27 Circadian Timing

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Living organisms are submitted to day-night cycles that are imposed by the Earth's rotation within a 24-h period. Biological clocks running with a period of about 24 h are named circadian clocks (from Latin: *circa* (about); *diem* (day)). A circadian clock is present in most living organisms, including prokaryotes, and temporally controls a wide range of physiological and behavioral functions. Circadian clocks have three important properties: in constant environmental conditions they usually tick with a period of about 24 h (free running), they keep a near-constant period over the range of physiological temperatures (temperature compensation), and they synchronize to environmental cycles (entrainment). A clock provides the organism with the capacity to anticipate environmental daily changes, such as those affecting light, temperature, or humidity. It also provides a temporal reference to compensate for the changing position of the Sun used for orientation and navigation. The internal timing system also allows the temporal organization of physiological functions.

In metazoans, clocks are present in most tissues and are able to tick autonomously in individual cells. The clock that controls sleep-wake and body-temperature rhythms in mammals is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The SCN clock synchronizes to day-night cycles by receiving photic inputs from the retina. Circadian photoreception uses rods and cones photoreceptors as well as a subset of retinal ganglion cells that act as photoreceptors thanks to the presence of the melanopsin photopigment. Cellular clocks outside the SCN do not sense light directly but are entrained by day-night cycles through the SCN, which acts as a master clock of the body. Body temperature and humoral signals act as synchronizing cues between the SCN and peripheral clocks. Many insects use a more decentralized clock organization, with peripheral clocks directly sensing light through the blue light-sensitive molecule cryptochrome that is expressed in all clock cells. In addition to cryptochrome, the brain clock neurons use retinal inputs to synchronize rest-activity rhythms to day-night cycles.

Cellular circadian clocks rely on molecular oscillations of clock proteins that result from transcriptional feedback loops. In both insects and mammals, transcription factors activate the expression of transcriptional repressors, which accumulate with a delay to finally antagonize the transcriptional activators. The delay is mostly due to posttranslational modifications that tightly control the stability of the repressors, to tightly control the 24-h period of the molecular feedback loop. This 24-h transcriptional cycle controls a large body of clock-regulated genes, which represent about 5 % of the genome and largely differ among cell types. Genetic polymorphism in the circadian gene network defines molecular chronotypes that shape our individual physiology and behavior. The control of sleep-wake cycles results from the interaction between the circadian clock and a homeostatic process, the sleep pressure, which builds up during wake. Sleep will thus occur when the circadianly driven arousal is low and sleep pressure is high. The cellular and molecular mechanisms underlying sleep remain poorly understood. Recent studies have uncovered the

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existence of sleep-related states in insects and begin to provide molecular insights into sleep mechanisms.

Circadian and sleep-wake cycles are the best-studied examples for neurally controlled rhythms, but others also exist: lunar, seasonal, circannual, and endogenous cycles to name but a few, controlling humoral, behavioral, cognitive, and developmental aspects of neural organization. For the sake of simplicity we focus on circadian rhythms and sleep in this chapter.

27.1 Circadian Clocks

The first experimental evidence for a circadian clock came from the French astronomer Jean-Jacques d'Ortous de Mairans who reported his observation to the "Académie Royale des Sciences" in 1729 [1]. Mimosa plants open their leaves during daytime in day-night cycles and still show a 24-h rhythm of leaf movement when placed in complete darkness conditions. They thus need to have some time-keeping mechanism to control the day-night cycle in the absence of light cues. During the following 200 years, similar experiments were carried out with different rhythms in various plant and animal species. In particular, the observation of circadian periods slightly deviating from the usual 24 h supported the existence of an internal pacemaker rather than a response to a physical factor linked to the rotation of the Earth. However, the possible contribution of an uncharacterized external factor fueled the debate up to the 1960s. The extensive characterization of circadian rhythms by Erwin Bünning (1906–1990) in plants, by Colin Pittendrigh (1918–1996) in *Drosophila*, and by Jürgen Aschoff (1913–1998) in rodents and humans set the ground for a biological clock being a key component of the physiological organization of organisms living on our 24-h-rotating planet.

27.1.1 Properties of Circadian Clocks

The **circadian period** illustrates the first property of circadian clocks: they persist running (they free-run) in constant conditions with a period of about 24 h. Experimental conditions are constant temperature and lighting, the later being either constant darkness (also named DD for Dark:Dark) or constant light (named LL for Light:Light), as opposed to Light-Dark (LD) or temperature cycling (Fig. 27.1).

A second property is **temperature compensation**. The free-running period of circadian clocks is largely independent of the external temperature, in the range of physiological temperatures. This holds not only for animals that maintain a constant body temperature (homeotherms) but also for those who do not (poikilotherms), such as reptiles, amphibians, and all invertebrates. A clock with a pace that would change with temperature would act as a thermometer rather than providing time information. However, the rate of biochemical reactions strongly increases with rising temperature (Q_{10} is usually around 2, meaning that reaction rates increase by about two-fold for a 10 °C temperature increase). Circadian clocks thus need to include a compensating mechanism in order to maintain a constant free-running period over a wide range of temperatures. This was nicely demonstrated by C. Pittendrigh in the 1950s using the emergence of the young adults of the *Drosophila pseudoobscura* from their pupal cases, at the end of the metamorphosis process (Fig. 27.2). Between 16 and 26 °C the circadian period of emergence slightly decreased from 24.5 to 24.0 h, indicating a strong although not perfect temperature compensation.

A third property of circadian clocks is their ability to synchronize to the day-night cycles that are imposed by the rotation of the Earth. In contrast to their endogenously defined period in constant conditions, the phase of circadian rhythms is determined by the day-night cycles or the LD cycles in laboratory conditions. In other words, environmental cues entrain the circadian clock. Such cues have thus been named **Zeitgebers** by J. Aschoff. In addition to the light and temperature changes that are associated with day-night cycles, environmental changes such as food availability or social cues also act as Zeitgebers for the entrainment of animal circadian rhythms. Light and to a lesser extent temperature are the most potent Zeitgebers for behavioral rhythms and have been used in the majority of the experiments aimed at understanding circadian entrainment. Since endogenous circadian periods are often not exactly 24 h, clocks would be quickly out of phase with day-night cycles if not entrained. If one takes sleep-wake cycles as an example (see Fig. 27.1), a 25-h clock would results in diurnal animals falling asleep 1 h later everyday, thus going from nighttime sleep to daytime sleep (12-h

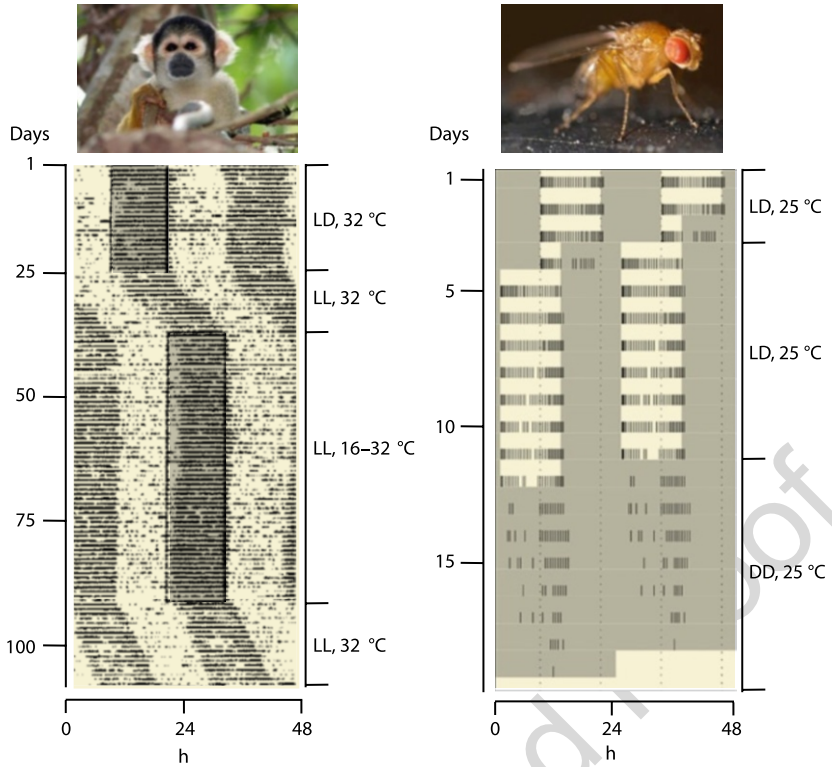

Fig. 27.1 Sleep-wake cycles in squirrel monkeys and fruit flies. Sleep distribution is shown by activity graphs or actograms. *Vertical black bars* of the actograms are proportional to animals' activity, which is recorded by an infrared photocell. The activity of each day is plotted twice (i.e., day 2 activity is shown on the *right part* (24–48 h) of row 1 and on the *left part* (0–24 h) of row 2, etc.). Squirrel monkey *(Saimiri sciureus)*: activity in light-dark cycles (lights-ON is *boxed*), constant light (*LL*), or constant light with temperature cycles (high temperature is *boxed*). Drosophila *(Drosophila melanogaster)*: activity in light-dark cycles (lights-ON in *white*) and constant darkness. The temporal distribution of activity shows that sleep-wake cycles can be synchronized by light or temperature. Their persistence in constant conditions indicate that they are driven by an endogenous clock (Squirrel monkey data from Aschoff et al. [31] with permission)

shift) every 12 days. The entrainment of the clock allows the animals to keep a stable phase angle between the sleep-wake rhythm and the environmental day-night cycle. To adapt the sleep-wake rhythms to the 24-h period of the day-night cycles, light needs to delay fast (period <24 h) clocks and to advance slow (period >24 h) clocks. This is the consequence of circadian clocks responding differently when hit by light at different phases of their cycle. This property is best described by phase-response curves obtained from experiments where animals are exposed to a short light pulse a particular time of the day (hence of the circadian cycle) in constant darkness (Fig. 27.3). Light pulses given in the middle of the subjective day elicit no responses, pulses given in the early night induce phase delays, and late-night pulses induce phase advances. A short-period clock exposed to a 24-h LD cycle will enter its nighttime phase when light is still ON outside and will thus be delayed to better fit the LD cycle. In contrast, a long-period clock will see outside light before ending its nighttime phase and will be advanced to resynchronize to the LD cycle. These phase-response curves are similar in all species, as expected for such an important adaptive process [2]. The entrainment capacities show limits, which can be defined by recording the sleep-wake rhythms in light-dark cycles of various lengths. When the difference between the free-running period of the animal and the length of the day-night cycles exceed a few hours, the clock will start free-running. This can be used to estimate the free-running period of animal or human subjects, using a so-called forced

