Cell clocks

Wolfgang Engelmann
Institut für Botanik, Tübingen

Tübingen 2009

Published at Tobias-lib, University library Tübingen:
URL: http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3797/

2. edition 2009
The first edition appeared in 2009 at http://www.uni.tuebingen.de/plantphys/bioclox,
in the 2. edition text and figures were updated

This book was typeset using LyX, a powerful document processor using the LaTeX typesetting system (http://www.lyx.org). Vectorgrafic illustrations were produced with xfig under Linux. For diagrams PyXPlot was used. I am thankful to Dr. Klaus Reutter, Anatomical Institute of the University of Tübingen, for allowing me to use several figures of preparations.

Contents

List of Figures

1 Introduction

Rhythmic processes are widespread in nature. If you want to inform yourself on rhythms in organisms, I have written the following books, where you find further references: *Rhythms of life:* Engelmann (2007), *How plants grow and move:* Engelmann (2004a), *Flower clocks, time memory and time forgetting:* Engelmann (2008), *Flying Clocks - The clocks of Drosophila:* Engelmann (2009c), *Bio-Calendar - The year in the life of plants and animals:* Engelmann (2009a), *Clocks which run according to the moon - Influence of the moon on the earth and its life:* Engelmann (2009b), *Rhythms in structures of organisms:* Engelmann (2004c), *How to stop a biological clock: Point of singularity:* Engelmann (2004b), Our internal clocks - Biological timing in humans and other mammals: Engelmann (2009d)

In this book rhythms in cells are presented. Cells are the basic units of organisms. Unicellulars consist of a single cell only, whereas in multicellulars several or many cells form a functional unit. Whereas unicellulars have to cope with all demands in just one cell (for instance food uptake, moving around, propagation), most of the cells of multicellulars have specialized to certain tasks and lost their independence. Such diverse cell types form tissues and organs. Cells have different sizes. Normally they are between 1 and 30 micrometers, but an *Acetabularia* (see figure 4.1) reaches several centimeters, the egg cell of an ostrich even beyond 7 centimeters. The egg cell in humans is 110 to 140 micrometers large and the only cell of our body, which is visible with the naked eye.

I present in the first four chapters rhythms in *Cyanobacteria*, the dinoflagellate *Lingulodinium*, the alga *Acetabularia*, and the green alga *Chlamydomonas*. The period length of these rhythms is under day/night conditions exactly 24 hours, under so called freerun conditions[1] about 24 hours. This rhythm is called circadian[2]. Circadian rhythms are synchronized to the 24-hour measure of the day by time cues and their period lengths are only slightly different in the normal temperature range.

In chapter 6 we will get to know the somewhat unusual rhythm of a marine myxamoeba. It reminds of a circadian rhythm at a temperature of 20° Celsius, but at higher temperatures it is much shorter and at lower temperatures much longer. It can, furthermore, not easily be synchronized to the daily cycle. In this very chapter we will elaborate on the yeast *Schizosaccharomyces*, which exhibits not a circadian, but a much shorter rhythm. These rhythms are called ultradian[3].

However, this oscillation is, like a circadian rhythm, temperature-compensated, that is, the period length is at various temperatures the same. In this respect it differs from the normal ultradian rhythms.

Next we have a look at the circadian rhythm of a fungus, the red bread mold

[1] for instance under continuous light or continuous darkness, constant temperature. There are no further time cues, which would provide informations concerning the daylength

[2] from *circa*, Latin about, and *dies*, Latin day

[3] *ultra* Latin below. *dies* day

Neurospora crassa. This mold forms a tubular branched structure with numerous nuclei, but without cell walls (called syncytium). It is a kind of giant cell. Numerous molecular-biological studies have been performed on this fungus. Therefore the molecular mechanism of the circadian clock is quite well understood. We could furthermore deal with circadian as well as ultradian rhythms in various animal unicellulars such as *Paramecium*, *Tetrahymena pyriformis* and *Acanthamoeba*. Perhaps I will add this in a later edition.

Finally in chapter 7 the daily rhythms in the SCN of mammals, eye clocks, oscillators in the pineal organ, in fibroblasts and other cells of mammalian tissues and organs are treated. In this chapter we have among others a look at the control of processes such as glucose transport, gluconeogenesis, lipolysis, adipogenesis and oxidative phosphorylation in the mitochondria by circadian clocks.

If the timing of tissues and/or organs is disturbed in humans, if the time of food uptake, of activity and of sleep is altered, diseases such as sleep disturbances, diabetes and obesity might occur. It is therefore important, to understand the synchronization of these tissue clocks (Kohsaka and Bass (2007)). This will be treated in some examples in chapter 8.

2 Cyanobacteria

Prokaryonts possess circadian rhythms. They show up for instance in photosynthesis, nitrogen fixation, carbohydrate synthesis and cell division. A luciferase-expressing reporter gene was introduced into the chromosome, which allowed to recognize easily the circadian clock controlled rhythm by the bioluminecence. Numerous mutants were found, in which the circadian rhythm was altered or lacking. Genes were identified, which participate in the clock work. In this way the mechanism of the clock could be studied and a model put forward. Such mutants were also used for testing the adaptive significance of circadian rhythms. Time cues such as the light-dark cycle are perceived by receptors and transferred to the clock, in order to synchronize them with the day-night rhythm.

If we want to study the mechanism of circadian control systems and to understand it finally, it is advantageous, to use a system as simple as possible. It should, of course, possess a circadian clock work, but should not be complicated, in short, it should be a 'minimal system´. This organism should have a simple structure and physiology. It should be easy to cultivate and to experiment with. Furthermore it should be amenable to molecular-biological methods. Finally the hands of the clock should be easily recordable.

Cyanobacteria possess all these properties and are therefore especially well suited for circadian studies. The cells of prokaryonts are smaller, build more simple and the metabolism is much less complicated as compared to the eukaryonts. There is only one ring-like chromosome and the genome is smaller. The regulation of transcription and translation is simpler and better known in respect to eukaryonts.

Many laboratories study *Cyanobacteria* with molecular biological methods. The smaller genome allows saturating mutagenesis of the genes, which are necessary for the function of the circadian clock (Mackey and Golden (2007)). Furthermore, in the meantime the genome of some cyanobacteria has been sequenced completely (Kaneko et al. (1996), general information on prokaryonts in Lengeler and Drews (1998)). Numerous mutations in the circadian system have been found.

2.1 Circadian rhythms

Circadian rhythms in cyanobacteria were detected first in *Oscillatoria* (figure 2.1 and Stal and Krumbein (1985a)) and later studied intensively in *Synechococcus*- (see figure 2.2) and *Synechocystis*-species. Nitrogen fixation[1] and photosynthesis of these cyanobacteria occur in a light-dark cycle in a way as shown in the left part of figure 2.3 (from Mitsui et al. (1986)). Nitrogenase-activity is high during the night, where photosynthesis does not take place. This

[1] numerous cyanobacteria are able to fixate nitrogen of the air. They play therefore an important role in the nitrogen-cycle between water/soil, plants, animals and the atmosphere. However, quite a number of cyanobacteria separate nitrogen fixation from photosynthesis by fixating nitrogen in special cells, the heterocysts.

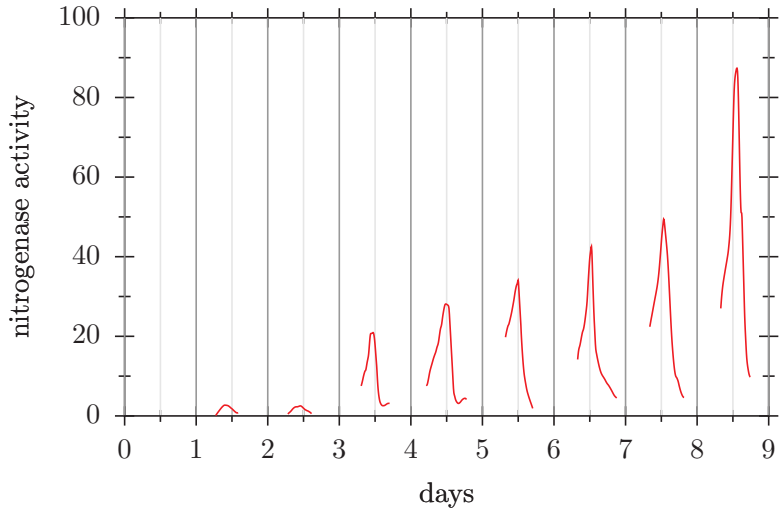


Figure 2.1: *The nitrogenase-activity of an* Oscillatoria *species was recorded for eight days in continuous light by measuring the acetylene reduction. A circadian rhythm was found. After Stal and Krumbein (1985b)*

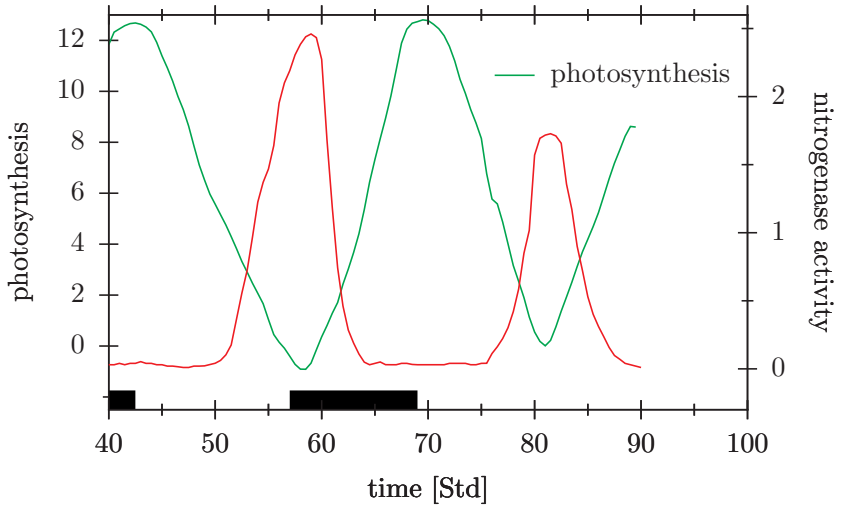


Figure 2.3: *Circadian course of oxygen production during photosynthesis (green curve) and nitrogen fixation by nitrogenase (red curve) in* Synechococcus *under a 12:12h light-dark (left, dark period: black bars) and under continuous light (right). Nitrogenase-activity is high, while photosynthesis is low*

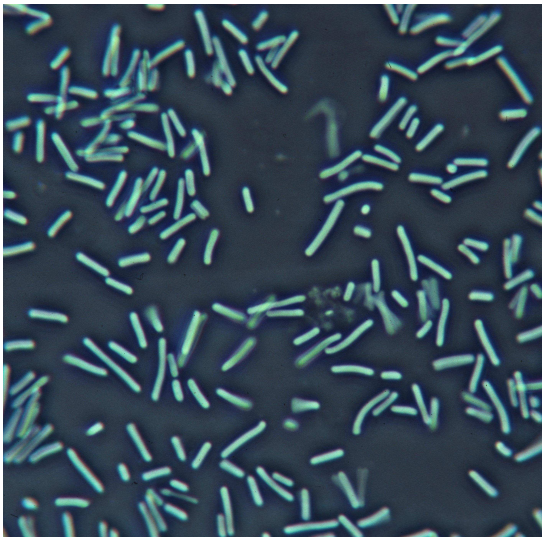


Figure 2.2: *Cells of* Synechococcus elongatus PCC 7942, a cyanophycea. *Figure was kindly supplied by Takea Kondo, Nagoya University, Japan.*

is understandable, since the enzyme nitrogenase, which is responsible for the nitrogen fixation, is inhibited by oxygen, which occurs during photosynthesis. Interestingly the nitrogenase activity is also high under continuous light (right part of figure 2.3), while photosynthesis is weak, namely at phases of the light-dark cycle at which night would prevail. Apparently in *Synechococcus* a circadian clock takes care, that even under continuous light both events are separated from each other. Likewise carbonhydrate synthesis occurs rhythmic under constant light conditions (weak continuous light), that is, it is controlled by a circadian clock (Mitsui et al. (1986)).

Finally cell division is under control of a circadian clock (figure 2.4 top). This poses an interesting question: Many cyanobacteria divide under optimal conditions faster than 24 hours. Does the circadian control of division continue to run in spite of this? That is indeed the case, as shown in figure 2.4 for *Synechococcus*. Although the cells double every 11.8 hours, the *circadian* rhythms continue.

A further hand of the circadian clock can be recorded with a pH-meter. The rhythm can, however, be detected only by using a special mathematical treatment: The medium is acidified by *Synechococcus* in a circadian pattern (Kippert and Lloyd (1995), figure 2.5). This occurs stepwise, whereby the steps occur in about 24 hour intervals; the medium is thus more and more acidified (in the figure the trend was removed and only the differences plotted). The acidification could be caused by the activity of proton pumps or could rely on other proton transport mechanisms.

Transport processes are influenced also by a circadian clock in the uptake of various amino acids by *Synechococcus*. The uptake rates fluctuate in a circadian manner (Chen et al. (1991)).

All these various oscillations possess already the typical properties of circadian rhythms of eukaryonts: The show freerun under constant conditions, are synchronizable by time cues (for instance a 12:12 hour light-dark cycle), and they are temperature-compensated (the period lengths depends only slightly on the environmental temperature).

2.2 Luciferase-expressing *Synechococcus*

If a suitable hand could be found for the circadian clock, cyanobacteria such as *Synechococcus* or *Synechocystis* would be almost ideal organisms for searching for mutants in the circadian system. The circadian hands of *Synechococcus* known so

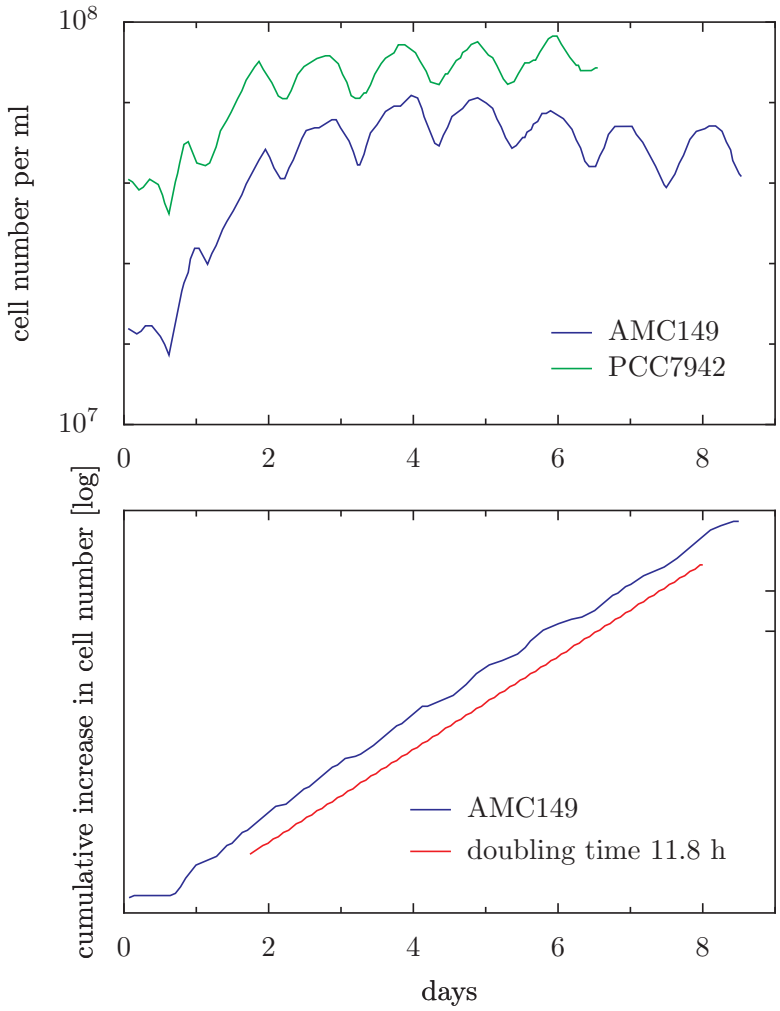


Figure 2.4: *Cell division in* Synechococcus *PCC7942 (green curve upper diagram, period lengths 24.0 hours) and AMC149 (blue, period lengths 25.2 hours). Lower diagram: Blue curve from the upper diagram redrawn as cumulative increase in cell number ('logistic growth curve', calculated from the dilution rate and the cell number). Red curve: Doubling time of cell division. Curves show, that in spite of a doubling time of 11.8 hours only,* the circadian *rhythms continue to run (steps in the blue curve). After Mori et al. (1996)*

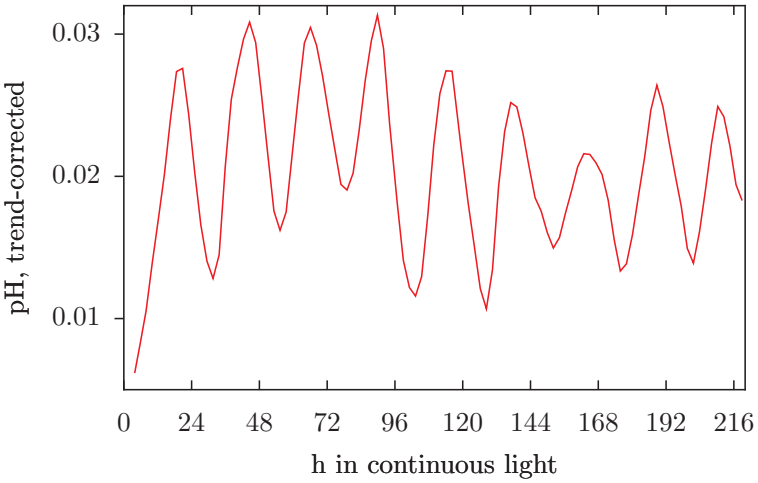


Figure 2.5: *The pH of the medium is modulated by* Synechococcus *in a circadian pattern. The pH values were trend-corrected and the differences plotted. From time 0 onward continuous light was given, before time 0 a 12:12 hour light-dark cycle. After* Kippert and Lloyd (1995)

far (last section) were, however, too elaborate for recording. Therefore an elegant trick was used, which has been applied successfully already in other organisms for studying the circadian clock: Behind a promoter, which is controlled by the circadian clock (originally the promoter of the psbA1 gene, which expresses a main component of the reaction center of the photosystem II; later a large number of other promoters) a reporter gene was appended, which codes for a bacterial luciferase. In this way rhythmic luminescent cyanobacteria were obtained (Kondo et al. (1993)). Now the running of the circadian clock could be followed in quite a number of populations simultaneously: The cells were grown in Petri dishes and mounted on a turntable ('Kondotron', Kondo and Ishiura (1994)). They could be photographed with a light-sensitive camera in equal intervals. Golden (2007) used a Packard lumimometer for recording the luminescence. The images were analyzed by an imaging program, where the intensity of the luminescence reflected the circadian rhythm. Using a microscope, even rhythms of individual cells of *Synechococcus elongatus* could be recorded (Mihalcescu et al. (2004)).

With the help of this new hand of the circadian clock a number of important questions could be clarified. For instance two cultures were kept in light-dark cycles, which were phase shifted against each other by 12 hours (while one of the cultures was in the light periode, the other one was in the dark period). Afterward both cultures were transferred into continuous light and the luminescence rhythm recorded. As seen in figure 2.6, the rhythm is indeed endogenous and not just brought about by an external time cue (which was perhaps present in spite of the continuous light). This rhythm was detected also in continuous darkness (Aoki et al. (1997)). Normally the rhythm would damp out rapidly in the dark, but if glucose is added to the substrate, it is running for at least 7 days. With light pulses given during

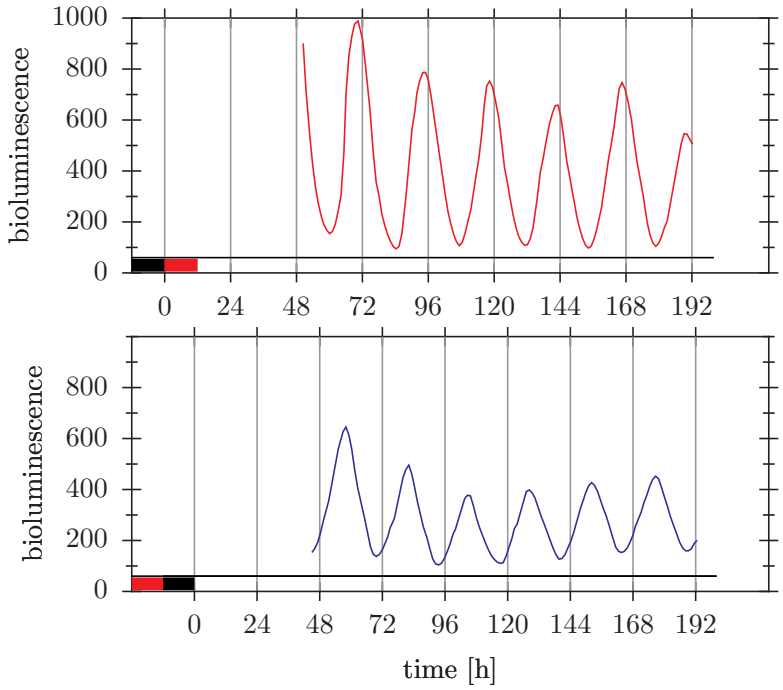


Figure 2.6: *Two cultures of* Synechococcus *were reared in an opposite 12:12 hour light-dark cycle at* 30^0C *in such a way, that one of the cultures (red) was illuminated, while the other one (blue) was in the dark. Therefore the former culture entered the continuous light 12 hours earlier (upper time axis). The luminescence in the cultures fluctuated with a 12 hour phase shift between each other. Under constant light conditions the phase shift of the luminescence rhythm is preserved. After* Kondo et al. (1993)

the continuous darkness the rhythm of the cultures is shifted (figure 2.7, Aoki et al. (1997)). With appropriate mutants the transduction path of the light can be followed, by which finally phase and amplitude of the clock are influenced.

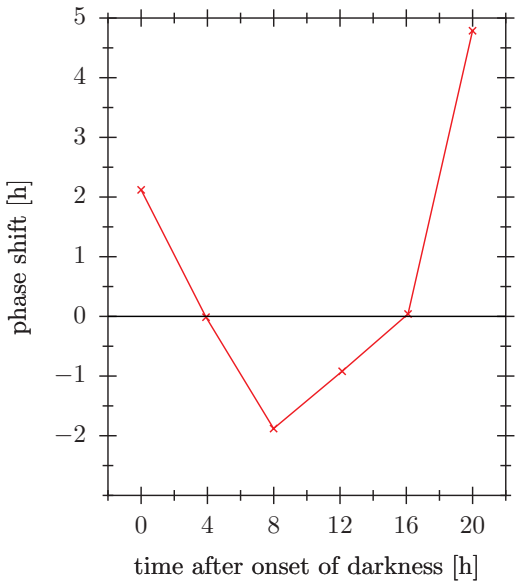


Figure 2.7: *Light pulses shift the circadian rhythm of bioluminescence* of Synechocystis *in continuous darkness differently, depending on the time of application. Advances of the rhythm above, delays beyond the zero line. After* Aoki et al. (1997)

The genome of *Synechocystis* has been sequenced in the meantime entirely (Aoki et al. (1997)). It contains several molecules, which could be responsible for the transduction of the light: Photoreceptors (for instance a phytochrome), a two-component-system, which transfers a signal, and adenylat cyclase. If certain genes are knocked out or over-expressed, it can be found out, whether the genes, coded by these molecules, are involved in the transduction path of the light.

At different environmental temperatures the period length of the circadian oscillator of *Synechococcus* is almost identical (Kondo et al. (1993), Sweeney and Borgese (1989)). The circadian clock of this cyanobacterium is thus temperature-compensated (figure 2.8). The Q_{10}-value is around 1.1 and thus in a range, which is characteristic for daily rhythms of eukaryonts.

2.3 Finding mutants

To find clock mutants, firstly mutations have to be induced by treating cyanobacteria with a mutagenic substance and colonies of many treated individual cells reared on agar plates. Secondly the circadian rhythm has to be measured. For this purpose the bioluminescence (see section 2.2) is recorded with a sensitive video camera every 30 minutes and evaluated for each colony separately (Kondo and Ishiura (1994)). Most of the clones do not show any change in the properties of their circadian clock. But some of them exhibited different period lengths or amplitudes of the circadian luminescence (figure 2.9).

2.4 The clock work of the circadian system

How does the circadian clock of these prokaryonts work? Three things facilitate the attempt, to find out, how the clock work operates: The simple method for recording the circadian rhythm, the clock mutants obtained, and the molecular genetic techniques.

Originally it was assumed, that similar to the circadian clocks of plants, fungi and animals the circadian system of cyanobacteria consists of a negative feedback loop,

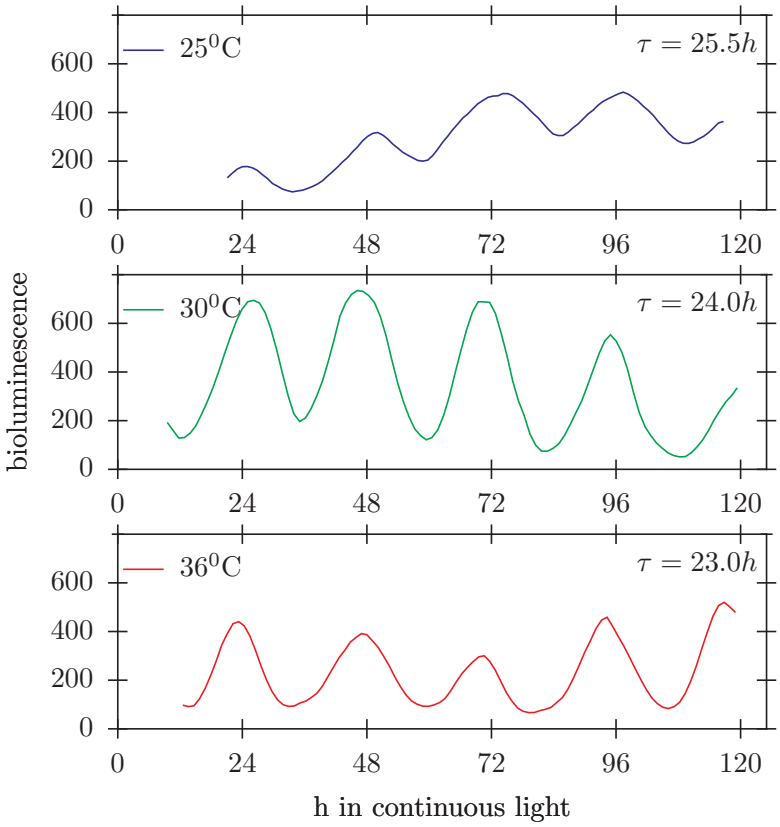


Figure 2.8: *Luminescence rhythm of transgenic* Synechococcus *cultures in continuous light (after 12:12 hour light-dark cycle) at various temperatures of the sea water (upper curve:* 25^0 *C, second curve:* 30^0C*, bottom curve:* 36^0C*). Although the amplitude is smaller at lower and at higher temperatures, the corresponding period lengths (25.5, 24.0 and 23.0 hours) differ only little. The bioluminescence rhythm is thus temperature compensated. After* Kondo et al. (1993)

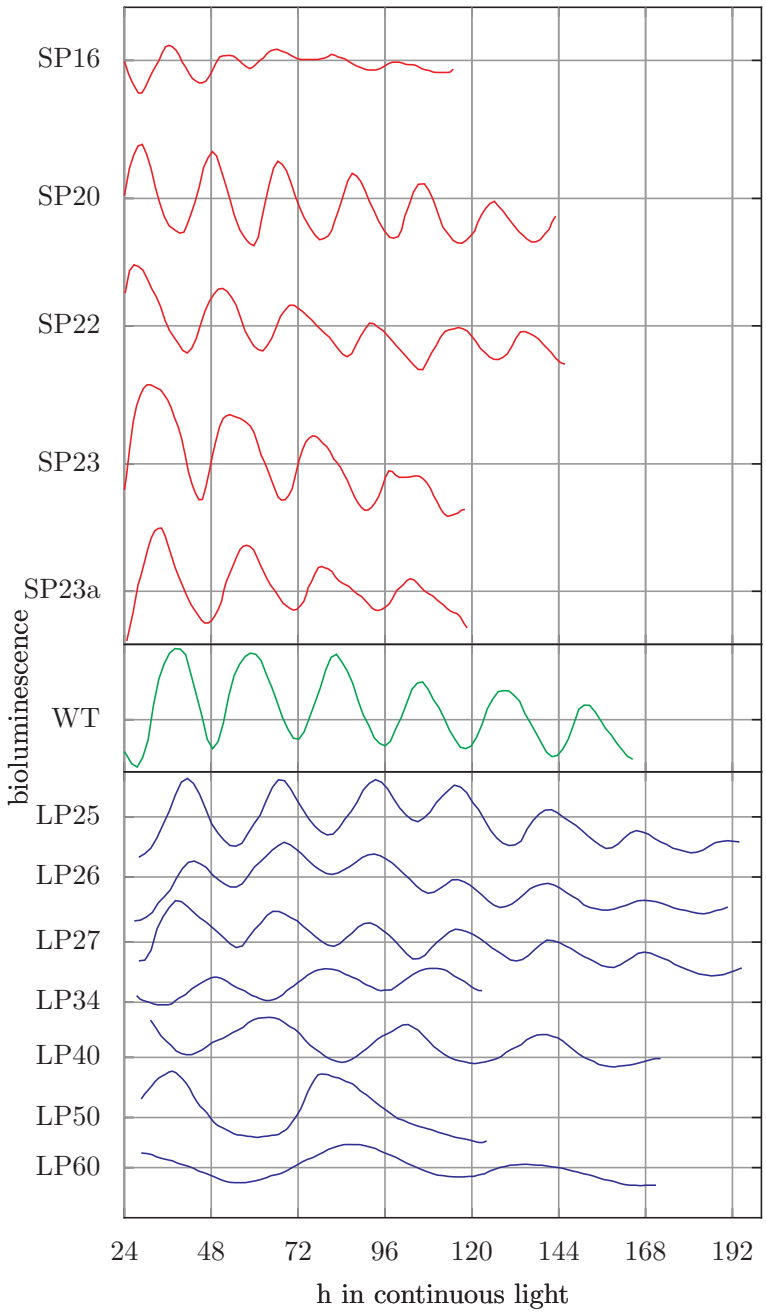


Figure 2.9: *Circadian rhythm of the bioluminescence of transgenic* Synechococcus *cyanobacteria ('wild type', green curve) and of mutants with different period lengths (from 16 to 60 hours length; red curves shorter, blue curves longer periods as compared to the wild type). After* Kondo et al. (1994)

in which the products of the genes kaiA, kaiB and kaiC inhibit the transcription of these genes (Ishiura et al. (1998), Golden (2007)). It turned out, however, that the Kai proteins themselves form the circadian clock, which operates *without transcription and translation* (Xu et al. (2003), Nakajima et al. (2005)).

The molecular clock mechanism is shown in figure 2.10 (Ivleva et al. (2005), Iwasaki and Kondo (2004), Golden and Canales (2003), Akiyama et al. (2008)). The phosphorylation and dephosphorylation of KaiC occurs also *in vitro* with a 24 hour period and without damping for at least three cycles[2], if Kai-proteins and ATP are present. KaiA causes KaiC to auto*phosphorylate*. KaiB prevents this, causing KaiC to auto*dephosphorylate*. KaiC phosphorylation and dephosphorylation constitutes the molecular clock of the circadian system of *Synechocystis*. The circadian rhythm in metabolism and in physiology are thus produced by the Kai-*Proteins* and not by the kai-*promotors*[3]. The molecular structure of the three Kai-proteins is known, and so is their dynamic interaction. However, acording to studies of Kitayama et al. (2008) transcription and translation seems also to be important for materializing the rhythm: At lower temperature the circadian rhythm is found only, if the phosphorylation cycle of KaiC *and* the transcription/translation are in step. Furthermore KaiC is also rhythmic in KaiA-overexpressing mutants and mutants without phosphorylation. Thus, for a robust and precise rhythm a multiple coupled system is responsible, which is based on the biochemical properties of KaiC (Brunner et al. (2008)).

