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Do Biological Clocks Age like Their Owners?

VIJAY KUMAR SHARMA*
Chronobiology Laboratory, Evolutionary and Organismal Biology Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, P.O. Box. 6436, Bangalore 560 064

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Aging of biological clocks that time various biochemical, physiological and behavioural functions in organisms, may be reflected in its modified expression in aged animals compared to the young ones. Traditionally sleep disorders have been considered to be the major symptoms of aging of biological clocks, which in turn cause disruption in other functions such as digestion, mood, fatigue, and decrease in alertness. These disorders are treated using hypnotics, which are well known for their side effects. Although the aging of biological clocks has been studied to some details, our understanding about the mechanism remains in its infancy. It has been reported that circadian rhythms in aged individuals are less precise, shorter in period, smaller in amplitude, slow in resynchronization to shifted light/dark cycle, advanced in phase compared to the younger individuals and that finally, just before death the rhythms disappear altogether. There is hardly any evidence as to whether these overt changes in the biological rhythms are due to impairment of the biological clock itself, and/ or the entrainment pathways, and/or in the pathways downstream to the biological clock. At the molecular level, it is now known that the levels of some clock genes and its products in mice (*mPer1*, *mCry1* and *mClk*) do not change with age. The level of the *Period* gene *mPer2*, however, was demonstrated to change with age. Therefore, the *mPer2* gene and its protein product should be studied in great details both in young and old animals to understand the molecular mechanisms behind aging of biological clocks in mice. Some studies also indicated that the undesirable modifications of biological rhythms at old age might be caused mainly due to modifications of the processes downstream to the central clock mechanisms in the suprachiasmatic nucleus (SCN). In order to decelerate the aging of the biological clocks, several non-pharmacological treatments like administration of bright light of appropriate strength at appropriate phases of the circadian cycle have been recommended in the place of the several hypnotics which are associated with severe side effects.

Key Words: Circadian rhythms, Biological clocks, Aging, Sleep-wake cycle, Melatonin, SCN, Light/dark cycle

Introduction

Aging of biological systems is a continuous process of an organism's life and is not limited to a certain (late) stage (Schriefers 1993). Therefore, studies to understand the aging of biological systems should not be restricted to a particular age. In this review I shall discuss the continuous aging process of those biological systems that time our biochemical, physiological and behavioural processes in a way that they occur precisely at the same time everyday. These time keeping devices are ubiquitous, occurr in all organisms ranging from primitive cyanobacteria to complex human beings and are also referred to as *biological clocks*. The periodicity of such biological clocks under conditions which do not in any way influence the oscillations is called *free-running* or *endogenous period*. The free running periods of the rhythms they control are approximately 24 hr (*circadian*). Although I will focus more on the aging of human biological clocks, its relationship with sleep and its modification during aging, occasionally I shall digress to classical research on aging of biological clocks in other non-human mammals. The motivation behind studying sleep disturbances as an important symptom of aging of biological clocks is a general belief that, aging affects sleep in humans. Sleep in turn affects some of the key physiological functions, like heart rate, immune functions, mood, attention span, and alertness and presents a serious detriment to well

E-mail: vsharma@jncasr.ac.in

being. The phenomenon of sleep has been reported in organisms ranging from fruit flies to human beings and therefore age-related changes would remain a matter of concern for organisms of all orders of complexity.

One may ask whether such symptoms of aging of biological clocks are merely a result of the biological aging process; or whether they are the outcome of the aging of the physiological mechanisms, which times daily events?

Most of the studies on the aging of the biological clocks have been done on mammals and therefore I shall restrict myself to the aging of mammalian biological clocks.

Clock Parameters Reflecting Aging of Biological Clocks

A number of age-related changes in biological clock controlled phenomena, which are described below, are comparable across mammalian species. The first step towards the study of aging of biological clocks is the identification of the symptoms that can reliably reflect its state. It is therefore useful to assay the key parameters of the biological clocks like *amplitude*, *phase*, and *period*. Besides these, another feature, which has helped us in understanding the age-related changes of biological clocks, is its sensitivity to time cues (both biotic and abiotic).

