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Review

Synchronization of the Molecular Clockwork by Light- and Food-Related Cues in Mammals

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The molecular clockwork in mammals involves various clock genes with specific temporal expression patterns. Synchronization of the master circadian clock located in the suprachiasmatic nucleus (SCN) is accomplished mainly *via* daily resetting of the phase of the clock by light stimuli. Phase shifting responses to light are correlated with induction of *Per1, Per2* and *Dec1* expression and a possible reduction of *Cry2* expression within SCN cells. The timing of peripheral oscillators is controlled by the SCN when food is available *ad libitum*. Time of feeding, as modulated by temporal restricted feeding, is a potent '*Zeitgeber*' (synchronizer) for peripheral oscillators with only weak synchronizing influence on the SCN clockwork. When restricted feeding is coupled with caloric restriction, however, timing of clock gene expression is altered within the SCN, indicating that the SCN function is sensitive to metabolic cues. The components of the circadian timing system can be differentially synchronized according to distinct, sometimes conflicting, temporal (time of light exposure and feeding) and homeostatic (metabolic) cues.

Key words: Circadian clock / Clock gene / Entrainment / Food restriction / Suprachiasmatic nucleus.

Introduction: Hierarchical Organization of the Circadian Timing System

Circadian rhythms are behavioral and physiological endogenous rhythms driven by an internal timing system with a period close to, but generally not equal to, 24 hours. The circadian timing system in mammals is thought to be organized in a hierarchical way. The master clock is located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Takahashi *et al.*, 2001). Because single neurons of the SCN are able to generate and maintain self-sustained circadian oscillations, they can be defined as circadian pacemakers or clock cells (Welsh *et al.*, 1995). The retina is also capable of maintaining self-sustained oscillations *in vitro* (Tosini and Menaker, 1996). The retinal clock may gate photic inputs and modulate the SCN clock (Yamazaki *et al.*, 2002). Indirect evidence also suggests the existence of a food-entrainable clock outside the SCN (Mistlberger, 1994; Stephan, 2001). The location of this clock within the body is still a matter of debate, and knowledge of the mechanisms underlying food-entrainable oscillations is very limited. Apart from the main SCN clock and retina, there are also circadian oscillators in other brain regions and peripheral organs. In contrast to the SCN and retina, peripheral oscillators show rapid dampening in oscillations when isolated *in vitro* (Yamazaki *et al.*, 2000). Moreover, circadian rhythms of peripheral organs are no longer detectable in SCN-lesioned animals (Sakamoto *et al.*, 1998). Therefore, the SCN can be viewed as a master clock that controls rhythmicity of slave brain and peripheral oscillators (Yamazaki *et al.*, 2000), the latter being supposed to play a role in local timing of physiological and biochemical processes (Balsalobre, 2002).

Regulation of Molecular Oscillations in the SCN Clock

The molecular core of the circadian clock located in the SCN is considered to involve self-sustaining transcriptional/translational feedback loops based on rhythmic expression in the mRNA and proteins of clock components (Reviewed in Okamura *et al.*, 2002; Reppert and Weaver, 2002; Figure 1). Three mammalian orthologs of the *Drosophila Period* (*i.e.*, *Per1, Per2* and *Per3*) containing a Period-Arnt-Single-minded (PAS) domain important for protein-protein interactions have been cloned; their mRNA oscillate with peak levels during the (subjective) day (Albrecht *et al.*, 1997; Shearman *et al.*, 1997; Sun *et al.*, 1997; Tei *et al.*, 1997; Zylka *et al.*, 1998). Heterodimers of two basic helix-loop-helix (bHLH)-PAS transcription factors, CLOCK and BMAL1 (also known as MOP3), activate the transcription by binding E-boxes in the promoter region of *Per1* and *Per2* (Yamaguchi *et al.*, 2000; Travnickova-Bendova *et al.*, 2002). While *Clock* mRNA levels do not markedly oscillate in the SCN neurons (Shearman *et al.*, 2000), *Bmal1* mRNA levels show daily variations with nocturnal peak and daytime trough (Abe *et al.*, 1998). The CLOCK/BMAL1 heterodimers also

