Neurobiology of Circadian Rhythm Regulation

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KEYWORDS

• Circadian • Pacemaker • Suprachiasmatic nucleus • Entrainment • Clock genes

KEY POINTS

- The suprachiasmatic nucleus (SCN) of the anterior hypothalamus has been firmly established as the master circadian pacemaker in mammals.
- The SCN circadian pacemaker is synchronized (entrained) by environmental light-dark cycles via photoreceptors and neural pathways distinct from those mediating visual perception.
- The cellular-molecular basis of circadian rhythm generation involves several circadian clock genes expressed not only in the SCN but also throughout the brain and peripheral tissues and organs.
- The SCN serves as a central pacemaker atop a hierarchically organized, anatomically distributed circadian timing system and entrains downstream circadian clocks via neural and neuroendocrine pathways.
- System-wide circadian coordination is necessary for optimal physiologic function and maintenance of physical and mental health.

IDENTIFICATION OF THE SUPRACHIASMATIC NUCLEUS CIRCADIAN PACEMAKER

The initial demonstrations that lesions of the SCN severely disrupt or abolish circadian rhythms in behavioral and endocrine functions were published in the early 1970s.[1,2] Following these initial demonstrations, extensive subsequent research involving lesions, in vivo and in vitro electrophysiology, functional metabolic mapping, fetal tissue transplant, and molecular analyses revealed that the SCN is capable of autonomous, self-sustained circadian rhythmicity at both the single-cell and tissue levels. These now-classic studies are summarized in the published report of a meeting held to evaluate the state of SCN research on the 25th anniversary of its discovery.[3]

SUPRACHIASMATIC NUCLEUS: A NETWORK OF CLOCK CELLS

Studies using a variety of in vitro models, including electrophysiological recording and optical monitoring of SCN cell and tissue cultures, have provided compelling evidence that circadian oscillation is fundamentally a cell-autonomous process, expressed in many, but probably not all, individual SCN neurons.[4–7] Nevertheless, individual SCN clock cells normally interact to produce coherent circadian signals at the tissue (and behavioral) level.[8–10] Despite the capacity of individual SCN neurons for autonomous rhythmicity, recent studies have revealed that neuronal network interactions increase the frequency of rhythmic cells detected in culture, as well as the amplitude of their

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oscillation, and contribute to the overall robustness of SCN pacemaker function.[11,12]

Early studies suggested that SCN clock cells could maintain intercellular synchrony in the absence of sodium-dependent action potentials,[13,14] suggesting that gap junctions, glial coupling, calcium-dependent signaling, or local diffusible signals might be responsible for synchronizing the network of clock cells.[15] In contrast, however, more recent studies have found that blocking action potentials in SCN tissue slices or cell cultures can disrupt intercellular phase synchrony,[10] thus reviving interest in the possible synchronizing role of synaptic transmission. Both γ-aminobutyric acid (GABA) and vasoactive intestinal peptide (VIP) neurotransmission, among other signaling mechanisms, have now been implicated in the maintenance of coupling among SCN clock cells, as well as among subpopulations of SCN clock cells.[16,17]

MOLECULAR BASIS OF THE SUPRACHIASMATIC NUCLEUS CIRCADIAN PACEMAKER

A critical role for protein synthesis in the mammalian circadian pacemaker was established in the late 1980s,[18,19] and elucidation of the fundamental molecular genetic oscillatory mechanism began in earnest about 10 years later. The first mammalian circadian clock gene, *Clock*, was identified in a forward-genetics mutagenesis screen,[20] and this discovery was followed quickly by the identification of several other core molecular clock components, some of which were homologous to previously discovered circadian clock genes in the fruit fly.[21,22] In addition to *Clock*, other recognized mammalian clock genes include the 3 period (*Per*) genes (*Per1, Per2, Per3*), 2 cryptochrome genes (*Cry1* and *Cry2*), *Bmal1* (also known as *Arntl1 and Mop3*), *CK1e* (*Casein kinase 1 epsilon*), *Rev-erba,* and *Fxbl3*, all of which are expressed in SCN neurons. The specific functions of these various genes within the interlocking molecular feedback loops that generate circadian signals at the cellular level have been reviewed extensively elsewhere, and are not discussed here.

Mutations or deletions of any of these genes produce alterations in circadian phenotype at the behavioral level. The most devastating effects on clock function are seen in *Bmal1* knockout mice, which express immediate loss of rhythmicity in the absence of a light-dark cycle.[21,22] In contrast, the original *Clock* mutation, which codes for a dominant-negative CLOCK protein, dramatically lengthens free-running period and often leads to a gradual loss of rhythmicity under long-term free-running conditions.[21,22] Surprisingly, however, unlike the original *Clock* mutation, *Clock*-null (knockout) mice express robust and persisting circadian rhythms, with only a modest shortening of circadian period[23]; it was subsequently found that NPAS2 can substitute for CLOCK as a dimerization partner for BMAL1 within the SCN, thus maintaining circadian pacemaker function.[24] Regarding the Per genes, several distinct mutations have been studied by different laboratories, but in general, *Per1* or *Per2* disruption shortens circadian period and reduces the robustness of free-running rhythms.[25,26] Similarly, *Cry* mutant mice also exhibit alterations in the free-running period, whereas *Cry1*/*Cry2* double mutants are rendered arrhythmic.[27,28] In contrast to other clock genes, the circadian clock function of *Ck1e* was discovered by genetic analysis of a spontaneous single-gene mutation that dramatically shortens free-running period in the hamster, originally called the *tau* mutation.[29] Cloning of the *tau* gene revealed its identity as *Ck1e*, and subsequent transgenic insertion of this allele into mice recapitulated the hamster short-period phenotype, whereas deletion of *Ck1e* in mice lengthened the circadian period.[30] More recently, mutations of the *Fxbl3* gene have been shown to lengthen the free-running period.[31,32] Like *Ck1e, Fxbl3* influences circadian period by regulating the posttranslational stability of other clock proteins such as PER and CRY.[33] Of course, in order for the molecular clock to drive circadian rhythmicity in physiology and behavior, clock gene expression must be linked to intracellular signaling pathways regulating neuronal membrane potential, and ultimately, firing rate. Remarkably, recent research demonstrates that ionic events at the cell membrane influence the molecular clock via some of the same intracellular signals that convey clock signals to the membrane, and in some cases, these ionic currents may be necessary for self-sustainment of the molecular clock.[34] Such results—at a minimum—serve to blur the distinction between the core clock mechanisms and the so-called hands of the clock.

In addition to their effects on circadian behavior, some circadian clock gene mutations also affect sleep-wake homeostasis, and several forms of affective behavior, suggesting possible molecular links between the circadian, sleep regulatory, and motivational systems of the brain.[35–37]

FUNCTIONAL ARCHITECTURE OF THE SUPRACHIASMATIC NUCLEUS

Although the SCN was initially characterized as being composed of distinct ventrolateral and