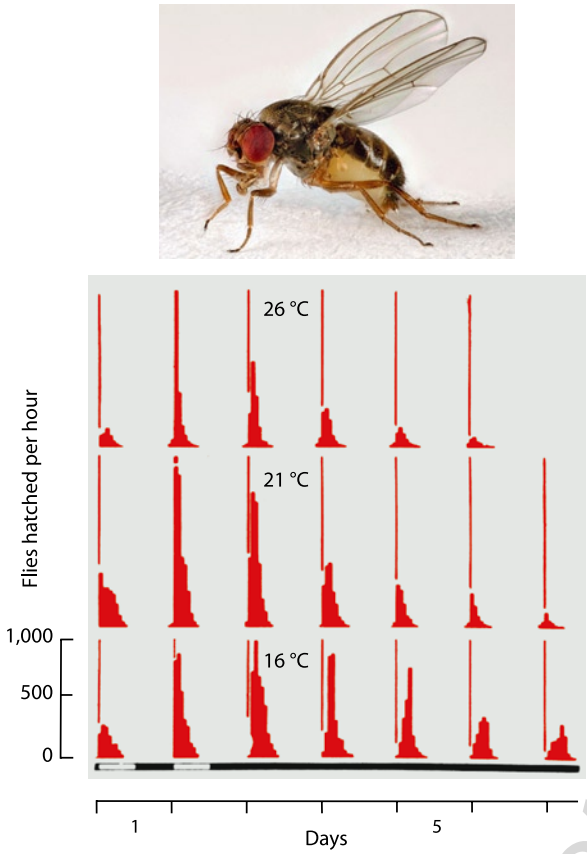

Fig. 27.2 Eclosion rhythms of fruit flies at different temperatures. *Drosophila pseudoobscura* cultures are kept in LD cycles and then released in constant darkness. The number of adult individuals emerging from pupal cases is plotted as a function of time. The very small change of period through a wide range of temperatures shows that the circadian clock is temperature-compensated (Adapted from Pittendrigh [32] with permission)

desynchrony protocol with, for example, a 6-h:6-h LD cycle.

Besides laboratory experiments, circadian clocks do not experience phase shifts of the LD cycle or light pulses in natural conditions. However, they are submitted to the seasonal variation of day length, which advances or delays morning and evening light-dark transitions. Within the 24-h cycle, day length is 12 h all year round at the Equator but increasingly varies from the Equator to the Poles between the June solstice (North Pole maximally tilted towards the Sun) and the December solstice (South Pole maximally tilted towards the Sun). For example, at 50 °C North of latitude (Brussels), day length varies from about 16 h (June 21) to about 8 h (December 21). Animals active during the early day or early night would miss dawn or dusk during summer if keeping the winter phase, but the daily resetting of the clock by light allows them to track dawn or dusk from solstice to solstice.

27.1.2 What Is a Circadian Clock for?

The presence of a circadian clock in most organisms indicates its strong adaptive value for living on Earth. Living organisms need to adapt their physiology and behavior to these daily environmental changes, in particular light and temperature variations. Having an internal clock allows to anticipate and thus prepare the organism for these changes, rather than simply responding to them. For example, young adult *Drosophila* have a very thin and permeable cuticle (protective layer all over the insect body) when emerging from their pupal case at the end of metamorphosis and are thus very susceptible to desiccation. The circadian clock sets the time of emergence in the morning when humidity is higher and prevents them from being exposed to the hot Sun of the afternoon, which would have deleterious effects especially on wing expansion [3]. Since the emergence process takes a few hours, it has to be started during the night, and the circadian clock allows the fly to anticipate sunrise. It is also important to be in time for available food sources, as nicely illustrated by the synchrony between flowers and their pollinators. Some flowers open each day during a few hours only, with different species opening at different times, as illustrated by the famous flower clock of Linné (1707–1778). This time specialization increases the chance to get pollen from the same species and is controlled by the circadian clock. On the other side, bees can learn where to go at a given time to find an open flower and collect nectar. Famous experiments initiated by Karl von Frisch (1886–1982) have shown that they can do so in the absence of sky light and thus use their internal clock to associate time with the learned location of the food source. The clock thus allows to predict remote events that the bee cannot see or smell (see Chap. 28). Time and space are even more related if one considers navigation. The apparent course of the Sun in the sky throughout the day requires migratory birds and insects to time-compensate their orientation.

Indeed, G. Kramer (1910–1959) studied migratory birds in the laboratory and showed that their attempts to fly during migration periods were orientated in a

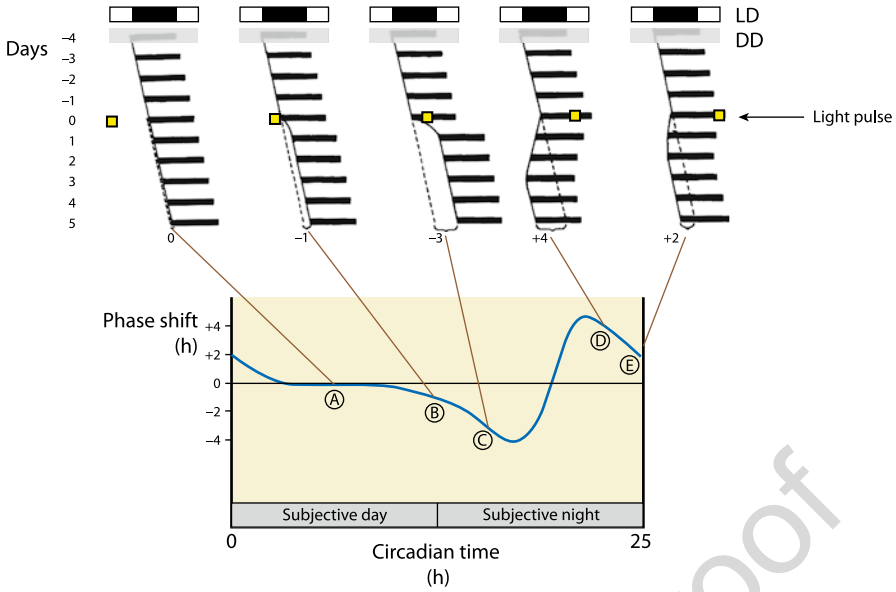

Fig. 27.3 Photic entrainment of sleep-wake rhythms: the phase-response curve. Five experiments (**a**–**d**) are done with individual rats, whose night locomotor activity is reported as a *black bar*. Each animal is entrained in 12-h–12-h light-dark cycles (LD, *white*: light, *black*: dark) and released in constant darkness (DD, *gray*). From day −4 to day −1, the animals display free running rhythms in DD with a circadian period of 25 h. On day 0, rats are submitted to a short (≤1 h) light pulse (*yellow square*), which is applied at a different time of the 25-h circadian cycle for each individual. The animals are then left in DD from day 1 to day 5. Depending of the time at which the pulse was given, the rat will show either no change or a phase shift of its activity rhythm after the pulse. Pulses applied during the subjective day (**a**) produce no response, pulses applied at the beginning of the subjective night (**b**, **c**) produce phase delays, and pulses applied at the end of the subjective night (**d**, **e**) produce phase advances. These phase shifts are plotted against circadian time to derive the phase response curve that is shown at the bottom (Modified from Moore-Ede et al. [1] with permission)

manner that depends on the time of the day. Imposing a phase-shifting to the clock (see below) altered the orientation with a predictable angle. Similar experiments were conducted with monarch butterflies, which migrate over ~3,000 km from northeast America to central Mexico during the fall. When submitted to light conditions that abolish the function of their brain circadian clock, they lose time-dependent orientation and orient randomly in the flight simulator. A circadian clock thus allows animals to keep a constant direction as the Sun moves in the sky from east to west.

The circadian system may stop timing some clock outputs in the absence of cycling environmental conditions. Reindeers living far above the Arctic Circle show robust sleep-activity rhythms in spring and autumn but they lose behavioral rhythms during either summer days when the Sun does not set or winter days when it does not rise [4]. In some cases, evolution has profoundly changed the properties of the circadian system to adapt it to specific ecological niches. Some organisms such as fish species living in subterranean caves never see daylight.

As opposed to its surface counterparts, the eyeless Somalian cavefish *Phreatychtis andruzzii* does not show activity rhythms in light-dark cycles [5]. This appears to be the consequence of mutations that have accumulated in the genes encoding the photopigments involved in the synchronization of the clock by light. Providing food at the same time everyday, induces activity rhythms that persist when the animals are starved, indicating that a food-entrainable internal clock is running but has lost the ability to synchronize to light-dark cycles. Having a clock not only allows to anticipate environmental cycles, but also provides a temporal reference for the internal synchronization between physiological processes. A well-known example in humans is the opposite phasing of sleep and urine production, which is very low at night. Also critical is the circadian phasing of nitrogen fixation and photosynthesis in some prokaryotic cyanobacteria. Photosynthesis produces oxygen during the day, which

inhibits the nitrogenase that reduces atmospheric nitrogen to ammonia. The circadian clock of these photosynthetic bacteria restricts the expression of the gene encoding the nitrogenase to the night, thus temporally separating two incompatible biochemical pathways.

The fitness value of circadian clocks was experimentally tested in a few studies. Spectacular results have been obtained in cyanobacteria, whose cell division time (about 24 h in LD 12:12 cycles) allows competition studies to be performed easily [6]. Equal numbers of wild-type or mutant cells with either a short or a long circadian period were mixed and then grown in LD cycles of different durations. In all conditions, the strain with the circadian period best fitted to the LD cycle outcompeted the other strain in less than 20 generations. A circadian clock thus confers a strong fitness advantage in cycling environmental conditions. In an attempt to estimate the adaptive value of the clock in mammals, the survival in the wild of either squirrels or chipmunks in which the SCN were surgically ablated was compared to sham-operated controls. The mortality by predation of SCN-lesioned animals was higher, suggesting that the circadian system provides an advantage, likely by restricting activity at times where predators were less actively seeking a meal [7].