Amplitude

The amplitude of some of the endocrine and neuroendocrine rhythms decreases and some others increases with age (Touitou & Haus 2000). Amplitude of circadian rhythm in hormone levels, in core body temperature, in sleep-wake (SW) cycle and in several other behavioural cycles is reduced progressively with age in humans and other mammals (Baerensprung 1851, Renfrew et al. 1987, Morin 1988, Monk 1989, Richardson 1990, Brock 1991, Touitou & Haus 1992, Satinoff et al. 1993). In rats, amplitude of activity/rest rhythm, body temperature rhythm (Yunis et al. 1974, Halberg et al. 1981, Refinetti et al. 1990, Satinoff 1998, McDonald et al. 1999, Weinert & Waterhouse 1999) and other daily rhythms (Paris & Ramlay 1974, Weinert et al. 1990, Schuh et al. 1993, Cai & Wise 1996a,b) decreased with advancing age. Similar decrease in amplitude of activity rhythm was also reported in hamsters (Penev et al. 1997, Scarbrough et al. 1997, Davis & Viswanathan 1998) and in mice (Weinert & Schuh 1984a,b, Welsh et al. 1986, Valetinuzzi et al. 1997, Weinert & Weinert 1998). However, the rhythms in LH, FSH, insulin and C-peptide exhibited an increase in amplitude (Touitou & Haus 2000). Prinz et al. (2000) demonstrated that cortisol levels in humans also show an increase in amplitude with age. Impairment of sleep is believed to be due to increase in amplitude of cortisol level at a time when the amplitude of cortisol should be minimum. It appears that the opposite changes in endocrine and neuroendocrine systems with age are adaptive or compensatory. Amplitude of human body temperature rhythm was reported to reach its maximum during early childhood and then declined thereafter to about 50% of this value during senescence (Baerensprung 1851). Although decrease in amplitude of circadian rhythms with advancing age is common to rhythms in most of the functions, the age at which the amplitude starts to decrease differs among functions (reviewed by Brock 1991). The amplitude of rectal temperature rhythm in rats starts to decrease only after 300-450 days of age (Refinetti et al., 1990), whereas, the rhythms in brain histamine concentration (Orr & Quay 1977) and CO_2-output (Stupfel et al. 1986) are characterized by a decrease in amplitude at relatively early age. Most striking among these is the decline in the amplitude of plasma melatonin concentration in humans (Reiter 1992, Thomas & Miles 1990), and of pineal function in Syrian hamsters (Reiter 1992). The pineal hormone melatonin is considered as the "key variable" in aging of the biological clock. A decrease in its amplitude due to aging along with the finding that administration of melatonin can increase life expectancy in rats (Dilman et al. 1979) and mice (Pierpaoli & Maestroni 1987) by 20%, supports a general hypothesis that "aging is secondary to pineal failure". Human pineal gland shows age-related alterations in melatonin synthesis and increased pineal calcium deposition (Sack et al. 1986, Skene et al. 1990, Schmid 1993). Similar changes in pineal activity were also reported in aged hamsters and gerbils (Reiter 1992). However,

this hypothesis remains very controversial and is not believed by many people in the field (Reppert & Weaver 1995).