A-loops of the C-end of KaiC are the switches, which are relevant for the KaiC-activity. In the hidden state the autophosphatase, in the open state the autokinase of KaiC is effective. A dynamical state between both conditions determines the equilibrium. KaiA stabilizes the exposed state of the A-loop by a direct binding. Without this binding and stabilization KaiC is under-phosphorylated. KaiA and KaiB thus shift the dynamic equilibrium of the A-loops between an exposed and a hidden state, whereby KaiC is active either as an autokinase or as an autophosphorylase. The exposed A-loops get ATP closer to the phosphorylation sites. Details of the proposed mechanism in Kim et al. (2008), Brunner et al. (2008). The ATPase activity of KaiC is, by the way, extremely weak (Dong and Golden (2008)).

The period lengths is mainly determined by KaiC, since period mutants contain certain amino acid substitutions in the KaiC protein. The periods of these mutants range from 14 to 60 hours. KaiC forms together with ATP a homohexamer (which can be seen under the electron microscope (Mori et al. (2002)). A long period mutant kaiA1 strengthens the interaction of the Kai proteins. The biochemical function of the Kai proteins is not known. KaiC possesses two ATP/GTP binding domains, which play an important role in producing the rhythm (Nishiwaki et al. (2000)). The histidin kinase SasA interacts with KaiC and is necessary for a robust circadian rhythm (Iwasaki et al. (2000)).

This simple protein clock explains also, why and how a circadian timer in cyanobacteria can function even with a generation time of eight hours or less (Kondo et al. (1997)) and how cell division

[2] and can be measured by small angle x-ray scattering and low resolution shapes of KaiA-KaiC respectively KaiB-KaiC (Akiyama et al. (2008))

[3] even an *Escherichia coli* promotor works, provided it possesses enough RNA polymerase activity

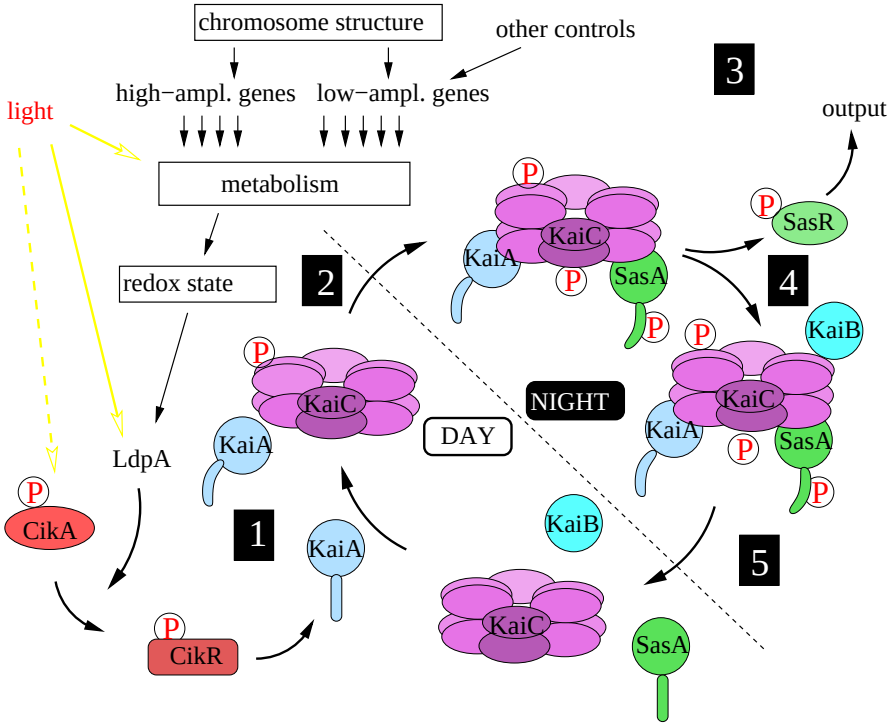


Figure 2.10: *Model of the circadian clock of cyanobacteria: 1: Light influences the metabolism and via plastoquinone (in strong light reduced, in weak light or in darkness oxidized) the redox state (alternatively light is sensed by LdpA directly and/or by CikA). A signal chain LdpA/CikA/CikR influences the sensor domain (straight tail) of the KaiA, which interacts with KaiC and stimulates its autophosphorylation and hexamer formation. 2: In the early evening SasA combines with the KaiA/KaiC complex and KaiC stimulates the phosphorylation of SasA. Its phosphate group is transferred to the response regulator SasR. 3: SasR and other proteins in the output transfer the time information from the oscillator (the KaiA/KaiB/KaiC complex) to the genome by influencing the chromosome structure (upper box) and the metabolism (below) via high amplitude- and low amplitude expressing genes (and other regulations). 4: In the late evening KaiB binds to KaiC and dephosphorylates it. Conformational changes of KaiA and SasA by signal transfer are indicated by straight or curved tails of the symbols. After Golden and Canales (2003) and personal communication with Takao Kondo, Nagoya University*

can still be controlled by a circadian clock (Mori et al. (1996)).

The circadian oscillator controls the entire metabolism and is based on the integration of the cellular metabolism (Nakahira et al. (2004)). The global circadian control of gene expression in *Synechococcus* affects at least two classes of clock-regulated genes: About 80% of the tested promoters are day active with a maximum at the end of the day. In the smaller group the expression has an opposite phase and is maximal in the evening and in the night, while the chromosomes are compact. These genes could for instance code for oxygen-sensitive enzymes, which would function optimally during the night, at which time photosynthesis does not take place. A specific phase element does not exist. Instead the chromosome dynamic or the DNA topology seems to determine phase (Min et al. (2004)).

An important question is, how this clock mechanism allows a global circadian gene expression. The circadian clock seems to determine also the condensation or the supercoiling condition of the chromosomes (Nakahira et al. (2004), Thanbichler et al. (2005)) and to control in this way the access to the promoter elements (Smith and Williams (2006)).

Since cyanobacteria differ so heavily from eukaryonts, the question is, whether the circadian rhythm of both groups is based on the same mechanism. According to the endosymbiont hypothesis the chloroplasts arose from cyanobacteria. If circadian clocks were transferred in this way, the circadian systems of plants and cyanobacteria should be more alike as compared to fungi and animals. If the functions of the genes involved will be better known, this question might perhaps be answered. According to the present state it is, however, more likely, that circadian rhythms did not evolve from a common ancient mechanism. It is more likely, that they were invented several times during various periods of the evolution.

2.5 Inputs of the clock

What are the inputs of the circadian clocks? Circadian clocks have to be synchronized with the periodic environmental factors by time cues. We expect, that the daily light-dark cycles, but also temperature cycles and perhaps also other 24-hour-rhythms of the environment can synchronize. Light-dark-cycles (Aoki et al. (1997)) and temperature-cycles (Lin et al. (1999)) do indeed synchronize the *Synechocystis*-clock. A three hour light pulse offered during continuous darkness advances or delays the rhythm, and the amount depends on the strength of the rhythm and on the phase of application. The results of this kind of experiments served to establish an action spectrum for phase shifts (Inouye et al. (1998)). Red and blue light are the most effective ones (Kaneko et al. (1996)). Light synchronizes probably via the redox state in the metabolism, which is adjusted by photosynthesis: If plastoquinone is oxidized, LdpA becomes active. At higher light intensities plastoquinone is reduced and LdpA inactivated. LdpA interacts with the clock proteine KaiA (Ivleva et al. (2005), see also figure 2.10). The signal chain of the light which synchronizes the clock is not yet known, but there are several candidates derived from the genome sequences such as a phytochrome, a two component system and adenylate cyclase. Furthermore bacteriophytochrome CikA (cir-

cadian input kinase, a histidine proteine kinase) seems to be a step on the path of the light signal to the oscillator (Schmitz et al. (2000)), which is perhaps a redox sensor (Golden (2007)). This sensor is important for phase shifts. The mutant is blind for dark pulses. Furthermore a bacterial cryptochrome was found (Hitomi et al. (2000)). Some genes, which are involved in the photosynthesis of *Synechococcus* and *Synechocystis*, are regulated by light (Golden et al. (1995), Kumar et al. (1999)), as for instance the rbcL gene, the catalytic subunit of the key enzyme of the light dependent CO_2-fixation (Chow and Tabita (1994)). Maximal nitrogen fixation occurs during the night, as mentioned already. In the dark period nitrogenase is synthesized, but in the light it is rapidly dismantled, perhaps by a protease.

2.6 Outputs

Circadian clocks control various processes at a transcriptional, translational, biochemical and physiological level. Some of these events in cyanobacteria were already mentioned. Others are known, many have still to be discovered. Thus it would be interesting, to study movements of cyanobacteria in respect to a circadian rhythm. Vertical movements, which occur in some cyanobacteria with the help of gas vacuoles, have been mentioned. It would be worth to look for a circadian modulation of these movements.

The circadian clock controls in cyanobacteria globally the genes by regulating the transcription via the chromosome status (compaction) (Golden (2007)). Thereby SasA and RpaA play a role (Dong and Golden (2008), the *Functional Genomic Project* Holtman et al. (2005). In addition there must be transfactors, which take care of the different phase positions and amplitudes (see figure 2.11, but see also page 14).

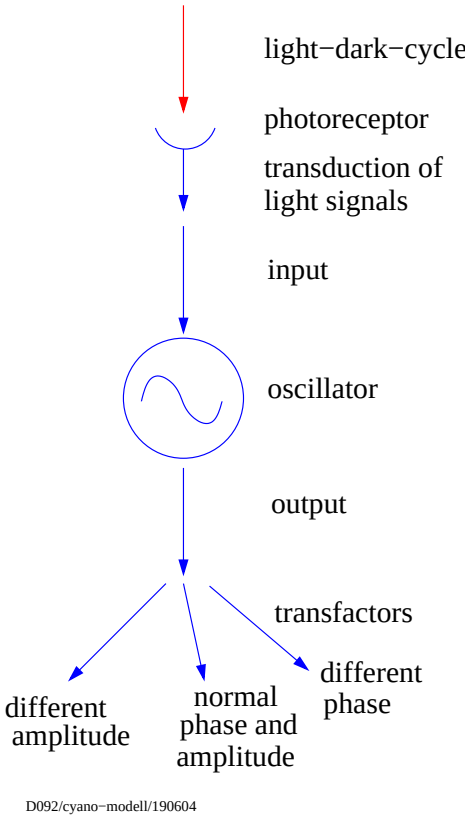


Figure 2.11: *Model of the circadian gene expression of* Synechococcus. *The circadian oscillator is synchronized by time cues such as the light-dark cycle. An output of this oscillator influences the transcription globally (normal phase and amplitude). Trans-factors can additionally modify the amplitude and phase position of the rhythm. After Ishiura et al. (1998)*

Genes, which are not part of the circadian clock, but controlled by it, are for instance rpoD2. It codes for a sigma 70 transcription factor[4]. RpoD2 seems to be a fac-

[4]transcription factors are made by 'switch genes'

tor, which increases the amplitude of the circadian oscillation of some genes (Tsinoremas et al. (1996)).

In 1994 in more than ten polypeptides of *Synechococcus* RF-1 a circadian regulation was demonstrated (Huang and Pen (1994)). In the meantime there are many more (Holtman et al. (2005)). However, this regulation is complicated and not yet well understood. The global regulator gene ntcA, which codes for a DNA binding protein NtcA, is a transcriptional activator of genes, which are under circadian control and have to do with nitrogen assimilation (Bradley and Reddy (1997)).

2.7 Adaptive significance of circadian rhythms in *Cyanobacteria*

What is the adaptive significance of a circadian clock in *Cyanobacteria*? In nitrogen-fixating *Cyanobacteria* it takes care that photosynthesis and nitrogen fixation occur at different times of the day. This is important, since oxygen, which is produced during photosynthesis, inhibits nitrogenase, the key enzyme of nitrogen fixation. However, this separation in time does not seem to be necessary in all cases (Ortega-Calvo and Stal (1991), Roenneberg and Carpenter (1993)). Diazotrophs are thus able to use completely different mechanisms, in order to protect nitrogenase from oxygen (see Gallon (1981) and Gallon (1992)).

An important function of the circadian clock in *Cyanobacteria* is probably 'warning of light'. The photosynthetic apparatus of the *Cyanobacteria* is especially susceptible to light damages. Therefore it is advantageous to be protected by events, which are controlled by the circadian clock.

It was shown, that mutants with shorter period lengths as compared to the wild type (the period of which lies around 25 hours), are rapidly supplanted in a 25 hour day (12.5 hours light, 12.5 hours darkness) by the latter, whereas in a 22 hour day the mutant outscores the wild type (see Johnson et al. (1998) and figure 2.12).

and influence the transcription of other genes, such as for instance genes which are involved in the clock mechanism.

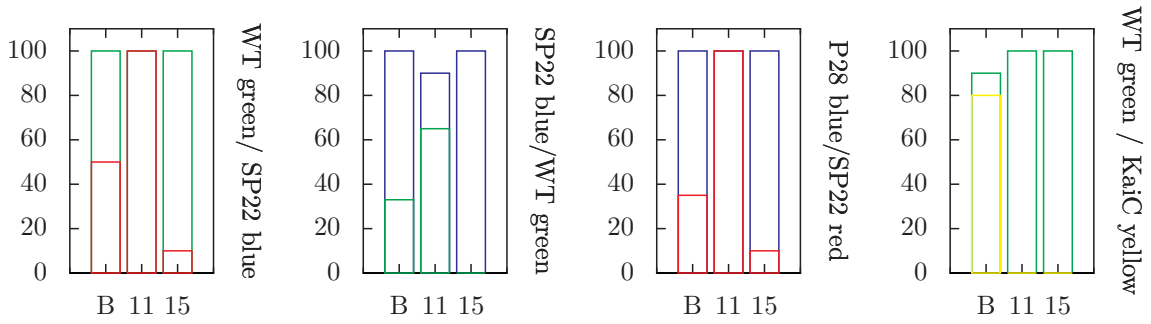


Figure 2.12: *Selection experiments of Johnson et al. (1998). Left: Mixture of wild type (freerun period 24 hours) and period mutant SP22 (freerun period 22 hours) kept in 22- (11:11 hour LD) and 30-hour-days (15:15 hour LD). After 27 cycles of 22-hour days the wild type was more numerous as compared to the mutant. In a 30-hour day the mutant had supplanted the wild type almost completely. Next diagram: Mutant P28 (freerun period 28 hours) has outscored the wild type in a 30-hour day after 27 cycles almost completely, in a 22-hour day however it was supplanted by the wild type. Third histogram: P28- and SP22-mutants mixed with each other. In 22-hour cycles SP22 outscores the mutant P28, in a 30-hour day it was reversed. Right: The arrhythmic mutant KaiC is outscored by the wild type both in 22-hour- as well as in 30-hour days. Abscissa: Ratio of the mixed groups in percent. After Johnson et al. (1998)*

3 Rhythms in *Lingulodinium*

In the unicellular alga Lingulodinium *some circadian rhythms were studied such as the bioluminescence, the aggregation of cells, cell division, and photosynthesis. Metabolism and a number of enzymes are under control of the circadian clock. Light synchronizes these rhythms.*

Various dinoflagellates as for instance *Lingulodinium* emit light during the night. These marine algae belong to the division of *Dinophyta* (armored algae), and here to the class of the *Dinophyceae* and to the order of *Peridiniales*. The algae are 1/20 mm in diameter and possess a cellulose skeleton with an equatorial and a longitudinal rim (figure 3.1). In each rim is a flagella,

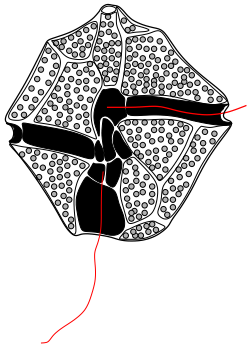


Figure 3.1: Lingulodinium polyedrum *cell with theka (cellulose armour), a transverse and a longitudinal rim, with a flagella in each of them. Ventral view. Diameter 40 µm. After Schussnig (1954) and an electron microscopic image from* Hastings (2006)

which is used by the alga to move laterally and vertically.

The bioluminescence is observable also under laboratory conditions, if the cultures are reared in glass bottles (figure 3.2).

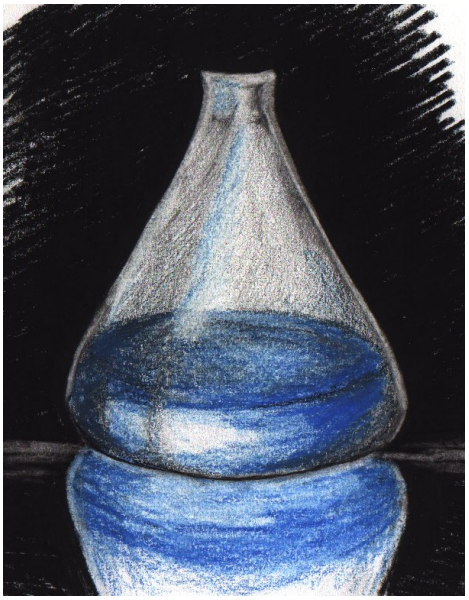


Figure 3.2: *The bioluminescence of* Lingulodinium polyedrum *was photographed in the dark briefly after shaking the Erlenmeyer bottle, standing on a glass plate. Drawn by Mareike Förster after a photography of Taylor in Hastings (1994)*

In a 12:12 hour light-dark cycle the bioluminescence occurs only during the night[1]. But even under constant environmental conditions the cultures continue to emit light rhythmically. Apparently an internal clock controls it.

This rhythm was studied in several lab-

[1] during the light period the light has to be switched off briefly for observing the bioluminescence of the culture

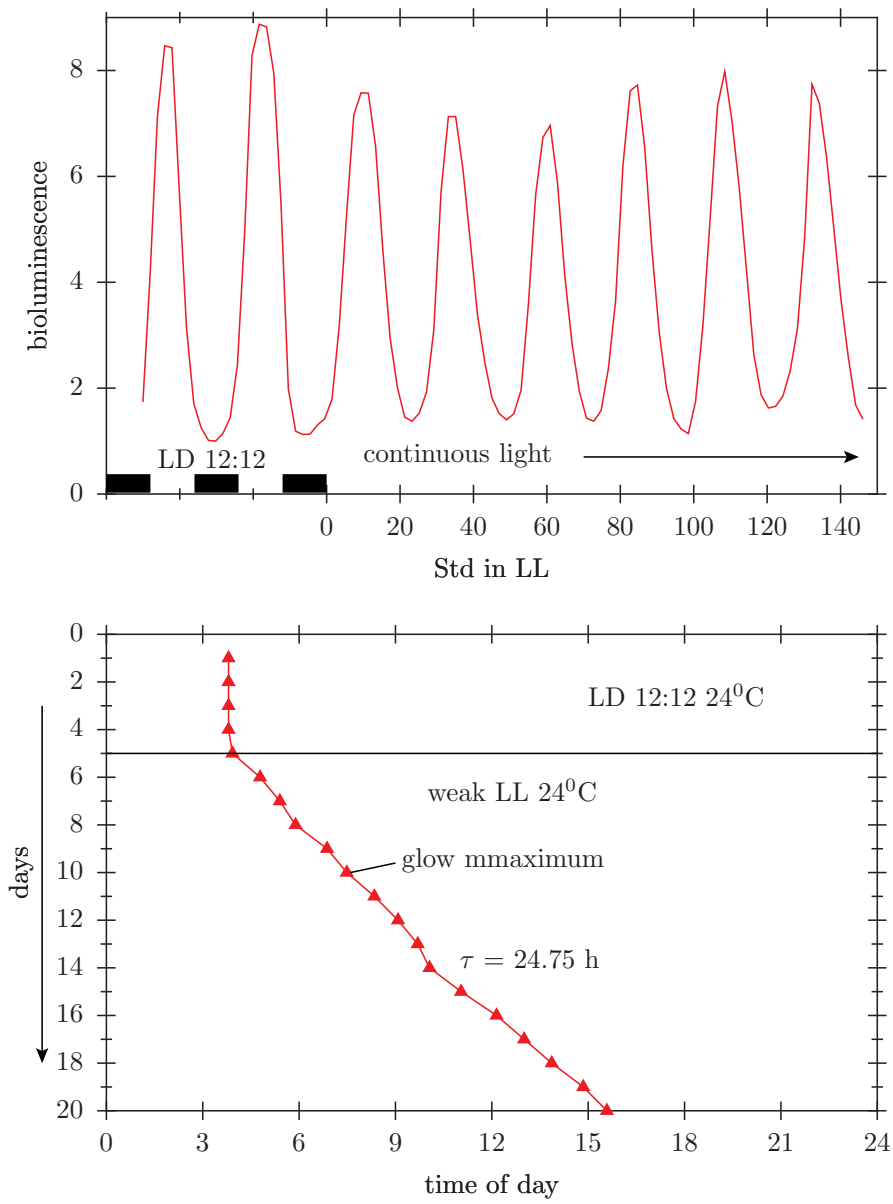


Figure 3.3: *Upper curve: Glow rhythm (y-axis: bioluminescence) of a culture of* Lingulodinium polyedrum *in a 12:12 hour light-dark cycle (until hour 0) and afterward in weak continuous light. Lower curve: glow rhythm of a culture of* Lingulodinium polyedrum *at constant temperature of* 24^0C *in a 12:12 hour light-dark cycle (to the fifth day) and afterward in weak continuous light. Only the maximum of the light intensity of the glow rhythm is indicated for each day by triangles. The period lengths of the rhythm in the light-dark cycle is 24 hours (synchronized) and in continuous light 24.75 hours (freerun). After Hastings (1960)*

oratories intensively (overview Sweeney (1984), Hastings (1959), Roenneberg and Rehman (1998)). The bioluminescence can be recorded automatically for longer time spans and in many vials simultaneously. The bioluminescence consists of two phenomena: a series of flashes caused by mechanical or chemical disturbances and a weaker glow observable in undisturbed cultures (figure 3.3). The bioluminescence of the flash rhythm is maximal at the middle of the dark period and occurs only during a few hours per day. A flash takes just 100 ms, and 10^7 to 10^{10} light quanta are emitted per cell. The glow rhythm, however, has its strongest bioluminescence at the end of the dark period.

The bioluminescence rhythm is relatively in dependant of the environmental temperature (figure 3.4, Sweeney and Hastings (1960)), as is characteristic for circadian rhythms. At higher temperatures the clock of *Lingulodinium* runs a bit more slowly, the Q_{10} (see glossary) is 0.85 (Hastings and Sweeney (1957)). Temperature compensation can be explained by two chemical reactions with the same temperature dependence, but one reaction product inhibits the other reaction. In this way the clock is buffered against fluctuations in the temperature of the environment.

The bioluminescence rhythm is quite precise. It can amount to 2 minutes per day for the population (0.015%, figure 3.5). For the individual cell the variability of the period lengths is 18 minutes per day (1.36%) (Njus et al. (1981), Morse et al. (1990)). Under constant conditions the rhythm of bioluminescence lasts for a long time; however, synchrony declines with time, and therefore the maxima broaden.

Why the rhythm damps gradually, can have two reasons. Either the period lengths of the clocks, which drive the rhythm, are very similar. Therefore it takes a long time until the population rhythm damps out. Or the cells communicate with each other and synchronize themself mutually. Against a chemical communication speak experiments, in which cultures with different phases were mixed with each other. They behaved after mixing in such a way as one would expect, if they would *not* influence each other (Sulzman et al. (1982)).

As in most organisms light is the strongest time cue for synchronizing the *Lingulodinium* clock. Light influences furthermore the period length of the bioluminescence rhythm. How the period is changed, depends on the light quality[2] and the amount of light[3].

Bioluminescence occurs in special spherical organelles, the scintillons (Lapointe and Morse (2008)), which lie in the vicinity of the cell membrane. Whereas during the light period only about 40 scintillons are found per cell, there are about 400 during the night (figure 3.6, Fritz et al. (1990), Johnson et al. (1985)). They protrude as pockets in the vacuole and are connected with the cytoskeleton. The scintillons can be seen under the microscope as bright spots and can be detected also with gold particles and an antibody for luciferase immunocytochemically. Scintillon extracts flash, if

[2] under continuous red light period is longer than 24 hours and increases further with higher intensities. Under continuous blue light it is shorter and decreases further with higher intensities

[3] the period length amounts to 24.4 hours at 1200 lux of continuous light. At 3800 lux the period is 22.8 hours and the rhythm damps. At intensities above 10000 lux the bioluminescence rhythm disappears. In continuous darkness the period is 23.0 to 24.4 hours and the rhythm is also damped

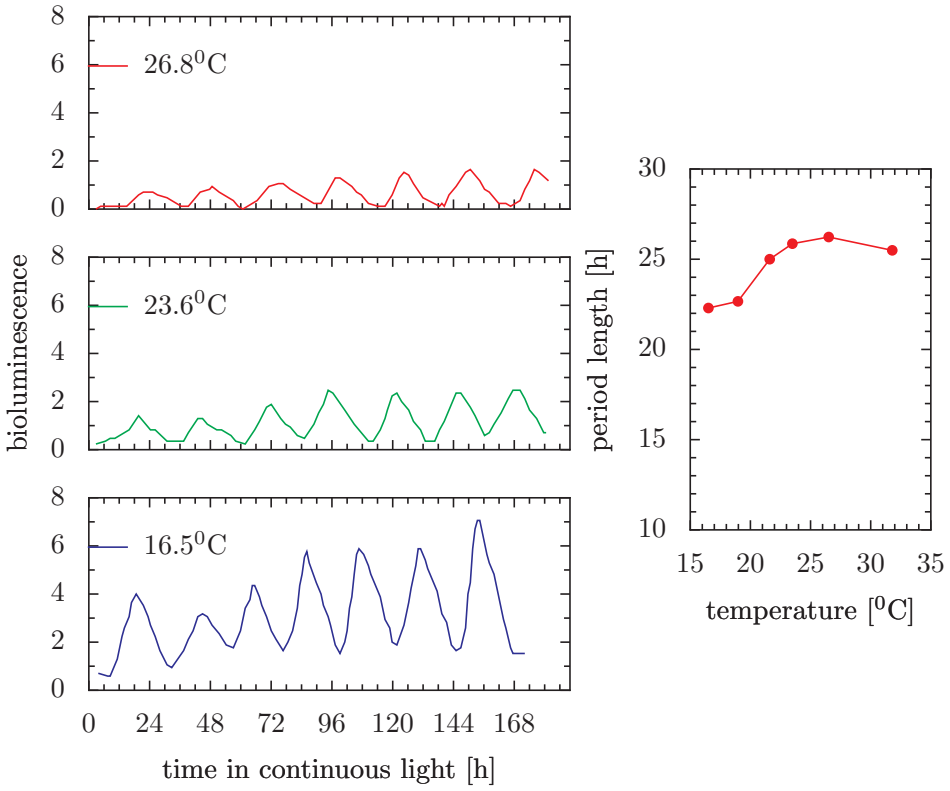


Figure 3.4: *The period length of the flash rhythm of* Lingulodinium polyedrum *is only slightly dependent on the temperature of the seawater ('temperature-compensated'): The course of the bioluminescence (in weak continuous light) is plotted for various temperatures (top left curve:* 26.8^0*, middle left curve:* 23.6^0*, bottom left curve:* 16.5^0*C. 26.8). y-axis: light intensity. Right curve: period lengths (in hours) of the glow rhythm of bioluminescence in* Lingulodinium *as a function of the temperature of the medium. After Hastings and Sweeney (1957)*

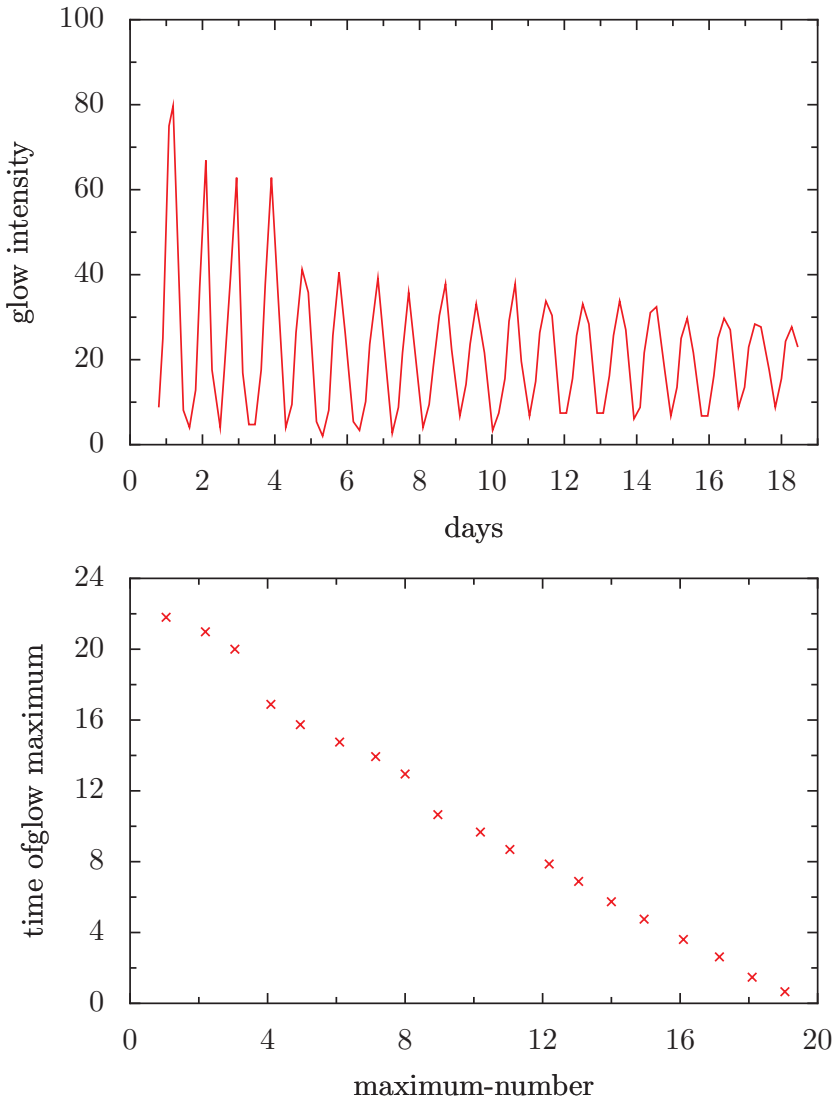


Figure 3.5: *The circadian glow rhythm of bioluminescence in* Lingulodinium polyedrum *was determined in a culture, which was kept first in a 12:12 hour light-dark cycle and a constant temperature of* 19^0C. *At the time 0 (x-axis) the algae were transferred to weak continuous light (upper part of figure). The precision of this rhythm is demonstrated in the lower part. Here clock time of the bioluminescence maxima is plotted against the number of the maxima. The precision is even higher, if the maxima of the bioluminescence are connected by three straight lines (the cause of the two phase shifts is not known). After Morse et al. (1990)*

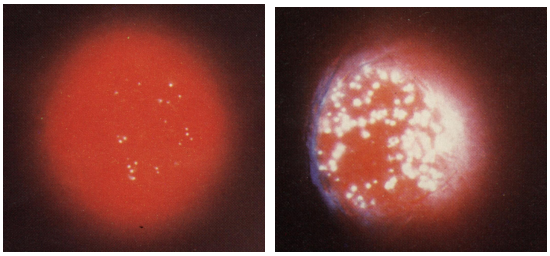


Figure 3.6: *Scintillons are organelles for producing the bioluminescence in* Lingulodinium polyedrum. *During the night a cell contains about 400, during the day only about 40 scintillons. They protrude from the cytoplasm as bubbles of the tonoplast into the vacuole. Diameter about 0.5 µm. Shaking the culture or other stimuli of the cells evoke an action potential. This triggers an H^+ efflux from the acid vacuole in the less acid scintillon. As a result bioluminescence starts (see figure 3.8)*

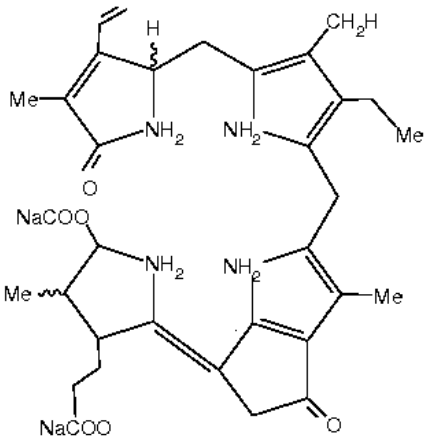


Figure 3.7: *Tetrapyrrole-structure of the luciferin of* Lingulodinium polyedrum. *Me: Methyl groups. After Nakamura et al. (1989)*

transferred from a pH of 8 into a pH of 6.