27.2 Molecular Mechanisms of Circadian Oscillators

27.2.1 Finding the Clock in *Drosophila*

The first genetic mutations affecting sleep-wake cycles were described in fruit flies, in a pioneer genetic screen led by Konopka and Benzer [8]. *Drosophila* cultures issued from mutagenized individual flies were tested for their eclosion rhythms and mutants either arrhythmic or rhythmic with an altered circadian period were obtained. The mutants were then tested for their rest-activity rhythms. This can be measured by placing individual adult flies in small food-containing glass tubes that are crossed by an infrared beam. When the fly is moving, it intercepts the beam and its activity is defined by the number of times the beam is crossed during a time interval of usually 5–30 min. The period of the circadian clock is determined by measuring rest-activity rhythms in constant darkness, and the clock mutations identified by Konopka and Benzer were found to affect similarly eclosion and activity rhythms, indicating that they are driven by the same molecular clock. The three mutations were found to affect the same gene, named *period*. After the molecular cloning of the *period* gene in 1984, the first molecular studies unraveled the negative feedback loop mechanism as a core engine of the circadian oscillator [9]. The power of *Drosophila* genetics soon led to the discovery of the small set of genes that participate in the negative autoregulatory loop taking place in each individual clock cell [10] (Fig. 27.4). In the evening, two transcriptional activators, CLOCK (CLK) and CYCLE (CYC) associate to induce the expression of the *period (per)* and *timeless (tim)* genes, whose transcripts reach a peak in the first half of the night. The PER and TIM proteins accumulate during the night, and reach a peak in the early morning, a few hours after the mRNA peak. This delay is a key feature of the circadian oscillator. Indeed, PER and TIM proteins interact in the cytoplasm and enter the nucleus in the middle of the night, where they induce the repression of the CLK-CYC transcriptional activity. As a consequence, *per* and *tim* mRNA levels drop to low levels. This defines a transcriptional negative feedback loop. The delayed protein accumulation thus creates a transcriptional oscillation, with active transcription in the early night and repression in the late night.

The delayed accumulation of PER and TIM is a consequence of posttranslational mechanisms, which finely control the stability, the subcellular localization and the activity of the clock proteins through their phosphorylation, ubiquitylation, and degradation by the proteasome. Various genetic screens have identified key enzymes involved in these steps, which include kinases such as Casein kinase 1 [11] and 2, phosphatases, and ubiquitin ligases that determine the speed of the molecular oscillator, hence the period of the sleep-wake cycles. In addition to the *per* and *tim* genes, the transcriptional feedback loop controls two other transcription factors which regulate the expression of the *clk* gene itself, thus building a secondary loop. How do these cycling molecules transmit cyclic information within the cell? Large-scale transcriptional studies have revealed that about 5 % of the *Drosophila* transcripts display circadianly regulated levels in the fly head and all of them stop cycling in the absence of either CLK or PER. It thus appears that, in addition to the clock genes themselves, the transcriptional feedback loop controls hundreds of downstream

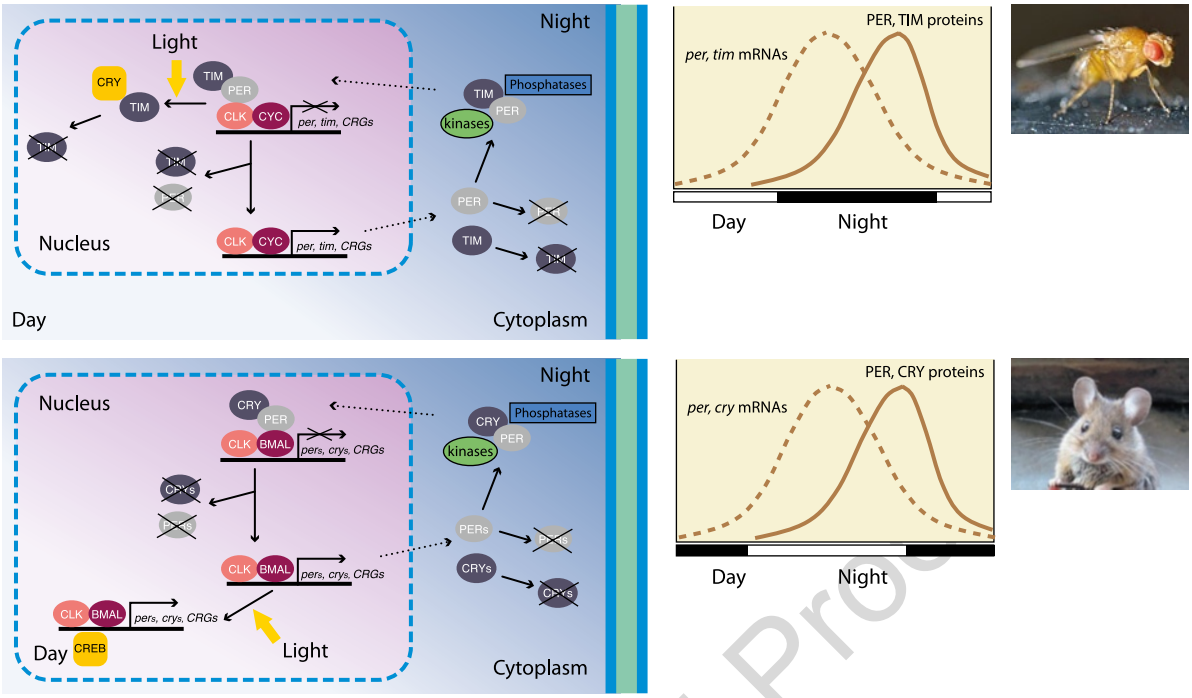

Fig. 27.4 The molecular feedback loop model of the circadian clock. In mammals and insects, major clock genes are involved in a negative transcriptional feedback loop. In *Drosophila*, the *per* and *tim* genes are activated by the CLK and CYC proteins at the end of the day. Post-translational modifications destabilize the PER and TIM proteins and delay their night accumulation. PER and TIM proteins repress the activation of the *per* and *tim* genes in the morning. Degradation of the PER and TIM repressors during the day allows a new cycle to resume. A similar feedback loop occurs in the mouse, where multiple PER and CRY proteins repress the expression of the three *per* genes and the two *cry* genes that is induced by the CLK and BMAL activators. The feedback loop also controls a large number of downstream circadianly-regulated genes (*CRGs*) that are involved in various aspects of circadian physiology. In *Drosophila*, CRY acts as a photoreceptor to induce the light-dependent degradation of the TIM protein. In mammals, light resets the clock by inducing a CREB-dependent increase of *per* genes' transcription. Crosses indicate protein degradation or transcriptional repression

genes that are involved in many aspects of cell physiology. Importantly, blocking electrical activity of clock cells appears to abolish molecular cycling, suggesting that neuronal activity feeds back to the molecular oscillator [12].

27.2.2 Molecules in the Mammalian Clock

The first identified mammalian clock genes were isolated in 1997, on the basis of their similarity with the *Drosophila* genes *(per)* or through a sleep-wake cycle behavioral screen using a running wheel as an activity monitor [13]. The architecture of the molecular clockwork is very similar in flies and mammals and the two clocks share several components (Fig. 27.4). The two transcription factors, CLK (or a similar protein named NPAS2) and BMAL (the *bmal1* gene is the mammalian ortholog of *cyc*) activate the expression of the genes encoding the repressors of their activity. There are three *per* genes in mammals, and two of them act as repressors in the transcriptional feedback loop, whereas the role of the third one remains elusive. In contrast to *Drosophila*, the mammalian PER partner does not seem to be TIM but CRYPTOCHROME (CRY). CRY was first characterized as a blue light-sensitive photoreceptive molecule in plants and plays a role in *Drosophila* circadian photoreception (see below). There are two *cry* genes in mammals, and surprisingly, mice devoid of the two *cry* genes are behaviorally arrhythmic but are not impaired in any type of photoreception tested so far, indicating that CRY acts as a bona-fide clock protein and not as a photoreceptor. Similarly to *Drosophila* PER/TIM complexes, PER/CRY complexes enter the nucleus and repress CLK/BMAL-dependent transcription.

The repressing complex recruits enzymes that modify the chromatin-structural histone proteins in the promoter region of the CLK-BMAL target genes, thus changing their ability to be transcribed. Why is CRY a photoreceptor in the *Drosophila* brain clock and a transcriptional repressor in the mammalian clock? The availability of the genome sequence in several insect species has shed light on this evolutionary mystery. In some insects such as butterflies, two *cry* genes were found, with one more similar to the *Drosophila cry*, whereas the other one is closer to the mammalian variant. Other insects such as bees only have the mammalian-type *cry* gene. Biochemical experiments indicate that the *Drosophila* type CRY proteins act as photoreceptors, whereas the mammalian-type CRY proteins act as transcriptional repressors. Sequence comparisons suggest an evolutionary scenario where gene duplications followed by divergence generated two *cry* gene families, with some lineages keeping either the photoreceptor-encoding gene or the transcriptional repressor-encoding gene, and other lineages keeping both. Intriguingly, the absence of a *tim* gene ortholog in bees suggests that their molecular clock machinery might be closer to the mammalian one than to *Drosophila* [14]. Insects devoid of photoreceptive cryptochrome are likely to use their compound eyes as the only light input or yet unknown photoreceptive pathways.

In contrast to flies, the mammalian secondary feedback loop mostly controls *bmal1* expression and relies on a different pair of transcription factors, which belong to the nuclear receptor family. The pace of the two feedback loops depends on posttranslational modifications of the transcription activators and repressors by kinases, phosphatases, and ubiquitin ligases, with several of them being identical in flies and mammals. Such modifications are also sensitive to the cell metabolism and a growing number of studies show that the cell metabolism strongly influences the feedback loop mechanism, thus fine tuning the speed of the clock.