Free-running Period

The free-running period of a biological rhythm is its periodicity under constant conditions, wherein the time cues that can possibly influence the functioning of the animal's biological rhythms are absent. The results of a number of studies in humans (Richter 1967a, Aschoff 1994), in rats (Richter 1967a,b, van Gool et al. 1987, Rietveld et al. 1988, Satinoff et al. 1993, Witting et al. 1994), in hamsters (Pittendrigh & Daan 1974, Pittendrigh & Daan 1976, Morin 1988, 1993, Rosenberg et al. 1991, Aschoff 1994), in squirrel monkeys (Richter 1968) and in deermice (Pittendrigh & Daan 1974) suggests that the period of the circadian clocks decreases in older animals compared to younger animals. This is contrary to the expectations based on observations made on several mammals that the level of activity decreases with advancing age (reviewed by Brock 1991) and therefore the period of activity rhythm should increase with advancing age. According to the hypothesis that the activity exerts feedback on the biological clock (Mrosovsky 1989), an increase in activity should decrease the period and a decrease in activity should increase the period (Daan & Aschoff 1982, Mrosovsky 1989, Turek 1989, Edgar et al. 1991). On the contrary, most of the studies which recorded period of activity rhythm in various organisms for long duration, invariably report either a small but significant decrease in period or no change in period. Period lengthening associated with advancing age was reported only in very few studies. It was reported that in mice (Davis & Menaker 1981, Welsh et al. 1986, Possidente et al. 1995, Mayeda et al. 1997, Valentinuzzi et al. 1997) the period of biological clocks in older animals was shorter than that in younger animals, whereas, no change in clock period was observed in hamsters (Davis & Viswanathan 1998), in mice (Wax 1975) and in humans (Weitzmann et al. 1982, Monk 1989). However, available reports with regards to the age-related changes free-running period of biological clocks are inconsistent in that depending upon the species the period may decrease or increase or remain unchanged in older individuals as compared to the younger ones (Brock 1991). We have carried out long-term experiments with male nocturnal field mice *Mus booduga*. Pregnant females were introduced into constant darkness in the laboratory wherein they delivered on average 6 pups. The pups were kept along with their mothers in constant darkness till they attained an age of about one and half months. These animals were then introduced into activity running wheels and their activity rhythm was monitored until they attained an age of ca. 18 months. The picture, that emerged was that the period of the locomotor activity rhythm in young animals started decreasing drastically until the animals attain an age of about 100 days. Thereafter, the period did not change much with age (Sharma & Chandrashekaran 1998). Davis and Viswanathan (1998) and Czeisler et al. (1999) also reported similar stability in the periodicity of biological clock in adult hamsters and humans respectively.

Sensitivity to Light Stimuli

The sensitivity of the biological clocks is estimated as the change in the phase of the rhythms it controls and is represented as a phase response curve (PRC), a plot of change in phase *vs.* the phase at which stimuli are presented. In mammals the amplitude of light pulse PRC decreases with age. However, there are a few contradictory sets of data claiming an increase in amplitude of PRC with age. In hamsters no difference in PRC amplitude was recorded between the ages of 3 and 12 months (Pohl 1984), but in another study relatively larger amplitude was observed in 'old' animals compared to 'young' ones (Rosenberg et al. 1991, Rappold & Erkert 1994). Some support for an age-related reduction in the PRC amplitude also comes from the observations that entrainment to *Zeitgeber* (periodic time cues of the environment) periods longer than 24 h is lost in old animals as compared to the young ones. This suggests that the sensitivity to light stimuli and therefore the amplitude of PRC could be reduced with age. Entrainment (synchronization) to Zeitgeber was lost faster in old than in young hamsters (Morin 1988). Besides loss of entrainment, a decrease in the amplitude of the PRC both in the delay as well as in the advance

regions were observed in hamsters (Aschoff 1994), rats and mice (Sutin et al. 1993, Zhang et al. 1996, Benloucif et al. 1997). In a separate study, the delay portion of the PRC reduced in amplitude in older hamsters whereas the advance portion remained unchanged (Provencio et al. 1994). The differences in the above mentioned results may be due the lack of any objective definition of "old animal". It may be that the receptors and/or the biological clock's sensitivity to light stimuli starts increasing from 3 months and attains its maximum at an age of 12 months in these rodents. A decrease in sensitivity to light stimuli might be seen only at relatively older ages than those used typically in experiments. In rats, the molecular responsiveness of the biological clock to photic stimulation was found to be reduced in aged rats (Sutin et al. 1993). The response of immediate early genes (IEGs) which are part of the entrainment pathways (Wollnik et al. 1995), was less in the SCN of old animals, which was consistent with the age-dependent decrease in the phase-shifting effect of light pulses.