As in all bioluminescing processes the light emission of *Lingulodinium*-cells consists also of a reaction of a substrate (luciferin) with an enzyme (luciferase).[4] There are about $2.7 * 10^{12}$ luciferase molecules per cell. The luciferin of *Lingulodinium* is a tetrapyrrole, a small molecule (molecular weight <1000; figure 3.7). It is heat stable, whereas the luciferase is heat sensitive. By oxidation luciferin emits light and enters the singlet state. In the scintillons is furthermore luciferin binding protein LBP found. It binds luciferin, if the pH is 7.5 (the normal pH of the cytoplasm) or higher. At a pH of 6.5 or less the configuration of the LBP changes, the luciferin is being freed and reacts with O_2 via luciferase. Low pH activates also the luciferase.

$$LBP - LH_2\,(pH7.5) \Rightarrow (H^+)\,LBP + LH_2\,(pH6) \Rightarrow (O_2, Lfase) \Rightarrow h\nu + L \Rightarrow O + H_2O$$

Controlled in a circadian manner are the *translation* of the luciferin binding protein, the luciferin (LH_2) and the luciferase (Morse et al. (1989)). The mRNA of LBP is, however, constant. Likewise the translation of the mRNA is the same during all phases of the cycle. Thus, the rhythm is not based on a fluctuating transcription. Instead it is controlled translationally. Therefore the circadian rhythm of bioluminescence can be influenced by translational inhibitors, but not by transcriptional inhibitors. Perhaps trans-acting factors play a role in the clock controlled LBP synthesis (Mittag (1998)). This translational control contrasts with the circadian control in the crucifer *Arabidopsis*, in which *transcription* is controlled by the circadian clock.

[4] Luciferase has at a pH of 8 a molecular weight of 140 kDa and is a dimer (each of 70 kDa). At a pH of 6 the molecular weight is 35000 to 40000. At a pH of 6.4 it is maximally active. A 4.1 kb mRNA produces the luciferase. Its cDNA was cloned. It does not contain any intron and differs from all luciferases known so far

Translational control of regulatory clock-proteins (CP1, CP2, CP3) was proposed as a part of the circadian mechanism. CP1 cancels the repression of the synthesis of CP2 by interacting with the repressor of mRNA-2. CP2 interacts with the regulatory region of mRNA-3, which is responsible for the synthesis of CP3. Thus a cascade takes place, in which each protein inhibits its own synthesis, until the rhythm has come to an end.

The luciferase concentration parallels the bioluminescence of intact cells. At midnight it is 10 times higher as at noon. The maximum occurs 6 hours after onset of darkness. Under continuous light this rhythm continues with low amplitude. The rhythmic course of luciferase-activity could be the result of the modification of the enzyme by phosphorylation, methylation, activation or inhibition, respectively. Alternatively the amount of enzyme might fluctuate in a circadian manner. That was indeed found (Dunlap and Hastings (1981), Johnson et al. (1984)). Thus, either the synthesis or the degradation of luciferase, or both fluctuate in a circadian pattern.

A mechanical or chemical (Ca^{2+}, NH_4, K^+, H^+) stimulation leads to an action potential. This travels via the tonoplast to the scintillons and depolarizes them. As a result, the H^+-Ions enter the scintillons. Due to the rapid change in pH (from pH8 to 6) the LBP emits luciferin and this reacts with luciferase. Light is emitted (figure 3.8). After the stimulus luciferin is bound again to LBP and a new stimulus is possible (Fogel and Hastings (1971)). Perhaps the circadian fluctuations of the various reactants are the result of the destruction of the scintillons in each cycle and its resynthesis. The spontaneous bioluminescence (the glow rhythm) might take place during the destruction of the scintillons. How this takes place is not yet clarified. Either the scintillons separate and are demolished in the vacuole, or they are emptied into the cytoplasm. The latter possibility is more likely. Scintillons are removed in the early morning.

3.1 Circadian control of bioluminescence, mechanism of the clock

After discussing the function of the biochemical machinery we should have a look at the circadian control of bioluminescence. The circadian clock regulates periodically the synthesis and (possibly) the degradation of luciferin, luciferase and LBP (figure 3.9). The *activity* of luciferase and the rate of phosphorylation stay, however, constant.

Protein synthesis is involved in the bioluminescence rhythm, since inhibitors of protein synthesis (cycloheximid, puromycin, anisomycin) influence the period (Taylor et al. (1982)). Cycloheximid pulses shift the rhythm of bioluminescence as a function of the phase of the oscillator. Thus protein synthesis influences also the clock (Schröder-Lorenz and Rensing (1987), see however also Thorey et al. (1987)). Probably here, as in other circadian systems (NATF in the pineal organ, tyrosine aminotransferase in the liver, ß-hydroxy-ß-methylglytaryl-CoA reductase), enzymes with short half life (0.5 to 1 hour) are involved, which limit the metabolic rate. Other enzymes possess a half life of several days. Protein synthesis plays a role for the circadian rhythm also in other organisms, for instance in *Aplysia* (Jacklet (1981)) and *Acetabularia* (Schweiger (1977)).

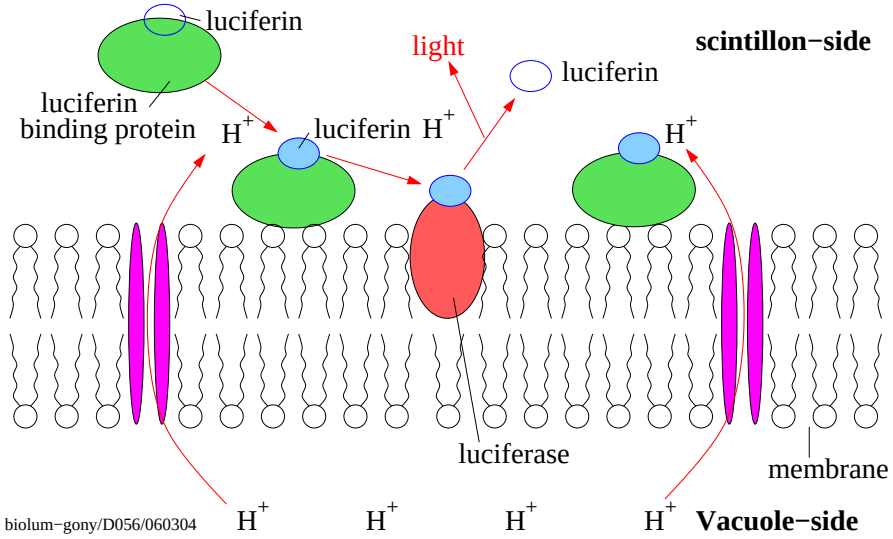


Figure 3.8: *Mechanism of light production in* Lingulodinium polyedrum *in the scintillon. A small part of the scintillon membrane is shown (vacuole below, interior of the scintillon on top). If H^+-ions enter the scintillon due to depolarization (for instance as a result of shaking) (bent red arrows), the interior of the scintillons becomes acid. This frees the luciferin, which had been bound to the luciferin binding protein. It is oxidized by luciferase. Light is emitted during this reaction. After Dunlap et al. (1981)*

Details of the circadian control of bioluminescence are not yet known. A few observations speak in favor of two clocks: For instance, light pulses can influence the phase of the flash- and the glow rhythm differently. Although the same luciferin and the same luciferase are used, the responsible reactions differ. They probably occur in different compartments. Under certain conditions the period lengths of the glow- and flash rhythm differ (23.8 versus 23.6 hours) and consequently the phase relationship between both rhythms has changed (figure 3.10, Heyde et al. (1992)). The optimal light intensity differs for the two bioluminescence rhythms ($6\,\mu\,Einstein/cm^2sec$ for the flash rhythm, ($90\,\mu\,Einstein/cm^2sec$ for the glow rhythm). Likewise, the temperature influences flash- and glow rhythm differently. Finally the bioluminescence rhythm and the aggregation rhythm can exhibit different period lengths (Roenneberg and Morse (1993)). There are thus good indications, that two clocks control the circadian system of *Lingulodinium* (Heyde et al. (1992), Morse et al. (1994)).

3.2 Significance of bioluminescence

Bioluminescence is often found among organisms. The reasons for emitting light vary, depending on the species in which it is found: Male and female animals can recognize and find each other (glow worms), swarms can be formed, territories marked. Fishes can illuminate their visual field and lure prey (*Anomalops* in Japanese waters). Bioluminescence might serve for protec-

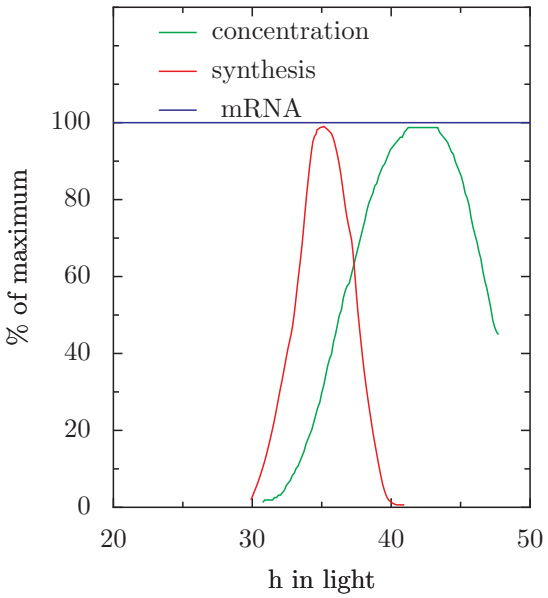


Figure 3.9: *The luciferin binding protein of* Lingulodinium polyedrum *is synthesized in a circadian manner (red curve, maximum of synthesis about 35 hours after onset of the continuous light following a 12:12 hour light-dark cycle). The LBP protein reaches after 43 hours maximal concentration (green curve). Afterward it is degraded. The mRNA which is responsible for the LBP-synthesis is continuously present and active (blue curve). Thus the circadian control of LBP-synthesis occurs on a translational level. After Morse et al. (1990)*

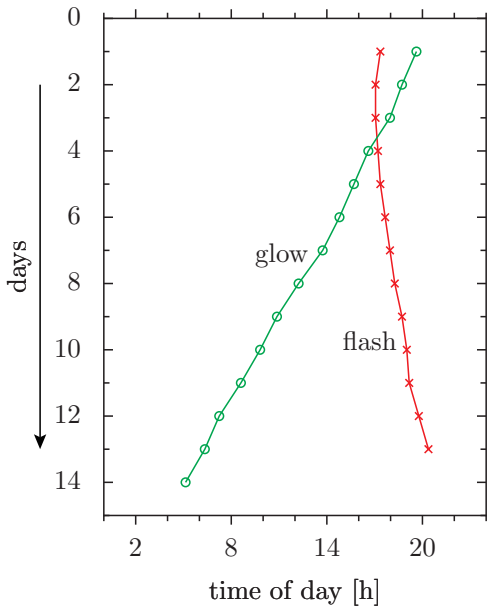


Figure 3.10: *Different period lengths of flash- and glow rhythm are found in* Lingulodinium polyedrum *under continuous light at* 21^0*C. The daily maxima of the glow rhythm (green x) and the flash rhythm (red crosses) were plotted beneath each other and connected with a curve. The period length of the glow rhythm is 22.9, the one of the flash rhythm 24.2 hours. After Heyde et al. (1992)*

tion. Enemies can be deterred or distracted from the front ends (worms), it might serve as camouflage.

But why does *Lingulodinium* exhibit bioluminescence and why is there a flash- and a glow rhythm? Bioluminescence could be a side product of the metabolism. It serves perhaps to get rid of protons under circumstances, where acceptors for protons are scarce or lacking. Other reasons have been proposed, why this organism exhibits bioluminescence. Thus, the flash of bioluminescence might frighten fishes, discouraging them from feeding the algae.

Another question is, why scintillons and its machinery has such a short life duration. Isn't it a waste of energy, if they have to be renewed each day? But for an alga, which has enough energy to its disposal, it might be more advantageous, to produce these organelles anew and to use the nitrogen of the scintillon proteins for other enzymes, since nitrogen is a limiting factor.

3.3 Rhythms of aggregation, phototaxis, vertical migration and mobility

In *Lingulodinium* cultures the cells aggregate to swarms. They can be observed in Petri dishes (figure 3.11 and Roenneberg et al. (1989)). This behavior is a population rhythm, which occurs endogenously for a few weeks before it desynchronizes. During the day the population stays close to the surface, in the night it sinks to the bottom.

The aggregation rhythm changes its period length with light intensity. But the period depends also on the wavelength of the light. In red light the period increases with higher light intensity, in blue light it decreases (figure 3.12). Probably two different photoreceptors and possibly also two clocks are involved (Roenneberg et al. (1988), Roenneberg and Morse (1993), Morse et al. (1996)). In favor of this assumption is, that light pulses affect bioluminescence and aggregation differently. The B-oscillator, which controls bioluminescence, reacts to blue light, the A-oscillator, which regulates aggregation, reacts to blue *and* red light. In green light (550 nm) the cells are blind (Morse et al. (1994)).

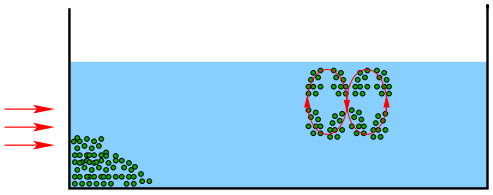


Figure 3.11: *Swarm formation in* Lingulodinium polyedrum *in a Petri dish, which is laterally illuminated with light of 120* $\mu E/m^2 sec$. *During the day swarms are formed at the surface of the sea water, whereby the cells in the middle of the aggregation move slightly down and move up again at the margin of the aggregation. In the night the cells settle at the side of the dish which was originally closest to the light. After Roenneberg et al. (1989)*

3.4 Chloroplast rhythms

Circadian differences are found also in the chloroplasts of *Lingulodinium*. The ultrastructur of the thylakoid assembly varies, as shown in figure 3.13. During the subjective night (CT 18) the thylakoides lie closer together (upper part of figure) as compared to the subjective day (CT6, lower part of figure, Herman and Sweeney

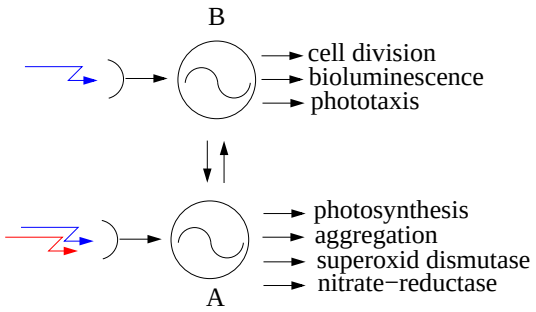


Figure 3.12: *The circadian system of* Lingulodinium *is supposed to consist of an A- and B-oscillator. The A-oscillator (below) controls among others aggregation of the cells, the B-oscillator, however, regulates besides cell division and phototaxis the bioluminescence. Blue light synchronized the B-oscillator, blue- and red light the A-oscillator. After Morse et al. (1994)*

(1975)). During the subjective day the thylakoides consist of two lamellae, during the subjective night of three. Furthermore the photosynthetic unit in the thylakoid membrane differs: During the subjective night it is partly decoupled from the electron transfer. Association and dissociation of the antennae of photosystem II fluctuates rhythmically. Thereby the excitation energy between photosystem I and photosystem II is distributed differently. Samuelsson et al. (1983) studied the causes of the circadian oxygen production. They found by using the electron acceptor methylviologen, that the electron flow in the photosystem I is constant, but fluctuates in photosystem II. Therefore only fluctuations in photosystem II are responsible for the photosynthesis rhythm in *Lingulodinium* (figure 3.14, see also next section).

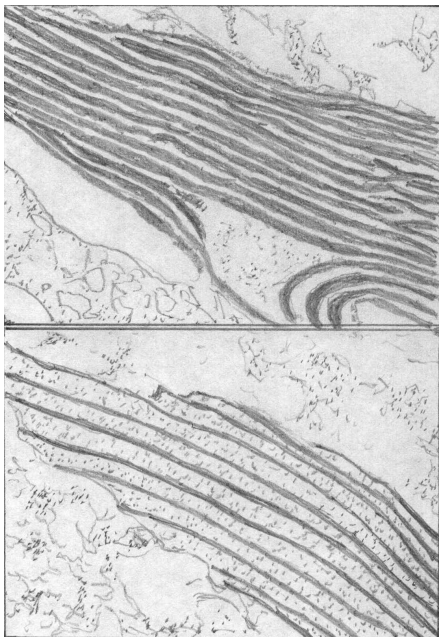


Figure 3.13: *The thylakoids in the chloroplasts of* Lingulodinium polyedrum *display circadian differences. During the subjective night (CT 18) they lie closer together (upper part of the figure) as compared to the subjective day (CT6, lower part of the figure). Drawn by the author after a figure in Herman and Sweeney (1975)*

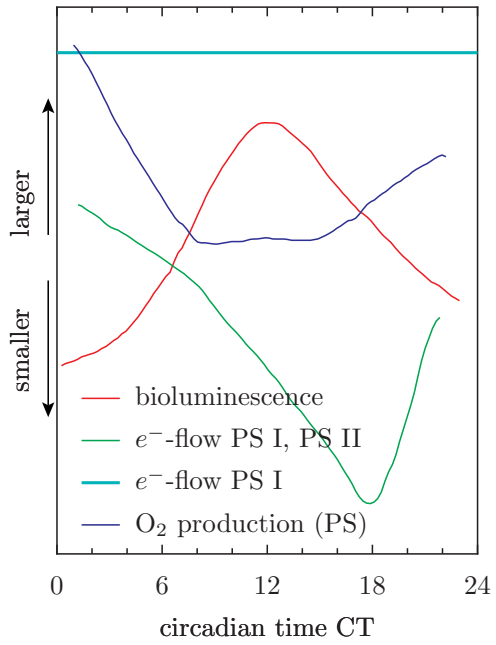


Figure 3.14: *Causes of the photosynthesis-rhythm in* Lingulodinium polyedrum. *Photosynthesis (PS, blue curve) fluctuates in a circadian manner (oxygen production measured). It has low values during the night phase, when bioluminescence is strong (red curve). Using the electron acceptor methylviologen, it was shown, that the electron flow in photosystem I is constant (PS I, bluegreen line), but in photosystem I and II it fluctuates in a circadian manner (PS I, PS II, light green curve). Thus only fluctuations in photosystem II are responsible for the photosynthesis rhythm of* Lingulodinium. *The x-axis reflects the circadian time CT. The measured variables are not given at the y-axis. See Samuelsson et al. (1983)*

3.5 Circadian rhythms in photosynthesis

Structural variations in the photosynthetic devices show already, That circadian rhythms influence photosynthesis. A number of events is involved in the case of *Lingulodinium*: CO_2-uptake (Hastings et al. (1961)), light reactions in photosystem II (Knoetzel and Rensing (1990)), chlorophyll fluorescence and -degradation (Sweeney (1981)) are examples. However, photosynthesis itself is not a part of the clock: If the electron flow in photosystem II is inhibited with DCMU, the clock continues to run (Sweeney et al. (1979)).

CO_2-fixating enzymes do not show a rhythm. Likewise the O_2 *uptake* is constant. Respiration is thus not responsible for the circadian rhythm. In the middle of the subjective light period O_2-*emittans* is high, in the middle of the subjective dark period low (figure 3.15).

Since the density of a cell changes with photosynthesis, a circadian rhythm in a single *Lingulodinium*-cell could be demonstrated by using a Cartesian diver (figure 3.16, Sweeney (1960)). Here too the rhythm disappears at high light intensities. The damping is, therefore, not caused by a desynchronisation of the rhythms between the individual cells of a population.

3.6 Cell division rhythm

Cell division occurs in a population of *Lingulodinium* mainly during the morning in a light-dark cycle and under continuous light at the subjective morning (the time at which light normally begins). It is thus under circadian control. Generation time of a cell amounts under the used light intensity to 6-7 days as an average (figure 3.17,

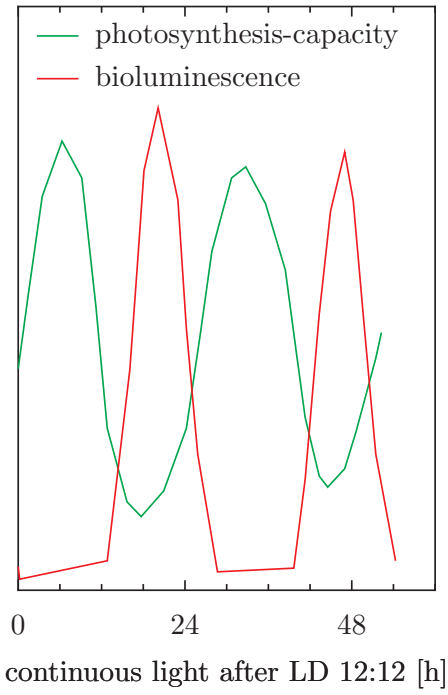


Figure 3.15: *Photosynthetic capacity in* Lingulodinium polyedrum *(green curve): Samples were treated at various phases for 15 minutes during strong light with $C^{14}O_2$. The amount of C^{14} taken up was determined. Additionally the course of bioluminescence is shown (red curve). After Hastings et al. (1961)*

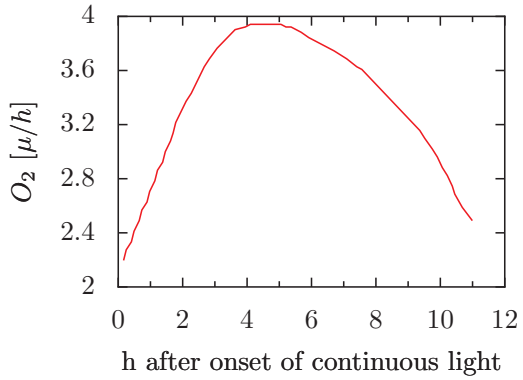


Figure 3.16: *The course of photosynthesis of an individual* Lingulodinium*-cell was determined during the light period with a Cartesian diver. The density of the cell depends on the oxygen production. Consequently the position of the Cartesian diver (in which the cell is housed) changes in the vial. After Sweeney (1960)*

Sweeney (1984)).

The division cycle in the cell is thus controlled by a circadian clock (Sweeney and Hastings (1958)), which allows division to occur only in certain gates or time windows (Vicker et al. (1988)). Mitoses occur usually towards the end of the dark period or somewhat earlier. Cytokinesis takes in *Lingulodinium* an hour. The cell division rhythm is not directly connected with the bioluminescence rhythm: cells, which do not divide, do still exhibit a circadian bioluminescence, and so do colchicin-treated cells.

The cell cycle can be synchronized by selecting cells according to their size by using sieves (Homma and Hastings (1988), Homma and Hastings (1989)). After cell division the phase of the circadian rhythm is transferred to the daughter cells (Homma et al. (1990)).

Under optimal conditions cell division (studied in *Euglena* and *Chlamydomonas*)

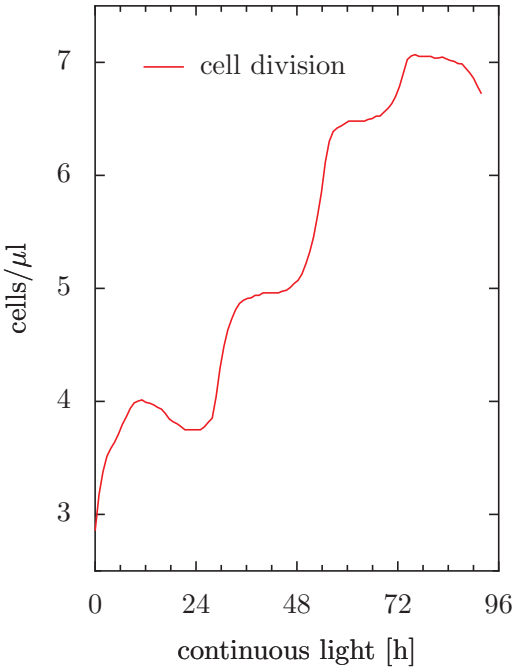


Figure 3.17: *Circadian rhythm of cell division of* Lingulodinium polyedrum*: The number of cells per µl increases, in spite of continuous light (3000 Lux), not continuously, but step-like. Plotted at the x-axis is the circadian time CT. Period lengths is about 24 hours. After Vicker et al. (1988)*

is solely determined by the mechanism of division. Under suboptimal conditions an ultradian clock controls division (Lloyd and Volkov (1990)). Under conditions, which allow only slow growth, the circadian clock comes into play (*gating*, Lloyd and Gilbert (1998), Lloyd and Gilbert (1998)). Various models were proposed, which simulate the cell cycle and its timing (Tyson (1987), overview Lloyd and Gilbert (1998)).

3.7 Circadian rhythms in metabolism and of enzymes

The tricarbonic acid cycle in *Lingulodinium* is rhythmically controlled. Probably on the protein level a NAD- or NADH-dependent form of the isocitrate dehydrogenase is the target of the control; this in turn regulates the metabolic flow through the TCA-cycle rhythmically (figure 3.18, Akimoto et al. (2005)).

Circadian rhythms were found in a number of enzymes. One of it is nitrate-reductase (figure 3.19 and Ramalho et al. (1995), Fritz et al. (1996)). It is the first enzyme of the nitrogen assimilation path and converts nitrate into nitrite. Its concentration fluctuates in a circadian way with a maximum in the (subjective) day phase. Another enzyme, superoxide dismutase, has its highest activity also during the day phase (figure 3.19 and Colepicolo et al. (1992)). It is not known, whether its concentration fluctuates in a circadian manner. This enzyme is a superoxide-anion scavenger. In the case of RUBISCO, the most frequent enzyme of the biosphere, because it takes up CO_2 during photosynthesis, the activity is circadian, whereas

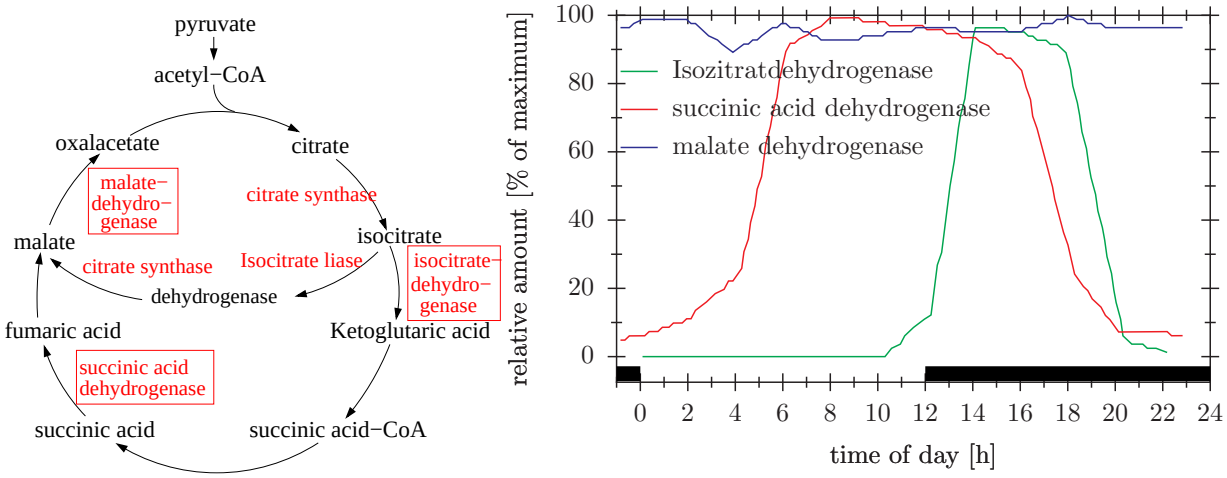


Figure 3.18: *Left: The tricarbonic acid cycle with enzymes (red) for intermediate steps. In red boxes the enzymes shown in the right diagram. Right: circadian rhythm of isocitrate dehydrogenase (green) and succinic acid (red). Malate dehydrogenase (blue) does not fluctuate much. After Akimoto et al. (2005)*

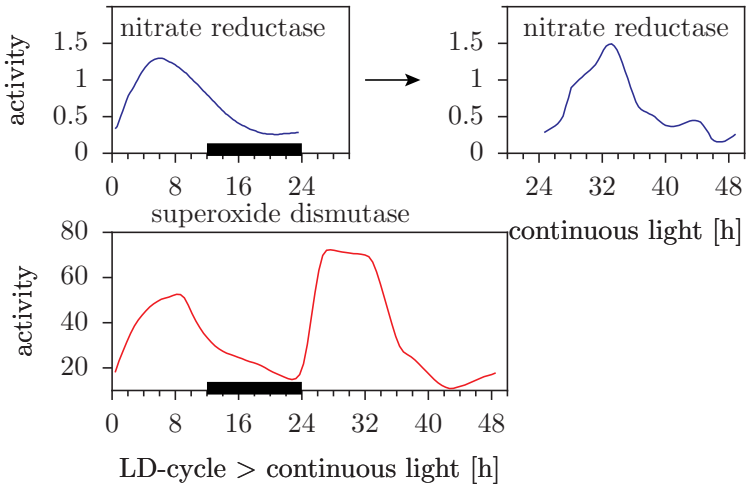


Figure 3.19: *Circadian rhythm of nitrate reductase (top) and superoxide dismutase in* Lingulodinium polyedrum. *Top left: In a light-dark cycle of 12:12 hours white light of 150 $\mu E/m^2 sec$, right under continuous light (hours after onset of continuous light of 35 $\mu E/m^2 sec$). Activity of nitrate-reductase in relative units per mg. After Ramalho et al. (1995). Superoxide dismutase (bottom) with high activity during the light period. During the dark period the activity declines and reaches a minimum at the end of the night. In the following continuous light the rhythm continues. It is thus circadian. After Colepicolo et al. (1992)*

the amount of the enzyme stays constant (Marcovic et al. (1996)). In contrast to these enzymes tyrosine-aminotransferase reaches its highest activity during the night (Gross et al. (1994)). The same is found in the case of the enzyme luciferase, which participates in the bioluminescence rhythm (Dunlap and Hastings (1981), Johnson et al. (1984). Generally the protein synthesis is higher during the day phase as compared to the night phase. Most heatshock proteins have a constant synthesis, but in some it is circadian with a maximum around CT15. Ribosomal proteins are phosphorylated in a circadian manner (Esch et al. (1995)).