The alteration of the core clock genes either abolishes sleep-wake cycles or alters their period. In LD conditions, where the clock is reset every cycle, a change in the period of the circadian clock will have consequences similar to a phase defect, namely sleep onset and/or offset will be advanced (short period) or delayed (long period). The familial advanced sleep phase syndrome (FASPS) is an autosomal dominant inherited trait that induces an early (up to 4 h advance) sleep onset and offset. Linkage analysis in a large family with several affected patients revealed that the FASPS phenotype was cosegregating with the chromosomal region where the human *per2* gene is located. Molecular analysis identified a mutation in the gene, which induced a serine to glycine amino acid change, thus affecting the ability of the PER2 protein to be phosphorylated by the CK1delta kinase [15]. Transgenic mice carrying the human mutation showed short period rhythms, strongly supporting a causal link between the *per2* mutation and FASPS in this family. In another family, a mutation was found in the *ck1delta* gene of a FASPS-affected individuals. These studies show that multiple CK1-dependent phosphorylation sites on PER2, differently influence the stability of the protein, hence the period of the circadian oscillator. Interindividual differences in human populations have been estimated by asking people about their sleep habits. In a survey more than 55,000 people filled out a questionnaire that was analyzed to calculate individual chronotypes (half-way time between sleep onset and end of sleep) (Fig. 27.5). A wide distribution of clock-dependent chronotypes was observed, with influences of age and sex. Importantly, the interaction between chronotype and social habits strongly influences sleep duration. Early chronotypes sleep less on free days since they go to bed later, thus missing the first part of their night sleep, whereas late chronotypes sleep less on work days since they have to wake up early and miss the end of their night sleep [16]. The survey also revealed that chronotypes follow solar time, especially those leaving outside of large cities: inhabitants on the eastern border of Germany wake up half an hour before those on the western border, even though official time and working hours are identical [17].

In addition to the core clock genes, hundreds of mammalian genes show circadian oscillations of their expression in mammals, and do so in a tissue-specific fashion. Many of these genes are involved in different metabolic pathways, thus generating oscillations of various metabolites. The physiological consequences of these oscillations are very important. For example, changes in amino acid levels affect the synthesis of neurotransmitters and are thus likely to modulate brain activity. A study has shown that the circadian clock controls the expression of the gene encoding monoamine oxidase A (MAO-A), which is involved in dopamine catabolism [18]. *per2* mutant mice not only are

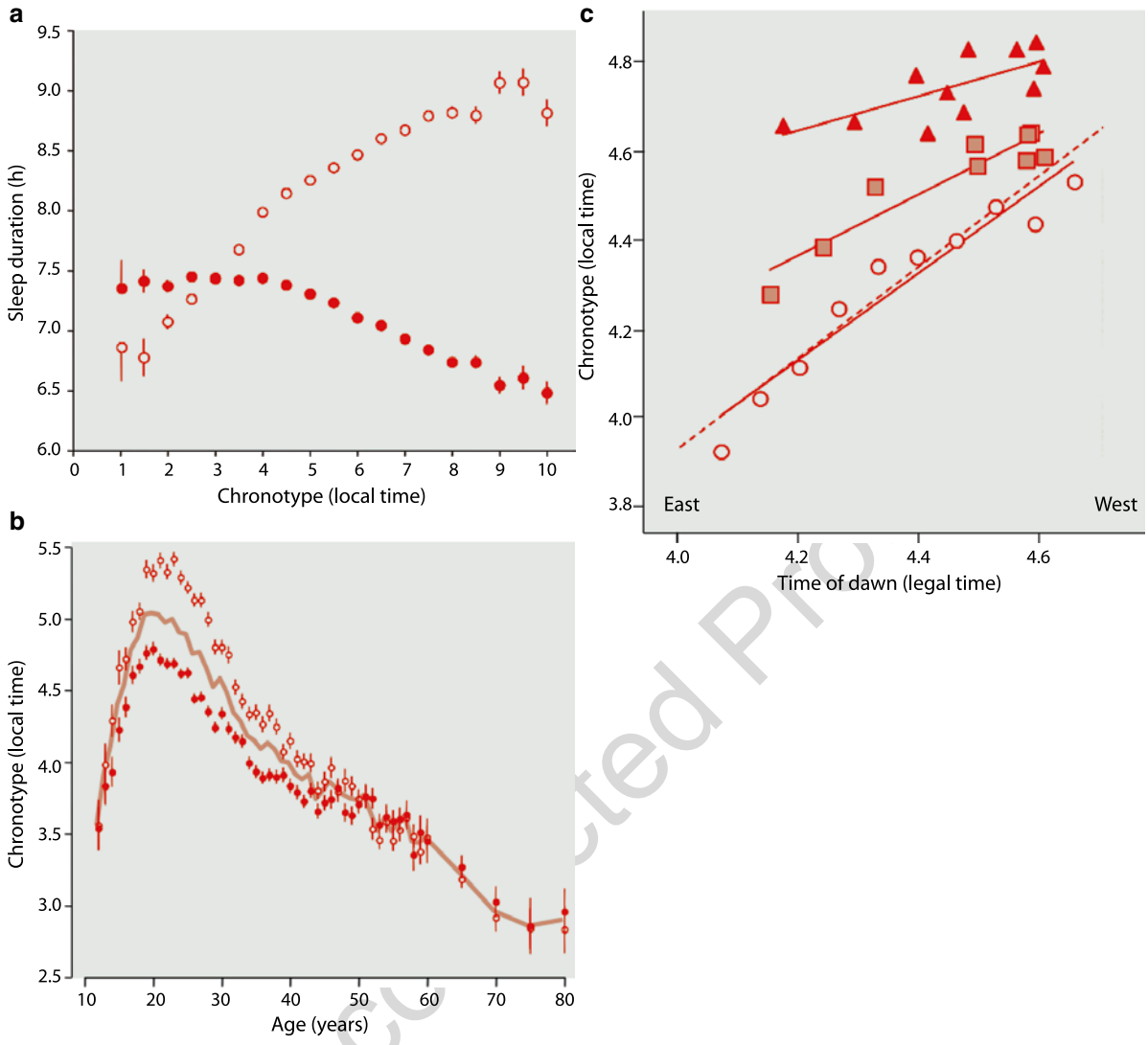

Fig. 27.5 Inter-individual variability of circadian clocks: human chronotypes. (**a**) Chronotype affects sleep duration in real life. Chronotype is defined by mid-sleep in free days (MSF), which is the half-way between sleep-onset and end of sleep. Sleep duration is reported for work days (*filled circles*) and free days (*open circles*). Early chronotypes sleep less on free days since they go to bed later. In contrast, late chronotypes sleep less on workdays since they wake-up earlier. People who sleep between 11:30 p.m. and 6:30 a.m. (MSF at 3:00 a.m.) show no difference in sleep duration between work days and free days. (**b**) Chronotype depends on age and sex. Children are early chronotypes and progressively delay their body clock with age. The maximum delay is reached in the early 20s, then sleep times become earlier with age. Women (*filled circles*) reach their later chronotype around 19.5 years of age, while men (*open circles*) delay until 21. Men are thus, on average, later chronotypes than women but the sex difference disappears around 50. (**c**) Chronotype depends on solar time rather than on social time. Average chronotypes of the German population follow dawn when going from East to West. Dawn time changes by 36 min between the eastern and western borders of the country. Chronotypes of people living in areas with up to 300,000 inhabitants (82 % of the population) are shown as *circles*, while those living in cities with 300,000–500,000 inhabitants and more than 500,000 inhabitants are shown as *squares* and *triangles*, respectively (a, b – From Roenneberg et al. [16]. c – From Foster and Roenneberg [17] with permission)

altered for sleep-wake rhythms but show high levels of dopamine and altered neuronal activity in the striatum. These animals behave abnormally in despair-based behavioral tests, which model human mood disorders. This suggests that genetic or environmental conditions (such as shift work) that perturb the circadian system may have significant consequences on mood regulation.

27.3 Neural Organization of the Fly Clock

27.3.1 A Network of Clock Neurons Controls Activity Rhythms in *Drosophila*

In order to locate the clock that controls *Drosophila* sleep-wake cycles, the brains of flies carrying a per^S allele (displaying 20-h behavioral rhythms) were introduced in the abdomen of per^0 null mutants (no PER function). Flies with a per^S mutation have strong short period (20-h) sleep wake cycles, whereas per^0 are behaviorally arrhythmic. The surviving transplanted individuals displayed short-period sleep-wake rhythms indicating that the brain carries the circadian pacemaker for sleep-wake cycles and that this pacemaker does not need synaptic connections to drive circadian rhythms [19]. Antibodies directed against the clock proteins identified about 150 brain neurons that show circadian oscillations of the clock proteins. These neurons are distributed in half a dozen clusters located in the lateral and dorsal regions of the brain (Fig. 27.6). When submitted to light-dark cycles in the laboratory, *Drosophila* flies display morning and evening activity peaks anticipating lights-on and lights-off. This bimodal pattern is often observed and defines a so-called **crepuscular behavior**. Activity records of rodents or insects in short (winter) or long (summer) days show that the morning and evening activity bouts partially follow dawn and dusk, suggesting that they reflect the behavior of somehow independent morning and evening circadian oscillators. The anatomically distinct groups of clock neurons in the *Drosophila* brain provide a cellular substrate for these oscillators. About 16 lateral neurons express a neuropeptide, the pigment-dispersing factor (PDF), which was previously identified in crustaceans where it controls the light-induced changes of screening pigment distribution in the cuticular chromatophores and in the retina (see Chap. 11). With the sophisticated *Drosophila* genetic tools, transgenic animals can be produced where a specific subset of clock cells is killed or synaptically silenced or is the only one running a functional clock in an otherwise clockless genetic background. Such cellular manipulations have shown that flies with a molecular clock restricted to the PDF cells display only morning behavior in light-dark cycles, whereas flies with a clock restricted to the PDF negative subset of lateral neurons display only evening behavior [20, 21]. Manipulations of some of the dorsal groups show that light and temperature conditions define whether or not the dorsal neurons will contribute to these activities. Light also controls the contribution of the lateral neurons with the morning cells driving sustained sleep-wake rhythms in constant darkness whereas the evening cells can do so in constant light. The adaptation of the activity pattern to various environmental conditions thus appears to result from light and temperature modulating the contribution of different subsets of the circadian neuronal network to the behavioral output. The opposite effects of light on different parts of the network appear to be important for adapting the sleep-wake cycles to the seasonal changes of daylength. By using transgenic flies running the clock at a different speed in the morning and evening cells, the contribution of the two oscillators to the sleep-wake cycle could be followed in short (winter type) and long (summer type) days. These results suggested that PDF-expressing morning neurons have a prominent contribution in short days whereas PDF-negative evening cells take the lead in long days.