Phase-relationship with Light/dark Cycle

Besides changes in the amplitude and the period of the expressions of biological clocks, another very important parameter, which gets modified due to aging, is the phase-relationship with the Zeitgeber, more commonly the light/dark cycle. The phase relationship of a biological rhythm with light/dark cycle is defined as the difference in time between a reliable phase of the biological rhythm and "dawn" or "dusk" of the light/dark cycle. The phase-relationship with the Zeitgeber became more advanced with advancing age. Pittendrigh and Daan (1974) reported phase advancement in the activity/rest cycle in older animals of three species of rodents *Peromyscus leucopus, Peromyscus maniculatus,* and *Mus auratus.* The SW cycle (Reilly et al. 1997, Duffy et al. 1998), body temperature rhythm (Monk et al. 1995, Duffy et al. 1998) and various hormonal rhythms (van Cauter et al. 1996) of old human subjects were also phase advanced compared to younger individuals. Such phase advancement in circadian rhythm was also reported in hamsters (Zee et al. 1992, Scarbrough et al. 1997) and in mice (Weinert et al. 1990, Weinert & Weinert 1998). The only exception to this was a study that reported phase delay in activity onset in C57Bl-mice with advancing age (Valentinuzzi et al. 1997). Another interesting aspect of phase advancement of circadian rhythms due to old age is that different circadian rhythms phase advance at different ages. Plasma insulin and corticosterone rhythms for example were already phase advanced in one year old animals, whereas the activity rhythm was phase advanced in mice that were approximately one and half year old, while the body temperature rhythm was almost unchanged (Weinert et al. 1987, Weinert & Schuh 1988). In a separate study a small phase advance of the body temperature rhythm at an earlier age as compared to the phase advancement of activity rhythm was reported in rats, mice (Yunis et al. 1974) and in humans (Duffy et al. 1998).

Loss of Temporal Order

Another important symptom of aging of biological clocks is the loss of temporal order in its behavioural expression. A severely fragmented sleep-wakefulness pattern was reported in elderly people (Sanford 1975, Rabins et al. 1982, Eastman et al. 1984, Lebert et al. 1996). The activity rhythm in rats (Weinert 2000), in hamsters (Penev et al. 1997, Scarbrough et al. 1997, Davis & Viswanathan 1998) and in mice (Weinert & Shuh 1984a, b, Welsh et al. 1986, Velentinuzzi et al. 1997, Weinert & Weinert 1998) not only damped but also became fragmented with advancing age. It is believed that one of the most important purposes of biological clocks is to maintain internal temporal order (Pittendrigh 1960, 1961, Aschoff 1979, Moore-Ede & Sulzman 1981). Temporal order is provided by both, the entraining signals from the zeitgebers and the coupling among various oscillators generating circadian rhythms (Pittendrigh 1974). Therefore, the loss of temporal order as reflected by fragmented rhythmicity, can be interpreted as a loss of coordination among many interdependent oscillating systems, which are responsible for the generation of circadian rhythmicity (Samis 1969). When circadian rhythm disappears (for reasons mostly unknown), ultradian rhythmicity remains or may even become more strongly expressed (Albers et al. 1981, Gerkema & Daan 1985, Honma & Honma 1985), a sequence of events which might also be characteristic for aging (Aschoff 1992).

More convincing evidence of an age-related circadian disorder is generally observed in continuous light. The circadian activity rhythms underwent splitting in constant light more frequently in old hamsters than in young ones (Morin 1988). Another interesting characteristic of aging of biological clock is its stability, which may be related to decrease in amplitude of circadian rhythms and therefore can also be thought to stem from a loss of temporal order. It is generally observed that the day-to-day variability of circadian rhythms increases with age (Wever 1984, Aschoff 1994).

Internal Desynchronization

With advancing age, the biological clocks of humans and other mammals are progressively disturbed. Rhythmicity in various biochemical, physiological and behavioural factors which under normal conditions are coupled to each other and maintain a stable phase relationship, internally desynchronize after which they free-run with different periodicities (Monk 1989, Richardson 1990, Brock 1991, Touitou & Haus 1992). In humans maintained in isolation, the sleep-wake cycles and body temperature rhythms free-run with different periodicities resulting in desynchronization (Aschoff et al. 1967). Such internal desynchronization was more common in elderly people than in youth (Wever 1979, Zulley 1992).

Rate of Resynchronization after Shift in Light/dark Cycle

If a decrease in amplitude of circadian rhythms in various physiological functions reflects a loss in "stability" of the biological clocks driving it, then one should expect an increase in rate of resynchronization following phase shift in light/dark cycle in older organisms (Aschoff 1994). Such a hypothesis is based on the assumption that the sensitivity of receptors to zeitgeber signal and response of the biological clock do not change with age, which, however, has been rejected by the results of various experiments. The rate of resynchronization of circadian rhythms following a shift in the light/dark cycle was slower in old rats (Quay 1972, Buresova et al. 1990), old mice (Schuh et al. 1991, Weinert et al. 1995, Weinert et al. 1997) and aged humans (Reilly et al. 1997). The rate of resynchronization also depends upon the direction and duration of phase shift. In hamsters, the time needed for resynchronization after an 8-h phase advance was shorter in old than in young animals, while the reverse was true for 8-h phase delay (Zee et al. 1992). The rate of resynchronization to phase advances was faster and to phase delays was slower in old animals (Rappold & Erkert 1994).