3.8 Effect of substances on circadian rhythm, membranes

Various substances influence the circadian rhythm in *Lingulodinium*. As mentioned already, protein synthesis is participating in the mechanism of the circadian clock. But membranes seem to play also an important role. This is interesting, since the fluidity of membranes is temperature compensated. In this way the temperature compensation of the period lengths of circadian rhythms could be explained (see also figure 4.5). Membrane active substances such as K^+, Li^+, D_2O, valinomycin (Sweeney (1974)), alcohols (Sweeney and Herz (1977), Taylor et al. (1979)), vanillic acid (depolarizes membranes, Kiessig et al. (1979)) affect circadian rhythms. That speaks also for a significance of membranes for circadian rhythms. Alternatively, metabolic differences or protein synthesis could be influenced. To check for it, membrane properties were changed and measured with fluorescence-polarization techniques. No correlation between membranes and period lengths were found (Scholübbers et al. (1984)). The activity of membrane bound enzymes and membrane potentials could, however, have changed rhythmically.

Other substances such as acetate aldehyde (Taylor and Hastings (1979)) and catecholamine (Hardeland (1980)) do also influence the rhythm, whereas respiration inhibitors , photosynthesis inhibitors (Sweeney (1981)), inhibitors of the organelle ribosomes, of cAMP and of DNA-synthesis do not play a role.

Creatine, a storage form of ATP and conveyor of energy-rich phosphate between mitochondria and energy-consuming events, shorten the period from 23 to 18 hours (Roenneberg et al. (1988), Roenneberg and Taylor (1994)). It amplifies the phase shifting effect of blue light and phototaxis. Creatine is, however, not naturally found in *Lingulodinium*. Instead, the alga contains another substance, gonyauline, which shortens period (Roenneberg et al. (1988), Roenneberg et al. (1991)).

4 Rhythms in *Acetabularia*

Acetabularia *is another alga, in which several circadian rhythms can be observed. Because of the exceptional size rhythms can be measured in parts of this unicellular alga. Grafts can also be made. This allows, to find out, which significance the nucleus has for the circadian clock hat. Oxygen production, chloroplast migration and electrical potentials were used as a hand of the clock. A model of the circadian mechanism was proposed and tested.*

Another alga, which possesses a circadian rhythm, were studied intensively because of its exceptional size. It is *Acetabularia*, called mermaid's wineglass and belongs to the *Dasycladaceae*, a very old family, which existed already 500 million years ago. Depending on the species of this unicellular alga it is from a few mm up to 25 cm long (the latter is *Acetabularia major* in the Torres street of Australia and in Papua-Newguinea). The algae are tube like and possess a root-like rhizoid. In the finished state they have a hat ("umbrella" Italian, figure 4.1). Most of the Acetabularia occur in shallow zones of the coasts of tropical and subtropical seas.

Figure 4.1: *Various stages of the unicellular* mermaid's wineglass Acetabularia mediterranea. *The shortest is a germinating zygote (originating from the fusion of two gametes), which sticks to the soil with a rhizoid. It extends to a stalk and forms a whorl, which is later lost. Finally a hat is formed, which generate numerous cysts in many chambers. In the cysts gametes are formed. The alga reaches a length of 50 mm. It occurs in the mediterranean and the western Atlantic. After* Gibor (1966)

4.1 Daily rhythmic phenomena

In the laboratory *Acetabularia* can be kept in artificial sea water and studies can be made. Daily rhythms of oxygen production during photosynthesis (Terborgh and McLeod (1967)), of enzyme activities (Hellebust et al. (1967)), of chloroplast migration and of electrical potentials

(Schweiger and Schweiger (1977), Broda and Schweiger (1981), Koop et al. (1978)). The chloroplasts migrate during the night to the rhizoid at the foot of the alga and during the day to the upper parts of the alga. The form of the chloroplasts does also change rhythmically (round during the dark period, oval during the light period (vanden Driessche et al. (1976)) and in a circadian manner (vanden Driessche (1966), vanden Driessche (1967)). Furthermore the RNA-synthesis fluctuates in a circadian way (vanden Driessche and Bonotto (1969)).

These circadian rhythms are temperature-compensated and show a $Q_{10} < 1$ (Karakashian and Schweiger (1976), Berger et al. (1992)).

4.2 Role of the nucleus

The nucleus of the cell can be removed easily during the vegetative phase (Schweiger (1977)). Since the nucleus is located at that time in the rhizoid, one needs only to cut this part off. O_2-production during photosynthesis is even without nucleus under constant conditions rhythmic (figure 4.2). Thus the oscillator is in the cytoplasm. The integrity of the cell is not necessary for the circadian rhythm. Even smaller cell fragments show still a circadian rhythm. It turned out, that the mRNA of *Acetabularia* is stable for weeks, especially, if the nucleus is absent.

Since the nucleus is in the foot of the alga, grafting experiments could be performed, to find out the significance of the nucleus for the daily rhythm (Schweiger and Schweiger (1977), Sweeney and Haxo (1961), Schweiger et al. (1964), Karakashian and Schweiger (1976), Terborgh and McLeod (1967)). The nucleus o f another cell can be washed and implanted into the nucleus-free cell fragment (Hämmerling (1963)). If rhizoids of *Acetabularia* are grafted onto stalks of algae with a phase shifted rhythm of the O_2-production, the rhythm under continuous light is determined by the nucleus-containing rhizoid (figure 4.3, Schweiger et al. (1964)). To eliminate cytoplasmic effects (for instance via mRNA), only nuclei of phase shifted synchronized algae were implanted. Again the rhythm was determined by the nucleus. If the rhizoid and the upper part of an *Acetabularia* are exposed to opposite light-dark cycles, the rhythm of O_2-production has the phase of the rhizoid. vanden Driessche (1967) grafted arrhythmic stalks of *Acetabularia* onto rhizoids of rhythmic algae. Afterward a rhythm of O_2-production was observed again.

Thus there is a paradox: Although an *Acetabularia* shows a circadian rhythm even without nucleus, the phase of the nucleus is according to these authors determined by the nucleus. Since anucleated *Acetabularia* have a circadian photosynthesis, a continuous transcription of the nuclear genoms is apparently not necessary for oscillations to occur. It was also shown, that an inhibitor of transcription in the nucleus, actinomycin, does not prevent the rhythm of the algae (Mergenhagen and Schweiger (1975)). Inhibitors of transcription in organells such as rifampicin have also no influence on the circadian rhythm (vanden Driessche (1970)). On the other hand translation is necessary, since inhibitors of translation such as cycloheximid prevent circadian oscillations (Mergenhagen and Schweiger (1975), Karakashian and Schweiger (1976)).

From these observations Schweiger proposed a translation-membrane model (fig-

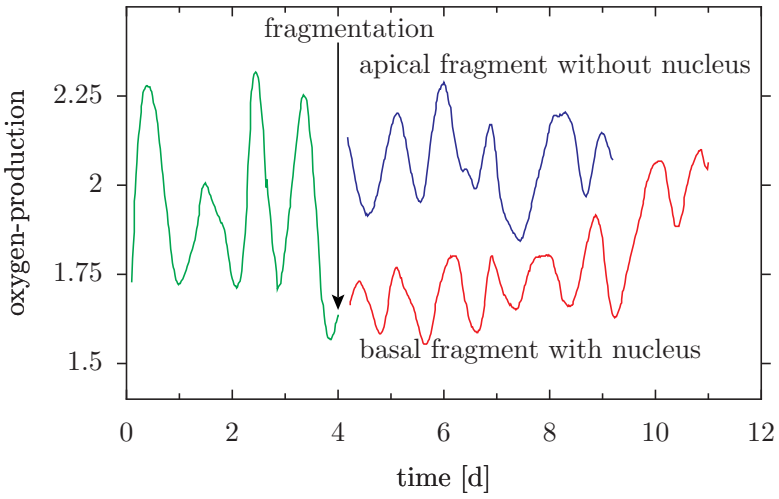


Figure 4.2: O_2 *rhythm before (green curve, until the vertical arrow) and after fragmentation of an* Acetabularia mediterranea *cell in an anucleated apical (blue curve) and a nucleus containing basal part (red curve). After Schweiger (1984)*

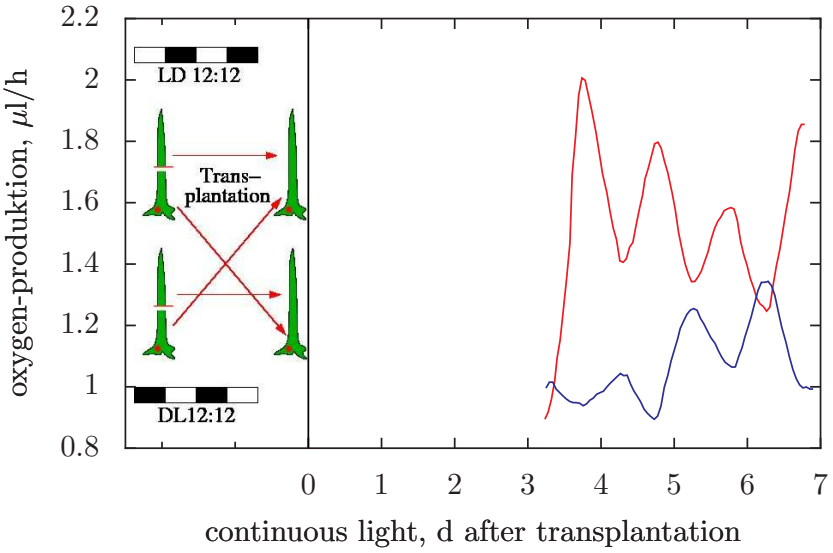


Figure 4.3: *Two* Acetabularia *algae were kept in an inverse light-dark-cycle (see LD 12:12 and the black and white bars before transplantation above and below the Acetabularia). The rhizoid with the nucleus (in the figure marked red) of the upper left alga was grafted with the stalk of the lower left alga, and on the rhizoid of the lower left alga the stalk of the upper left alga was grafted. The recording started after three days and shows opposite rhythms. Comparison with controls (not shown) shows, that the nucleus determines the phase of the rhythmic output of the graft. After Schweiger et al. (1964)*

ure 4.4). Central components of the oscilla-

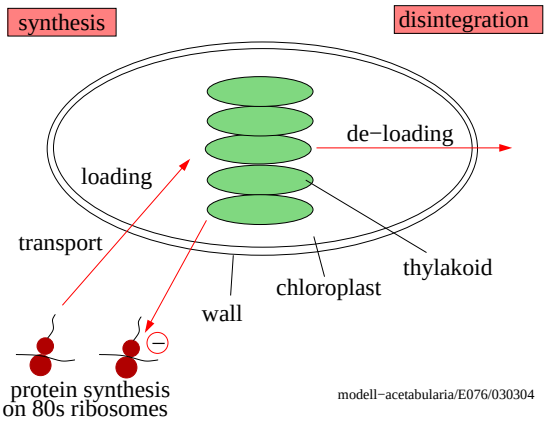


Figure 4.4: *Translational membrane model of the circadian mechanism of* Acetabularia *proposed by Schweiger and Schweiger (1977). Essential proteins are made by 80s ribosomes and transported via the cytosol into the chloroplasts. There they are incorporated into the thylakoid membranes. After this loading the protein synthesis is inhibited and the essential proteins degraded, until the thylakoids are deloaded (right part of figure). Now the assembly can begin again (loading, left part of figure). After Engelmann and Schrempf (1979)*

tor are essential membrane proteins in the thylakoids of the chloroplasts. They influence the permeability for ions. A negative feedback loop inhibits the translation of the membrane proteins at 80s ribosomes. The membrane proteins are gradually degraded and the permeability for ions changed. The inhibition of translation stops.

How is this model experimentally supported and how can certain properties of the circadian clock such as temperature compensation be explained? Hartwig and Schweiger (1986) found a nucleus-coded protein P230 in the chloroplast fraction of nucleated and anucleated *Acetabularia*. It is synthesized under constant conditions in a circadian manner. Cycloheximide, an inhibitor of protein synthesis at 80s ribosomes, inhibits the translation of this protein in a phase-dependend way. Cycloheximide furthermore shifts the circadian rhythm of photosynthesis, depending on the phase, at which it was administered. It might therefore be the essential protein of the model.

The temperature compensation of the circadian rhythm is explained according to this model in the following way (see figure 4.5): Translation of the essential membrane protein at 80s ribosomes has a Q_{10} of 2 to 3, but the integration of the protein into the membrane of the chloroplasts has a Q_{10} of less than 1 (because of the lower state of order at higher temperatures the integration is more difficult). Taken together a low dependency of the period lengths from temperature results.

Woolum (1991) tested these results by using nucleated and anucleated *Acetabularia*. Instead of O_2-emission he measured the circadian chloroplast migration in the rhizoid by using light beams as described by Schmid and Koop (1983) (figure 4.6). Instead of just two measurements per day as used by Schweiger et al. (1964) he obtained each minute a value and averaged over an hour. Algae with rhizoids showed a period lengths of 25.4 hours under constant conditions, algae without rhizoid 26.2 h. Thus, the nucleus does influence rhythm, although to a small extend. Controls show phase differences up to 4 hours between each other. Woolum was not able to reproduce the results of Schweiger by using differential illuminations of the upper part of the algae and of the rhizoids. The nucleus did not pass a phase information to the upper part of the algae. Apparently the oscillator needs a stable mRNA, but can do without mRNA synthesis. Acti-

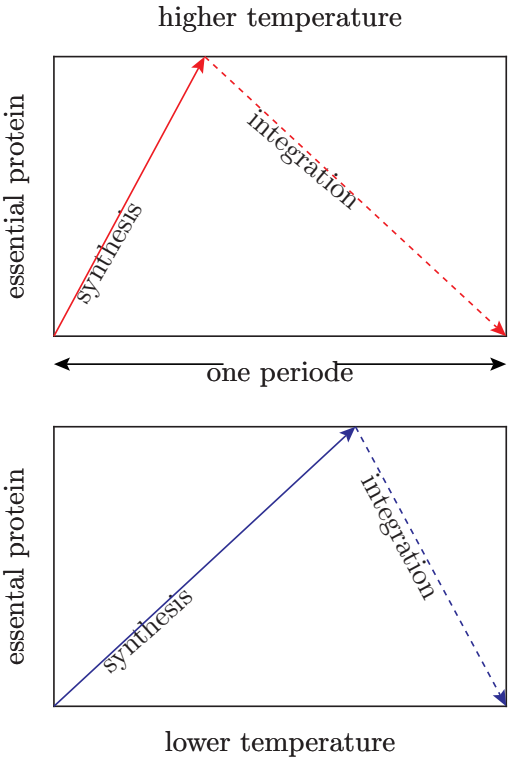


Figure 4.5: *Temperature compensation of the circadian rhythm of* Acetabularia. *At higher temperature the synthesis of essential proteins is faster, the integration into the thylakoides, however, slower. At lower temperatures the synthesis is slowed, but the integration speeds up. In this way the period length does only slightly depend on temperature. The rates are displayed by the slope of the straight lines. After Engelmann and Schrempf (1979)*

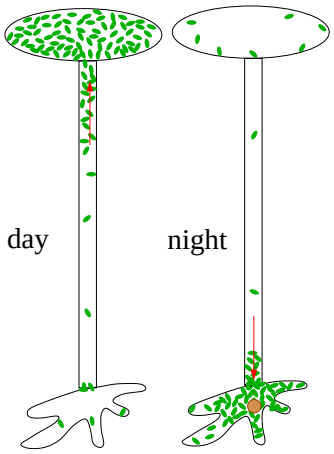


Figure 4.6: *Circadian chloroplast migration in an* Acetabularia mediterranea *cell. Accumulation during the day in the hat and upper stalk (left), during the night in the rhizoid and lower part of the stalk. After Schweiger (1984)*

nomycin, which inhibits mRNA synthesis, had no effect on phase. Only the amplitude of the rhythm was reduced (Sweeney et al. (1967), vanden Driessche (1970)).

4.3 Several oscillators?

Are the various circadian oscillations in *Acelabularia* all controlled by one clock? To answer this question, the chloroplast migration, the electrical potential and the oxygen production was recorded in the same cell simultaneously (Schweiger et al. (1983). They kept the same phase relationship to each other, even after changing the temperature of the water. Thus, either the various rhythms are driven by the same clock, or there are different oscillators, which are strongly coupled to each other. In favor of the latter is an observation of Schweiger et al. (1986): The circadian chloroplast migration as well as the circadian fluctuations of the electrical poten-

tials are normally both phase shifted by an 8 hour dark pulse under continuous light to the same amount. Occasionally, however, only one of the rhythms is shifted, the other one not. This would speak in favor of two different clocks which control one of the two rhythms, respectively.

4.4 Do cells interact?

Do *Acetabularia* cells interact (Mergenhagen and Schweiger (1974))? 50 cells, which were kept in a 12:12 hours light-dark cycle, were transferred to a vial with a single *Acetabularia* cell, which was kept in a light-dark cycle 12 hours phase shifted (that is, they had light, when the 50 cells were in the dark phase). Under subsequent constant conditions these cells did not influence the individual cell: It continued its rhythm as before (measured for 7 days). The same has been found in *Euglena* and *Lingulodinium*. They too do not mutually influence each other in their phase position.

5 Rhythms in *Chlamydomonas*

Chlamydomonas is an alga of the soil, which is also able to swim (figure 5.1). It is photosynthetic (Matsuo et al. (2005)), but possesses additionally many properties of animals (and has the corresponding genes). It is called 'green yeast', but resembles more a plants and an animal (Brunner and Merrow (2008)). An eye spot allows to receive light and to reflect it onto a membrane-bound photoreceptor (see Kateriya et al. (2004), Nagel et al. (2005), Oldenhof et al. (2006)), which contains rhodopsin (Grossman et al. (2007)). *Chlamydomonas* has more than 15 000 genes, and many of them code transporters (Matsuo et al. (2008)).

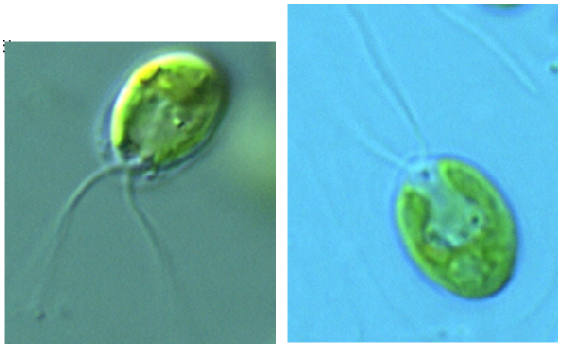


Figure 5.1: Chlamydomonas reinhardtii *is a flagellated green alga and belongs to the Chlorophytae. Images from Jens Bösger, kindly supplied by Maria Mittag, Institut für Allgemeine Botanik, Friedrich Schiller Universität Jena. Left with yellow eye spot, at right figure U-shaped chloroplast visible.*

Like animals, plants, fungi and cyanobacteria *Chlamydomonas* is equipped with a circadian clock, which developed, however, probably in all the groups mentioned independently from each other. The circadian clock was studied by Bruce (1970) using the phototaxis of *Chlamydomonas* (light induced movements of microorganisms are described in Sgarbossa et al. (2002)). He found various clock mutants. The cells show furthermore a rhythmic UV sensitivity (Nikaido and Johnson (2000), figure 5.2). The growth rate does also fluctuate in a circadian manner.

5.1 Clock mechanism

The circadian clock of *Chlamydomonas* utilizes phosphatases and kinases and is based on metabolic events such as phosphorylation (see also chapter 2). Phoscillators (Merrow et al. (2006)) interact with species specific transcriptional feedback loops (Mittag and Wagner (2003), Mittag et al. (2005)). Matsuo et al. (2006) transferred luciferase into the *Chlamydomonas* genome, which allowed to record the luninescence rhythm. By using forward gene screening[1] 105 clones were identified (Matsuo et al. (2008)). 32 of them showed different period lengths, phase positions and amplitudes of the rhythm. 78% were arrhythmic or showed a very low amplitude. The growth rhythm was influenced

[1] a genetic screen allows to select individuals with a certain phenotype. If one is looking for new genes, it is called 'forward genetics', whereas in 'reverse genetics' mutants of already known genes are searched for

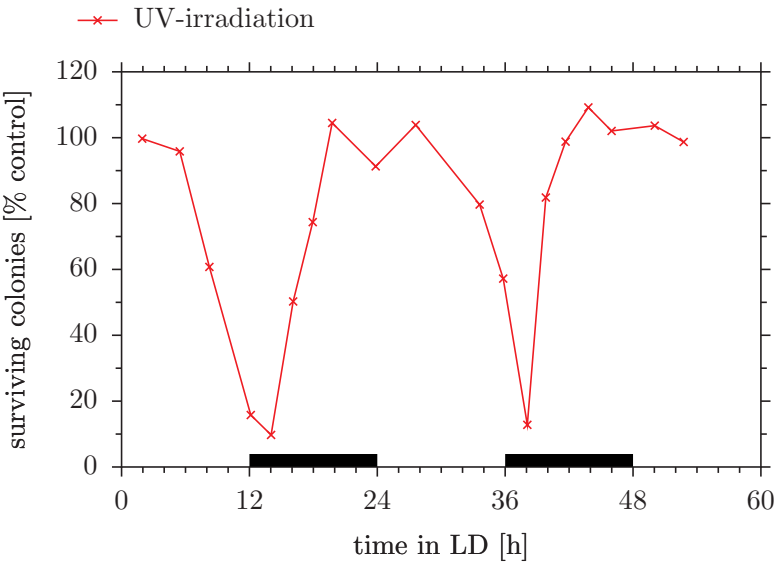


Figure 5.2: Chlamydomonas reinhardtii *colonies are rhythmically sensitive towards UV, if irradiated at different times of the day. After Suzuki and Johnson (2002)*

in the same way.

CHLAMY1 is an RNA-binding protein, which controls as a post-transcriptional regulator for instance protein turnover and ubiquitin-proteasomes. Phosphatases play also a role (Brunner and Diernfellner (2006)). The clock mechanism resembles partly that of *Arabidopsis thaliana*, the domains involved are, however, no true homologues (Schmidt et al. (2006), Wagner et al. (2005), Wagner et al. (2006)).

5.2 Photoperiodism in *Chlamydomonas*

Chlamydomonas growth faster under longday as compared to shortday. This is a photoperiodic reaction to environmental conditions (Suzuki and Johnson (2002)).

Under non-optimal conditions (low nitrogen supply) the haploid vegetative cells form gametes, which conjugate and form a diploid zygospore. This spore is dormant and does not divide. Unfavorable conditions such as darkness, dryness, and food deprivation can be better endured in this stage. The germination of these spores differs in the various daylengths (figure 5.3). However, not the germination itself is photoperiodically controlled, but an earlier event. The zygospores germinate synchronously, if they are kept for a day in continuous light, afterward six days in continuous darkness, and than again in continuous light (Harris (1998)). 12 to 16 hours later germination takes place. Meiosis occurs and divisions resulting in haploid vegetative cells.

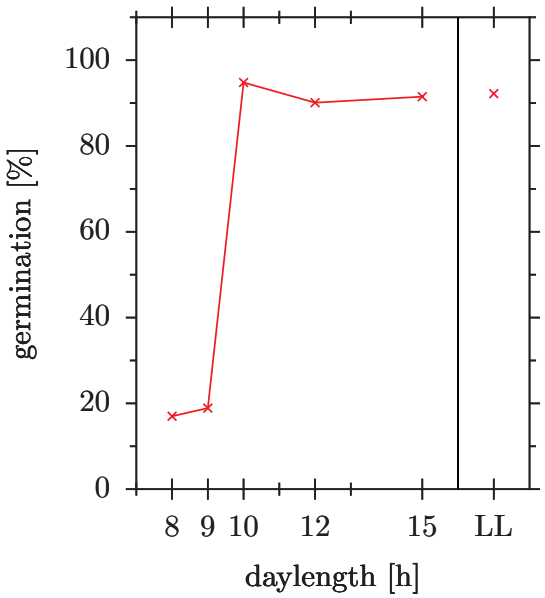


Figure 5.3: Chlamydomonas reinhardtii *zygospores were kept in various daylengths (8:16, 9:15, 10:10, 12:12, 15:9 hours L:D and continuous light LL). The percentage of germination 10 days after conjugation is shown. After Suzuki and Johnson (2002)*

6 Rhythms in amoebae, yeast and fungi

Here I could present a whole number of unicellulars with circadian rhythms, which do not photosynthesize. I restrict myself to three examples. In the first one a rhizopode is described, which exhibits an unusual rhythmic change between a resting stage and a mobile phase. The yeast as a second example oscillates in an ultradian rhythm, which is, however, temperature-compensated. The molt *Neurospora* possesses a circadian rhythm.

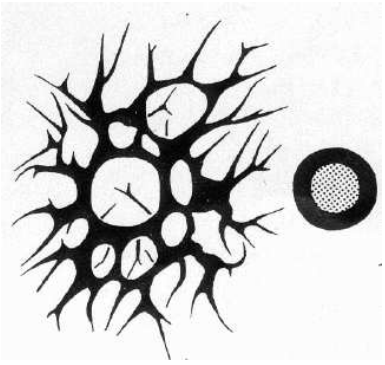


Figure 6.1: *Thalassomyxa australis* changes its form rhythmically between an active phase with pseudopodia (left) and a resting stage (right). After Grell (1985)

6.1 *Thalassomyxa*

Grell (1985) discovered at the west coast of Australia a marine rhizopod, which he named *Thalassomyxa australis*. It belongs to the naked amoeba. It changes its form rhythmically between a resting stage, in which it lies like a hat on the substrate, and a phase, in which it crawls with pseudopodia over the substrate, while feeding and digesting unicellular marine algae (6.1). The movie "The Change of Phases of *Thalassomyxa australis* (Promycetozoida)" shows its biology and this change in form (Grell (1987)). At a temperature of 22^0C the period length is 25 hours. At lower temperatures the period is considerably longer. At 10^0C, for instance, it amounts to 90 hours, at 28^0C only 18 hours (Silyn-Roberts et al. (1986)). The dependency is shown in figure 6.2. Temperature compensation is, however, regarded as a characteristic property of circadian rhythms. Furthermore the synchronization by a light-dark cycle and by a temperature change does not function in the usual way (figure 6.3, Förster and Engelmann (1988), Smietanko et al. (1988)). Shaking of the culture (as it occurs in the sea due to the tides) synchronizes only partly; this time cue is thus a weak one (Förster and Engelmann (1988)). Combined time cues (temperate change, light-dark cycle, periodical shaking as a simulation of low/high tide) synchronizes (figure 6.4). It is unknown, which time cues are effective in nature. We might be dealing with a precursor of a circadian clock, a kind of ancient clock, which does not yet possess all the characteristic properties of 'modern' circadian clocks. Whether and how they can be deduced from ultradian rhythms, which do not or do possess temperature compensation, is speculative (Silyn-Roberts and Engelmann (1986)).

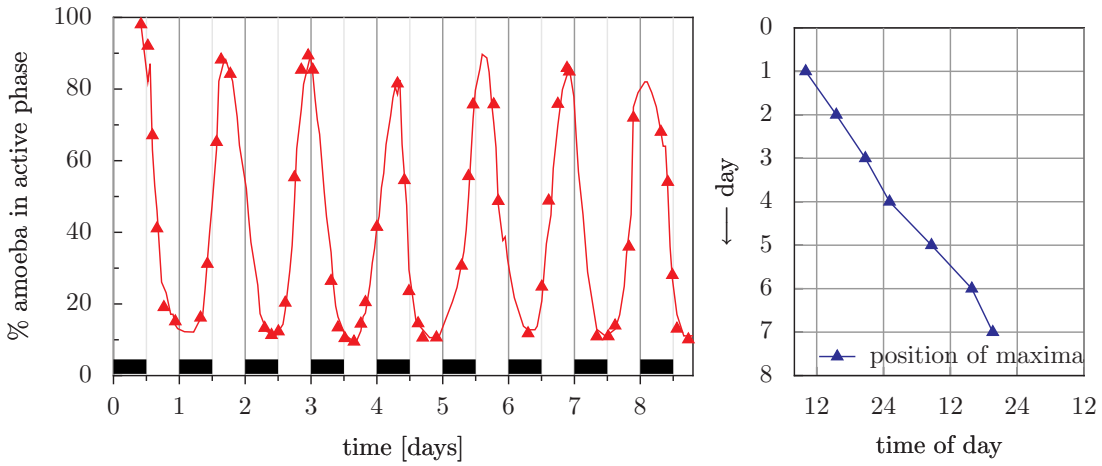


Figure 6.3: *Red curve in left part of figure shows percentage of* Thalassomyxa *amoeba in its active phase, with maxima shown as triangles (x-axis: days). The change in form of* Thalassomyxa australis *can not be synchronized by a 12:12 hour light-dark cycle (see the position of the maxima (triangles) to the bright/dark bars). That is shown in the right part, where the maxima (blue triangles) occur eachday by 5 hours later. If the rhythm would be synchronized, they should lie exactly below each other. After Förster and Engelmann (1988), Smietanko et al. (1988)*

6.2 Yeast, glycolysis oscillator

Cells are able to produce energy in three different ways: By photosynthesis, by respiration and by glycolysis. Glycolysis is used by organisms, which live without oxygen such as Yogurt fungi, bacteria in sauerkraut, parasitic worms, red blood cells, diving vertebrates.

During glycolysis glucose in converted to pyruvate. During the process ATP is generated as an energy carrier. Nine different enzymes are involved in this conversion (figure 6.5). Duysens and Amesz (1957) found, that glycolysis of the yeast does not occur uniformly, but under certain conditions also rhythmically. This is found during the fermentation of glucose by yeast. In the absence of oxygen alcohol is produced. The biochemical steps of the glycolysis oscillations in the yeast *Saccharomyces* are well known. If the various enzymatic reactions are combined in equations, oscillations occur in some of the reaction steps. Oscillations are indeed found also experimentally, if to a suspension of yeast cells glucose is added as a substrate (Betz and Chance (1965)). The easiest way of recording the oscillations is the measurement of the fluorescence of NADH (figure 6.6). Depending on the conditions the period lengths lies between two and 70 minutes. It depends strongly on temperature.