The PDF neuropeptide likely acts at different levels in the *Drosophila* circadian system. In agreement with the expression of peptide in the morning cells, pdf^0 mutants lose robust rhythms in constant darkness and morning activity in LD cycles. However, they also show advanced evening activity in LD, suggesting that the peptide might be a synchronizer between day-night cycles, morning cells, and evening cells. In agreement with such a role, clock protein oscillations tend to desynchronize among neuronal groups in pdf^0 mutants. This synchronizing function is supported by the expression of the PDF receptor in most clock cells and its requirement for maintaining a proper phase in the PDF-negative neurons. Similar synchronizing functions have been assigned to PDF in other insects such as cockroaches and crickets.

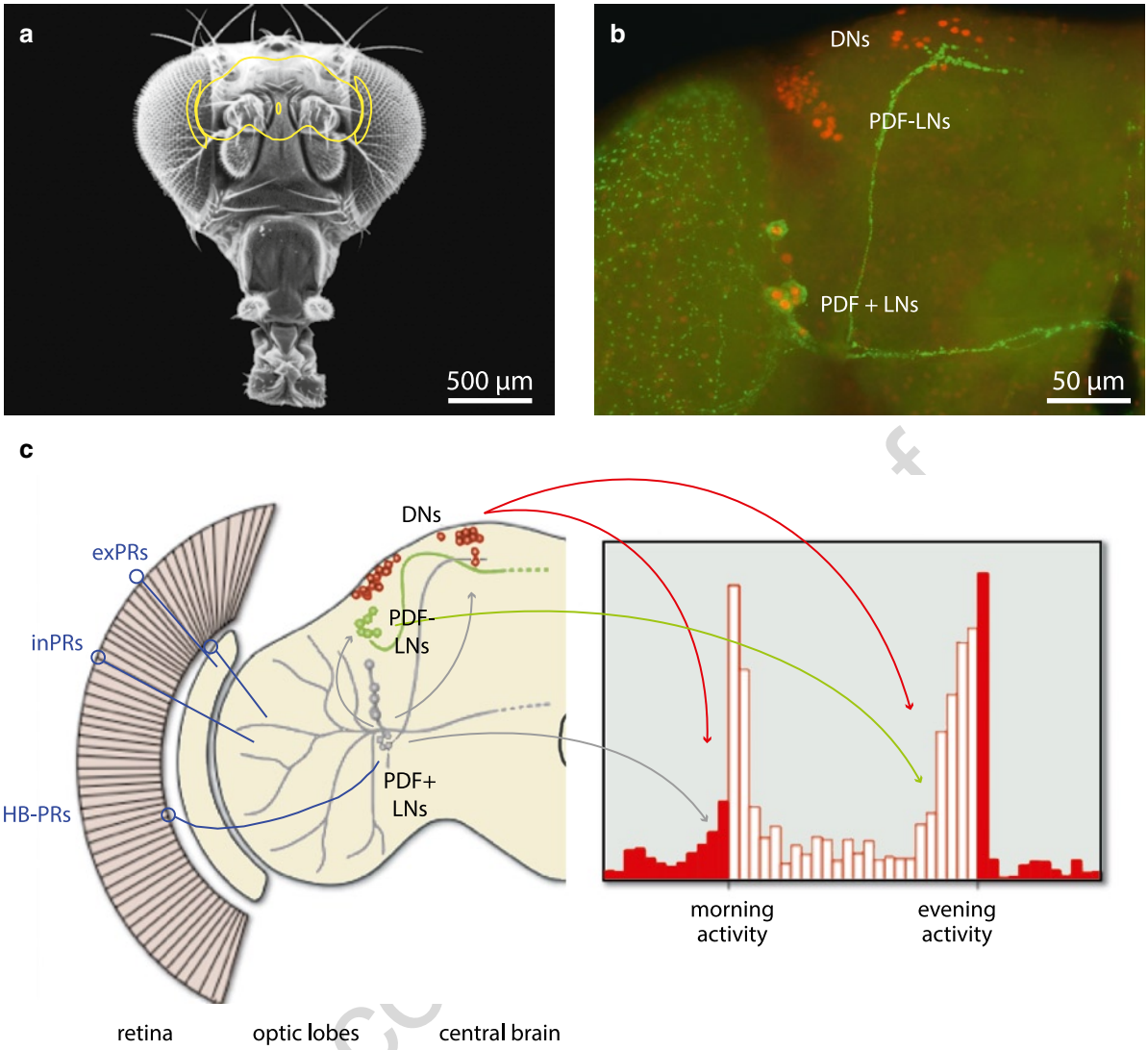

Fig. 27.6 Several groups of clock neurons control sleep-wake rhythms in *Drosophila*. (**a**) The *Drosophila* brain contains about 150 neurons that show clock protein cycling. (**b**) A brain hemisphere collected in the morning is labeled for the PER protein (*red*, all clock neurons including lateral (LNs) and dorsal neurons (DNs)) and the PDF neuropeptide (*green*, subset of lateral neurons). (**c**) The behavioral function of several subsets is partly identified. LNs that express PDF (*gray*) are key players in the generation of morning activity whereas PDF-negative lateral neurons (*green*) play a major role in the control of evening activity. Morning cells also provide time information to evening cells. Additional neurons located in the dorsal brain (dorsal neurons, *red*) regulate morning and evening activities, as a function of light and temperature. The brain clock neurons receive light inputs from the visual system through different types of photoreceptor cells (*blue*): internal (*inPRs*) and external (*exPRs*) photoreceptors in the retina, as well as extra-retinal Hofbauer-Buchner photoreceptors (*HB-PRs*)

27.3.2 Sleep-Wake Cycles in *Drosophila*

Robust rest-activity rhythms are observed in *Drosophila*. Is the fly's rest similar to mammalian sleep? Although there is no correlate to the different sleep states defined by electrophysiology in mammals, behavioral features very similar to mammalian sleep behavior have been described in flies (Fig. 27.7). First, insects have episodes of rest without any detectable movements. Sleep recording is done by counting how many times an individual fly will move in the activity monitor during a 1-min interval. A fly will be considered asleep when not moving for at least 5 min. In laboratory LD conditions, flies sleep at night and take

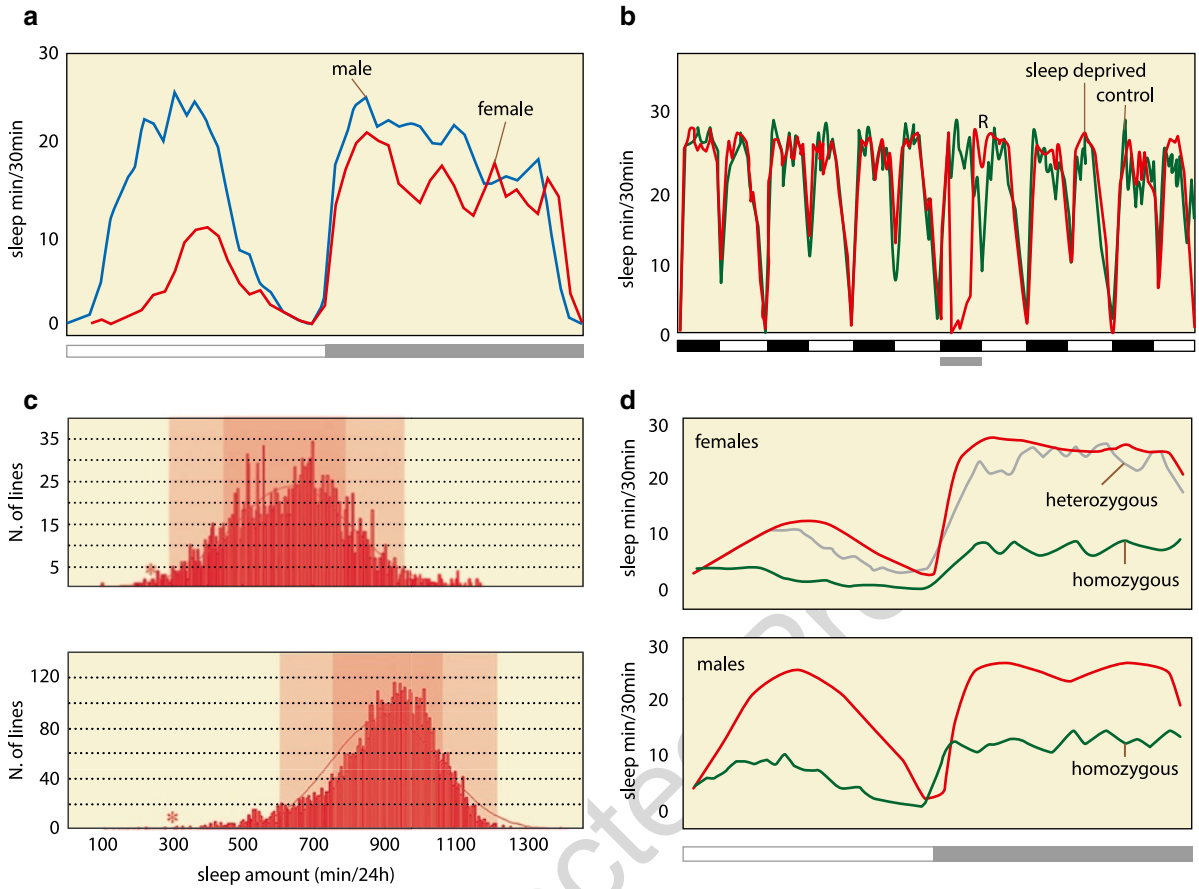

Fig. 27.7 Defining sleep in *Drosophila*. (**a**) Temporal distribution of sleep in wild-type *Drosophila melanogaster*. In 12 h–12 h light-dark laboratory conditions, sleep follows a bimodal distribution with peaks at night and during the middle of the day. Females (*red*) show much less day sleep than males (*blue*). (**b**) When sleep-deprived at night (*gray bar*) fruit flies sleep more (*red line*) than nondeprived controls (*green*) the following day. *R* indicates the sleep recovery period in the sleep-deprived animals. (**c**) Sleep mutants can be identified by genetic screens. Sleep amounts in 9,000 *Drosophila* male (*bottom*) and female (*top*) mutant lines. Mean daily sleep duration is about 900 min in males and 600 min in females. *Shaded areas* show one and two standard deviations from the mean. *Asterisks* show the *minisleep* mutant. (**d**) Daily distribution of sleep amount in wild-type (*red*) and *minisleep* flies (heterozygous in *grey* and homozygous in *green*). *White* and *gray bars* indicate light and dark periods respectively (Modified from Cirelli et al. [22] with permission)

a nap in the middle of the day. Since males show a much stronger mid-day siesta than females, they accumulate a total sleep duration of about 15 h/day, whereas females only sleep for about 10 h/day. Second, responses to mechanical stimuli are reduced during sleep episodes. Third, flies respond to sleep deprivation by increasing their time of inactivity in the following hours, indicating the homeostatic nature of sleep control in insects as it is in mammals.

