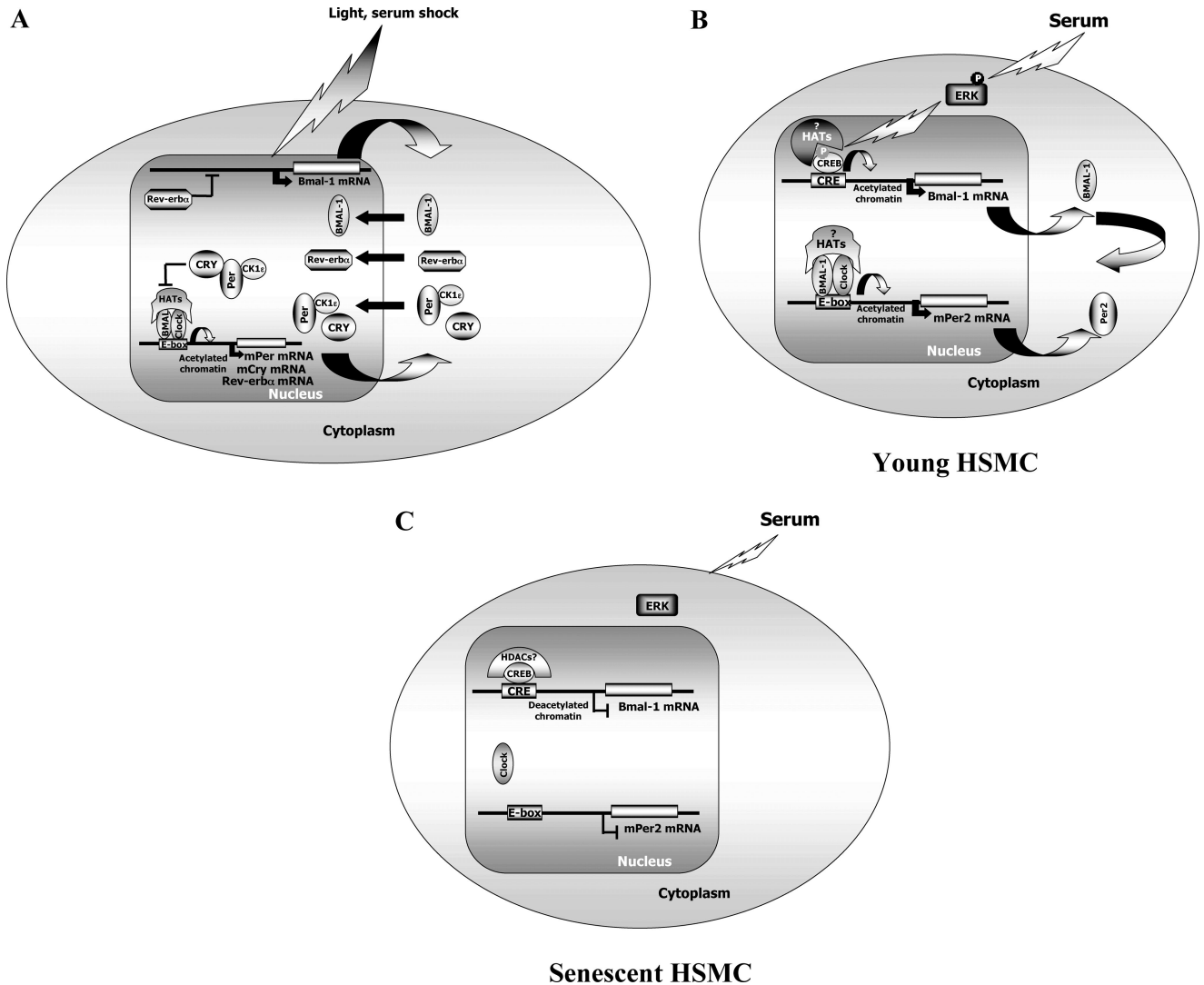
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Circulation Research is available at <http://circres.ahajournals.org>
DOI: 10.1161/01.RES.0000214331.90904.aa



Molecular model of circadian regulation of clock gene expression in young and senescent HSMCs. A, Schematic representation of Clock genes circadian regulation. In response to light or to other living conditions (eg, an abrupt change in feeding time), BMAL-1 associates to the constitutively expressed Clock protein allowing *Per*, *Cry*, and *Rev-erbα* genes transcription. In the cytoplasm, *Per* protein products are phosphorylated by CK1ε and associate with *Cry* proteins. *Per*/CK1ε/*Cry* complex shuttles to the nucleus to repress their own transcription by binding to BMAL-1/*Clock* activator complex. On the other hand, *Rev-erbα* protein, translocate into the nucleus to repress BMAL-1 gene expression. B, In young HSMCs, serum shock activates ERK/CREB pathway, allowing BMAL-1 gene transcription. BMAL-1 protein products translocate from the cytoplasm into the nucleus to form heterodimers with the constitutively expressed clock protein, allowing transcription of *Per2* mRNA. C, In senescent HSMCs, a deficient response of ERK to serum stimulation impairs the signal transduction cascade described in young cells. An involvement of the chromatin modifying enzymes, histone acetyltransferases (HATs), and deacetylases (HDACs) in this phenomenon is envisaged.

signal-regulated kinase (ERK) (see Figure, panel B). Indeed, ERK activation is lower in senescent HSMCs compared with young cells. Because it has been demonstrated the nitric oxide–cyclic guanosine monophosphate (NO/cGMP) pathway is important for the circadian responses to light in the SCN *in vivo*²⁶ and given the essential role of NO in the vascular system, whose ability to produce NO declines with age,²⁷ it would be of interest to investigate whether in aged HSMCs a dysregulated response to NO may account, at least in part, for the impaired signaling and circadian oscillation of clock genes expression.

Finally, the authors have demonstrated that senescence inhibits circadian oscillation of clock genes *in vivo*. In transplantation experiments, in which fat tissue from young

and old mice was implanted into young recipient, they showed a decreased clock gene expression in the fat tissue from old mice, reporting also an impairment of *Per2* and *Bmal-1* expression in the old hearts. These results may explain the higher susceptibility of aged hearts to myocardial ischemia, because vascular clock genes have been suggested as unique players of a novel molecular mechanism of the morning onset of myocardial infarction.²⁸

Disregulation of clock genes have been shown to occur in several diseases, including diabetes²⁹ and cancer.³⁰ The search for molecular therapeutic targets for age-associated pathologies cannot exclude clock genes, because an alteration of the endogenous day/light cycle is one feature of aging. Kunieda and coworkers propose in their study the regulation

of the ERK/CREB pathway as a therapeutic strategy to counteract age-associated impairment of circadian rhythms. However, before a circadian clock-based therapeutic approach may be suitable, the high complexity of the genetic clockwork and the absence of specifically targeted drugs should be taken into account. Future studies addressing the role of posttranslational modification of clock proteins^{15,16} and histones^{22,25} should integrate the current paucity of information about this system and provide a better elucidation of peripheral clocks response to specific stimuli.^{19,20}

Acknowledgments

We thank Dr Antonella Farsetti, from the Neurobiology and Molecular Medicine Institute, CNR, Rome, Italy, for critical reading of the manuscript.

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KEY WORDS: senescence ■ gene expression ■ chromatin ■ circadian rhythm ■ cardiovascular