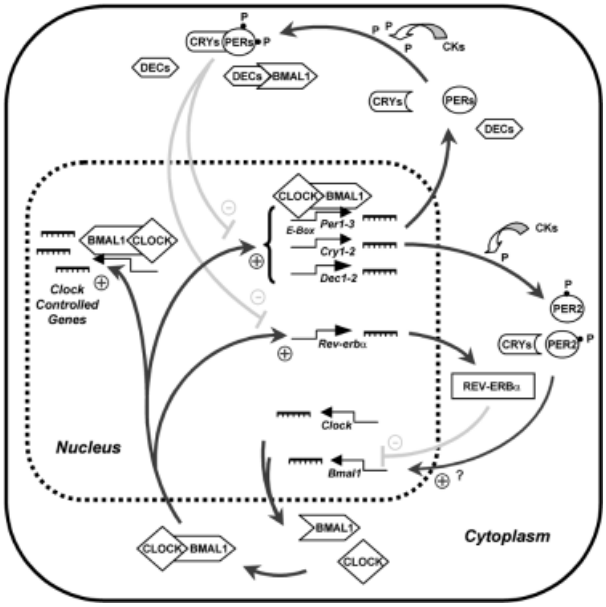

Fig. 1 Model of Core Feedback Loops in the Mammalian Circadian Clock.
For details, see text. BMAL1, brain and muscle Arnt-like protein 1; CK, casein kinase; CRY, cryptochrome protein; DEC, differentiated embryo chondrocyte protein; P, phosphorylation; PER, period protein; REV-ERBα, reverse viral erythroblastis oncogene product, an orphan nuclear receptor encoded by the opposite strand of the α-thyroid hormone receptor gene.

drive the transcription of two *Cryptochrome* (*i.e.*, *Cry1* and *Cry2*) genes and the orphan nuclear receptor *Rev-Erbα* (Okamura *et al.*, 2002; Preitner *et al.*, 2002; Reppert and Weaver 2002). In turn, REV-ERBα inhibits *Bmal1* transcription. Several proteins including CRY1 and CRY2 as well as DEC1 and DEC2, two bHLH transcription factors, inhibit the rhythmic transcription of *Per*, *Cry* and *Rev-Erbα* through binding to CLOCK/BMAL1 heterodimers and/or competition for E-boxes (Kume *et al.*, 1999; Honma *et al.*, 2002; Preitner *et al.*, 2002). PER2 has been suggested to activate *Bmal1* transcription (Shearman *et al.*, 2000). Casein kinases Iε and Iδ play a post-translational role in the clock mechanism by phosphorylating PER proteins, thereby regulating their nuclear translocation (Lowrey *et al.*, 2000; Akashi *et al.*, 2002; Figure 1).

A topographic compartmentalization of *Per* expression during the daily cycle has been shown in the SCN of rats. During the (subjective) day, both *Per1* and *Per2* are markedly expressed in the dorsomedial part of the SCN, a region containing AVP neurons, while only low levels of *Per* mRNA are detected in the ventrolateral part of the SCN in which VIP and GRP neurons are found (Dardente *et al.*, 2002; Yan and Okamura, 2002). Also, high levels of *Bmal1* mRNA are found in the AVP region of the SCN (Hamada *et al.*, 2001). These data add a degree of complexity in the functional organization of the SCN, which can be viewed as a heterogenous structure in terms of clock gene regulation.

Regulation of Molecular Oscillations in Extra-SCN Brain and Peripheral Oscillators

Characterization of clock components in the SCN has led to the unexpected finding that clock genes are also expressed in other brain regions, like cerebral cortex and hippocampus (Shearman *et al.*, 1997; Abe *et al.*, 1998; Wakamatsu *et al.*, 2001), and various peripheral organs including liver, heart, lung and muscle (Albrecht *et al.*, 1997; King *et al.*, 1997; Shearman *et al.*, 1997; Sun *et al.*, 1997; Tei *et al.*, 1997; Sakamoto *et al.*, 1998; Lee *et al.*, 2001). Moreover, mRNA and protein products of clock components in peripheral organs were found not only to be present, but also to show circadian expression (Sakamoto *et al.*, 1998; Zylka *et al.*, 1998; Yamazaki *et al.*, 2000; Lee *et al.*, 2001). Furthermore, even fibroblasts cultured *in vitro* can produce circadian oscillations of clock components in response to a serum shock (Balsalobre *et al.*, 1998; Yagita *et al.*, 2001).