The possibility of doing large genetic screens in *Drosophila* has been exploited to search for genes involved in sleep control. One of the first isolated sleep mutation was *minisleep*, which reduces sleep duration to about 4 h in females and 5 h in males and also reduces lifespan. The *minisleep* mutation affects the *shaker* gene, which encodes a voltage-dependent potassium channel involved in synaptic transmission [22]. Further studies have revealed that modulators of Shaker channel activity also affect sleep. Other pathways include dopamine and serotonin receptor signaling in the mushroom bodies, a brain structure involved in learning and memory (see Chap. 26). Circadian and sleep pathways intersect in a particular subset of PDF neurons, which increase action-potential firing and behavioral arousal in response to light. This PDF-induced

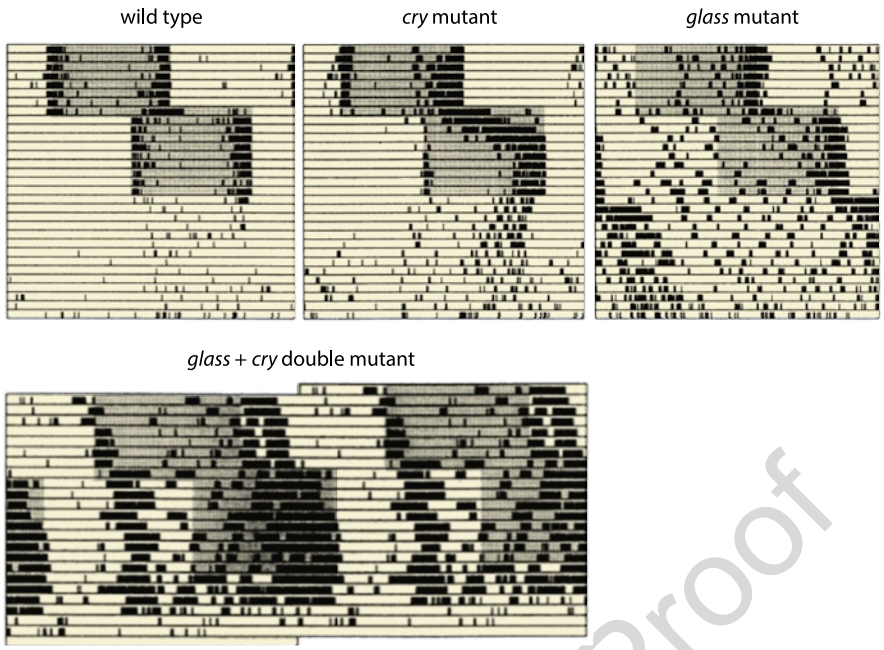

Fig. 27.8 Visual system and cryptochrome synchronize the *Drosophila* clock. Individual actograms of wild-type and mutant flies submitted to an 8 h delay of the environmental light-dark cycle (*dark gray* is lights-ON). A wild type fly synchronizes its sleep-wake rhythm to the new light regime within a day, whereas mutant flies with no CRY protein (*cry* mutant) or no visual system (*glass* mutant) need a few days to complete the resetting of their clock. In contrast, a fly devoid of both the visual system and CRY (double mutant) is not able to synchronize its sleep-wake rhythm to the new LD cycle (Modified from Helfrich-Förster et al. [23] with permission)

arousal is inhibited by GABAergic inputs indicating that light and GABA compete to control PDF-dependent arousal in flies.

27.3.3 Light Entrainment of the Fly Clock

How do day-night cycles synchronize *Drosophila* sleep-wake cycles? A temporal shift of the LD cycle (for example, an 8-h delay would mimic a transatlantic flight from Moscow to New York) will induce a resetting of the sleep-wake cycle that will take place within a day in wild-type flies (Fig. 27.8). A mutation that prevents the differentiation of all photoreceptor cells slows the resetting, but these blind flies nevertheless synchronize to the new LD cycle within a few days. Mutant flies devoid of the cryptochrome protein show a similarly slow resetting. In contrast, double mutants without CRY nor photoreceptors do not seem to synchronize: they appear to be circadianly blind to light-dark cycles [23]. The blue-light-sensitive CRY protein is expressed in most clock neurons. Light-activated CRY binds TIM protein, which then recruits the JETLAG ubiquitin ligase. This will lead to the fast proteasome-dependent degradation of TIM, thus resetting the molecular oscillations. Recent experiments indicate that this cell autonomous mechanism may not be the only way for CRY to reset the clock driving the sleep-wake rhythm, but its nonautonomous function has not yet been characterized. CRY is also expressed in clock cells outside the brain (peripheral clocks), where it acts as a cell autonomous circadian photoreceptor.

Body parts from transgenic *Drosophila* carrying a luciferase reporter gene driven by a *per* gene regulatory region were cultured separately for several days in LD cycles, and showed robust bioluminescence rhythms (bioluminescence is produced by luciferase in the presence of luciferin in the culture medium). In DD, the oscillations of the peripheral clocks quickly dampen, but applying new LD cycles restarted the oscillations in phase with the new LD cycle [24]. CRY thus allows insect peripheral clocks to perceive light inputs and synchronize to LD cycles independently of the brain and the visual system.

Photoreceptors in insects are distributed in several organs. The *Drosophila* compound eye is the main

visual structure and contains about 350 ommatidia that each include six external photoreceptors and two internal ones, in addition to support cells (see Chap. 18). The external photoreceptors express one type of rhodopsin, whereas the internal ones express four different types that are believed to confer color vision. The eye photoreceptors send axonal projections to the optic lobes of the brain, which are in part innervated by the arborization of the PDF neurons. Flies also have a tiny photoreceptive organ, the eyelet, with four photoreceptor cells expressing only one type of rhodopsin. The eyelet is located underneath the retina and sends axons to the optic lobe area where the PDF neurons spread their dendritic arborization.

Finally, three ocelli on the top of the head are involved in horizon detection during flight and express a sixth type of rhodopsin. Experiments using different types of light quality and intensity indicate that the compound eye and the eyelet both contribute to the synchronization of sleep-wake rhythms. Invertebrate photoreceptors use histamine as neurotransmitter, but how the histaminergic signals reset the molecular oscillations in the clock neurons is unknown. The complexity of the *Drosophila* photoreception system suggests that the different cellular oscillators of the circadian network may receive different types of photic information, possibly related with the changes of light quality during the course of the day.

Similarly to light, temperature cycles efficiently reset insect circadian clocks [3]. Although the entrainment pathways remain uncharacterized, recent data indicate that the chordotonal organs (known as mechanical sensory organs) act as sensory organs for temperature entrainment of the behavioral clock. Two groups of clock neurons respond better to temperature entrainment whereas others are more responsive to light. How temperature and light inputs are integrated by the clock is not known yet.

27.4 Neural Organization of the Mammalian Clock

27.4.1 A Master Clock Resides in the Suprachiasmatic Nuclei

In mammals, the circadian clock that controls sleep-wake rhythms resides in the suprachiasmatic nuclei (SCN) of the hypothalamus. The SCN is a bilateral structure that contains about 10,000 neurons in each side of the brain. The SCN is located above the optic chiasma close to the third ventricle of the brain, and receives retinal inputs though the retinohypothalamic tract (RHT) (Fig. 27.9). Lesioning this small region in rodents disrupts the temporal pattern of sleep-wake cycles, as well as body temperature rhythms [25, 26]. Furthermore, the fortuitous finding of a *tau* mutant in hamsters which displays a 20-h circadian period provided a nice tool to perform SCN transplantation studies between animals with different behavioral periods. Grafting an SCN from a short-period *tau* hamster to a lesioned wild-type animal (24-h period before lesion) restored a 20-h sleep-wake rhythm to the arrhythmic host [27]. Similarly, a wild-type mouse SCN graft transplanted into a genetically arrhythmic animal restores 24-h rhythms. A clock in the SCN is thus sufficient to drive sleep-wake cycles in the absence of any other clock-running tissue and to define the behavioral period over all the other clocks of the organism. SCN grafts embedded into semipermeable capsules that prevent neural outgrowth also restored sleep-wake rhythms, indicating that diffusible factors could drive rhythmic behavior [28]. Most SCN neurons are GABAergic neurons, but neurochemical and anatomical analyses have divided the nuclei in two regions. The ventral-lateral region (often named core) receives most of the afferences (in particular those from the visual system), whereas most of the efferences originate from the dorsomedial region (often named shell). The two regions also show differences in their neuropeptide content. Arginine-vasopressin peptide (AVP) is expressed in many shell neurons, whereas the core is enriched in gastrin-releasing peptide (GRP) and vasoactive intestinal peptide (VIP) neurons, with a small region expressing calbindin (CalB). How molecular oscillations control electrical activity in SCN neurons is not clear but circadian regulation of several ion channels at the transcriptional or posttranslational level has been observed, suggesting a possible link. Electrical activity also feeds back to the molecular machinery, as reported in *Drosophila* clock neurons. Electrophysiological recordings indicate that SCN neurons have high electrical activity during the day and low electrical activity at night, in both diurnal and nocturnal animals. Similarly, the SCN shows the same phase of molecular oscillations in diurnal and nocturnal species, although the link between clock protein oscillations and neuronal firing oscillations