Neurobiology of Biological Clocks and Consequences of Aging

The mammalian biological clock, SCN is a small collection of neuronal cells in the basal part of the anterior hypothalamus just dorsal to the optic chiasma on either side of the third ventricle. Under normal conditions these neurons behave like a collection of mutually synchronized pacemaker cells (Miller 1998). Labeling the SCN immunocytochemically with antibodies against neuropeptides such as vasopressin (VP), vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), or neurotensin, can help recognize the SCN neurons (Mai et al. 1991, Moore 1992, Reuss 1996, Swaab et al. 1996). Photic information may have a synchronizing effect on the clock mechanism of the SCN by inducing changes in the functional activity of certain groups of neurons. Some of these cell groups exhibit distinct changes in peptide content related to circadian time or photoperiod. Vasopressin, for example, one of the most abundant peptides in the SCN, exhibits circadian rhythm under light/dark conditions and in continuous darkness (Södersten et al. 1985, Yamase et al. 1991, Tominaga et al. 1992), indicating that biosynthesis of this neuropeptide is under the control of the biological clock. This is also suggested by findings that the VP gene expression and the vasopressin peptide levels in the SCN are increased during daytime *in vivo* and *in vitro* (Gillette 1991, Inouye & Shibata 1994, Shinohara et al. 1994). In contrast, VIP neurons located mainly in the ventral part of the SCN (Mai et al. 1991, Moore 1992, Mikkelson et al. 1994, Hofman et al. 1996), respond to photic stimuli but do not show an endogenous circadian rhythmicity under contact light (Morin et al. 1991, Yang et al. 1993, Duncan et al. 1995). Receptors for photic information in mammals lie

only in the retina (Morin 1994). The biological clock of mammals is synchronized to the environmental light/dark cycle by photic information from the retina to the SCN via direct neural pathways, the retino-hypothalamic tract (RHT) and via two indirect projections which pass the intergeniculate leaflet (IGL), the geniculo-hypothalamic tract (GHT) and pretecto-hypothalamic tract (PHT) (Rusak 1992, Rea 1998, Card & Moore 1991, Morin 1994, Meijer et al. 1996). Several afferent fibers predominantly terminate in the ventral part of the SCN, where they make synaptic contacts with VIP neurons (Morin 1994, Reuss 1996). In addition, efferent projections from the SCN to various areas of the hypothalamus and to other central brain structures control several body functions and are part of the circadian timing system (LeSauter & Silver 1998). The VIP subdivision of the SCN is therefore thought to play a prominent role in the mediation of photic information to the circadian timing system.

Circadian rhythmicity in mammals is produced by core pacemaker (biological clock) located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Weaver 1998). The aging biological clocks in mammals are generally characterized by decrease in neuronal activity in the SCN (Swaab et al. 1985, 1992). Changes in the SCN and other parts of the circadian timing system is believed to underlie some of the sleep disturbances among elderly people (van Someren et al. 1993, van Someren 1997), a symptom common in sleep disturbed human subjects. Increased stimulation of the brain can improve or even restore some of neuronal inactivity which is caused by aging of the SCN (Swaab 1991).