The oscillations of clock genes in extra-SCN brain and peripheral cells and their reciprocal phase relationships close to those in the SCN cells suggest that they function as circadian oscillators (Balsalobre, 2002). Compared to the SCN clockwork, only few differences have been observed. For example, daily expression of *Clock* may cycle in the liver (Lee *et al.*, 2001) while it seems to remain constant throughout the day in the SCN (Shearman *et al.*, 2000). Also, PER1 has been proposed to play a specific role in peripheral oscillators because a targeted mutation in *Per1* induces a delay in clock gene expression of peripheral tissues only (Cermakian *et al.*, 2001; Pando *et al.*, 2002). NPAS2 (also known as MPO4) is a bHLH-PAS transcription factor sharing high homology with CLOCK (King *et al.*, 1997; Reick *et al.*, 2001). In contrast to *Clock*, *Npas2* is not expressed at detectable levels in the SCN (Shearman *et al.*, 1999), but is present in several regions of the forebrain, like cerebral cortex and hippocampus (Reick *et al.*, 2001). Because NPAS2 can dimerize with BMAL1 to activate rhythmic transcription of *Per* and *Cry*, NPAS2 may be a specific component of the forebrain oscillators (Reick *et al.*, 2001).

The timing of clock gene oscillations appears to be similar between various peripheral organs like liver, heart and kidney (Damiola *et al.*, 2000; Balsalobre, 2002). In comparison to what occurs in the SCN, however, there is a 6–8 h delay in the temporal pattern of clock gene expression within peripheral oscillators (Damiola *et al.*, 2000). In the cerebral cortex of mice fed *ad libitum*, the daily peaks of oscillations of *Per1* and *Per2* are delayed by 7 and 2 h, respectively, as compared to those observed in the SCN (Wakamatsu *et al.*, 2001). The delay between the central SCN clock and extra-SCN brain regions or peripheral tissues may be related to the lag necessary for nervous (Buijs and Kalsbeek, 2001) and/or humoral signals (*e.g.*, McNamara *et al.*, 2001) to reach and reset peripheral timing from time giving cues provided by the SCN. As a whole the SCN and extra-SCN oscillations

are not identical, although they share clear similitude. The critical reason why the molecular clockwork in the SCN cells is the only one capable of generating self-sustained oscillations is still unknown.

Besides endogenous circadian oscillations, another critical feature of the circadian timing system is its ability to be reset by external cues, which allows the organisms to be in phase with the environmental variations they are exposed to. The aim of this article is to review the ways by which light and food availability affect the phase of the molecular clockwork in the SCN and peripheral tissues.

Synchronizing Effects of Light

Phase Response Curve to Light

The major environmental synchronizer (*Zeitgeber*) of the SCN clock is the light-dark cycle. Photic synchronization is achieved by a daily resetting of the phase and period of the master circadian clock. Photic resetting depends on the time of the daily cycle when light is perceived by the retina. The phase response curve to light in mammals is characterized by phase delays during the early subjective night and phase advances during the late subjective night, whereas light has little or no phase-resetting effect during most of the subjective day (Daan and Pittendrigh, 1976). Under laboratory conditions, photic synchronization can be obtained by daily light pulses applied at proper circadian times depending on the endogenous period. Daily light exposure applied in the late subjective day/early subjective night is necessary to synchronize individuals with an endogenous period lower that 24 h, while those with a period longer than 24 h need to be phase advanced by light in the late night/early subjective day in order to be synchronized to 24 h.