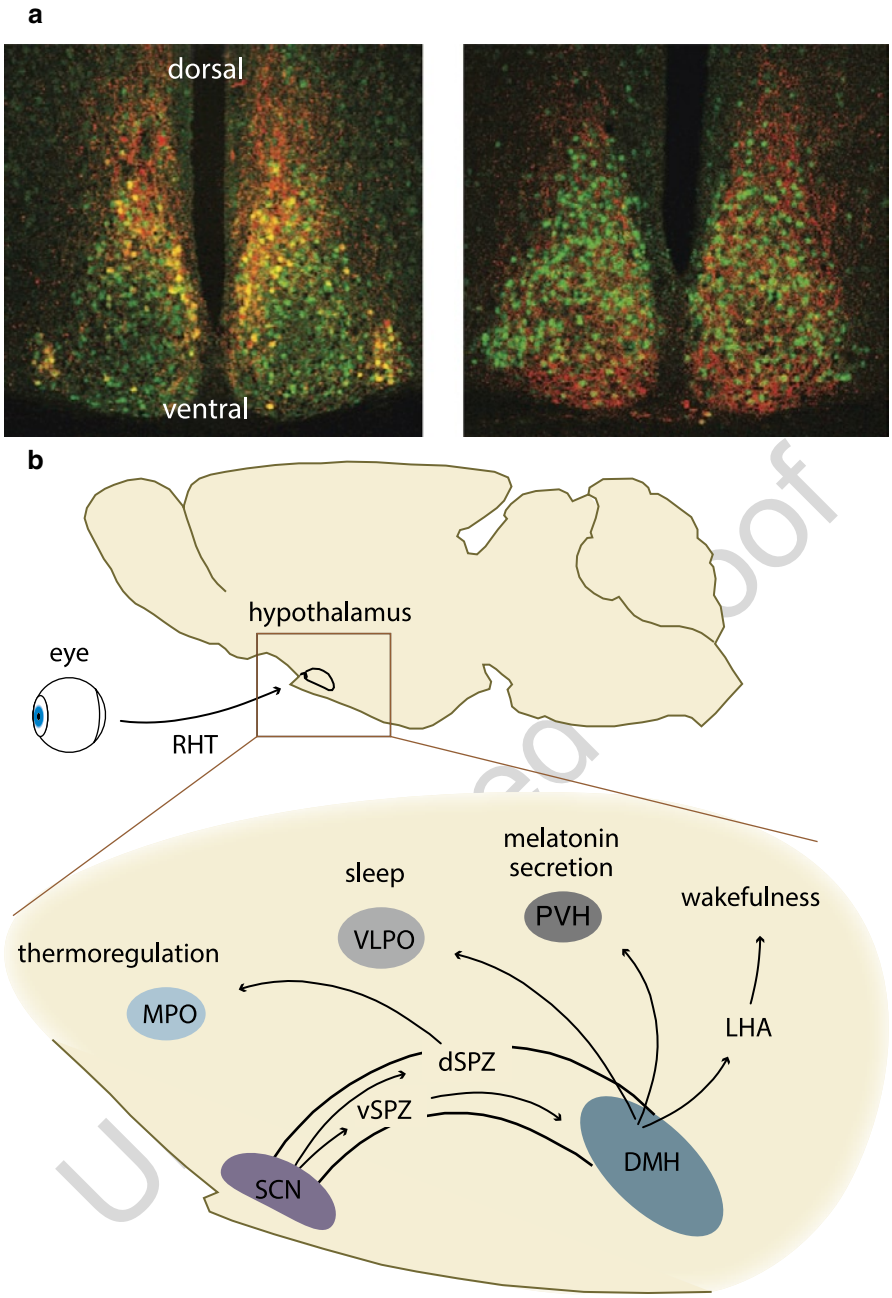

Fig. 27.9 The suprachiasmatic nuclei (*SCN*) control sleep-wake rhythms in mammals. The SCN are two paired nuclei of the hypothalamus located above the optic chiasm. (**a**) The mouse SCN each comprise about 10,000 neurons. Different subsets of SCN neurons can be identified according to the expression of clock genes as well as several neuropeptides. *Green color* identifies neurons that express the CLK protein. *Red* identifies neurons that express either the arginine vasopressin (*AVP*, *left*) or the vasoactive intestinal polypeptide (*VIP*, *right*). *Yellow* indicates co-expression of CLK and the neuropeptide. VIP is more abundant in the ventrolateral SCN, which receives afferences from the retinohypothalamic tract (*RHT*). AVP is prominent in the dorsomedial SCN, which sends efferent fibers. (**b**) SCN outputs mostly target the ventral (*vSPZ*) and dorsal (*dSPZ*) subparaventricular zone. The dSPZ neurons are required for body temperature rhythms through projections to the medial preoptic area (*MPO*). Outputs from the vSPZ are integrated in the dorsomedial nucleus of the hypothalamus (DMH), which send projections to the ventrolateral preoptic area (*VLPO*) to drive sleep-wake cycles. The DMH also targets the paraventricular nucleus (*PVH*) to control melatonin secretion by the pineal gland through an indirect pathway, and send projections to the lateral hypothalamic (*LHA*) neurons to control wakefulness (orexin neurons) and feeding cycles (SCN photograph drawn from Mohawk and Takahashi [33] with permission; drawings adapted from Saper et al. [34] with permission)

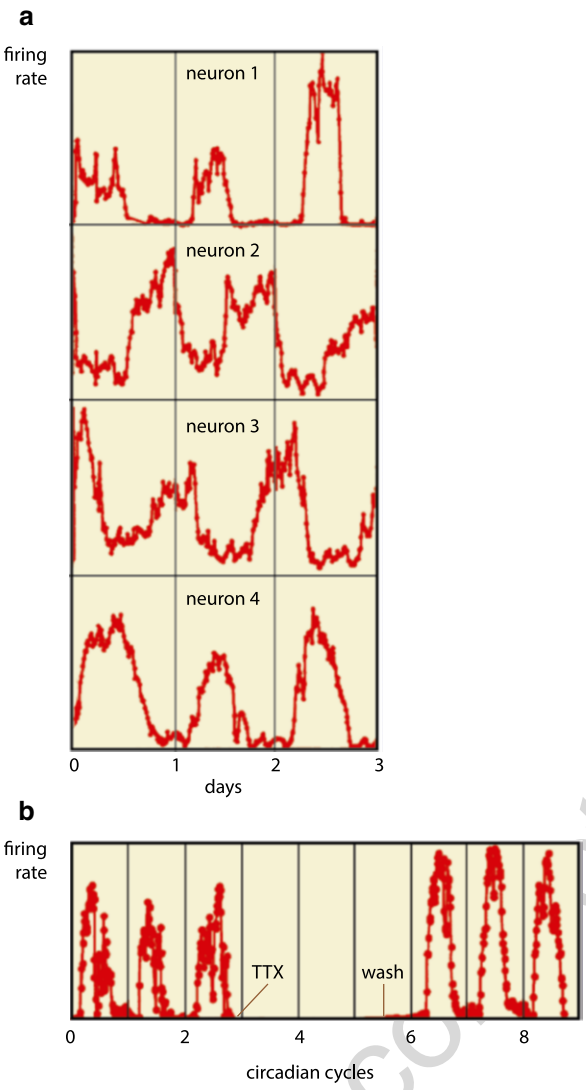

Fig. 27.10 The SCN is made of coupled individual circadian oscillators. (**a**) Recording dissociated SCN neurons in culture shows that individual cells display circadian firing rhythms with different periods and phases. (**b**) After a reversible blockage of neuronal firing with tetrodoxin (*TTX*), isolated SCN neurons resume their firing rhythm with the same phase, showing that the circadian clock keeps ticking in each individual neuron in the absence of firing and synaptic transmission (Modified from Welsh et al. [35] with permission)

is unknown. High SCN activity thus corresponds to behavioral activity in diurnal species and to resting in nocturnal species, indicating that the rest/activity control is defined downstream of the clock.

Isolated SCN slices show firing rhythms in vitro, indicating that it behaves as an autonomous oscillator. The finding of circadian clocks in cyanobacteria or unicellular eukaryotes indicates that a single cell is able to run a molecular clock. Are brain clocks networks of individual oscillators? The answer is yes, **brain clocks contain multiple oscillators that are coupled** (synchronizing each other) to generate synchronous oscillations of neuronal activity.

First evidence for a neuronal clock being made of individual oscillators came from the eye of the sea snail *Bulla gouldiana*, where a circadian clock controls the firing frequency of a small population of retinal neurons. Recording isolated neurons at different times of the day, indicated that each individual neuron displayed a circadian rhythm of membrane electrical conductance, which was higher at night than during the day. Similarly, isolated SCN neurons express rhythms of neuronal firing and keep doing so in the absence of synaptic transmission between cells, indicating a cell autonomous clock property (Fig. 27.10). When isolated, SCN neurons show variable periods and phases of their firing rhythms, indicating that some coupling occurs within the tissue to keep cells in synchrony. Behavioral recording of mice defective for the genes encoding either VIP or the VIP receptor VPAC2 show strongly altered sleep-wake rhythms. The analysis of individual SCN neurons in such animals indicates that some neurons stop oscillating whereas other cells still cycle, but not in synchrony. VIP/VPAC2 signaling is thus a key component for keeping the clock running in some neuronal populations and coupling between the individual oscillators in others. Recent SCN imaging experiments indeed show that in wild-type animals, not all SCN neurons have the same phase, revealing that regional subnetworks may play different roles in the SCN circadian function.

27.4.2 The SCN Controls Sleep-Wake Cycles in Mammals

The output pathways through which the SCN controls sleep-wake rhythms remain poorly understood. SCN efferent projections reach different areas of the hypothalamus where they modulate many neuroendocrine and autonomic pathways. SCN neurons densely project to the area located dorsocaudal to the SCN (Fig. 27.9). This region contains the subparaventricular zone (SPZ) and the dorsomedial hypothalamic (DMH) nucleus, the latter receiving direct innervation

from the SCN as well as indirect SCN outputs through the ventral SPZ. Lesions of the dorsal SPZ specifically disrupts body temperature rhythms. Lesioning ventral SPZ or DMH strongly alters the temporal control of sleep-wake rhythms with affected animals showing several sleep-wake cycles per day (ultradian rhythms). The DMH sends inhibitory GABAergic projections to the ventrolateral preoptic area (VLPO) and excitatory glutamatergic projections to the lateral hypothalamic area (LHA). The VLPO promotes sleep while the orexin-secreting neurons of the LHA promote wakefulness. Since the disruption of the SCN or its hypothalamic targets reduces wakefulness, it suggests that the circadian system promotes wakefulness during the active period [29].