Age-related deterioration in the circadian timing system in human beings (Vitiello & Prinz 1990, Czeisler et al. 1992) coincided with neuronal degeneration of the SCN in senescence (Zhou et al. 1995, Hofman et al. 1996, Hofman 1997). The SCN of patients with Alzheimer's disease, who frequently suffer from sleep disruption, show dramatic loss of VP and neurotensin neurons. The number of VP-immunoreactive neurons in the human SCN exhibits a marked diurnal oscillation in young people (< 50 years of age), but not in elderly people (>50 years) (Hofman & Swaab 1994). It might be that the absence of a distinct diurnal rhythm in the VIP-expressing neurons in the human SCN, which might be due to the artificial lighting conditions to which most terminal patients are exposed at the end of their lives. It could also be that the sensitivity of the VIP neurons was too low to be able to respond adequately to the environmental illumination. Although low light intensities (< 500 lux) have been reported to have a synchronizing effect on the SCN (Laakso et al. 1993, Boivin et al. 1996), other studies have demonstrated that higher light intensities (>1000 lux) are required to produce a direct biological effect on the human circadian pacemaker (Brainard et al. 1988, Wever 1994, Czeisler 1995, van Someren et al. 1996). Gradual age-related deterioration of the SCN itself (Swaab et al., 1985; Hofman et al. 1996, Hofman 1997) and other parts of the circadian timing system, including the retina and optic nerves, may lead to the reported disruptions of circadian rhythms in elderly people. Different organs produce their own intrinsic rhythms, and the SCN is believed to control or synchronize them. When organisms age, each cell may have reduced ability to be rhythmic and the ability of SCN to transmit timing information may also deteriorate (Krajnak et al. 1998). The gradual deterioration of the SCN might cause damping of circadian amplitude, which is then expressed as an altered circadian rhythm. This has been concluded from the results of experiments conducted to study rhythms in glucose utilization (Wise et al. 1988) and the level of VIP mRNA (Kawakami et al. 1997) and of neuronal firing rate in rats (Satinoff et al. 1993) and hamsters (Watanabe et al. 1995). The question then arises as to whether a single neuron in the SCN affects these changes in generation of circadian rhythm? It can also be that a comparatively less number of normal neurons and/or a weaker coupling between them results in decreased amplitude of circadian rhythm. It was established using SCN cell lesion that the volume of neurons in the SCN is directly proportional to the circadian period of locomotor activity rhythm in the hamsters (Davis & Gorski 1984). These partially SCN lesioned animals show a pronounced reduction in the amplitude and phase advancement of daily activity, drinking and eating rhythms (Davis & Gorski 1984, Pickard & Turek 1985). However, Edgar et al. (1993)

reported that less than 10% of the normal number of cells are sufficient to maintain circadian rhythms, even though the amplitude of rhythm decreased in old animals. The total number of neurons in the SCN of rats does not change with age (Roozendaal et al. 1987, Madeira et al. 1995). Therefore it is less likely that the disruption in expression of biological clocks in mammals is due to reduction in the number of cells in the SCN. It is also known that the mammalian SCN is composed of three different types of neurons: neurons expressing vasopressin (VP), vasoactive intestinal polypeptide (VIP), neuropeptide-Y (NPY), or neorotensin, (Mai et al. 1991, Moore 1992, Reuss 1996, Swaab et al. 1996). Although the total number of neurons in rat SCN did not change with age, the number of neurons expressing the neuropeptides arginine vasopressine (AVP), and VIP, which is believed to play an important role in time keeping mechanism decreased in old rats (Roozendaal et al. 1987, Chee et al. 1988, Lucassen et al. 1995, Li & Satinoff 1998) and in humans (Swaab & Hofman 1994, Hofman et al. 1996). Another study reported a decrease in the number of AVP-immunopositive cells, in aging common voles that coincided with a loss of precision of the circadian locomotor activity rhythm (van der Zee et al. 1999).

That disturbances in circadian rhythms in old organisms are due, at least in part, to aging of SCN, is also demonstrated by experiments with fetal SCN grafts. Transplants into intact old rats restored circadian rhythms of body temperature, locomotor activity, and drinking (Li & Satinoff 1998) and the rhythm in hypothalamic corticotrophin releasing hormone (CRH) and POMC mRNA (Cai et al. 1997). In another elegant experiment, fetal SCN grafts were transplanted into young and old SCN-lesioned hamsters. The free-running period of activity/rest cycle was similar both in young and old animal hosts; before SCN ablation, the free-running period was shorter in older hamsters (Viswanathan & Davis 1995). When grafts were allowed to age, the mean free-running period becomes shorter, as in intact, aging hamsters. Therefore the aging of biological clocks of mammals appears to be independent of the typical aging process and therefore chances of reversal to their normal functioning becomes greater.