To observe the phenomenon, a yest suspension is starved (no sugar offered). If the NADH fluorescence is constantly low, glucose is added, so that the concentration is 100 mM. Afterward potassium cyanide KCN is added. The cells are now without oxygen. Glycolysis occurs now at 20^0C

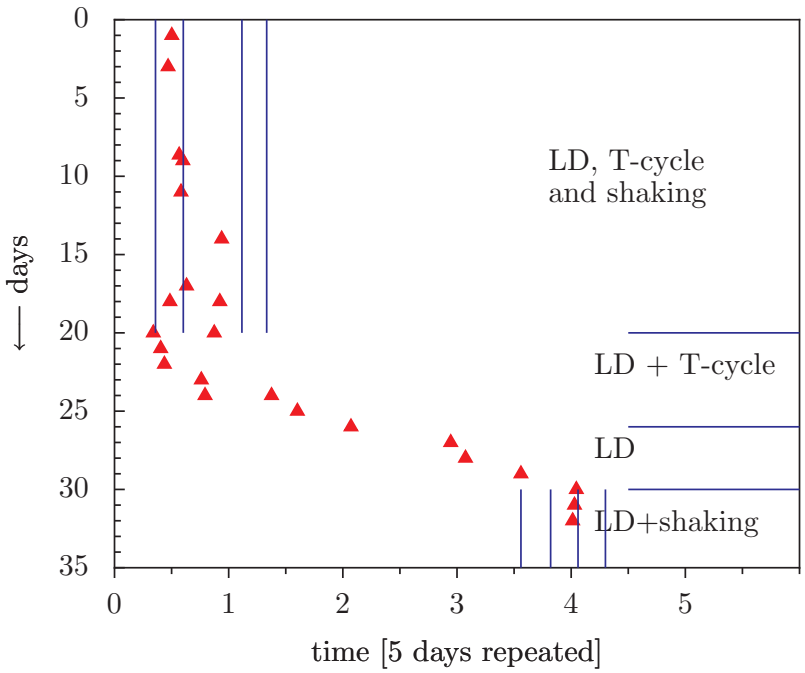


Figure 6.4: *Combined time cues (light-dark cycle, temperature-change and shaking periods in a 6-hour-measure, upper part of figure) synchronizes the rhythmic change of form in* Thalassomyxa australis, *whereas neither the combination of a light-dark-cycle and a temperature cycle (LD and T cycle) nor a light-dark-cycle allown (LD) synchronize the rhythm. Shaking combined with a light-dark cycle, however, does synchronize. After Förster and Engelmann (1988)*

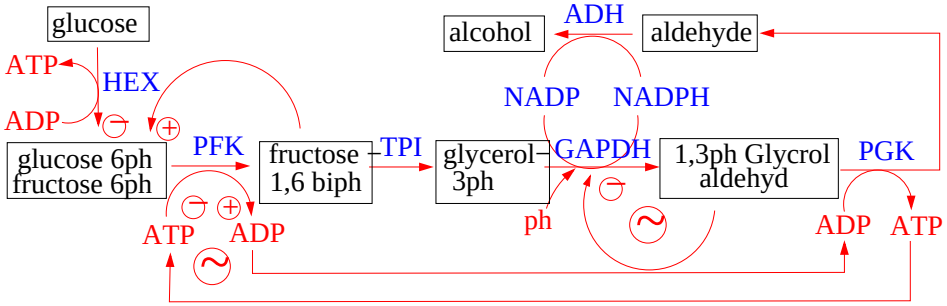


Figure 6.5: *Course of glycolysis in yeast and feedback of substrates on enzymes. With ~ the positions are marked, at which oscillations occur. →: flow. Bent arrows: Feedback pathes. +: Activation, -: inhibition. Enzymes involved: HEX: Hexokinase, PFK: Phosphofructokinase, TPI: Triosephosphate-isomerase, GAPDH: Glycerine aldehyde phosphate-dehydrogenase, ph: Phosphate, PGK: Phosphoglycerokinase, ADH: Aldehyde-dehydrogenase, NADP: Nicotinamide-dinucleotide-phosphate. ATP: Adenosine triphosphate, ADP: Adenosine diphosphate. After Chance et al. (1967)*

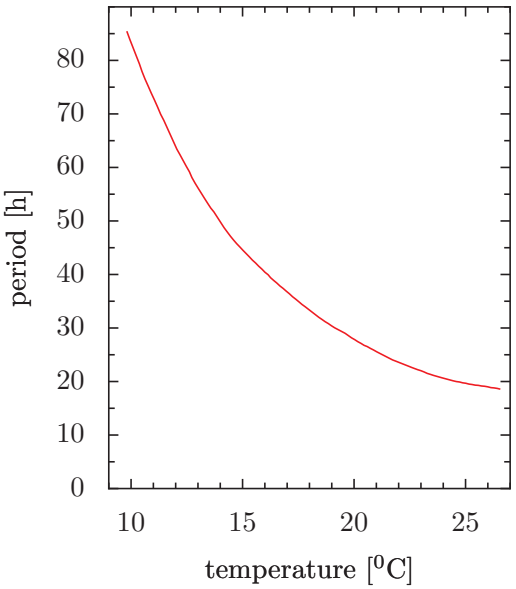


Figure 6.2: *The change between an active phase and a resting stage of* Thalassomyxa australis *depends on the temperature of the sea water. Period lengths is plotted on the y-axis, temperature of the sea water on the x-axis, in which the amoebae were kept. After Silyn-Roberts et al. (1986)*

to cycle with a period lengths of about a minute.

The oscillations occur in a critical range of the flow rate, because than feedback takes place in the course of the reactions (figure 6.5). Goal of the glycolysis is, to produce ATP. ADP controls thereby the activity of the phosphofructokinase (PFK) by binding to a specific receptor of the enzyme. This alters the configuration of the enzyme and it works hundred times faster. At lower ADP-concentrations (that is high ATP content) the glycolysis is inhibited. In this way oscillations occur. The glycolysis oscillations are also observable in cell free extracts (Hess and Boiteux (1971)).

For the control and coordination of metabolic events organisms use also ultradian clocks, which might proceed like circadian clocks *temperature-compensated* . They have, however, a higher frequency and can not be synchronized by time cues in a 24-h-measure. An example is *Paramecium*. If the movement of individual *Paramecium* cells is observed under the microscope, periods are found, in which these unicellulars swim longer distances more or less straight and choose seldom another direction. After a certain time, however, the pattern of swimming changes. Now they swim only short distances in a straight way and change direction more frequently. This behavior occur in periods of 45 minutes (Lloyd and Kippert (1987)). The same periods are found also at higher and lower temperatures. Thus, this ultradian rhythm shows like circadian rhythms a temperature compensation. It might imply a function as a clock. In the meantime further examples for temperature-compensated ultradian rhythms have been found (overview Lloyd and Rossi (1992)).

An ultradian clock for instance controls

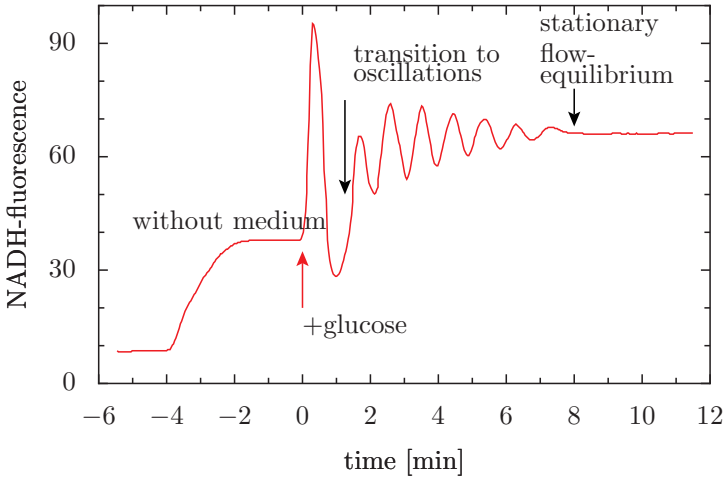


Figure 6.6: *Induction of glycolysis oscillation in yeast suspension. At time 0 (blue arrow) glucose was added to the starved suspension. The stationary flow equilibrium is reached after an oscillatory transition. Glycolysis was recorded by the fluorescence of the NADH (y-axis). After Antkowiak (1987)*

in rapidly growing cells of *Schizosaccharomyces pombe* cell division. The rhythm has a period lengths of 40 to 44 minutes (figure 6.7). This rhythm is temperature-compensated and independent of the growth rate (Kippert and Lloyd (1995)). It continues to oscillate for at least 18 hours without damping. This speaks in favor of an intercellular communication. The CO_2 formation in fermentation (figure 6.8) and also in respiring cultures fluctuates with the same period as O_2 uptake and acidification of the culture medium. If cell division is blocked, the other three rhythms continue to tun. They are thus not a direct consequence of the cell division rhythm. An ultradian clock seems to exhibit a general control of metabolic events (Kippert and Lloyd (1995)).

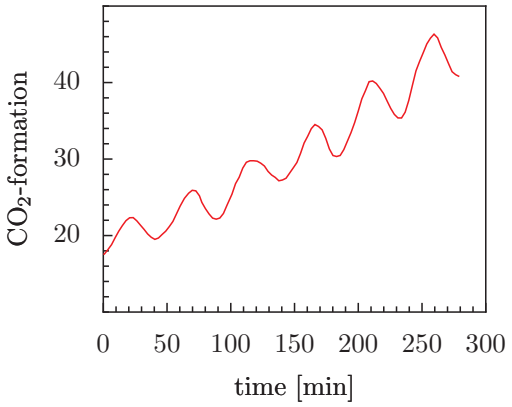


Figure 6.8: CO_2*-formation in a fermenting* Saccharomyces pombe *culture at* 30^0C *after 4 temperature cycles of 30 minutes* 30^0 *and 15 minutes* 26^0C *(not shown). Measurements in 5 minute intervals in three different and independent experiments. The data were averaged and smoothed. After Kippert and Lloyd (1995)*

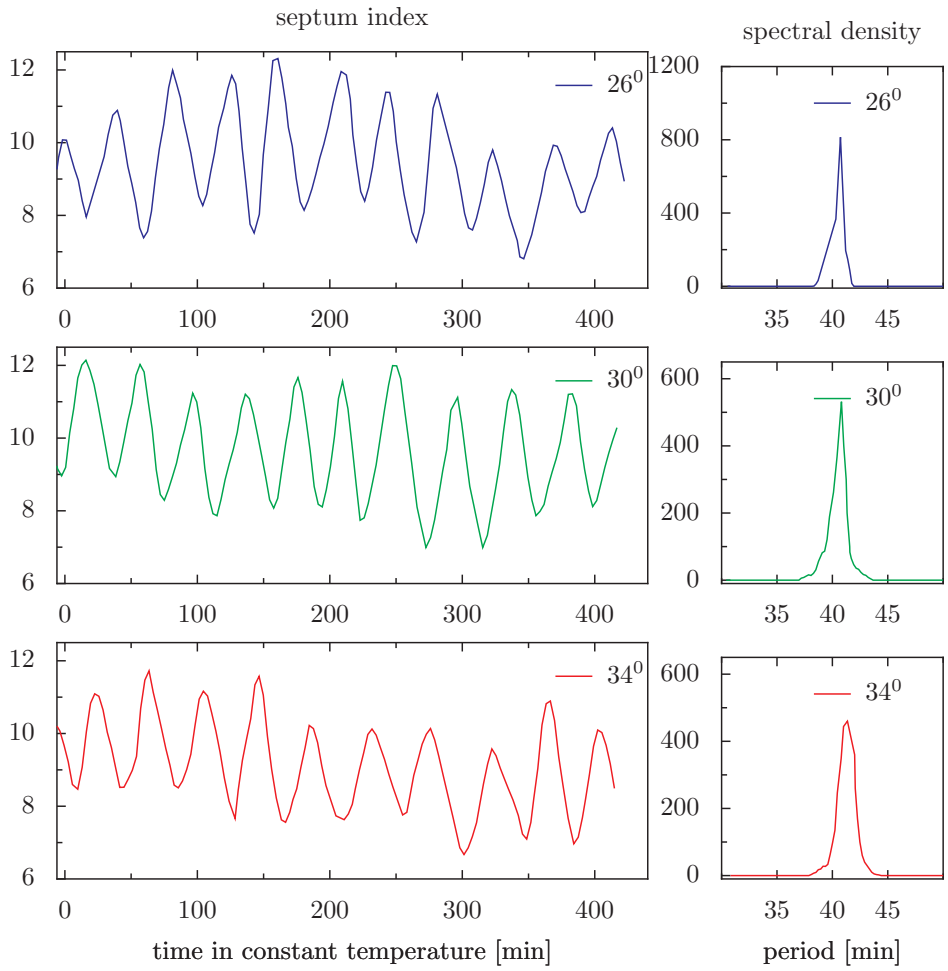


Figure 6.7: *Cell division in Saccharomyces pombe cultures. The culture was first synchronized by temperature cycles of 30 minutes* 33^0 *and 15 minutes* 27^0*. Afterward the cultures were transferred into constant temperature conditions of* 26^0 *(upper diagram),* 30^0 *(diagram below) and* 34^0C *(lower diagram). The percentage of cells, which form a cross wall (septum), was determined as the septum-index. The results of time series analysis using maximum entropy spectral analysis (MESA) are plotted at the right of the curves (spectral density plotted against period lengths). They show at all three temperatures the same period lengths of about 40 minutes. The period lengths is thus independent of the temperature of the medium. After Kippert and Lloyd (1995)*

6.3 *Neurospora*

Neurospora belongs to the Ascomycetes. The fungus forms a mycelium, in which the cells are fused to a syncytium. We are dealing with a kind of giant cell.

Filamentous fungi are more closely related to animals, as shown by newer studies (Wainright et al. (1993)). Therefore studies of daily rhythms in fungi are of significance and might possibly help, to understand also the mechanisms of circadian rhythms in animals. Fungi are furthermore genetically and biochemically well studied. This is especially true for *Neurospora crassa*, an Ascomycetes. It is originally a native of tropical and semi-tropical areas, but is in the meantime a cosmopolitan. Its developmental cycle and alternation of generations is described in figure 6.9. There is a sexual and an asexual propagation cycle. Ascospores are formed in asci, which are in perithecia. *Neurospora* produces furthermore also asexual conidia. For this the mycelium differentiates into aerial hyphae, and they produce conidia.

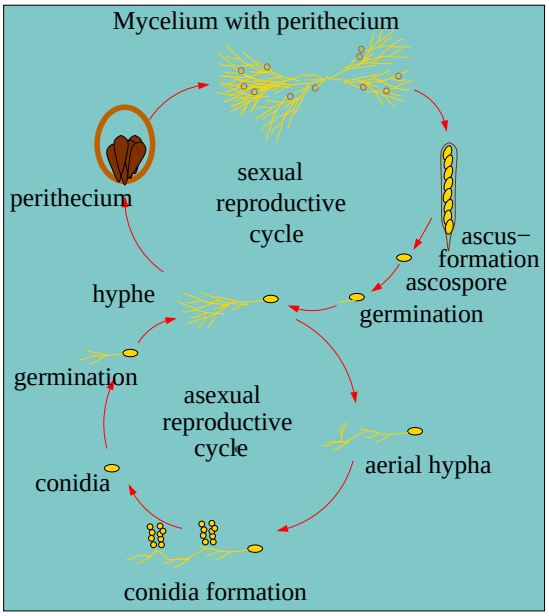


Figure 6.9: D*evelopmental cycle and alternation of generations in* Neurospora. *Top: Sexual propagation cycle. Ascospores are formed in Asci, which are in perithecia. After germination an ascospore forms a mycelium (coenocytic, that is, many nuclei in the common cytoplasm). Perithecia are formed via protoperithecia. In a perithecium asci are made, which produce ascospores, out of which again mycelia develop. Now the sexual propagation cycle is terminated. Bottom: Asexual propagation cycle. The mycelium forms asexual conidia (‘macroconidia´): Aerial hyphae arise, which produce later conidia. They germinate and form new mycelia (the pattern formation of* Neurospora crassa *was described by Deutsch et al. (1993)). The switch between undifferentiated mycelium and aerial hyphae is triggered by a circadian clock. After Springer (1993)*

6.3.1 Circadian rhythm of conidiation and other events

The switching between undifferentiated mycelium growth and formation of aerial hyphae is controlled by the circadian clock. The period lengths of the rhythm is 21.6 hours in the wild type (Dunlap (1993)). Conidiation serves as a hand of the clock and is easy to measure. During the daily alternation of light and dark these asexual spores are produced in the late night and the early morning. During the conidia formation a dense mycelium is formed. It is more ramified and aerial hyphae grow out of the medium. On

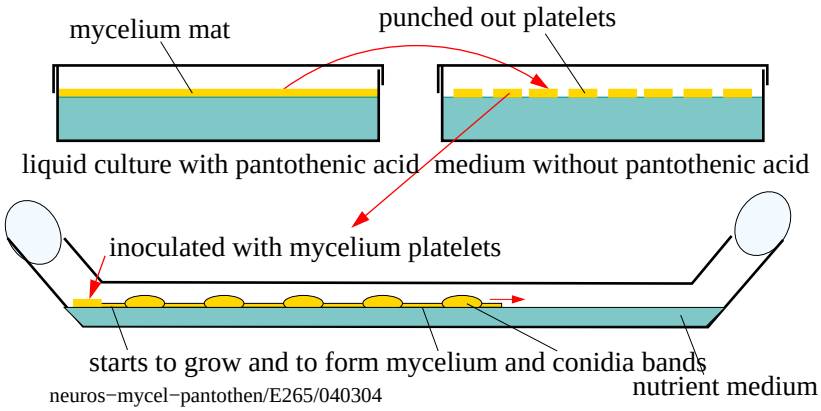


Figure 6.11: *Circadian rhythms occur also in liquid cultures of* Neurospora crassa. *A mutant with a panthothenic acid defect was reared with panthothenic acid, until at the surface mycelium mats had formed (top left). With a cork borer circular pieces were punched out and transferred in a liquid culture without panthothenic acid (top right). In this way growth is prevented. In spite of it the circadian rhythm continues: On agar of a* running tube *containing panthothenic acid circadian bands are formed (bottom)*

their tips conidia are later formed (figure 6.10). Macroscopically they can be recognized easily as yellow bands. Since growth is constant in spite of the conidia formation [1], the rhythm can be recorded with a ruler in the running tube and the intervals between the center of subsequent conidia bands determined (see running tube in figure 6.11 and a video clip of growing *Neurospora crassa* showing protoplasm streaming (Fungal Genetics Stock Center's World Wide Web Site, http://www.fgsc.net/)).

However, a special mutant, *bd*, has to be used, which forms conidia also in closed tubes. Otherwise the accumulating CO_2 would suppress conidia formation. The mutant *bd*

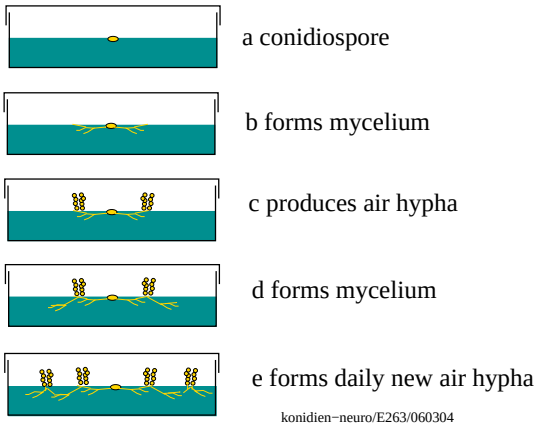


Figure 6.10: *Conidiospores of* Neurospora crassa *germinate and the hyphae on the substrate form the mycelium. After some time aerial hyphae are produced, which grow out of the substrate as conidiophores and produce the conidiospores. Afterward they grow again as a normal mycelium on the substrate, until the next conidia band* is formed. *This process reiterates each day. After Rensing (1993)*

[1] see, however Lakin-Thomas et al. (2001): With time lapse video photography it was found, that the band/interband -commitment at the growth front is not tightly bound to the subsequent synchronization of the conidiospore differentiation. This could indicate, that two circadian oscillators are present, which control these two events and does also mean, that growth is not always constant.

growth furthermore about 30% more slowly as compared to the wild type.

At a constant temperature and under physiological darkness (weak red light) the freerun period is 21 to 22 h. The rhythm is temperature-compensated between 18^0 and 30^0C. It can be phase shifted by light pulses.

Although the conidia formation is tightly coupled with the circadian oscillator, the latter functions also without the conidia rhythm. Hands of the oscillator are in this case for example biochemical rhythms such as the amount of nucleic acid and -synthesis, protein content, enzyme activity (Hochberg and Sargent (1974)), or the ratio of ADP to ATP in the mitochondria. Furthermore some fatty acids of membranes fluctuate periodically.

Other events which are connected with differentiation are under circadian control. The signals, which control these metabolic processes and differentiation, are not yet known. Such a signal could be cAMP, which fluctuates in a circadian manner (Hasunuma et al. (1987)). The circadian clock of *Neurospora* can be observed also in individual cells (freshly germinated conidiospores, Lindgren (1994)).

6.3.2 Time cues and temperature compensation

In the same way as light pulses temperature-pulses can also shift the circadian rhythm of *Neurospora crassa* . Temperature cycles are even stronger time cues in *Neurospora* as compared to light-dark-cycles (Liu et al. (1998)). Depending on the strength and duration strong and weak reactions occur. These effects are found in cultures on agar as well as in liquid cultures. Heat shocks are more effective than cold pulses. Already a difference of 2^0 given in a 24 measure synchronize the rhythm of *Neurospora crassa* (Francis and Sargent (1979)).

If a circadian clock is functioning correctly, it should not run with different speeds at different environmental temperatures. The circadian conidia formation of *Neurospora crassa* is temperature-compensated only between 18^0 and 30^0 ($Q_{10} = 0.95$) (Gardner and Feldman (1981)). The temperature compensation of the circadian clock does also in other organisms function only in certain limits. Outside of these 'allowed temperatures' the temperature compensation does not work any more or the circadian clock stops and stays in a special phase. At higher temperatures the Q_{10} is 1.3.

Various models were proposed for explaining the temperature compensation. According to studies of Dunlap et al. (1998) it is the result of the expression of different amounts of two different kinds of FRQ (sFRQ and lFRQ) at higher respectively lower environmental temperatures (Liu et al. (1997)). At higher temperatures more sFRQ is made, at lower temperatures more lFRQ. Thus the ratio of the two FRQ' depends on temperature. Both FRQ specimens are degraded by phosphorylation.

6.3.3 The circadian clock of *Neurospora*

How do circadian clocks control gene expression and how do they interact with the environment? The underlying mechanism of these circadian rhythms will be discussed briefly in the following. It is quite complicated. In the case of the *Neurospora* system circadian control, control by light, metabolic control and control of the development interact.

In the following I will present first some of the known essential components of the

circadian clock work.

The product FRQ of the *frq*-mRNA of the *frq* gene is one of the major players in the circadian game of *Neurospora crassa*. The *frq* gene was cloned and is a 7.7 kb DNA consisting of two transcripts (4 and 4.5kb). If one of the two transcripts are deleted, it fails to function. FRQ is in *Neurospora* responsible for the period lengths *and* for the temperature compensation of the clock.

Two further gene products are important, White Color WC-1 and WC-2. They are exressed by the *wc*-1 and *wc*-2 genes. Both genes were cloned. *wc*-mutants have a low *frq*-expression in the dark and do not show a circadian rhythm. Likewise temperature is not capable to induce a rhythm in these mutant. That shows, that WC-1 and WC-2 are components of the clock or are tightly connected with factors of the clock. WC-1 and WC-2 are transcription factors with DNA binding positions, trans-activating domains and PAS domain es for protein-protein interactions. PAS domains are found in many regulatory proteins, which play a role for signal transfer and -perception of various stimuli (light, chemical compounds, oxygen). In the the present case the PAS domain recognizes binding positions of light regulated promoters and serves perhaps, to interact between receptors and signal-transduction components.

WC-1 and WC-2 dimerize with each other and form the White Color complex WCC. The PAS domain is used for dimerization. WCC binds at two locations to the promoter of the *frq* gene. This activates the expression of the *frq* gene. The primary transcripts are spliced in a complex way, which has strong effects on the produced protein. WCC transfers light signals onto light-sensitive and clock-controlled genes (arrows to *frq*, *wc*-1 and *ccg* in figure 6.13). WC-2 is a frequent, constitutive nuclear protein, which serves as a scaffold, on which FRQ and WC-1 (which are out of phase with each other) interact (Denault et al. (2001)).

A further player in the circadian system of *Neurospora* is VVD, a protein, which is transcribed by the vivid gene (vvd). It has been cloned and characterized (Heintzen et al. (2000)). Light induces is rapidly, but is independently controlled by the circadian clock. It is small protein with a PAS domain. It affectsinput *and* output of the clock, without being a part of the clock-mechanism (vvd null-mutants are still rhythmic).

Other players must be involved in the circadian system of *Neurospora*, since *frq*-null mutants are still rhythmic (although not in a circadian pattern). These players are so far unknown (see subsection 6.3.3).

The product FRQ of the *frq*-gene is an essential component (a state variable) of the circadian oscillator of *Neurospora*. mRNA and protein production of the *frq*-gen are parts of the feedback system, which constitutes the circadian clock. The products of the *frq*-locus regulate their own production (Aronson et al. (1992), Aronson et al. (1994)). That seems to be generally the case in circadian oscillators. The underlying principle is shown in figure 6.12 as a molecular feedback-oscillator.

A more specific model of the circadian clock of *Neurospora* was proposed by Dunlap and his group using molecular-biological studies. It is shown in figure 6.13. According to this model the product FRQ of the *frq* gene is an essential component of the circadian oscillator. The mRNA and the FRQ protein of the *frq* gene are parts of the feedback system, in which FRQ controls its own expression via

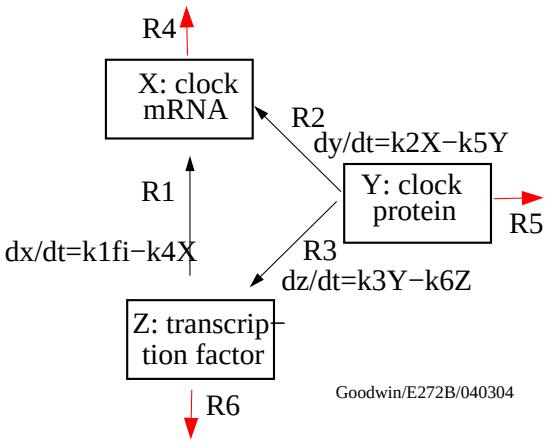


Figure 6.12: *Molecular feedback-oscillator according to Goodwin. The clock protein Y inhibits its own transcription from the clock-mRNA (X) via the transcription factor Z. The production rate of the intermediate products Y and Z are linear functions of the concentrations of X respectively Y. Without inhibition the X production occurs with a constant rate. It is, however, inhibited by Z due to the inhibiting factor f_i. Each intermediate product I (X, Y or Z) is produced and degraded in the following way $\rightarrow k_s \rightarrow I \rightarrow k_d \rightarrow$I (S), whereby* k_s *is the synthesis rate term* (k_1,k_2,k_3) *and* k_d *the degradation term* (k_4,k_5,k_6). *I in (S) fluctuates between high and low values, depending on whether synthesis reactions occur or, due to inhibition by Z, do not occur. The reaction chain (S) says, that the relaxation time (time scale of approximation to the equilibrium in I) depends on* k_d *only, and that the period lengths of the oscillation is determined by* k_d *only. Reaction R1: Formation of X, R2: Synthesis of Y, R3: Production of Z, R4 to R6 (red arrows): Degradation reactions. Inhibiting factor* $f_i = 1/1 + z^9$. *After Ruoff et al. (1999), based on Goodwin (1965) and Murray (1993)*

the *white color* complex WCC (Lee et al. (2000)). FRQ would thus be a state variable in the circadian system (Aronson et al. (1994)).

Light influences the circadian system by activating the expression of *frq* via the WCC complex. The complex is formed by dimerization of the WC-1 and WC-2 proteins. Figure 6.13 illustrates the way, in which light affects the clock work.

It was proposed (Loros (1995)), that light influences the circadian clock by switching off the negative feedback of FRQ on its own synthesis: Repression by FRQ is canceled (or: in spite of the presence of FRQ induction takes place). This effect of light can abolished by inhibition of the protein synthesis or of the mRNA production. The model explains the effect of single light pulses on the rhythm of conidia formation under continuous darkness, the behavior under light-dark changes and under skeleton photoperiods. It furthermore clarifies, how a light signal advances or delays the rhythm as a function of the phase, at which it was applied (figure 6.13).

WCC transfers light signals also to light sensitive and clock-controlled genes (arrows to *frq*, *wc*-1 and ccgs in figure 6.13, Arpaia et al. (1993)) independently of effects on the clock. There are further genes, which are controlled by the clock as well as directly by light.

Finally the vivid gene (vvd) participates in the game. It influences inputs and outputs of the clock. It is induced by light, but additionally controlled by the circadian clock, without being a part of the clock mechanism (see figure 6.14).

Temperature effects are induced in *Neurospora* by post-transcriptional control (Liu et al. (1998)). As mentioned, two different FRQ forms are made during the translation by two initiation codons, namely

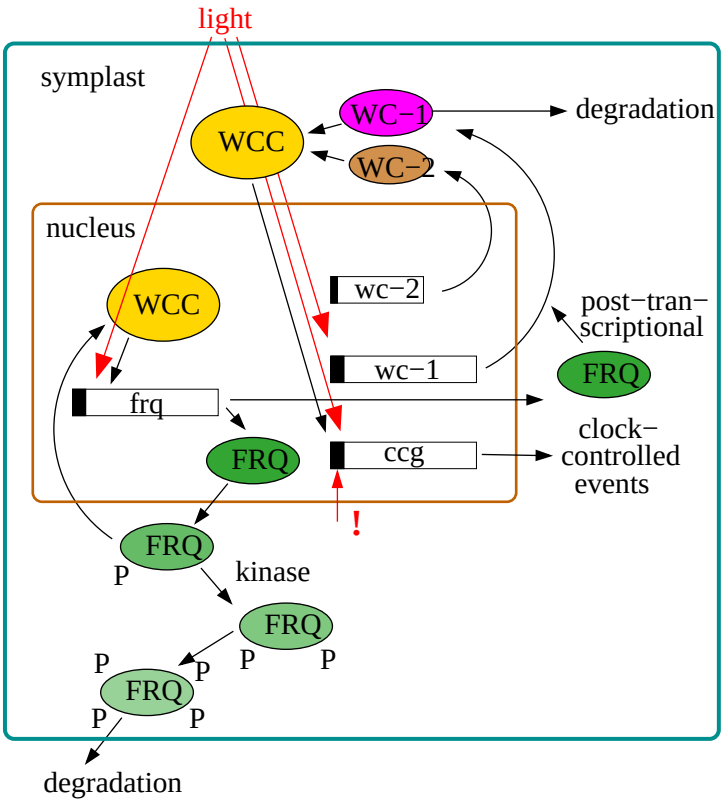


Figure 6.13: *Model of the feedback -oscillator of* Neurospora crassa. *mRNA and FRQ protein production* of the frq-*gene are parts of the* feedback *system in the circadian clock work. FRQ plays several roles. It regulates* the frq-*mRNA via trans-effecting factors of circadian controlled elements (CCRE's) and causes in this way a specific transcription at certain times of the day. It furthermore activates directly or indirectly genes, which are in this way controlled by the circadian clock and are therefore called 'clock controlled genes' (ccg's).*
Additionally the effect of light is shown as the most important time cue. Light influences the transcription of the frq *gene, and the protein WCC plays a role in transducing the light signal. In the late night and early morning a light pulse advances the rhythm. The advance is maximal, if much* frq *mRNA is present. In the late day and early night a light pulse delays the rhythm. At this time the concentration of FRQ declines. Since, however, the light pulse leads to more* frq *mRNA, it takes longer, until the mRNA and FRQ are reduced. Light affects furthermore clock-controlled genes (ccg's) directly. Thereby WC-2 plays a role. It is re-activated in the dark. After Dunlap et al. (1998)*

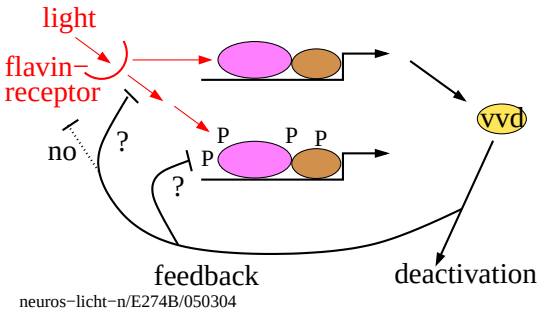


Figure 6.14: *The vivid gene vvd is induced by light, which it receives through a flavin-photoreceptor via the transcription factor WCC (consists of WC-1, violet, and WC-2, brown, top). It feeds back to the input path of the light via the transcription factor WCC of the* frq *gene (center) (right ?) or to the light signal transduction path of the photoreceptor to the transcription factor WCC* of the frq *gene (left ?), but not (no) to the photoreceptor (dashed path). The vivid gene is thus induced by light, but independently controlled also by the circadian clock, without being a part of the clock mechanism. After Linden et al. (1999)*

sFRQ and lFRQ. Each form leads to an oscillation at certain temperatures, but for a robust rhythm both are needed (Liu et al. (1997).

The setting of the rhythm by a temperature step reflects also a post-transcriptional regulation. Although the oscillations are alike at different environmental temperatures, the mean levels, around which the FRQ values fluctuate, differ. At a higher temperature the level is higher (illustrated in figure 6.15).