Photoreceptors of the Circadian System

In contrast to the visual image system which is well known to be mediated by rod and cone photopigments, the putative photoreceptors relevant for the circadian timing system are still a matter of debate, although they are most likely located in the retina (*e.g.*, Wee *et al.*, 2002). The retinal ganglion cells innervating the SCN express melanopsin and are photosensitive (Hattar *et al.*, 2002). Recent studies using mice knocked out in different photopigments raise the possibility that not only melanopsin, but also rod and cone opsins, and possibly cryptochromes contribute to some extent to photic synchronization of the circadian system, masking, and nonvisual irradiance detection (Thresher *et al.*, 1998; Okamura *et al.*, 1999; Bellingham and Foster, 2002; Panda *et al.*, 2002; Ruby *et al.*, 2002; Van Gelder *et al.*, 2003). The fact that none of the putative circadian photopigments is indispensable suggests the existence of redundant photic inputs to the SCN clock.

Effects of Light on the SCN Clock through Per1 and Per2 Induction

Synchronization of the SCN clock to photic cues has been associated with transcriptional mechanisms involving induction and inhibition of clock gene expression (Figure 2). Light pulses applied during the night lead to increased levels of *Per1* and *Per2* in the SCN (Albrecht *et al.*, 1997; Shearman *et al.*, 1997; Shigeyoshi *et al.*, 1997). Components of the signaling pathway involved in *Per1* induction are the following: nocturnal light exposure induces release of glutamate from the retinohypothalamic terminals, activation of *N*-methyl-D-aspartate receptors, and calcium influx within the SCN cells (Gillette and Mitchell, 2002). Light-induced increase in intracellular calcium is considered to trigger rapid phosphorylation of cAMP-responsive element binding protein (CREB) which, in turn, binds to cAMP-responsive elements (CRE; Ginty *et al.*, 1993) located in the promoter of *Per1* upstream of the E-boxes (Gau *et al.*, 2002; Travnickova-Bendova *et al.*, 2002; Tischkau *et al.*, 2003). Therefore, the light-induced increase of *Per1* mRNA differs from the circadian CLOCK/BMAL1-induced activation of *Per1* transcription which is, as noted above, mediated *via* E-boxes (Travnickova-Bendova *et al.*, 2002).

An acute light-induced response of *Per1* occurs in the early and late night, while *Per2* expression is only strongly stimulated by light in the early night (Figure 2). This pattern in the SCN is observed not only in nocturnal mammals, like mice and rats (Albrecht *et al.*, 1997; Shearman *et al.*, 1997; Shigeyoshi *et al.*, 1997; Yan *et al.*, 1999; Miyake *et al.*, 2000), but also in a diurnal species, *Arvicanthis ansorgei* (Caldelas *et al.*, 2003). In addition to the temporal difference between *Per1* and *Per2* expression in response to nocturnal light pulses, there is also a spatial difference in photic induction of *Per1* and *Per2* in the SCN. In the early night (*i.e.*, in the phase-delaying portion of the phase response curve to light), a light pulse leads to increased levels of *Per1* mRNA only in the VIP- and GRP-containing region while levels of *Per2 m*RNA are increased in both VIP/GRP and AVP-containing regions in rats (Dardente *et al.*, 2002; Yan and Okamura, 2002) and mice (Yan and Silver, 2002). In the late night (*i.e.*, in the phase advancing portion of the phase response curve to light), a light pulse does not activate *Per2* expression, but produces an increase of *Per1* mRNA levels in both ventrolateral and dorsomedial parts in the SCN of mice (Yan

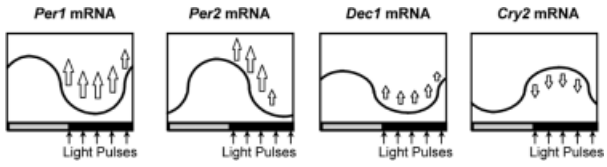

Fig. 2 Schematic Representation of the Changes (White Arrows) Induced by Nocturnal Light Pulses (Black Arrows) on *Per1*, *Per2, Dec1* and *Cry2* mRNA Levels (Curves) in the Suprachiasmatic Nuclei of Rodents.

and Silver, 2002). These differential patterns of expression point out a functional, yet unexplained, compartmentalization in photic phase resetting.