Molecular Biology of Biological Clocks and Markers of their Aging

The recent discovery that several mammalian genes encode the inter-linked core elements of the biological clock (van der Horst et al. 1999, Shearman et al. 2000), provides a new dimension to the investigation of the aging of mammalian circadian pacemaker and towards the understanding of the underlying mechanism behind it. Mammalian SCN oscillators are composed of interlocked feedback loops involving both positive and negative elements (Shearman et al. 2000). The mouse *Period* genes (*mPer1, mPer2, and mPer3*) are structural homologues of the *Drosophila period* gene (*Per*), and all three showed a robust rhythmic expression in the SCN, the mammalian biological clock. The *mPers* peak during the subjective day in constant darkness. *mPer1* and *mPer2* oscillate with very high amplitude of circadian expression (the mRNA peak/trough ratio of more than 5). The amplitude of these two *Period* genes is much higher than expression of other putative clock genes including *mClk*, *Bmal1*, *mTim*, *mCry1*, and *mCry2*. The *clock* gene in mice (*mClk*), which encodes a DNA-binding protein mCLK, is believed to be directly regulating transcription of mouse *Period (mPers)* and *Timeless (mTim)* genes. The mouse *cryptochrome* (*mCry1*, and *mCry2)* genes are also believed to be the components of the clock mechanism, because *Cry* deficient mice do not exhibit circadian rhythms of locomotor activity in constant darkness (van der Horst et al. 1999, Vitaterna et al. 1999). The mPER3 is believed to be acting as a dimerizing partner of mPER1 and mPER2, which helps them to enter the nucleus. *mPer1* and *mPer2* are rapidly induced by light in a time-dependent manner. The amplitude of induced *mPer1* was correlated with the phase resetting of the overt locomotor activity rhythm. Further, the two *mPer* genes coexpress in a single SCN cells. Therefore, it appears that the oscillation in the levels of *mPer1* and/or *mPer2* constitute the central pacemaker. The circadian oscillators of mice are believed to be composed of three interacting molecular loops:

(i) *mCry* genes and their proteins comprise one loop which is a true auto regulatory negative feedback loop, in which the protein products feedback to turn-off their own transcription.
(ii) The second loop is that manifested by three *mPer* genes and some clock controlled genes (CCGs). This loop is driven by the same positive elements (CLOCK: BMAL1) as the *mCry* loop, but transcription is not turned-off by the respective gene products. Instead the protein mCRY acts as its negative regulator, leaving the protein products of *mPer* for other actions. Thus mPER2 positively drives transcription of the *Bmal1* gene, mPER1 may function to stabilize protein components of the loop and CCG products functions as output signals. CRY plays an important role to stabilize mPER2.
(iii) The rhythmic regulation of *Bmal1* is the third loop, which is controlled by the cycling presence and absence of a positive element dependent on the protein mPER.

In addition, rhythmic activation of CCGs such as pre-pro-AVP is thought to transduce the clock information downstream. The comparison of the expression of the gene expression of these clock genes and the clock controlled genes in the SCN of both young and old animals could help identify the molecular mechanisms behind the aging of the mammalian biological clocks. In an elegant study targeted to understand the molecular mechanism of aging of the biological clock in mice, Weinert et al. (2001) compared the expression of clock genes in the SCN of young and aged mice at two phases of the animal's circadian cycle, CT7 (circadian time 7, i.e. 5 hours before the onset of activity of a nocturnal animal) and at CT21. These two phases (CT7 and CT21) are the phases of expected maxima and minima of the *mPer* mRNA expression in mice. In the SCN of young animals the *mPer1* and *mPer2* expressions were rhythmic as expected, with a high level at CT7 and low at CT21. On the other hand, the expression of *mClk* in the SCN did not show any circadian oscillation. The level of *mPer1*, *mCry1* and *mClk* did not change with age. However, the level of *mPer2* was demonstrated to change with age.

An important point, which emerges from this study, is that the SCN of the mice continues to show circadian oscillation at the molecular level till a very old age, which to certain extent is consistent with the observation, made at the overt level. It therefore appears that the decrease in amplitude of circadian rhythms at old age is caused mainly because of the processes downstream to the molecular mechanisms in the SCN (Weinert et al. 2001). The basis for such hypothesis was that old animals, despite their changes in activity rhythm, exhibit a body temperature rhythm as pronounced as that in young adult animals (Weinert & Waterhouse 1999). In a separate experiment the rhythmic expression of *mPer2* in the mice SCN was affected due to age (Albrecht et al. 1997, Zylka et al. 1998). However, such changes may be due to a change in phase of the molecular *mPer2* oscillation and therefore CT7 and CT21 no more represent the maxima and minima of the expression. In order to unequivocally establish age-related changes of the biological clocks the gene *mPer2* should be studied extensively. The mRNA and the protein products of this gene should be sampled and compared at several phases both in old and young animals.

