THE MOLECULAR BASIS OF THE CIRCADIAN CLOCK AND WHY WE EXPERIENCE JET LAG

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Nearly all organisms have a circadian clock. Plants and animals need this clock to synchronize their physiology and behavior to the daily light-dark rhythm caused by the rotation of the earth. Although evidence for the existence of clocks dates back a couple of centuries the nature of the circadian clock has long remained elusive. For example, it was once believed that the clock period of circa one day (hence the name ‘circadian’) was imposed by the natural light-dark rhythm. It was later found that the circadian clock operates with high accuracy (the inaccuracy is < 0.5%) even if organisms are kept for weeks under constant darkness; evidently the circadian clock runs autonomously and is self-sustained. Nonetheless, the circadian clock can respond to environmental cues as is evident from its ability to adjust to an altered light-dark cycle. This occurs when one crosses time zones, which initially evokes a “jet-lag” that is eventually compensated as the circadian clock adjusts to the new light/dark schedule.

The circadian clock is a gene-based machinery. Work over the past 15 years has identified the major components (genes and proteins) of this clock. Nearly every tissue in the organism expresses canonical clock genes and rhythmic expression of these genes indicates the presence of multiple peripheral clocks throughout the body. Even cultured cells can be induced to express clock genes and they do so with a 24-hour periodicity. Thus it appears that having a circadian clock is a fundamental property of the majority of cells, similar to metabolism and cell division. Because the circadian clock works as a pacemaker, the underlying genes form a molecular oscillator. Stated in a slightly simplified form this oscillator runs in the following way: Two helix-loop-helix transcription factors (Bmal and Clock) activate the transcription of the Period genes Per1 and Per2. The resulting Per mRNAs are translated in the cytoplasm. As the amount of Per1 and Per2 proteins augments in the cytoplasm in the course of the day, some of Per1 and Per2 molecules are degraded while others are imported into the nucleus where they inhibit (assisted by cryptochromes 1 and 2) the activation function of Bmal and Clock resulting in a shut-down of
Per1 and -2 transcription. This negative feedback persists as long as there is sufficient Per protein in the nucleus. Once this concentration lies below a certain threshold, transcription of Per1 and Per2 is initiated and this starts a new circadian clock cycle.

Per genes were discovered in the mid nineties and they show the above-described circadian expression profile in the suprachiasmatic nucleus (SCN). This nucleus, located in the ventral part of the hypothalamus, is a key neuronal component of the mammalian circadian clock since SCN ablation results in arrhythmicity. The SCN also drives peripheral clocks. One focus of our laboratory concerns the regulation of peripheral clocks. For example, the mammalian forebrain houses such a clock that is involved in the timing of feeding behavior. For the most part canonical clock genes operate this particular clock and our recent work now indicates a role of noradrenalin in regulating the forebrain clock. Noradrenalin is produced by the locus coeruleus (LC) of the midbrain and is then released through nerve fibers projecting into the forebrain area. The chief evidence of the role of noradrenalin in regulating the forebrain clock comes from studies in which the LC was partially ablated in the mouse by genetic means. LC development is regulated by a cascade of transcription factors which is disrupted in Ear2-/- embryos as revealed by a three-fold reduction in the number of LC progenitor cells. In the adult Ear2-deficient mouse the LC is, therefore, greatly diminished in size, especially in regions that send axonal projections to the forebrain. Circadian behavior experiments demonstrate that Ear2 deficient mice adapt less efficiently to daytime feeding schedules.

As noted above, the circadian clock adjusts to changing light conditions. Thus exposing mice to a 15 min light pulse during the early part of the night will delay the circadian clock by as much as 90 min. In part, light responsiveness is mediated by CREB, a transcriptional regulator. However, other factors also play a role. We recently found that mice deficient for protein kinase C alpha (PKCa) show a marked reduction of the light-induced clock delay. We discovered that light induces a rapid phosphorylation of PKCa in the SCN and at a cellular level, it is a controlling factor of the nuclear import of Per2 protein and in this way seems to be a component of the negative feedback loop of the circadian clockwork.

Taken together, a growing body of data including the work summarized above uncovers an intricate network of molecules that constitutes the circadian machine. One can think of this machine as a microprocessor whose primarily role is that of a self-sustaining oscillator. This oscillator controls output signals (e.g. delays or advances the daily behavioral and physiological routine of animals) and is also capable to respond to environmental input (e.g. light pulses). To some degree, the circadian machinery provides a paradigm for other neuronal networks that operate on similar premises.