Phase shifting responses to light of the locomotor activity rhythm are correlated with induction of *Per1* during the night (Shigeyoshi *et al.*, 1997). Intracerebroventricular injections of antisense oligodeoxynucleotides to *Per1* inhibit light-induced phase delays in mice (Akiyama *et al.*, 1999). Mice with a mutated *Per1* gene exhibit altered light-induced phase advances, while *Per2* mutant mice display impaired light-induced phase delays (Albrecht *et al.*, 2001). Moreover, the investigation of sustained photic stimulation in the mouse SCN at the protein level gives support to the involvement of PER1 and PER2 in photic synchronization (Field *et al.*, 2000). These findings, therefore, indicate the likely participation of *Per1* and *Per2* in light-induced phase shifting. A few results, however, raise the possibility that *Per1* and *Per2* do not play a crucial role in photic phase resetting. Targeted disruption of *Per1* has been found to keep light-induced phase shifts (both delays and advances) normal (Cermakian *et al.*, 2001). Also, in mice lacking pituitary adenylate cyclase activating polypeptide type 1 receptor, light-induced phase delays are larger than those of wild-type mice whereas light induction of *Per1* and *Per2* is markedly reduced in the mutant SCN (Hannibal *et al.*, 2001).

In view of the lack of phase resetting effect of light during the (subjective) day in nocturnal rodents, photic activation of retinal cells is commonly supposed not to affect the circadian clockwork during that period. However, experiments performed in our laboratory indicate that prolonged light exposure during the subjective day increases *Per1* and *Per2* mRNA levels in the mouse SCN, as shown by a higher amplitude of the daily variations of *Per* in mice exposed to a light-dark cycle compared to animals maintained only for one cycle in constant darkness or, to a lesser extent, to individuals synchronized to a skeleton photoperiod (*i.e.*, with daily dawn and dusk 1-h light pulses). Thus, these data suggest that the presence of light during the daytime can potentiate the amplitude of the circadian oscillations within the SCN (Challet *et al.*, 2003). A similar effect has been observed in rats which show a higher amplitude of *Per2* oscillation in the SCN when kept under a light-dark cycle compared to those housed in constant darkness (Yan *et al.*, 1999). It remains to be determined if other clock components mediate or influence these light responses during the day.

Effects of Light on the SCN Clock through *Bmal1* Modulation

Bmal1 is another clock gene possibly involved in photic resetting because its expression in the rat SCN has been shown to be stimulated by light in the early subjective day (Abe *et al.*, 1998). Moreover, *Bmal1*$^{-/-}$ (*i.e.*, *Mop3*$^{-/-}$) mice show altered synchronization to a light-dark cycle, as assessed by bouts of wheel-running activity anticipating the time of lights off (Bunger *et al.*, 2000). In diurnal *Arvicanthis*, however, *Bmal1* mRNA levels in the SCN do not seem to be directly modulated by light pulses applied at different circadian times (Caldelas *et al.*, 2003). Nevertheless, BMAL1 is reduced, and possibly degraded, in the SCN of rats exposed to light pulses applied either in early or late night (Tamaru *et al.*, 2000). Further studies will be useful to shed light on whether and how the regulation of *Bmal1* transcript and/or BMAL1 protein is involved in photic synchronization of the SCN clock.

Effects of Light on the SCN Clock through *Cry2* Downregulation

Only few studies have investigated the putative photic sensitivity of *Cry2* expression in mammals. Okamura and colleagues noticed that light can decrease the levels of *Cry2* in the SCN of mice exposed to light in late night (Okamura *et al.*, 1999). More detailed information has been obtained in a diurnal rodent, *Arvicanthis*. In this species, *Cry2* mRNA levels are reduced after light exposure at different times of the subjective night (Caldelas *et al.*, 2003; see Figure 2).

Effects of Light on the SCN Clock through *Dec1* Induction

Besides *Per1* and *Per2*, *Dec1* is another clock gene induced by light during the night (Honma *et al.* 2002). The temporal pattern of photic induction is similar to that of *Per1*, albeit with a lower amplitude. By contrast, expression of a close component, *Dec2*, is not sensitive to nocturnal light pulses (Honma *et al.* 2002). It is, however, premature to onclude that *Dec1* is critical in photic phase resetting.