Treatments to Reverse Aging of Biological Clocks

Most researchers generally use core body temperature rhythm as a marker of the phase and amplitude of biological clocks. It was also argued that the neuronal activity of sleep-related structures in the brain are sensitive to the circadian variation in body temperature (van Someren 2000). Core body temperature rhythm is believed to result from the interaction of rhythm in heat production and that of heat loss. Heat loss is accompanied by increased blood flow to the peripheral tissues, which in turn increases skin temperature. Increased blood flow and consequently skin temperature accomplish heat loss. Discerning the two out-of-phase rhythms of brain and skin temperature and their individual impacts on arousal-related structures in the brain appears useful for understanding the relation between temperature and sleep. In a model of this relation, the age-related changes in thermoregulatory capacities accurately predict age-related changes in sleep. The model also

shows how appropriately timed manipulations like passive heating, bright light, exercise, and melatonin effectuate changes in the nocturnal temperature profiles that have a sleep-improving effect (van Someren, 2000). Traditionally sleep disorders, which in turn cause disruption in other functions such as digestion, mood, fatigue, and alertness are treated using hypnotics, which are well known for their side effects. Improvement of the SW rhythm of elderly people has been demonstrated by the application of a variety of potent modulators of the circadian timing system like bright light (Campbell et al. 1993, Murphy & Campbell 1996, Murphy & Campbell 2001). Contrary to treatment with hypnotics, the improvement of sleep following these treatments is without adverse effects and even results in improvement of mood, performance, daytime energy, and general quality of life (Hanger et al. 1992, Sörensen & Brunnström 1995, Murphy & Campbell 1996, Wilkinson et al. 1997). The pineal hormone melatonin has also been used to treat impairment in SW rhythms in elderly people (Garfinkel et al., 1995, Haimov et al. 1995, Wurtman & Zhdanova 1995, van Someren et al. 1997). Although the treatment of sleep related problems by light has proven to be a great success, the same cannot be said about melatonin, because the efficacy and safety of melatonin were not yet confirmed in a large-scale clinical trials.

The overt SW rhythms (Witting et al. 1993) and the rhythm in cell function in the SCN (Lucassen et al. 1995) in aged rats can be restored by enhancing the stimuli the circadian timing system normally uses for synchronization of the SW cycle.

Conclusions

Aging of biological clocks that time various biochemical, physiological and behavioural functions of mammals can be reflected through its modified expression in aged animals compared to the young ones. We do know now that the circadian rhythms in older individuals are less precise, shorter in period, smaller in amplitude, slow in resynchronization to shifted light/dark cycle, advanced in phase compared to the younger individuals and that finally just before death the rhythms disappear altogether. Although, the expressions of some clock genes (*mPer1*, *mCry1* and *mClk)* and its products did not show any change with age, the level of another gene *mPer2* changed significantly with age. However, it is too early to conclude that the aging of the biological clocks in mammals is due to the changes in the level of mRNA and the protein product of *mPer2*. In order to unequivocally establish age-related changes in the expression of *mPer2*, the mRNA and its protein product should be sampled and compared at several phases both in old and young animals. Moreover, some studies indicate that the changes in the expressions of the biological clocks in mammals may not be due to modification of molecular mechanisms in the SCN, but downstream to it. It is also believed that the aging of biological clocks is independent of the classical process of aging of biological systems, which gives some ray of hope that these age-related changes in the biological clocks can be reversed to normal functioning. In order to reverse the aging of the biological clocks, several non-pharmacological treatments like administration of bright light of appropriate strength at appropriate phases of the circadian cycle can also be recommended instead of the several hypnotics which are associated with severe side-effects.

Rhythms in a number of biochemical, physiological and behavioural processes in a varied range of organisms, should be studied simultaneously both in presence and absence of environmental periodicities to understand the process of aging of biological clocks in living organisms. In mammals, long term study should be taken up to study the aging of the entrainment pathways, the SCN, and the output pathways from the SCN. Activities of the single neurons of the SCN should also be recorded in younger and older animals to understand whether aging of the mammalian biological clock (SCN) is due to the aging of the individual SCN neurons or due to the lack of synchronization between them.

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