FLO oscillator The role of FRQ was recently newly interpreted twofold: One group doubts, whether it is indeed an essential constituent of the circadian clockwork (that is, a wheel in the clock work). This group claims, that FRQ participates only in processes, which lie before the oscillator and affects (via lipid signals?) the actual oscillator (Roenneberg and Rehman (1998), Lakin-Thomas (2000)). It was proposed, to separate transcription and feedback of the protein on its own mRNA formation from the actual oscillator (figure 6.16).

The other group adds another oscillator (or perhaps even more?) to the FRQ oscillator (so called FRQ-less oscillator FLO). Although the FRQ oscillator is needed for the circadian rhythm, it is probably not sufficient (Iwasaki and Dunlap (2000)).

Reasons for assuming an additional oscillator are earlier reports on the frq9 mutant (Loros and Feldman (1986), Loros et al. (1986)). This mutant shows still a rhythm, however several characteristic properties of a true circadian rhythm are lacking. Thus, the rhythm is shown only in a part of the cultures in the running tubes, the period lengths is quite variable (12 to 35 hours), it can not be synchronized by

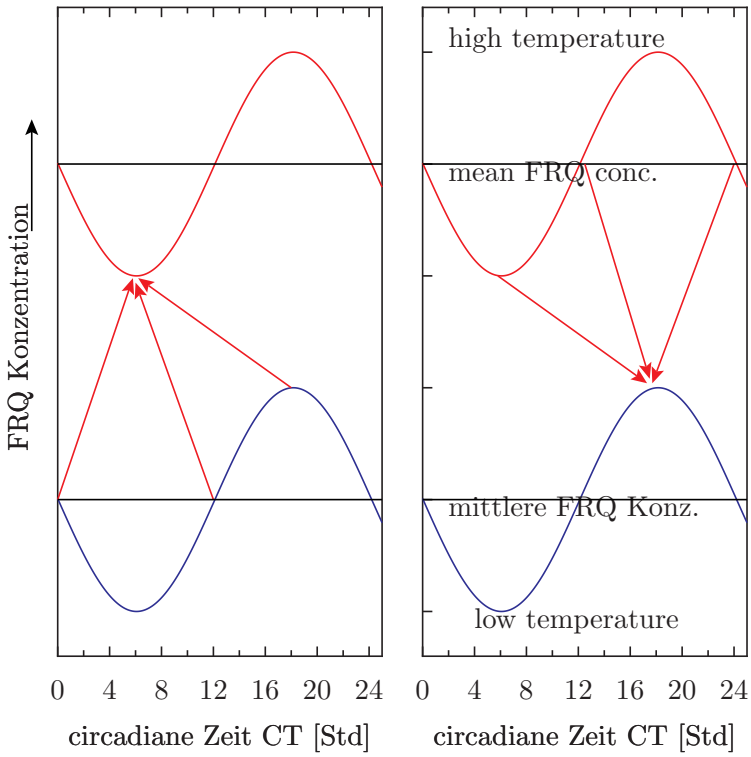


Figure 6.15: *The clock of* Neurospora *is shifted towards evening by an upward temperature step (left, red arrows), where the FRQ level is low (upper curve). Temperature steps downward (right) shift the rhythm to a morning phase, where the FRQ level is high, independent on the phase of the cycle, at which the step occurred (origin of the corresponding red arrow in right part). After Dunlap (1999)*

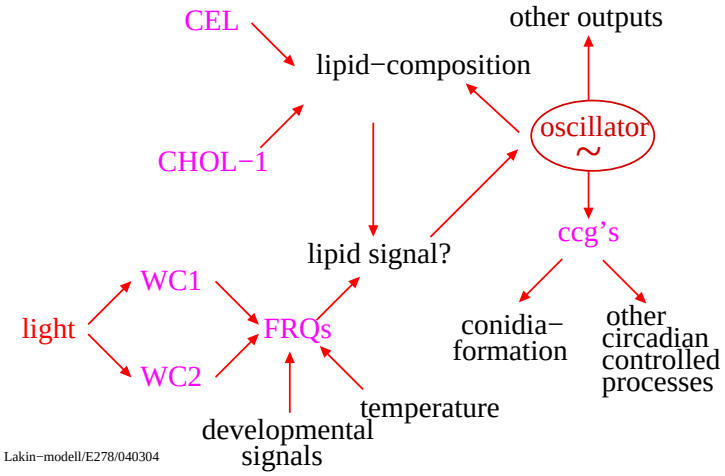


Figure 6.16: *After Lakin-Thomas FRQ is not directly an essential part of the circadian oscillator, but rather a part before the oscillator. Light acts via WC-1 and WC-2 on FRQ (temperature and developmental signals influence FRQ too). FRQ influences the circadian oscillator via lipid signals. Experiments with the mutant* cel *and* chol-1 *speak in favor of this interpretation. CEL and CHOL-1 affect the lipid composition and thus the lipid signal and the oscillator. The circadian oscillator controls 'clock controlled genes' (ccg's), conidia formation and other processes. The circadian oscillator has outputs, one of which feeds back to the lipid composition. After Lakin-Thomas (1998)*

light cycles, and it is not temperature- and nutrient compensated. It reminds of the strange rhythm of *Thalassomyxa australis* (see section 6.1) and represents perhaps a developmental rhythm.

Significance of the circadian system

The circadian system represents a reliable clock, because positive and negative acting feedback loops are interacting, which do not only determine the period length of the circadian clock, but confer also robustness and reliability (Yang et al. (2001)). This clock does not only run under continuous light or continuous darkness and constant temperature conditions and controls tributary events, but has to be synchronized in nature also to the 24 hour day. Otherwise it would rapidly get out of step with the day-night cycle and would become unreliable. Photoreceptors and transduction pathes to the clock must therefore be present. Temperature cycles are in *Neurospora* even stronger time cues as are light-dark cycles. That could be important for a molt which grows often on substrate, which is not exposed to the day light. Additonally the circadian clock mechanism of *Neurospora* possesses a temperature compensation, which is essential for a reliable clock.

It was also discussed, whether an annual rhythm in spore production, which is often found in fungi, is present also in *Neurospora* and whether it is photoperiodically controlled (Roenneberg and Merrow (2001)).

6.3.4 Outputs of the clock, clock-controlled genes

The outputs of the clock and the mechanism, how the observed rhythms are achieved, are also important parts of the circadian system. The best studied circadian rhythm of *Neurospora* is the switch of the hyphae from growing on and beneath the surface to growing into the air and the subsequent conidia formation. Rhythmic conidia formation shows up only at the growth front of the mycelium, while growing on solid medium. There it is decided, whether aerial hyphae, conidiospores and carotinoide are produced or not.

Many biochemical rhythms are connected with this developmental switch: The number of hyphae and aerial hyphae, the hyphal branching, septum formation, delivery of ripe conidia, division of the nucleus, glycolysis, lipid metabolism, the glyoxalate cycle, the tricarbonic acid cycle, the deposition of lipids, the synthesis of carbohydrates, CO_2 production, and the activity of a number of enzymes. First of all it has of course to be checked, whether these events are rhythmic only, because they depend on conidia formation. Alternatively these rhythms could arise also independently. To show, that for instance certain enzymes show a circadian rhythm independent of conidia formation, The connected morphological changes have to be prevented. This can be obtained by using for instance liquid cultures. Not only in studies using *Neurospora*, but also in those using other organisms it was found, that the circadian clock controls mainly enzymes at crucial points of the metabolism. This seems to be a general principle of circadian control.

Other events in the life cycle of *Neurospora* are under circadian control. Thus, the energy load fluctuates in a circadian way (Delmer and Brody (1975), Schulz et al. (1986)), the amount of heatshock proteins (Kallies et al. (1998)), and the delivery of ascospores are examples (Brody, unpublished). The period length of these rhythms corresponds to the one of the conidia formation.

Genes, which are rhythmically expressed also under constant conditions with a period lengths corresponding to the one of the genotype of the stock, are called clock-controlled Genes (‘ccgs´). If the function of these genes is lost, the clock is not affected. Their function is restricted to the output of the clock. They have to be distinguished from genes, which are developmentally regulated and from genes, which react to environmental changes. Clock-controlled genes are driven by the circadian clock via factors, which pass phase-specific time information to the target genes (see figure 6.17 and Loros and Dunlap (2001)). In the meantime quite a number of ccgs are known, and many will be added, if differential screening and microarray analysis are used.

How the time is read from the clock is not yet understood.

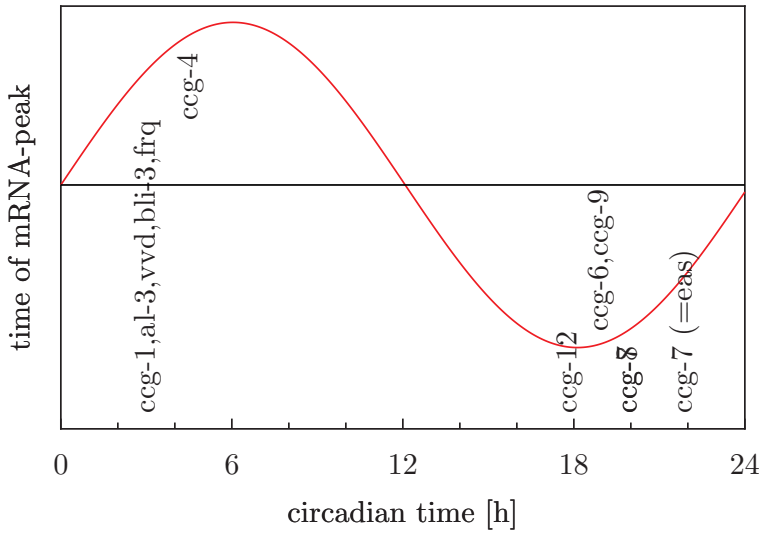


Figure 6.17: *Maxima of mRNA of various clock-controlled genes of* Neurospora. *During the late subjective night and the early morning:* ccg-*1 (identity unknown),* al-*3 geranylgeranyl-pyrophosphate synthase, vvd (vivid, light repressor),* bli-*3 (identity unknown),* frq *(clock-component, transcriptional co-repressor) and* ccg-*4 (identity unknown). During the subjective evening and the early night:* ccg-*12 (or* cmt*, cupper-metallothionein, ccg-6 (identity unknown) and ccg-9 (trehalose-synthase), ccg-8 (identity unknown), ccg-7 (glycerine aldehyde 3-phosphate dehydrogenase), ccg-2 or eas, hydrophobin). From table 2 in Loros and Dunlap (2001)*

7 Rhythms in mammalian cells

Cell clocks can be studied not only in unicellulars, but also in cells of tissue. In this chapter some examples for rhythms in the body of mammals are presented. In contrast to unicellulars the cells of mammals are, like in other multicellulars, specialized, in order to store and digest food, to eliminate toxic substances, to allow movement and much more. They form tissue (muscles, fat tissue) and organs (liver, kidney, brain). These cells in tissues and organs contain circadian clocks (see figure 7.2), and the phase position of their day/night-rhythm differs. Time cues for tissue can either act directly (light in the SCN) or indirectly (food in the liver, information from the SCN). Each tissue and each organ has its own pattern of rhythmically transcribing genes[1] (figure 7.1, Storch et al. (2007)). It reflects the various tasks and needs of the organs and allows, to function optimally under the rhythmic conditions of the environment existing since million of years by simulating these periodic fluctuations by an internal clock. This clock coordinates the transcription of genes for the most important metabolic pathes and directs the course of physiological processes and the behavior (Holzberg and Albrecht (2003)).

In the following we will get to know cell clocks in various tissues of mammals. It will be shown, how complex systems (metabolism, brain) interacts with the circadian center and the environment. It will furthermore turn out, that some diseases are caused by systemic malfunctions (see chapter 8).

In the hypothalamus of the brain is a center of rhythmic control, the suprachiasmatic nucleus (SCN, see section 7.1). It controls numerous areas of the central nervous system and the body, allowing to adapt their circadian rhythms to the optimal times. The SCN is provided via the eyes with information of the light-dark cycle in the environment (see section 7.3). It contains furthermore also information from the body about its conditions and needs (see figure 7.2).

Cells of peripheral tissues can generate circadian rhythms, but they are not directly synchronized by light (Schibler et al. (2003)). They have therefore to be set by nonphotic time cues to the correct time, and food uptake is the most important one. Feeding during the day inverts the phase of the expression of circadian clock genes in night-active rodents in the laboratory in many tissue such as liver, kidney and heart, but has no effect on the rhythm in the SCN. We have to assume therefore, that normally the SCN synchronizes the peripheral clocks mainly by the feeding behavior, which is then again caused by the sleep-wake behavior. Additionally the body temperature rhythm, which is also dependend on the pattern of the food up-

[1] only about 10% of the rhythmic genes are found in at least one other tissue, which is known to contain clock-genes. These common genes are probably tightly connected with the clock-mechanism or represent new clock components (studies of Duffield et al. (2002) using microarray technology)

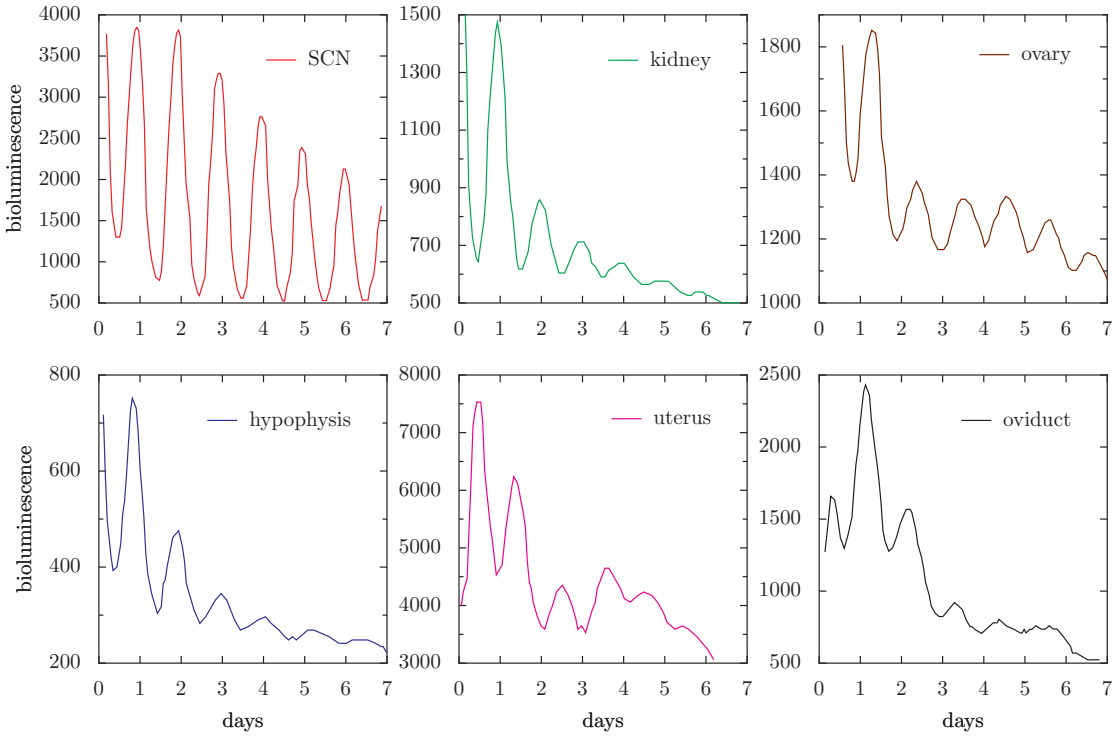


Figure 7.1: *Circadian rhythms in cells (with introduced luc-gene) of various organs*

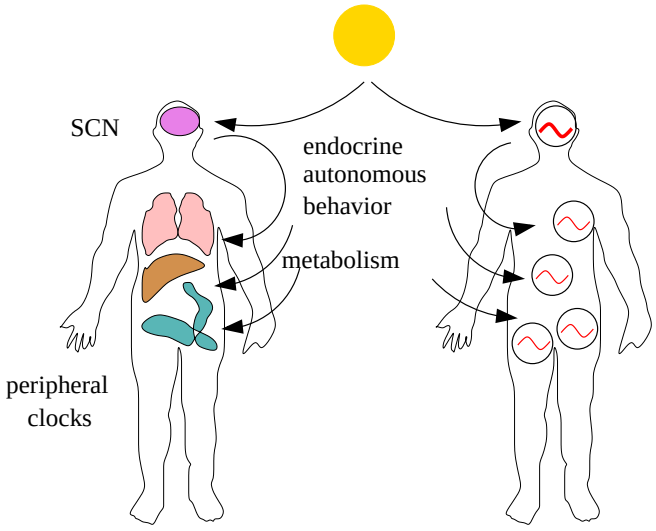


Figure 7.2: *The central clock in the SCN of the hypothalamus controls peripheral clocks in the tissues and organs of the body. The oscillators in the SCN are synchronized by the light-dark cycle and other time cues of the environment*

take, synchronizes the activity of circadian clock genes in vivo and in vitro.

The SCN can set phase of rhythmic gene expression in the peripheral tissue also via direct chemical signals. Whereas the SCN reacts only during the night to light, peripheral oscillators can be influenced by numerous chemical signals during the whole 24 hour day. Dexamethason, for instance, an antagonist of glucocorticoide receptors, restarts the rhythm of liver gene expression during any time of the day.

It is not yet clarified, how many rhythms of the peripheral organs and tissues are coupled and constitute a coherent system. To answer this question, Kurumiya and Kawamura (1991) measured the electrical activity in the SCN and outside of it in the lateral and the ventromedial hypothalamus. Blind rats with bilaterally destroyed SCN showed no more a circadian rhythm of the electric activity in the hypothalamic regions and no locomotor activity, if enough water and food was available, but it did under restricted food supply. Similar results were obtained in Guinea pigs (Kurumiya and Kawamura (1988)). Stokkan et al. (2001) studied the effect of cyclic feeding as a strong time cues on the gene expression in liver, lung and in the SCN. They used transgenic rats, the tissue of which produced luciferase in vitro. Thus the rhythm could be measured by using the luminescence of the tissues and organs. It turned out, that the rhythm in the SCN was still synchronized by the light-dark cycle, but feeding restricted to certain times shifted the rhythm of the liver within two days. The liver can thus be synchronized by food independent of the SCN and of the light-dark-cycle.

How the oscillators in the SCN control the locomotor activity and other events in a circadian manner, is so far only insufficiently known. Guo et al. (2005) used parabiosis between intact mice and mice with removed SCN. They found, that non-neural signals (behavior or blood-born) sustain the circadian rhythm of gene expression in the liver and the kidney, but not that of the heart, the spleen and the skeleton muscles. The SCN thus controls the expression of circadian rhythms in various peripheral organs via different pathways (Guo et al. (2005), Froy (2007), Froy et al. (2008), Guo et al. (2006), Hastings and Maywood (2000), Maywood et al. (2007b), Maywood et al. (2007a)).

How strong the rhythms in peripheral tissues and organs are, is shown by the fact, that they do not disappear after SCN lesions (Grundschober et al. (2001)). They are, however, not anymore synchronized with the LD. The SCN is thus needed for synchronization, but not for the preservation of peripheral rhythms.

7.1 SCN and its inputs and outputs

In all mammals the circadian system is dominated by the SCN. It consists of a paired aggregation of about 8000 to 10000 cells in the anterior part of the hypothalamus (figure 7.3). It is an autonomous rhythm generator, which operates via secretion of hormones and via the parasympathetic and sympathetic nervous system (Buijs et al. (2003), Buijs et al. (2006)). It prepares not only the body for changes to be expected in the activity rhythm, but also its organs for the hormone secretions connected with it. It controls rhythmically besides behavior (locomotor activity, sleep/wake) a large number of physiological processes (body temperature, Ruby et al. (2002), hiberna-

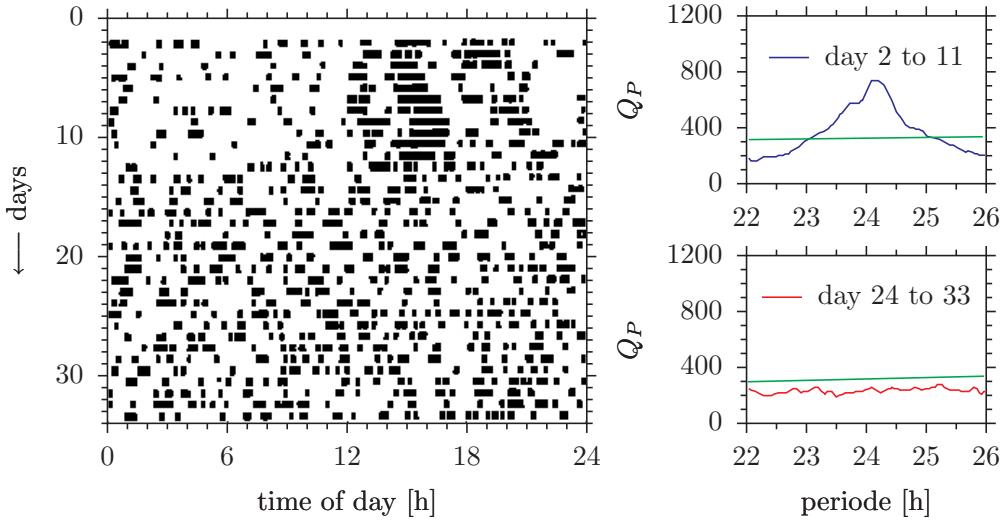


Figure 7.4: *The SCN of a rat was removed on the 28th day of freerun (running wheel activity). The activity became arrhythmic, as shown in the actogram (left). Shown at the right are the power spectra for the pre-lesion period (top) and the post-lesion period (bottom). The 25 hour rhythm, which was significant before the lesion (blue maximum above the significance line), disappears (red curve). After Wollnik (1995)*

tion, Ruby (2003), functions of the circulation and endocrine events (Hastings et al. (2007), Haus (2007), Kriegsfeld and Silver (2006), Vollrath (2002)). How important the paired SCN is, shows up, if this part of the hypothalamus is destroyed or removed. The animals become arrhythmic. They show, however, a circadian rhythm of the behavior again, if fetal SCN-tissue is implanted (Lehman et al. (1987) and figure 7.4 for rats). The SCN-tissue can also originate from other species (Syrian hamster, mice or rats). The induced period lengths corresponds to the one of the donors (Syrian hamster, mice, Sollars et al. (1995)). Cultured SCN-cells are also able to induce a circadian rhythm even after weeks in Syrian hamsters, in which the paired SCN was destroyed and which were therefore arrhythmic. The cells were implanted at the location in the brain, at which the SCN is normally situated (Silver et al. (1990)). The *structure* of the SCN must thus not be preserved. If the SCN-cells of two genotypes with different periods were implanted together, a coherent rhythm results. The cells are thus able to communicate and to compromise on an average period length (Ralph et al. (1993)).

The circadian rhythm is the result of clock-genes, which interact with negative and positive selfsustained transcription/translation-feedback loops. To these clock-genes belong also cryptochrome 1 and 2. The double mutant mCry1/mCry2 does therefore lack a rhythmic running-wheel behavior under continuous darkness. Besides the absence of a rhythmic cryptochrome-expression the circadian firing in SCN slices is also missing. Cryptochrome and thus an intact circadian clock work are necessary for the circadian electric activity in SCN neurons (Albus et al. (2002), see however

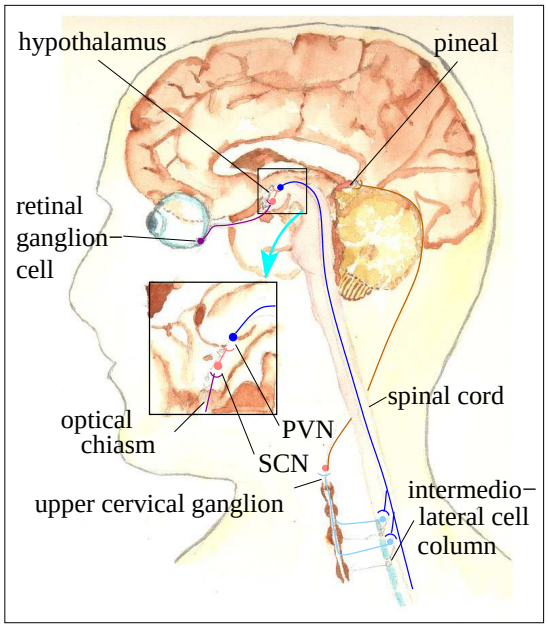


Figure 7.3: *Overview of the connections from the retina via the retino-hypothalamic tract to the SCN (magenta), from there to the paraventricular nucleus (pink) and (blue) to intermediolateral cell columns, to the upper cervical ganglion (light blue) and (brown) to the pineal organ. See also text. After Teikari (2006)*

Yamanaka et al. (2007) and Fan et al. (2007)).

Cells of neonatal SCN of rats were cultivated on microelectrode grids and the spontaneous action potentials of individual SCN-neurons recorded for days and weeks. They showed clear cicadian rhythms, which were, however, not synchronized with each other in spite of numerous functional synapses (Inagaki et al. (2007)). After reversible blocking of the action potentials for more than 2.5 days the rhythms reappeared with the same phase they had before. Thus, the individual cells of the SCN contain circadian oscillators. The synapses formed in vitro are neither needed for the functioning of the oscillators nor for their synchronization.

Herzog et al. (1997) used multimicroelectrode plates for recording extracellular action potentials in cultured SCN cells of mice simultaneously at various locations. Neurons in the isolated SCN showed for weeks circadian rhythms of spontaneous electrical activity. The same method was used by Welsh et al. (1995). They too were able to record electrical activities of individual SCN neurons in cultures for longer intervals. Within a culture cells with different phases and periods were found, although functional synapses had formed (Kohsaka and Bass (2007)). Klisch et al. (2006) examined the electrical activity of individual SCN neurons of one to two days old rats using multi-microelectrodes. With a low cell density in the culture the individual SCN neurons exhibited different circadian phases. In these cases synchronous firing in adjacent electrodes was seldom observed. The SCN neurons thus function as independent pacemakers. Melatonin pulses were able to shift the phase of the rhythm in individual pacemaker cells -without connection to other

neurons. Shifts of this kind occurred also at times, at which the pulse did not lead to phase shifts in individual SCN neurons in cultures of SCN *slices*. Thus, the neuronal network plays a large role in phase shifts.

The cells of the SCN interact with vasoactive intestinale polypeptide (VIP) via VPAC(2)-receptors. Mice without functional VPAC(2) receptors do not show a rhythm in behavior and likewise no rhythm in electrical signals in the SCN cultures (Brown et al. (2007)).

We have to elaborate on two questions. How does the activity rhythm evolve in day- and in night-active animals? And how are animals able to measure the daylength, in order to react photoperiodically to it (for instance by beginning to hibernate, if the days become shorter in the fall)? First the first question. The sleep-wake -rhythm of fetal and young rats was measured postnatal in P2[2], P8, P15 and P21-animals. Differences in the sleep-wake-activity were present already in P2. The nocturnal activity started to develop around P15 and was reliable in P21-animals. To test, whether the process of light-synchronization, which occurs during the course of the first postnatal week (in which the connections between the retinohypothalamic tract and the SCN arise), would lead later on to the nocturnal activity of the animals, P3 and P11 rats were blinded, that is, before and after synchronization by light. Whereas the P11 animals, which were tested in P21, were more active in the night (same as the controls with sham surgery), the P3 animals, which were also tested in P21, were more active during the day. Animals tested at P28 and P35 kept this daily pattern. Thus, pre- and postnatal experience leads to species specific circadian sleep-wake-patterns. The contact of the visual system with the SCN via the RHT affects the organization of the developing circadian system. Nocturnal activity is established in these animals (Gall et al. (2008)).

The metabolism in the SCN is circadian (Schwartz and Gainer (1977)) and independent of the animals being day- or night active, stronger during the days and weaker during the night. This rhythm occurs also in vitro in the isolated SCN (Newman and Hospod (1986)).

Before answering the second question, how the animals measure daylength for being able to react photoperiodically, we have to look at the structure of the SCN in more detail. It consists of a nucleus and a shell [3] with characteristic neurotransmitters of its neurons (Reuss (2003), Meijer and Schwartz (2003)), with different innervation (overview Bartness et al. (2001), Esseveldt et al. (2000), figure 7.5) and with different functions: Whereas the oscillators in the shell of the SCN react to signals of the retina caused by light, the oscillator cells of the nucleus do not. However, the oscillators of the nucleus and of the shell are mutually coupled and have therefore the same step (the same phase relationship). The electrophysiological activity of *horizontally* cut sections of a hamster SCN shows two specific oscillating components (Jagota et al. (2000)). They might reflect the activity of a *morning*- and of an *evening-oscillator*, which was predicted already before from behavioral studies (*Pittendrigh and Daan (1976)*, Illnerova and Vanecek (1982)). In night active rodents the morning oscillator would herold the end of the active period and would be synchronized

[2] Age of animals in days

[3] for the topographic situation see Yamaguchi et al. (2003)

by dawn, whereas the evening oscillator starts activity and is synchronized by dusk (Inagaki et al. (2007)). This would explain, how behavioral rhythms could keep their phase relation to the day/night-rhythm, although in the temperate and higher latitudes the daylength changes during the seasons. Photoperiodic reactions could also be explained by these oscillators.

How are theses individual oscillators in the SCN organized? To find out, Inagaki et al. (2007) performed quantitative imaging analysis using time lapse cameras, and in the brain slices a green fluorescent protein (GFP) with a short half life was used as a reporter for the circadian clock-gene period1 (per1). Per1-promoter rhythms were recorded on the level of the SCN and in individual neurons in the SCN and the temporal pattern determined in the light-dark-cycle and under continuous darkness. Additionally patch-clamp methods were performed on single neurons, in order to demonstrate electrophysiologically the relation between Per1-gene-expression and the neurophysiological reaction. In LD as well as under DD the total rhythm of the SCN is composed of the individual cell rhythms, which show maxima in distinct groups with 3 to 4 hour differences in phase. The phase relationship of the Per1-oscillation and the locomotor activity and the phase relationship between the individual neuronal oscillators in the SCN differ under LD and DD.

The oscillators in the posterior SCN were coupled at the end of the activity under three photoperiods (LD 18:6, 12:12 and 6:18). The oscillators in the anterior SCN were coupled with the begin of activity, showed, however, a bimodal pattern under the long photoperiod (18:6). This is caused by two cell groups containing circadian oscillators with an early respectively late phase position. Under LD 12:12 and LD 6:18 the pattern was unimodal. There are thus three oscillator groups in the SCN, and at least two of them are involved in photoperiodic reactions (Inagaki et al. (2007)).

7.1.1 Inputs of the SCN

Light is in mammals perceived by the retina of the eyes and a signal is transferred via the retinohypothalamic tract (RHT, figure 7.13) to the shell of the SCN. There the neurons are synchronized, the firing of which is under circadian control.[4]

Besides the RHT there are further inputs to the SCN, which derive from neuropeptid Y (NPY)-containing neurons of the intergeniculate leaf of the lateral geniculate complex (Shibata and Moore (1993)). This cord ends in the dorsomedial part, the core of the SCN. The inputs come from visual sources and the neurons show therefore rhythms, which are light-independent (references in Ibata et al. (1999)). Stimulation of these nerves or NPY-infusion into the SCN of intact animals leads to phase shifts, which differ from those of the RHT, but resemble the one, which are induced by perturbations of the locomotor activity. Phase shifts of the circadian rhythm in the SCN are thus evoked by activation of two different inputs and the physiological effect on the oscillators in the SCN differs a lot.

Rhythmic information are also transferred by diffussible substances.