Sleep-wake cycles result from the interactions between two independent processes. First, a homeostatic process, which controls the rise of sleep propensity during waking and its dissipation during sleep. Second, a circadian process, which defines a cycling level of the arousal state. At each time of the day, the behavioral state will depend on the level of these two parameters. Increasing awake time will increase sleep pressure, but sleep onset requires that the circadianly regulated arousal signal is sufficiently low (Fig. 27.11).

The SCN indirectly controls the nocturnal synthesis of melatonin in the pineal gland, with synaptic relays from the paraventricular nucleus of the hypothalamus to the thoracic spinal cord and superior cervical ganglia. A seasonal variation of neuronal firing has been observed in the SCN, with a compressed phase distribution during short days and a broad distribution during long days. The encoding of day length by the SCN thus relies on a variation of the phase distribution of individual neuron firing oscillations, suggesting that day length modulates the coupling between individual SCN neurons. After receiving a signal from the SCN, the pineal gland releases melatonin in the bloodstream, where the hormone reaches receptors in different brain areas, in particular in the SCN itself. Although melatonin has a clear sleep-promoting effect, the mechanisms of its action remain unclear. Furthermore, laboratory mice with virtually no melatonin do not show altered sleep, suggesting that other pathways play a more important role in the control of sleep-wake cycles. The clearest function of melatonin is controlling seasonal reproductive rhythms in many vertebrate species. Here, melatonin acts as a night hormone to interpret the day length (photoperiod) and induce physiological changes, in particular sexual rest or activity. In mammals, this control occurs through melatonin receptors that are located in the pars tuberalis of the pituitary gland.

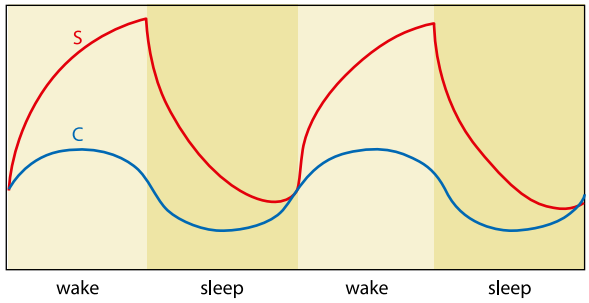

Fig. 27.11 The Borbely sleep regulation model. Sleep results from an interaction between the homeostatic sleep pressure *S* and the circadian process *C*. The sleep pressure builds up during wakefulness and quickly decreases during sleep. The circadian process drives alertness and is linked to the time of the day but does not depend on sleep duration. S and C oppose each other during the day. Sleep occurs when S is high and C is low (Adapted from Daan [36])

27.4.3 Light Entrainment of the Mammalian Clock

As in flies, light is the strongest Zeitgeber for the mammalian clock. Compared to image-forming visual photoreception, circadian photoreception requires higher irradiance and longer stimuli. The SCN gets inputs from the retina through the RHT. However, mice devoid of rods and cones keep synchronizing with LD cycles, suggesting that other photoreceptors can mediate light-driven circadian entrainment. The discovery that a subset of retinal ganglion cells express a new photopigment, **melanopsin**, and directly respond to light provided a candidate photoreceptor for circadian entrainment (Fig. 27.12a) [30].

Retinal ganglion cells receive inputs from rod and cone photoreceptors, which control their firing, and send the visual information to the brain by forming the optic nerve with their axons. The melanopsin-expressing intrinsically photoreceptive retina ganglion cells (ipRGC) represent a small fraction (<5 %) of the retinal ganglion cells which reach the SCN through the RHT. Mice devoid of rods and cones, on one hand, and melanopsin mutants, on the other hand, both synchronize to LD cycles, indicating that each of the two photoreceptive pathways can mediate entrainment of the SCN clock (Fig. 27.12b). In contrast to melanopsin

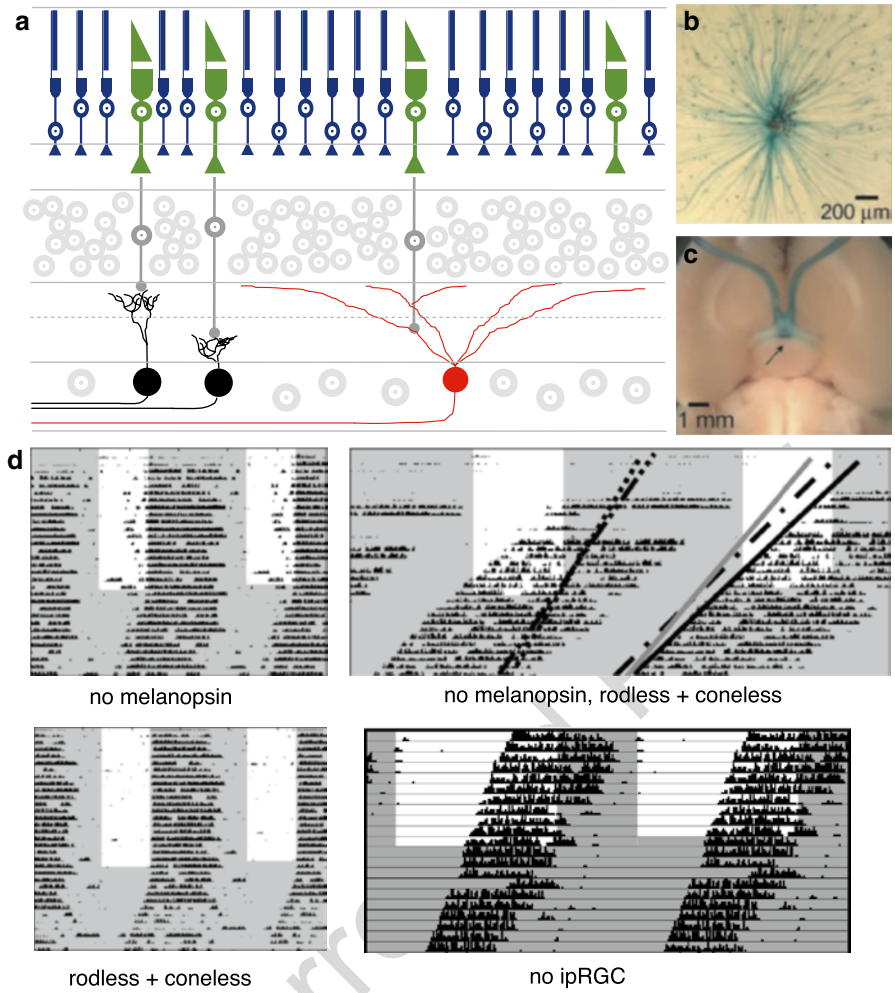

Fig. 27.12 Different types of retinal cells are involved in the synchronization of sleep-wake rhythms in mammals. (**a**) Light goes through the ganglion cells (*black*) and bipolar cells (*gray*) before reaching the rod (*blue*) and cone (*green*) photoreceptors. The rods and cones are connected to the bipolar cells, which then connect the ganglion cells that send their axons in the optic nerve (for better clarity, only cone connections are shown here). A small fraction of the retinal ganglion cells, the ipRGCs (*red*), express melanopsin and act as circadian photoreceptors. (**b**, **c**) Targeting a reporter gene in the melanopsin locus allows to observe ipRGCs and their projections. The ipRGCs (labeled in *blue*) axons gather in the center of the retina (**b**) and join the optic nerve to reach the SCN in the brain (**c**) (From Berson [30] and Hattar et al. [37] with permission). (**d**) Synchronization of sleep-wake rhythms to light-dark cycles in mutant mice devoid of melanopsin, rods and cones or ipRGCs. Opsins of the rods and cones, on one hand, and melanopsin of the ipRGCs, on the other hand, are sufficient to synchronize sleep-activity rhythms. Only animals devoid of the two pathways lose synchronization. However, light inputs going through rods and cones require ipRGCs since mice with no ipRGCs are unable to synchronize their rhythms (From Panda et al. [38] and Güler et al. [39] with permission)

mutants, mice genetically ablated for the ipRGCs do not synchronize anymore. This demonstrates the central role of ipRGCs in circadian photoreception: they receive inputs from rods and cones and have an intrinsic photoreceptive capacity. Different subtypes of ipRGCs have been characterized. Melanopsin is also involved in other non-image forming photoreceptive responses such as pupillary constriction and melatonin suppression. The RHT projects in other retino-recipient brain areas, providing the anatomical substrate for these responses. Recent results also indicate that melanopsin may participate in visual photoreception.

Melanopsin is a blue-light photoreceptor that is structurally and functionally related to invertebrate opsins. The phototransduction pathway that takes place downstream opsins in rods and cones involves the activation of the G protein transducin, which activates a phosphodiesterase, thus inducing a decrease of cyclic GMP levels. This triggers the closure of cyclic nucleotide-gated cation channels, hence the hyperpolarization of the photoreceptor cell. In contrast, invertebrate rhabdomeric opsins such as the *Drosophila* rhodopsins, activate a phospholipase C, which increases levels of the second messenger diacylglycerol. This leads to the opening of transient receptor potential (TRP) calcium channels, hence the depolarization of the photoreceptor cell. Although the melanopsin transduction pathway is not fully characterized, it clearly involves the opening of TRP-like channels that lead to depolarization. Glutamate is the main neurotransmitter of ipRGCs and triggers a cAMP pathway that activates the CREB transcription factor in the ventrolateral SCN neurons. CREB activates the transcription of the *per1* and *per2* genes, leading to a rapid increase in PER protein levels. This transient increase will alter PER oscillations, hence inducing a phase shift of the molecular oscillator.

As indicated above, circadian clocks are present in most cells, which are called **peripheral clocks** by comparison to the SCN. These peripheral clocks do not directly see light and thus require the SCN to be synchronized to LD cycles. How the SCN synchronizes body clocks is not fully understood but a large body of experimental work suggests that humoral factors are involved, in particular glucocorticoids. SCN outputs reach the autonomic nervous system, whose target organs thus receive indirect synchronizing signals from the light-entrained SCN. Recent work has identified body temperature to be a strong resetting signal driven by the SCN to synchronize peripheral oscillators. Some of the transcriptional components that translate the synchronizing signals into changes of the molecular oscillator in various peripheral tissues appear to be shared by several pathways, allowing the integration of various cues to orchestrate circadian timing in the body.

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