Light and Peripheral Oscillators

In contrast to what has been found in the transparent zebrafish where ambient light can act directly on peripheral oscillators (Whitmore *et al.*, 2000), light probably has only indirect effects on peripheral organs in mammals. In animals fed *ad libitum*, timing in peripheral organs is not directly modified by light exposure and thus is thought to be reset by output signals coming from the light-synchronized SCN (Sakamoto and Ishida, 2000). Another argument supporting this view is the observation that the liver is slowly resynchronized after an abrupt change in timing of a light-dark cycle compared to the rapid photic resetting of the SCN clock (Yamazaki *et al.*, 2000).

Synchronizing Effects of Food Availability

Food-Entrainable System

Restricted feeding affects the circadian timing system differently to photic stimuli. At a behavioral level, schedules of temporally restricted feeding (*i.e.*, supplying food for a limited daily period) in animals maintained in constant darkness usually only synchronize a bout of locomotor activity anticipating the time of feeding (so-called

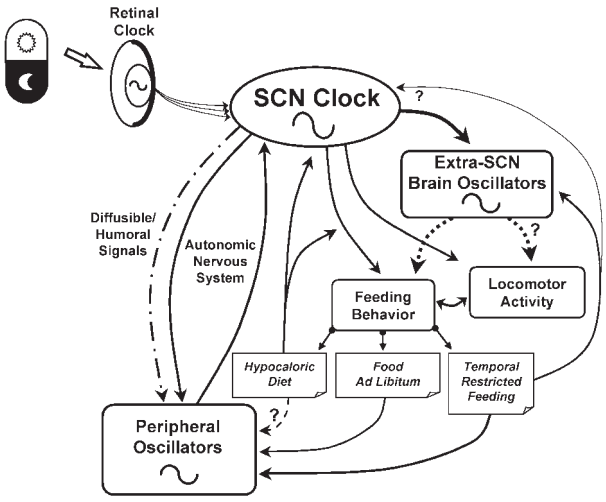

Fig. 3 Hypothetical Pathways Within the Circadian Timing System and Synchronization of Its Components to Light- and Food-Related Signals.

'food-anticipatory activity'), while the other circadian components of locomotor activity continue to free-run (Mistlberger, 1994; Stephan, 2001). The food-anticipatory activity is considered to be driven by a food-entrainable clock outside the SCN because it occurs in food-restricted animals with SCN lesions. Detailed reviews on the food-entrainable system and its coupling with the light-entrainable SCN can be found elsewhere (Mistlberger, 1994; Stephan, 2001). The molecular mechanisms driving the food-entrainable clock are not yet known. The fact that restricted feeding in heterozygous *Clock*$^{-/+}$ mutant mice does not alter the expression of food-anticipatory activity compared to that of wild-type mice suggests that *Clock* mutation does not markedly impair function of the putative food-entrainable clock (see Figure 3 in Challet *et al.*, 2000). It has been shown that restricted feeding in mice induces a phase advance in the circadian variations of *Per1* and *Per2* in the cerebral cortex of mice fed *ad libitum* (Wakamatsu *et al.*, 2001). Moreover, in the cortex of SCN-lesioned animals with a putative loss of rhythmicity in clock gene expression, restricted feeding leads to a day-night variation of *Per* in the cortex in phase with the time of food access, suggesting that the food-entrainable system modulates rhythmicity in the cerebral cortex (Wakamatsu *et al.*, 2001; Figure 3).