[4] Electrical stimulation of the optic nerves or the chiasm activates the RHT and leads to phase shifts in the activity of the SCN-neurons of hypothalamic slices in vitro. The phase response curve resembles the one of intact animals under continuous darkness, if the circadian rhythm is shifted with light pulses (Shibata and Moore (1993))

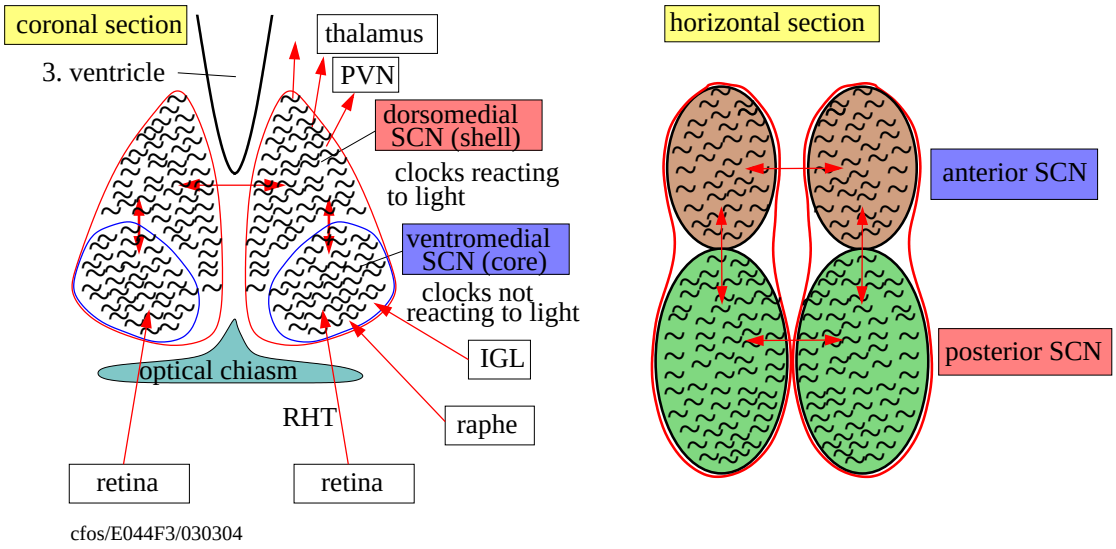


Figure 7.5: *Left: The suprachiasmatic nuclei (SCN) of mammals are paired structures at the lower end of the third ventricle and above ('supra') the optic chiasm in the hypothalamus. A coronal section through the SCN shows a dorsomedial part ('shell') and a ventrolateral part ('core'). Inputs into the SCN are from the retina via the retinohypothalamic tract (RHT), from the raphe nucleus and from the intergeniculate leaf (IGL). Outputs are to the thalamus, the paraventricular nucleus (PVN) and other areas of the brain. The shell consists of numerous cellular oscillators, which do not react to light inputs. The core, however, consists of cellular oscillators, which react to light signals.*
Right: A horizontal section shows the anterior SCN (brown), which consists of a population of cells, which are assumed to be morning-oscillators, and of the posterior SCN (green), which are thought to represent evening oscillator. Coupling between the various groups is indicated by the double arrows. After Shigeyoshi et al. (1997), and Dunlap (2000)

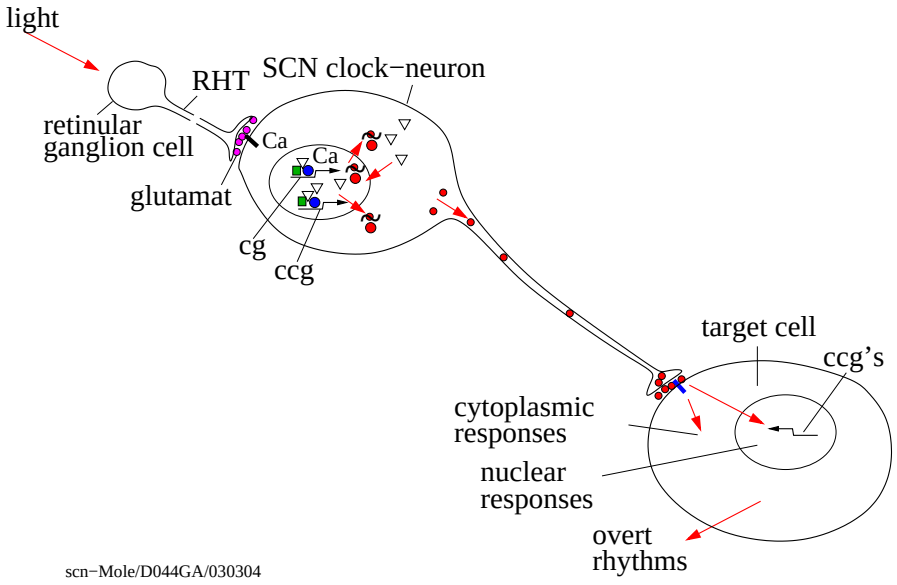


Figure 7.6: *Events between light perception, SCN clock neuron and target cell: Light is perceived in ganglion cells of the retina. Neurotransmitter glutamate (violet) is secreted and reacts with receptors (black bar). The expression of mPer and mCry of the clock-gene (cg) is turned on via a negative feedback loop with amplifying factors CLOCK (green) and BMAL (blue).* Ca^{2+} *is involved in it. Clock-protein-mRNA (red circles with ~) are produced, leave the nucleus and synthesize clock-protein (triangles) in the cytoplasm. It enters the nucleus, interacts with mPER and facilitates its translation by inhibiting the CLOCK-(green) and BMAL-(blue) dependent transcription: The mRNA concentration decreases. After a time delay negative acting complexes are inactivated and the gene expression begins again. The next round of negative and positive acting factors boost the rhythmic expression of clock-controlled genes (ccg´s). The product, clock-controlled proteins, contain information concerning time of day and pass it to the SCN-neurons and, via synaptic or paracrine signals, to target cells. Target-specific circadian outputs via cytoplasmic reactions or reactions in the nucleus affect secondary ccg´s. An example is the N-acetyltransferase. It controls the melatonin synthesis. After* Hastings and Maywood (2000)

Of the outputs of the SCN the projections to the pineal organ are well known. Synthesis and secretion of melatonin in the pineal is controlled by the SCN in a circadian fashion (see section 7.4 and Simonneaux and Ribelayga (2003)).

7.1.2 Outputs of the SCN

The outputs of the SCN signals to the hypothalamus use neuronal as well as hormonal mechanisms for communication. The same applies for the paraventricular nucleus, an important center for the integration of neuroendocrine, homeostatic and autonomic functions, as shown by studies of Tousson and Meissl (2004). They recorded the electric activity of SCN-neurons with electrodes and compared it with those of structures outside the SCN (for the anatomy see figure 7.7). In both cases the same phase position was found. If the SCN were removed, the rhythm in the hypothalamus disappeared. It could, however, be recovered by implanting SCN. Since the neural connections were absent, a hormonal factor must be responsible for the reappearing rhythm. Periodic application of arginin-vasopressin (AVP) is able to reconstitute the rhythm in the hypothalamus.

The outputs of the SCN were studied by Yamazaki et al. (1998). They used multiple electrodes in the SCN of freemoving Syrian hamsters to record neural activity and compared it with the running wheel activity under LD and continuous darkness. The circadian rhythm of the wildtype was in both cases about 24 hours long and in the homozygote tau mutant 20 hours. Neuronal activity rhythms were recorded also outside the SCN in the ventrolateral thalamic nucleus, the caudate putamen, the nucleus accumbens, the medial and the lateral septum, the ventromedial hypothalamic nucleus, the medial preoptic region and the stria medullaris. All these rhythms were out of phase with the electric rhythm in the SCN, but in phase with the rhythm of locomotor activity, and the maximum occurred during the night. Besides the circadian rhythms there were also ultradian rhythms. One of it with a period of about 80 minutes was in antiphase with a corresponding ultradian rhythm in the SCN and another one with a period of about 14 minutes was in phase with the one in the SCN. The periods of these ultradian rhythms were identical in the wildtype and in the tau-mutant. The Bett-nucleus of the Stria terminalis (BNST) seems to be tightly coupled with the SCN, because its circadian as well as its ultradian component was always in phase. Furthermore the electric activity in the BNST and in the SCN was suppressed, if the hamster was running in the wheel, whereas the activity in other areas was increased. It is likely, that we are dealing with here a main output of the SCN.

Another important output of the SCN is to the subparaventricular zone (SPZ, see figure 7.7). Lu et al. (2001) studied its significance in circadian processes such as sleep, body temperature and activity in rats. They used tightly confined lesions in the ventral or dorsal SPZ by using ibotenic acid, without affecting the directly adjacent paraventricular hypothalamic nucleus (PVH) and the SCN. Ventral SPZ-lesions reduced the circadian index of sleep by 90% and the locomotor activity by 75%, whereas the circadian index of the body temperature-rhythm was reduced only by 50%. Dorsal SPZ-lesions, however, reduced the circadian index of body temperature by 70%, the one of the

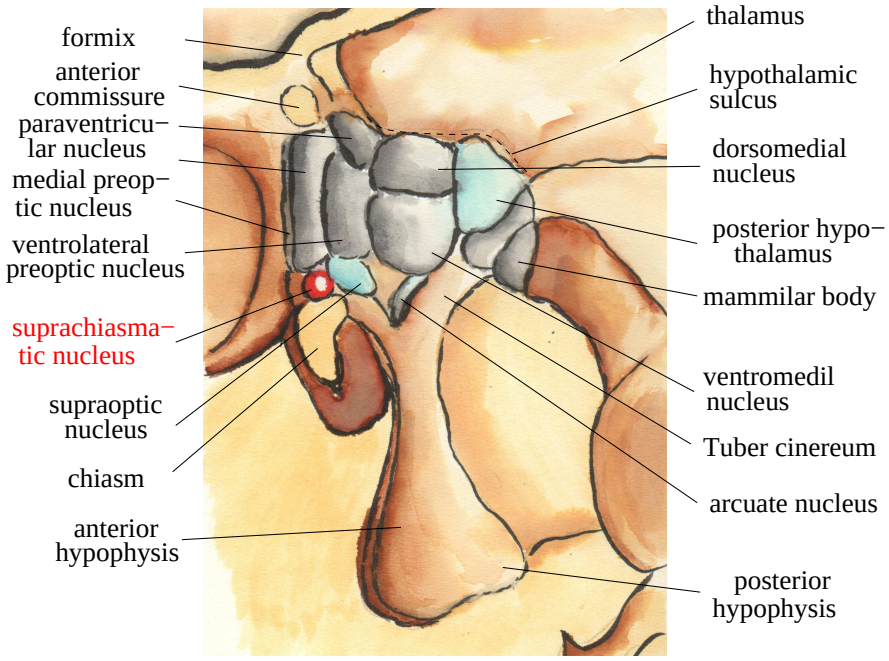


Figure 7.7: *The suprachiasmatic nuclei (SCN) of mammals are paired structures at the lower end of the third ventricle and are situated above ('supra') the optic chiasm in the hypothalamus. Below the hypothalamus are the pituitary, above the thalamus. After Teikari (2006)*

locomotor activity by only 45% and the one of sleep by 5% only. Instead ultradian rhythms of about 3 hours occurred in body temperature and in sleep. Lesions of the PVH directly dorsal of the SPZ affected none of the recorded circadian rhythms, and the basic temperature was not affected either. However, lesions of the PVH reduced strongly the fever reaction after injecting lipopolysaccharides. The circadian rhythm of sleep and of body temperature is thus controlled by different neuronal populations in the SPZ.

Another projection of the SCN was studied by Terazono et al. (2003) in mice. There are polysynaptic connections between the SCN and the liver. To clarify, whether the sympathetic nervous system is involved, adrenalin/noradrenalin was injected into the SCN, or the sympathetic nerve was stimulated, and tested, whether the mPer-gene is expressed in the liver. mPer1-, but not mPer2 was produced both in vivo and in liver slices in vitro. Electric stimulation of the sympathetic nerve or adrenalin injection increased the bioluminescence in the liver in transgenic mice containing mPer1-promoter-luciferase. If the SCN was destroyed, the daily rhythm of the mPer1-, mPer2- and the mBmal1-genes flattened. Likewise the noradrenalin rhythm of the liver damped out. By daily injections of adrenalin at a certain time of the day for 6 days a rhythm of mPer2- and mBmal1-gene expression could be re-initiated at the 7th day in the liver of the mice with SCN-lesions. Elimination of the sympathetic nerves by 6-hydroxy-dopamin flattened the daily rhythm of mPer1- and mPer2-gene-expression. Activation of the sympathetic nerves by noradrenalin- and/or adrenalin-application was also a factor, which controlled the peripheric clock.

Not much is known about the information flow from the periphery of the body to the SCN. The circumventricular organ in the brain has, like the ventricular area, only an imperfect blood-brain-barrier. Neurons in this area can therefore recognize chemical compounds in the blood such as peptide hormones, without having to rely on special transport systems, which allow these substances to pass the blood-brain-barrier. A number of hormones, neurotransmitters and cytokinines are effective in the ventricular and the circumventricular organ or are here secreted. The pineal organ, the hypothalamus and the pituitary lie also in the circumventricular area. Buijs et al. (2006) studied the arcuate nucleus in the hypothalamus, which is responsible for the homeostasis of energy and integrates long- and short-term signals of hunger and satiety. Receptors for insulin, leptin and ghrelin receive here information from the periphery and transfer them to the central nervous system. Neuroanatomical studies with injections of retrograde and anterograde tracers into the arcuate nucleus and into the SCN showed mutual connections between the SCN and the arcuate paraventricular nucleus (figure 7.7), in which signals connected to food are relayed to the SCN.

So far it was assumed, that the circadian system of mammals consists of the central oscillator, the SCN, and slave oscillators in most of the peripheral tissue and organs. Whereas the SCN-neurons are self-sustained oscillators, it was believed, that the rhythms of the peripheral oscillators damp our rapidly, if not any longer under the control of the SCN. However, it is perhaps not the damping of the rhythms in the individual cells, but a desynchronization among them. Welsh et al. (2004) observed therefore the circadian clocks of

individual rat fibroblasts for up to two weeks and showed, that they continue to run undamped. Changing the medium at the onset of recording synchronized the rhythms first, but since the periods of individual cells varied, a damping of the rhythm in the population would occur a few days later. Even adjacent cells in the culture exhibited different phases, indicating, that the cells are not functionally coupled[5]. Nagoshi et al. (2004) did also show, that NIH3T3 mice fibroblasts contain self exciting and cell-autonomous circadian clocks, as do SCN neurons. The circadian gene expression of the fibroblasts continues also during cell division, and the oscillator determines the time of cytokinesis.

7.2 Astrocytes

Astrocytes (astroglia) are star-like glia cells in the brain and the spinal cord (see figure 7.8).

In the brain there are ten times more than neuronal cells. They are electrically connected, support the brain structure, aid the metabolism and the activity of neuronal cells, regulate the ion concentration in the extracellular space[6], are plasma membrane transporter for neurotransmitters such as glutamate, ATP and GABA and can discharge them in vesicles. Astrocytes repair and scar over the brain after traumatic injuries and phagocytose necrotic neurons. There are various types of astrocytes, for instance protoplasmatic and nerve fiber -escorting once. The star-like protrusions envelope the synapses of neuronal cells.

Astrocytes nourish neurons by taking up glucose from the capillaries, converting it to lactate and by discharging it in the extracellular surrounding. From there it is taken up by the neurons and converted into energy in their mitochondria. Astrocytes store smaller amounts of glycogen. It serves as a reserve for cases, in which the metabolism of local neurons is exceptionally high. Astrocytes produce many proteins, which are important for survival, migration, differentiation and functioning of adjacent neurons. This role is particularly obvious during development, is, however, still preserved in normal adult brains and is fortified after injuries. The molecules involved are, among others, cytokines, chemokines and trophic factors. They can act as trophic or toxic substances and are -depending on the developmental stage and maturation of the brain- induced by signals, which influence the astrocytes. The molecules secreted by the astrocytes differ locally, allowing them to interact specifically with the adjacent neurons (Jean et al. (2008)).

Astrocytes are thus able to communicate with the neurons. If, for instance, the Calcium concentration in the astrocytes increases, they deliver glutamate. This reduces in most of the neurons the signal activity. The neurons, on the other hand, are able to communicate via glutamate with the astrocytes. The glutamate provokes the astrocytes to produce and deliver trophic factors. This makes it easier for neurons to process information for

[5] Rougemont and Naef (2007) developed a model, which explains the damping as desynchronization of the individual oscillators. It is based on a stable limit cycle (stiffness of the individual frequencies), fluctuations of the periods and of the intercellular coupling strength. The latter is too weak for synchronizing the cells

[6] active neuronal cells deliver potassium ions, which are taken up by the astrocytes. Otherwise they would depolarize the neuronal cells (epilepsy!)

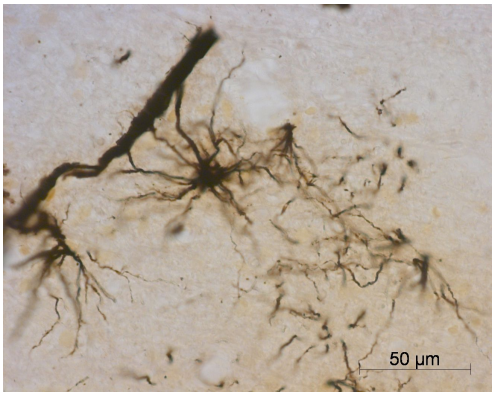


Figure 7.8: *Astrocytes close to a blood capillary of the brain, with starlike protrusions, osmiated. Image of a preparation of Dr. Klaus Reutter*

the memory. This might play a role in the Alzheimer disease. Other brain diseases could be connected with glutamate delivery by astrocytes. It has, for instance, been assumed, that due to the HIV virus or due to lack of oxygen after a stroke too much glutamate is discharged and that this causes the death of neurons. If the astrocytes could be induced by certain drugs to take up glutamate, the neurons would not die.

The SCN contains also astrocytes, as shown in mice by using immune reactions with a specific marker (glial fibrillary acidic protein GFAP). The reaction is more pronounced during the day phase. Astrocytes express cytokines and react to them. They transfer signals from the immune system to the SCN (Peters (2005)). In these astrocytes of mice- and rat cultures Per1 and Per2 is expressed in a circadian pattern, as shown by experiments of Prolo et al. (2005) using Per2::luc knock-in mice[7] and Per1::luc transgenic rats. The bioluminescence of gene activity measured in real time fluctuates in a genetically fixed period. The rhythm damps out after a few days, but can be re-initiated by changing the medium. Changing the medium *before* the complete damping out shifts the rhythm in a phase-dependend way. The rhythm can be synchronized by a daily temperature cycle of 1.5°C difference. This rhythm continues much longer, if the cells are co-cultivated with explants of the adult SCN. Cortical explants are, however, not able to overcome the damping. According to Scemes and Giaume (2006) various signals including a diffussible factor of the SCN are able to sustain the circadian oscillation in the astrocytes of the SCN.

Among the various kinds of communication between glia cells and neurons the synaptic interactions of the astrocytes play a special role. Structural and physiological studies have shown peripheral astrocyte protrusions close to the synaptic cleft. They contain the actin-binding ERM protein ezrin, which allows a rapid change of the protrusions. Its significance shows up in the SCN, where the form of the astrocytes alters depending on the activity of the animals (Derouiche et al. (2002)), thus reflecting the circadian time.

Various brain areas of the adult nervous system are able to change their structure according to the experiences they made. This morphological plasticity concerns not

[7] Knock-in mice are genetically modified by introducing a protein coded cDNA-sequence at a certain place of a chromosome. This allows to find out the function of the regulatory machinery (for instance of promoters), which leads to the expression of the natural gene: The new phenotype of the organism is observed.

In contrast, in knock-out mice (shortened to KO) the gene looses its function. Using this method, a gene, which has been sequenced, but the function of which is not or only partly known, can be better understood by finding out the differences between the knockout organisms and the normal one.

only neurons, but also glia cells (although they are able to change their location). This plasticity is observable under physiological conditions such as reproduction, sensory stimulation and learning and affects mainly the distal appendages, which surround the neurons. The changes occur in a few minutes. The geometry and diffusion properties of the extracellular space and the relations to the adjacent neuronal elements, especially the synapses, are influenced by it. Since astrocytes react to neuronal activities with ion channels, neurotransmitter receptors and transporters in the appendages, informations concerning the neuroactive substances are transmitted. In this way the interactions between the astrocytes and the neurons become highly dynamic. They modify the extracellular homeostasis of ions, the neurotransmission, glia-transmission and finally the neuronal function on the cellular and systemic level (Theodosis et al. (2008)).

Melatonin influences the intercellular communication between glia cells too (Peters et al. (2005)). The responses of the astrocytes towards melatonin differs in the various brain regions, and this seems to be based on different reactions of the melatonin receptors.

Astroglia seems to play a role also in depression (Theodosis et al. (2008)). There is a connections between diabetes and depression. In both cases the glucose metabolism in the brain is changed. In the brain only astroglia cells contain insulin receptors.

7.3 Eye clocks

Circadian clocks belong to one of the most fascinating adaptations of creatures to the environment. The organisms can thus not only predict the day-night cycle, but also use it for measuring the daylength and thus determine the season. Since the period length is not exactly 24 hours long, the clock has to be corrected each day by environmental signals (light, temperature). This synchronization by light is complicated by the fact, that the photoreceptors themselves are in many cases under circadian control (their transcription, feedback of the clock to inputs of the light, see Devlin (2002)).

To determine the season, the photoperiodic signals of the environment are transferred to the pineal organ in mammals, where they are decoded as melatonin secretion. Studies in mice mutants suggest, that two different molecular oscillators note the onset or the end of the light period and use it for measuring the daylength and to inform the organism (Oster et al. (2002)).

In marine snails such as *Bulla gouldiana,* which lives in warmer seas at the sandy bottom, circadian clocks are in special cells of the eyes, the basal neurons. They control the electrical activity of the neuronal cells (Block et al. (1995), Page (2001)). Consequently at the eye nerve a rhythmic compound action potential can be measured (Jacklet (1969)). The eyes (figure 7.9) can be isolated and kept for longer times in a suitable medium. In figure 7.10 the frequency and amplitude of the electrical impulses are plotted. According to Michel et al. (1993) even isolated basal neurons exhibit circadian rhythms for at least two days.

The circadian rhythm of the eyes is synchronized by light, but additionally by the brain. Serotonin serves as a neurotransmitter. Its interaction with light is complex (Colwell (1990)).

Circadian clocks have possibly evolved

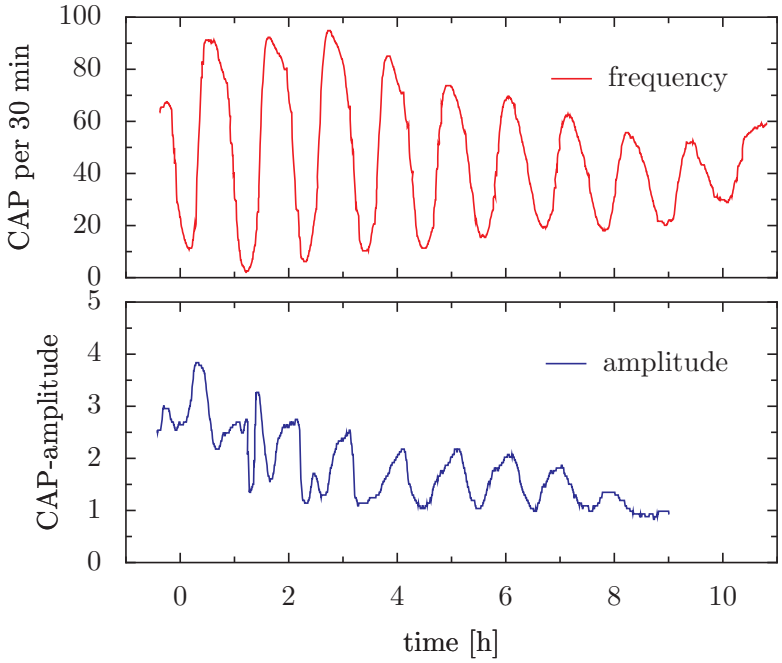


Figure 7.10: *Circadian rhythm of CAP amplitude (top, blue curve) and frequency (bottom, red curve) of isolated eyes in the dark. After Benson and Jacklet (1977)*

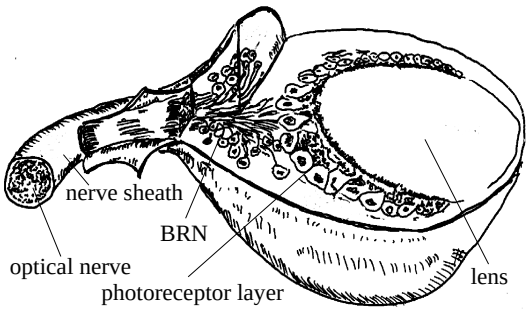


Figure 7.9: *Eye of* Bulla gouldiana. *From right to left: Lens, photoreceptor layer, basal retinal neurons (BRN) at the basis of the eye threedimensionally drawn, optic nerve with nerve sheath, partly cur open. After Roberts and Moore (1987)*

together with light-perceiving molecules long before photoreceptor cells and eyes specialized. Structural homologies between those molecules, which are the component parts of the clock-mechanism, and phylogenetically old photopigments suggest, that modern clock proteins evolved during the course of evolution from primitive light sensitive proteins (Crosthwaite et al. (1997)). The function of opsines can be altered, for instance, easily by exchanging single amino acids in such a way, that they adapt to the light situation in the environment. A circadian feedback system might have evolved from primitive photopigments, which were able to affect their own transcription. Such events might have occurred several times with different photopigments as outputs and might have been the cause for the various clock mechanisms, which are found among the differ-

ent lines of organisms.

Eyes evolved at least 40 times independently of each other in the course of the phylogeny (Land and Fernald (1992)). Among the vertebrates already the lampreys (*Petromycon marinus*) possessed eye clocks (Menaker et al. (1997)). Lampreys separated from other vertebrates already 450 million years ago.

Circadian oscillators are also present in the retina of mammals. They belong to the first circadian oscillators in mammals which were found outside the SCN (Tosini et al. (2008)). Morphological, neurochemical, electrophysiological and optical studies suggest such clocks (Remè et al. (1991)). These circadian clocks are not only present in the internal grain- and ganglion layer (Ruan et al. (2006)), but also in the photoreceptor layer (Tosini et al. (2007)). They, or more likely a network of hierarchically organized circadian clocks in the retina controles there many of the physiological, cellular and molecular rhythms (Morin and Allen (2006), Brainard and Hanifin (2005), Doyle and Menaker (2007)). Several (at least two) oscillator types seem to exist in the retina: Melatonin on the one hand and dopamin on the other hand are regulated in the photoreceptors and the internal retina in an antiphasic pattern (Green and Besharse (2004)). Melatonin is very likely synthesized in the cones. Similar to the situation in the pineal organ the synthesis is strong during the night and weak during the day. The retinal melatonin seems to act only locally as a neurohormone and/or as a neuromodulator. The melatonin synthesis occurs in a circadian pattern and has been established in vivo and in vitro. The rhythm is synchronized by light and is temperature compensated (Tosini and Fukuhara (2003)). Whereas in other vertebrates extraretinal photoreceptors are involved besides the eyes for synchronizing the cells of the SCN to the light-dark cycle of the day, in mammals only the retina of the eyes is used for this purpose (figure 7.11). Why other vertebrates use besides the retina also other photoreceptors deep in the brain (pineal organ), could play a special role in synchronization. They furthermore control direct reactions to light, the pupilary reaction and photoperiodic events (Doyle and Menaker (2007)).

As is well known, rods and cones are responsible for seeing forms. But a special population of retinal ganglia cells senses, whether light or darkness prevails in the environment (detectors of lightness). These bipolar cells (Landolts clubs) use melanopsin[8]as a photopigment. The cells are located in the outer nuclear layer of the retina and extend between the pigmented epithelium and the internal and external segments of rods and cones (Locket (1999), van Reeth et al. (1997), figure 7.12). They are distributed allover the retina and project via a special pathway, the retinohypotalamic tract (RHT)[9], to the SCN (but not to the visual ceters of the brain, references in Provencio et al. (1998), figure 7.13). Glutamate serves as the neurotransmitter and synchronizes the pacemaker in the SCN (see figure 7.6). They are connected with each other as adding or as averaging processors (Foster et al. (1993)). Besides synchronizing the rhythm to the light-dark cycle, the ganglion cells control also the pupilary reflex and modulate neu-

[8] if the melanopsin gene is knocked out, light is not able anymore to synchronize the circadian clocks, and the pupilary reflex is lacking (Brown and Robinson (2004))

[9] the neurotransmitter PACAP and glutamate transfer the signals of the light-dark cycle via the RHT to the SCN (Hannibal (2006))

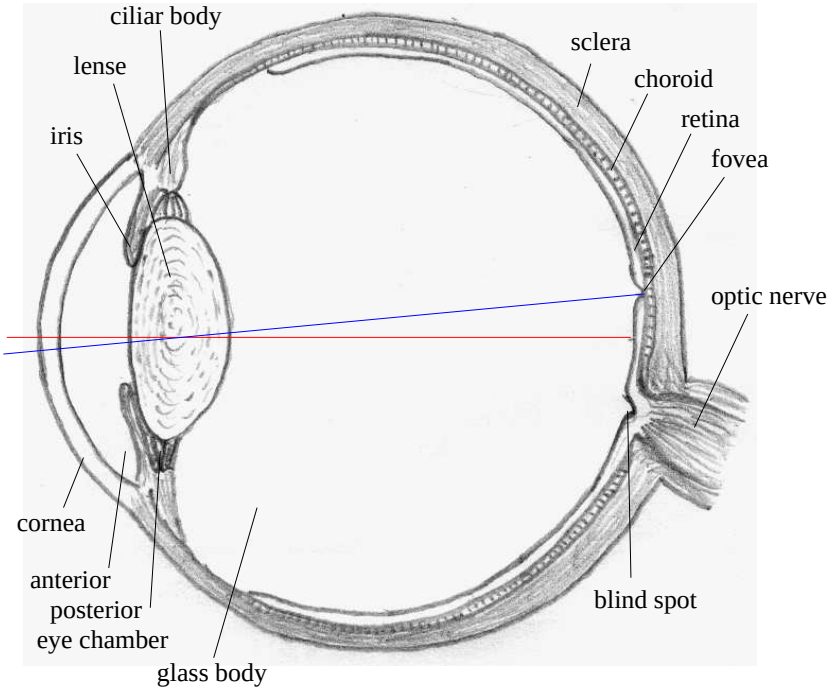


Figure 7.11: *Eye of a vertebrate (longitudinal section) with cornea, anterior eye chamber, iris, lens, ciliary body, glass body, retina, choroid, sclera, fovea, blind spot and optic nerve. Eye axis (red) and line of sight (blue) are drawn. Pigment cells. Drawn by the author after a figure in Mörike and Mergenthaler (1959)*

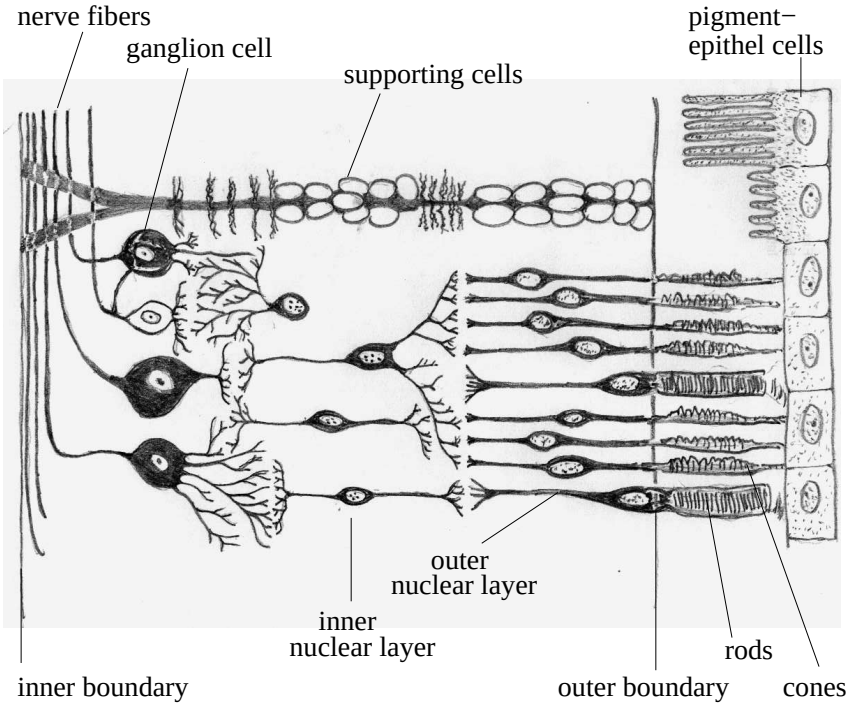


Figure 7.12: *Part of the retina with inner boundary, ganglia cells with nerve fibers, inner nuclear layer with bipolar and other neural cells, support cells, outer nuclear layer with nuclei of rods and cones, outer boundary, rods and cones, pigment epithelium and pigment cells. Glass body and lens would be at the left, the light comes thus also from the left. Drawn by the author according to a figure in Mörike and Mergenthaler (1959)*

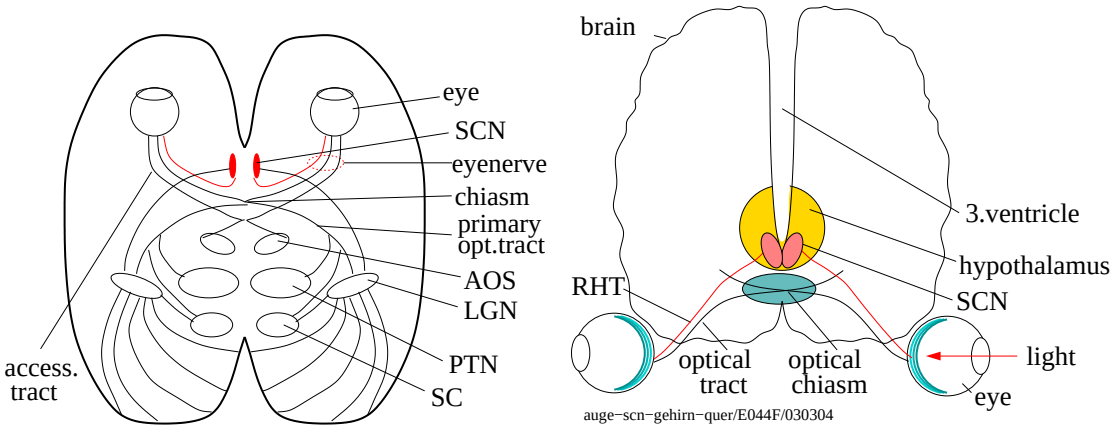


Figure 7.13: *Visual paths from the eyes to the brain in Syrian hamster. Left: brain from top. Right: Cross section through brain in the hypothalamic area and the SCN. Light is perceived via the retina of the eyes and the signals transferred via the primary optic tract, the accessory optic system (not shown) and the retinohypothalamic tract (RHT) to the visual cortex in the brain. The RHT ends in the suprachiasmatic nucleus (SCN). The SCN contains numerous 'pacemaker'-cells for producing the circadian rhythm. After Moore-Ede et al. (1982)*

roendocrine functions. As pigment they use melanopsin (Nayak et al. (2007)).