Effects of Food Restriction in Constant Darkness on the SCN Clock

In mice housed in constant darkness, a restricted feeding does not change the phase of cyclic clock gene expression in the SCN (Damiola *et al.*, 2000; Hara *et al.*, 2001; Wakamatsu *et al.*, 2001), indicating that restricted feeding does not have strong synchronizing properties for the master circadian clock. In contrast to schedules of restricted feeding that enable the animals to eat normal amounts of food, a timed caloric restriction (*i.e.*, when only a hypocaloric diet is given each day at the same time) applied in constant darkness is a potent *Zeitgeber* for the SCN clock, given that it synchronizes circadian rhythms of locomotor activity and body temperature in rats kept in constant darkness (Challet *et al.*, 1996). Furthermore, prolonged fasting (*i.e.*, no food available during several days) in free-running rats induces phase shifts of circadian rhythms of locomotor activity and body temperature, indicating that metabolic cues can, directly or indirectly, impact on SCN function (Challet *et al.*, 1997a). Current experiments in our laboratory have used the circadian expression of clock genes as markers of the phase of the SCN clock in calorie-restricted animals. Clock gene expression was determined over 24 h by sampling the animals according to the time of feeding. If timed calorie restriction has no synchronizing effect, expression of a given clock gene is expected to be non-oscillating due to random sampling of individuals with different free-running periods. Instead, oscillations of clock genes in the SCN of calorie-restricted animals were in phase with the time of feeding (I. Caldelas and E. Challet, unpublished data). These data demonstrate that a timed hypocaloric feeding in constant darkness acts as a *Zeitgeber* for the molecular clock in the SCN (Figure 3).

Effects of Food Restriction under a Light-Dark Cycle on the SCN Clock

When rodents are exposed to a light-dark cycle, restricted feeding during daytime (*i.e.*, daily food access limited to daytime) does not modify the temporal organization of the molecular loops within the SCN (Damiola *et al.*, 2000; Hara *et al.*, 2001; Stokkan *et al.*, 2001; Wakamatsu *et al.*, 2001). These findings are in accordance with behavioral data showing that the outputs of the light-synchronized SCN are usually not affected by restricted feeding (Mistlberger, 1994). In contrast, when rats and mice are subjected to a daytime restricted feeding coupled with calorie restriction, a marked alteration in the phase angle of photic synchronization is observed (Challet *et al.*, 1997b; 1998). Also, contrary to the lack of synchronizing effect of restricted feeding on the SCN, a timed calorie restriction under a light-dark cycle induces differential phase advances in the daily oscillations of clock genes and clock-controlled genes in the mouse SCN (C. Graff and E. Challet, unpublished data). These results demonstrate that repetitive food-related signals, in addition to a light-dark cycle, affect the synchronization of the molecular SCN clock (Figure 3). Binding of CLOCK/BMAL1 heterodimers to DNA can be modulated by the cellular redox state (Rutter *et al.*, 2001). This may provide an explanation for the more potent synchronizing effects of calorie restriction compared to restricted feeding without undernutrition. To obtain further insight into this hypothesis, it will be necessary to investigate whether redox status in the SCN cells is altered differently in the two paradigms.

Nonphotic signals, such as those mediated by novelty-induced running, applied during the subjective day in

constant darkness have been shown to act on the SCN both by inducing behavioral phase advances and by reducing *Per1* and *Per2* expression in the SCN (Maywood *et al.*, 1999; Maywood and Mrosovsky, 2001; Yannielli *et al.*, 2002). Because calorie-restricted rodents express an intense bout of wheel-running activity prior to the time of feeding (*i.e.*, food-anticipatory activity), the synchronizing effects of timed calorie restriction may depend, in part, on this daily behavioral activation. However, we did not observe any specific decrease of *Per* in the SCN of calorie-restricted mice under a light-dark cycle, suggesting other synchronizing mechanisms compared to those mediating nonphotic behavioral cues. Furthermore, light exposure at the end of a period of confinement to a novel running wheel has been shown to inhibit both the behavioral phase advances and reduction in *Per* expression induced by novelty-induced wheel running (Maywood and Mrosovsky, 2001), demonstrating that light inhibits nonphotic phase shifting and downregulation of *Per*. Because the synchronizing effects of timed calorie restriction observed at behavioral (Challet *et al.*, 1997b; 1998) and molecular (C. Graff and E. Challet, unpublished results) levels occur in spite of the presence of a normal light-dark cycle, timed calorie restriction has stronger synchronizing properties than novelty-induced running, and probably relies on different molecular mechanisms. Nevertheless, further work is needed to better understand the changes in the temporal pattern of transcriptional activity within the SCN cells of calorie-restricted animals. Such information will be useful to determine how the SCN cells integrate conflicting, photic and metabolic, cues.

Effects of Food Restriction on Peripheral Circadian Oscillators

Daytime food restriction in nocturnal rodents has been demonstrated to be a strong *Zeitgeber* of the molecular clockwork in peripheral oscillators (Figure 3) like liver, heart, kidney and pancreas, not only in constant darkness (Damiola *et al.*, 2000; Hara *et al.*, 2001), but also under a light-dark cycle (Damiola *et al.*, 2000; Hara *et al.*, 2001; Stokkan *et al.*, 2001). The gradual phase changes in circadian gene expression in liver have been shown to be faster than in other organs (Damiola *et al.*, 2000). Moreover, phase advances of molecular oscillations within the liver in response to daytime restricted feeding still occur in SCN-lesioned mice (Hara *et al.*, 2001). Taken together, these findings show that synchronizing processes of restricted feeding without calorie restriction appear to be essentially independent of the SCN, therefore indicating that peripheral oscillators become uncoupled from the master SCN clock under a temporal restricted feeding. By contrast, in animals fed *ad libitum* synchronizing cues of peripheral organs come, directly or indirectly, from the SCN because, as mentioned earlier, lesions of the SCN prevent oscillations of circadian gene expression in peripheral tissues (Sakamoto *et al.*, 1998).

Besides reduced food availability, other signals have been proposed to mediate synchronization of peripheral organs, such as glucocorticoid hormones (Balsalobre *et al.*, 2000) and temperature (Brown *et al.*, 2002). Moreover, these factors can interact because, for example, glucocorticoids inhibit uncoupling of peripheral oscillators from the SCN clock in response to restricted feeding (Le Minh *et al.*, 2001). Coupling between the SCN and peripheral organs could be achieved by nervous connections *via* the autonomous nervous system and/or by diffusible/humoral messages (Silver *et al.*, 1996; Sakamoto *et al.*, 1998; Yamazaki *et al.*, 2000; Buijs and Kalsbeek, 2001; Kramer *et al.*, 2001; McNamara *et al.*, 2001; Cheng *et al.*, 2002; Pando *et al.*, 2002; see Figure 3).

Conclusion

Light and restricted feeding are the most potent *Zeitgeber* of the SCN clock and peripheral oscillators, respectively. Light-induced phase shifts of the SCN clock are associated with changes in clock gene expression to reset the transcriptional-translational feedback loops. To date, light-induced changes in clock gene expression within SCN neurons concern almost exclusively changes in *Per1* and *Per2* expression. Despite considerable progress in understanding the molecular mechanisms of circadian oscillation, a number of fundamental questions remain concerning the mechanism of the SCN synchronization to light. In particular, the cellular and molecular substrates allowing the SCN clock to integrate synchronizing cues need to be identified. It seems unlikely that only the levels of *Per1* and *Per2* expression at a given time of the circadian cycle account for the amplitude and direction of phase shifts. Other candidate circadian genes, such as *Dec1* and *Cry2*, could also play a role in photic synchronization. Additional, yet uncharacterized, mechanisms may exist at the post-transcriptional level.

Light is considered to have only indirect effects on peripheral organs *via* light-synchronized signals coming from the SCN. On the other hand, restricted feeding can reset the phase of the peripheral oscillators while it has almost no effect in shifting the phase of the SCN clock. This paradigm therefore may uncouple synchronization of the SCN clock and peripheral oscillators. In contrast to temporal restricted feeding, a timed calorie restriction has a strong synchronizing influence on the SCN clock even in the presence of a light-dark cycle, as shown by altered temporal organization of clock gene expression. The mechanisms underlying synchronizing properties of daily hypocaloric feeding are far from being properly characterized. Taken together, studies on synchronizing properties of light and food availability highlight the plasticity in the hierarchical organization of circadian clocks and oscillators within the organism.

Further work using transgenic technologies coupled with physiological and electrophysiological approaches is needed to better understand how the circadian timing

system is differentially synchronized according to distinct, sometimes conflicting, temporal (times of light exposure and feeding) and homeostatic (metabolic) cues.

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