Although mammals are functionally blind at birth, because they do not yet react to the photoreceptors of the rods and cones, they are able to recognize light via retinal ganglion cells, because those are functionally connected already with the SCN (Sernagor (2005)).

7.4 Pineal organ and melatonin

The SCN of mammals projects to the pineal organ and synchronizes its melatonin production and -secretion with the light-dark cycle of the day (low concentrations during the day, high ones during the night). Reproduction of various mammals is controlled by the daylength (Baker and Ranson (1932), Bissonette (1932)), and melatonin serves as an indicator of the length of the dark period. Besides the reproductive system, bodyweight, fur color (Hoffmann (1973), Figala et al. (1973)) and body temperature are also photoperiodically controlled in the Djungarian hamster (Steinlechner and Heldmaier (1989)). Thus the pineal organ and its hormone melatonin transmits the photoperiodic signals of the environment to the neuroendocrine axis. These signals can be stimulating or inhibiting (Hoffmann (1981)). Melatonin feeds, however, also back to the SCN. Here it inhibits the neural activity and shifts the phase of the circadian rhythm (figure 7.15).

Although removing the pineal organ of mammals under continuous darkness conditions affects the circadian system only slightly, recent studies have shown, that this organ is still important. For instance, pinealectomy (PINX) in rats strengthens the rhythm-damping effect of continuous light. The reason might be, that these animals perceive the light in such a way, as if it has a higher intensity (at which the damping effect in con-

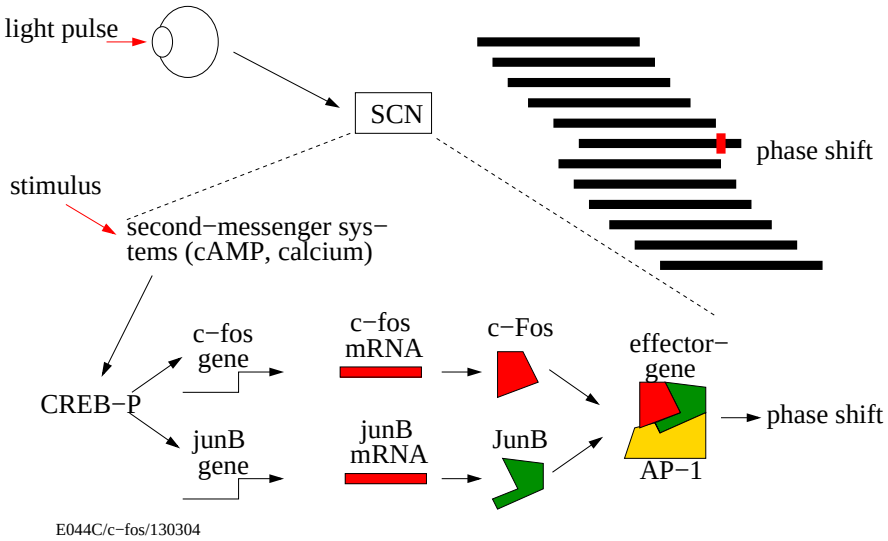


Figure 7.14: *Top: Light pulses are perceived via the retina of the eyes and transferred as signals to the SCN. There it shifts the phase of the circadian oscillators and as a result the locomotor activity rhythm (black horizontal rods for each day, red marking: Position of light pulse, which advances the rhythm). Below: Signal transduction cascade for synchronizing hamsters by light-dark-cycles. An external stimulus (a neurotransmitter or a hormone) activates a second messenger system (cAMP, Ca^{2+}). As a result cAMP response element binding proteins (CREB) are phosphorylated. This is the prerequisite for activating immediate early genes (IEGs) such as c-fos and junB. Transcription (→mRNA) and translation (→c-Fos, JunB) produce proteins of the Fos or Jun family. They form heterodimers and combine with the AP-1 region of other gene sections. Thereby their transcription is enhanced or inhibited. After Wollnik (1995)*

trol animals would also be stronger). Or the pineal organ is via its melatonin secretion involved in the coupling of components of the circadian systems. This coupling can take place on different levels, (1) between afferent nerves of the retinohypothalamic tract and the oscillator neurons of the suprachiasmatic nucleus (SCN), (2) between the SCN-oscillators, (3) between the SCN and its various outputs and/or (4) between the various circadian outputs. Warren and Cassone (1995) showed, that PINX rats possess at four different light intensities always longer periods as compared to the SHAM-operated rats. Furthermore, the differences in the period lengths are larger compared to PINX rats. That would be in favor of hypotheses (1). Not only the running wheel rhythm is more damped under continuous light in PINX-animals, but also the rhythm of general activity, of body temperature and of heart rate, and all these rhythms are affected in the same way. This speaks in favor of hypotheses (2). Hypothese (3) is wrong, since removing the eyes does not affect the binding of 2-iodin-melatonin in the SCN. [10]

In contrast to mammals, in which the cells of the pineal organ do not contain selfsustained oscillators, but are driven by the SCN, the pinealocytes of other vertebrates (birds, reptiles, amphibia, fishes) are equipped with circadian clocks and with photoreceptors. They can thus be synchronized by the light-dark cycle without the retina (Kumar et al. (1993)). In these vertebrates the brain is rather light translucid. Photoreceptors in the brain can thus perceive the light conditions in the environment.

The pineal of non-mammal vertebrates and their clocks, their function and light

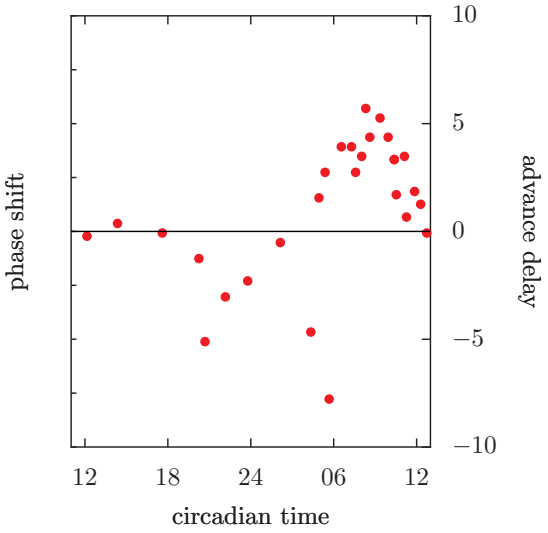


Figure 7.15: *Whether a melatonin pulse advances or delays the circadian rhythm in man and how much it shifts, depends on the time (x-axis, in circadian time), at which the pulse is given. The rhythm is not much affected between CT 12 to 18, delayed between CT 20 to 03, and advanced between CT 06 and 12. After Lewy et al. (1992)*

[10] a melatonin agonist shifts according to van Reeth et al. (1997) the circadian rhythm of mice (and Syrian hamsters) phase dependent

sensitivity as well as the signals of the outputs are recapitulated by Falcon (1999). Klein (2006) has discussed the relation between pinealocytes and retinal photoreceptors and proposes, how the pineal and the melatonin rhythm have developed. A central role plays the arylalkylamine N-acetyltransferase (AANAT). The melatonin production is always higher during the night. All vertebrates are able to use these signals, but in different ways. Melatonin has namely not just one biological effect: It facilitates sleep in some animals, but not in all, it inhibits reproduction and increases body weight, but not in all, it stimulates locomotor activity, but not in all. Melatonin is thus a time indicator, which is used by different animal species in various ways and in each case for their best advantage.

The circadian control of melatonin production allows vertebrates to become independent of the light-dark cycle in the environment, which is important for instance in rodents, because they spent the day in their subterranean caves. Due to the internal clock they are able to predict, when the day begins or ends and whether it is day or night. Physiological events can thus be started at the right time. The melatonin rhythm is synchronized by the LD cycle, which reflects also the seasonal changes of the daylength.

Common to the melatonin rhythm generating system of all animals is, that cyclic AMP influences two strongly conserved motives at the N- and C-end in the protein kinase A (Ganguly et al. (2005)). This stabilizes AANAT and increases the melatonin production. Light destabilizes AANAT and it will be degraded.

There are furthermore class- and species-specific differences in the pineal (Ekström (2003), Collin and Oksche (1981), Klein et al. (1998)). In non-mammalian vertebrates including fishes and birds, clock, photoreceptor and melatonin synthesis are localized in the pinealocytes (Cassone (1991), Falcon et al. (2003), Iuvone et al. (2005)), whereas in mammals the clock is in the SCN (Moore-Ede et al. (1982), Klein and Krieger (1979), Klein (1985)). In birds light is effective via the pinealocytes as well as via the retina and a homologue of the SCN (Cassone (1991)).

Many properties are common to pinealocytes and retinal photoreceptors. The pineal of fishes and amphibia are able to perceive light; this is true also for pinealocyte-*cultures* of birds and fishes. In the same way as the pineal the retina is able to produce melatonin (Iuvone et al. (2005)). Melatonin is said to facilitate the light adaptation of the retina. There are furthermore anatomical similarities between pinealocytes and photoreceptors (Klein (2004)). Genetic indications take the same line. The development of pinealocytes and of retinal photoreceptors occurs in a similar way. Both cell types stem originally from a light-sensitive melatonin synthesizing cell (figure 7.16). They

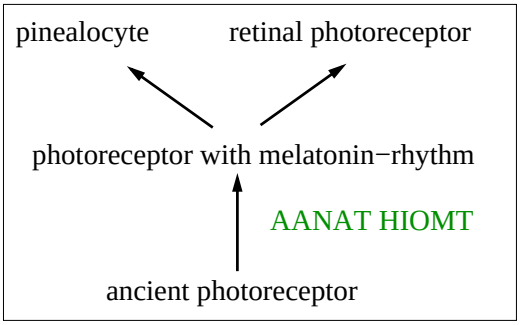


Figure 7.16: *Derivation of pinealocytes and retinal photoreceptors from a common ancient photoreceptor*

separated during the course of evolution

in two different cell types with different functions (Klein (2006), Yoshikawa and Oishi (1998)). In reptiles (*Iguana*) the pineal is responsible for the circadian control of body temperature, but scarcely for the one of the locomotor activity.

7.5 Fibroblasts

Fibroblasts compose the intercellular substance of the connective tissues. They develop later to fibrocytes (spindle cells, connective tissue cells). These cells can be reared from punched pieces of the skin (2mm diameter). In various tissues of mice the luc gene was fused with a circadian promoter by using a reporter-virus (Brown et al. (2005), Welsh et al. (2004), Yamazaki et al. (1998)), which allowed to utilize the emitted light as a hand of the circadian clock. Individual fibroblasts were recorded for one to two weeks and a robust, undamped circadian rhythm was found. The individual cells showed varying period lengths. Therefore the different cells run out of step after a few days, even if synchronized with each other by a change of the medium. The cells are thus not coupled with each other in the culture (Welsh et al. (2004)).

The synchronization in mammals is affected by diffussible signals of the SCN (Allen et al. (2001))[11]. Co-cultivated fibroblasts are synchronized via diffussible signals of the SCN, but with a phase angle of 12 hours in respect to the rhythm of the SCN. The gene expression of clock-genes can also be synchronized with a serum-shock, whereby the maximum of the mRNA of Per1 and Per2 is four hours earlier as that of Cry1 and Cry2. The activity of the metabolism is not synchronized by this treatment. Instead, SCN-specific outputs are needed, which are not the direct products of clock-genes (Allen et al. (2001)).

The intraindividual period length in the tissue fluctuates less than the interindividual one (between 23 and 26 hours). Brown et al. (2005) studied fibroblasts of 19 donors, the mean period length of whom amounted to 24.5 hours ($\pm$45 minutes standard deviation). The period corresponded by and large to the one, which was observed in physiological studies under timecue-free conditions, but the fluctuations were larger[12]. The standard deviation of the periods of samples of different persons was 48 minutes. There are thus significant differences in the fibroblast clocks even in small populations. Biopsies of clock mutants in mice showed periods, which corresponded to the one, which were obtained from behavioral studies or, in the case of arrhythmic mutants, which showed no rhythms. In most cases the periods of the fibroblasts were more variable as compared to the one determined from behavioral studies.

Nomura et al. (2008) found in fibroblasts of endogenous depressive patients a shortened period length. The SCN had also a short period, as shown by recording SCN controlled events. Antidepressives such as SSRI (*selective serotonin re-uptake inhibitor*), sertralin, fluoxetin, fluroxamin, citalopram and paraxetin shorten the period. Sertralin reduces the amplitude and lowers the damping rate of the fibroblasts

[11] only SCN2.2-cells showed a circadian rhythm of 2-deoxyglucose-uptake and of Per-expression

[12] Different measurements of the same sample gave standard deviations of 18 minutes, of samples, which were obtained from another virus infection, 25 minutes and the average value of various biopsies of the same person showed a standard deviation of 6 minutes

in a dose-dependend way.

Further literature: Nagoshi et al. (2004), Yagita (2004), Yagita et al. (2001).

7.6 Intracardial clock, cardiomyocytes, monocytes

Heart and circulation are exposed during the course of a day to a large number of external stimuli, all of which influence myocardial contraction (Bray and Young (2008)). The strongest influences are by the sleep-wake-cycle and the F/NF(food/non-food)-cycle. Wake periods are connected with increased physical activity, higher heart rate and heart performance and under rich food supply with higher food uptake. Without food supply the sleep phase can be prolonged by several days or even weeks. In this case different strategies are also chosen for the heart.

The myocardial metabolism fluctuates in a daily rhythm. It is a complex interplay of extracellular (neurohumoral) and intracellular factors (circadian clock). An intramyocardial circadian clock controls the metabolism directly (for instance triglyceride- and glycogen-metabolism) as well as indirectly (modulation of the reaction of the myocardium to loads, insulin, fatty acids). In this way environmental stimuli (changes in load, nutrial condition) can be recognized early enough. This synchronization between the myocardium and the environment is supported by regular food uptake. If the heart does not adjust its metabolism adequately, dysfunctions of the contractions might occur. As a result of disturbances in the circadian system diseases caused by high blood pressure, diabetes, shift work, apnoe and obesity might occur (see Young (2006), Durgan and Young (2008) and chapter 8). There are fluctuations in the oxidative and non-oxidative glucose- and fatty acid metabolism in the heart of the rat. An increased energy demand during the wake phase is foreseen by the heart and it can react to it correspondingly.

Circadian clocks were found in vascular smooth muscle cells of the heart as well as in cardiomyocytes. Cardiomyocytes of adult rats, which were cultivated without serum, exhibited strongly damped oscillations in bmal1 and dbp (Durgan et al. (2005)). Glucose, which reactivates circadian clock genes in fibroblasts, does not affect cardiomyocytes, be it with or without serum. A two hour treatment with the neurotransmitter norephinephrine (10 μM), however, induces the rhythmic expression of the circadian clock independent of serum. Circadian oscillations are also found in the metabolism-genes pyruvate-dehydrogenase kinase 4 (pdk4) and the decoupling protein 3 (ucp3). The circadian clock does thus run also in myocytes of the heart under standardized cell culture conditions and is able to control the myocardial metabolism.

According to Fahrenbach et al. (2007) the ratio between fibroblasts and cardiomyocytes changes in the sinus node of the heart as a function of age. This is possibly the cause of sinus node diseases. The non-excitable fibroblasts influence the excitability of pacemaker cells twofold: By coupling they depolarize the cardiomyocytes and by pure physical separation bradycardia is brought about, because the electrical excitation is reduced.

Circadian fluctuations in the cardiovascular system of the rat have been demonstrated by Davidson et al. (2005) by observing in vitro the activity of genetically inserted luciferase via the mPer1-gene-promoter in heart tissue cultures and in

a large number of veins. The tissue exhibits 3 to 12 circadian cycles of gene expression, before the rhythm damps out. Whereas the maxima of bioluminescence of the heart- and all artery cultures occur during the late night, the phases of the rhythms in the veins vary depending on their anatomical position.

Monocytes (see figure 7.17) are cells of

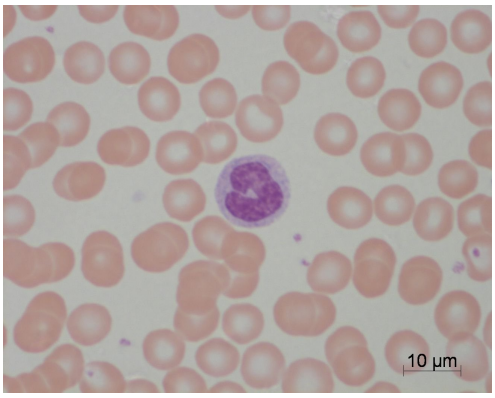


Figure 7.17: *A monocyte among numerous erythrocytes: Cell of the immune system and precursor of macrophages*

the immune system and precursors of the macrophages as well as a part of the dendritic cells. They destroy foreign structures in the body by phagocytosis and activate the immune defense. After one to three days they leave the blood and enter the tissues where they continue to live as macrophages for several weeks to months. They belong with a diameter of 5–20 µm to the largest of the white blood cells (leukocytes), contain a large nucleus and a small amount of cytoplasm.

Brown et al. (2005) infected 50 000 monocytes from the peripheral blood of two persons with luciferase in the same way as was done with fibroblasts and determined the rhythm by imaging analysis. Figure 7.18 shows a record. James (2007) studied likewise in peripheral blood mononuclear cells during a typical sleep-wake cycle in LD and during constant routine. Of six men and women the body temperature and the plasma melatonin was determined during a five day isolation without time cues; they served as hands of the central circadian pacemaker. The expression of the clock genes PER1, PER2 and BMAL1 was determined during a 72 h interval. PER1 and PER2 expression fluctuated in a circadian fashion with maxima during the early wake time, and this was identical under light-dark- and constant conditions. The BMAL1 expression varied more pronounced with a maximum during the middle of the wake time.

7.7 Kidney cells

A separate circadian oscillator has been found in the kidney (Ikonomov et al. (1998)). The authors studied, how the differences in day- and night-active animals comes about and which mechanisms are responsible for phase and amplitude of the circadian rhythms. They compared the peripheral rhythms of diurnal and nocturnal mammals, both activity-independent and activity-dependend. The maxima (acrophases) of the first group occurred in all mammals at about the same time and serve as internal time cues. The regulation of the activity-related circadian rhythms of behavior, of blood pressure and of kidney excretion follow, however, an other mechanism. It was proposed, that a passive hypothalamic oscillator coordinates the phase position of the circadian behavioral rhythms and is responsible for the high amplitude of these rhythms. A separate rostral hypothalamic network is involved in the regulation of the low-

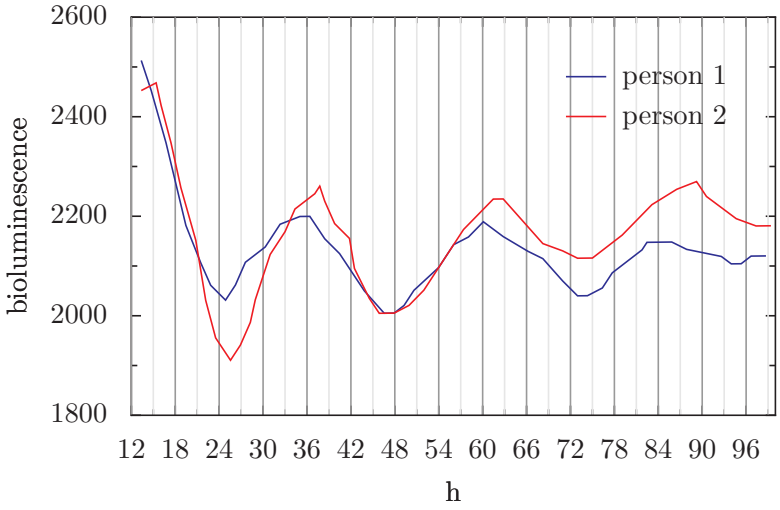


Figure 7.18: *Circadian rhythm of monocytes (with artificial bioluminescence) of two persons*

amplitude circadian rhythm of the blood pressure. Finally a circadian oscillator in the kidney causes the circadian rhythm of the electrolyte excretion. The authors propose a model, according to which activity is determined by the phase relationship between the circadian and the passive hypothalamic oscillator. Specific brain structures or peripheral circadian oscillators integrate circadian and other signals for the various activity-related circadian rhythms. The same hypothalamic areas modulate furthermore selectively circadian rhythms as reactions to homeostatic stimulation or stress, without involving the circadian oscillator.

Renal rhythms were studied also by Bray (1965).

7.8 Fat cells

Fat tissue is a fluffy connective tissue[13], which consists of fat cells (and other cells) (see upper part of figure 7.19). It consists of lipoblasts, in which energy is stored in the form of fat. It furthermore buffers and isolates the body. 20% of the body weight of a man and 25% of a woman are made up of fat. There are two types of fat tissue, white and brown (see lower part of figure 7.19). The latter occurs in babies (baby speck) and in hibernators for heat production. In white fat tissue fat is converted into fatty acids on demand. Insulin of the islets of Langerhans is docking on insulin receptors of the fat tissue and activates via a phosphorylation cascade the lipase. The fatty acids arrive in the blood, where they are first bound to albumin and glycerin and later delivered as free fatty acids serving as fuel for muscles (for instance in the heart muscle tissue) (see figure 7.20). Fat tissue is, however, also an important endocrine organ and produces adipocytokines[14]. They are signal proteins (cytokines[15]) acting between cells (hormones)

[13] its formation is induced by the adipose gene

[14] Greek adipo-, fat; cyto-, cell and -kinos, movement

[15] sugar containing proteins, which regulate growth and differentiation of body cells. This group

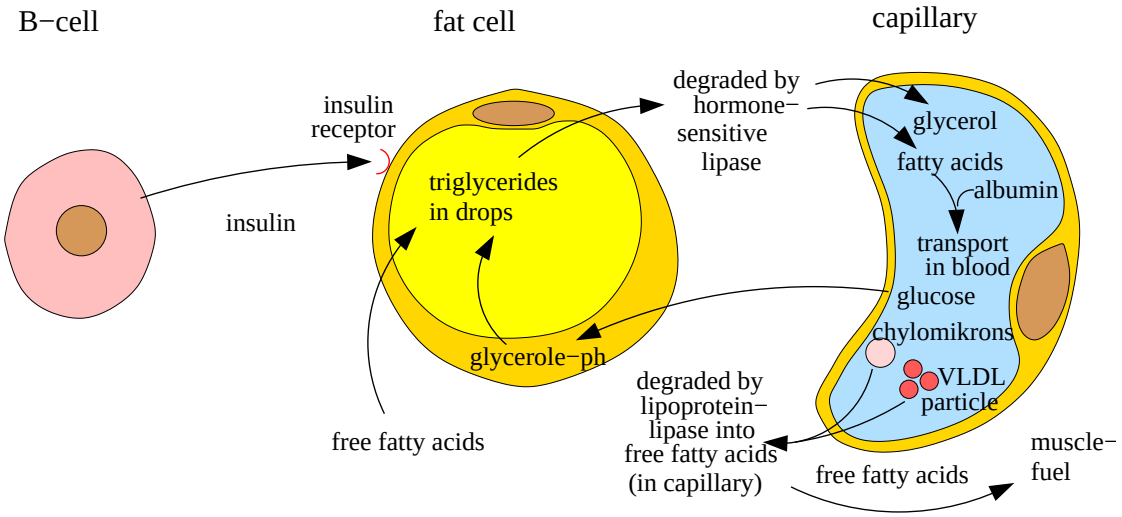


Figure 7.20: *Fat cells of the fat tissue deliver after docking of insulin on receptors triglycerides, which are degraded by lipase to fatty acids and transported in the blood to the locations where they meet the demand. Details in the text*

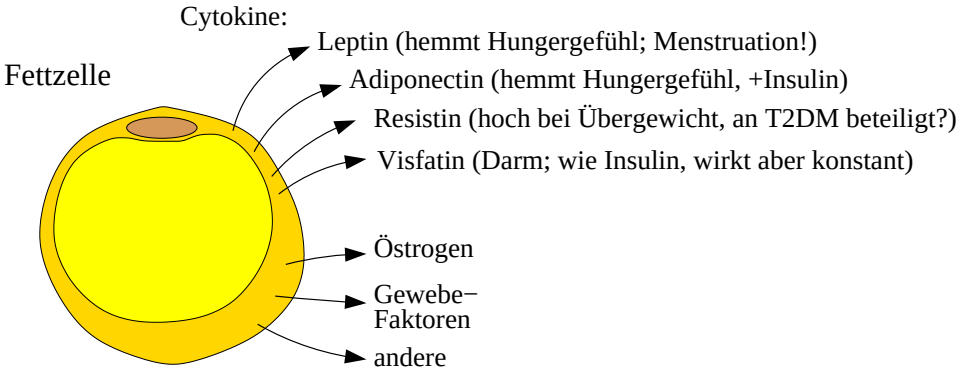


Figure 7.21: *Fat tissue as an important endocrine organ: fat cells produce adipocytokines, which serve as signals between cells. More details in the text*

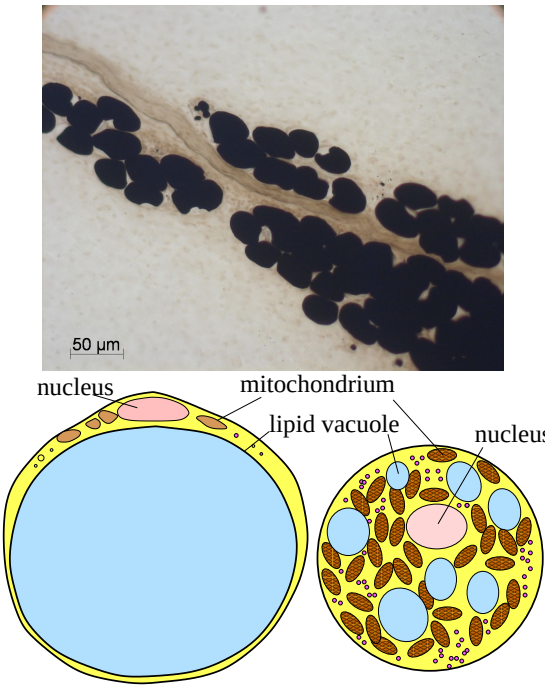


Figure 7.19: *Top fat cells (osmiated) close to blood vessel. Bottom left white, bottom right brown fat cell. The former contains only one large, the latter several smaller lipid vacuoles and numerous mitochondria. The top image was supplied by Dr. Klaus Reutter. Lower figures after a drawing of Dr. John Horwitz, U.C. Davis at http://sportsci.org/encyc/adipose/adipose.html*

such as leptin, resistin, adipsin, visfatin and TNFα (see figure 7.21). White fat tissue contains receptors for insulin, growth hormone, norepinephrine and glucocorticoides.

Leptin (Greek: leptos = thin) was discovered in 1994 by Friedman. It is a peptide hormone and is expressed by the „obese"-gene in fat tissue. Mice lacking the leptin gene *ob* were extremely fat. The obesity of these mice could be reversed by administering leptin. A leptin receptor was found in 1995 and belongs to the cytokine receptor family. Leptin receptors (OB-R) are found in the brain, especially in afferent satiety centers of the hypothalamus, and in peripheral organs (fat tissue, skeletal muscles, beta-cells of the pancreas, liver). It plays a role in the regulation of energy expenditure. In humans it exhibits a pronounced circadian secretion pattern with maxima around midnight. It interacts with insulin and cholecystokinine. Besides its physiological effect it can have pathological outcomes such as for instance arteriosclerosis based on overweight, oxidative stress and cancer (Anubhuti and Arora (2008)).

Leptin is released by fat cells into the blood proportional to the fat content of the fat tissue of the body. It reaches the brain via the blood-brain barrier, where it

of peptides induces or regulates the proliferation and differentiation of target cells. Some cytokines are therefore denoted as growth factors, others play an important role in immunologic reactions and could than be denoted as mediators. Essentially five main groups of cytokines are distinguished: Interferons, interleukins, colony stimulating factors, tumor necroses factors and chemokines. In the field of cell biology the significance of cytokines becomes more and more important. Several cytokines are nowadays already produced commercially as recombinant proteins.

reduces the appetite in the appetite control center of the brain[16] by affecting certain neurons. It furthermore stimulates the sympathetic nervous system, increases the blood pressure, the heart frequency and the heat production (by decoupling cell respiration from ATP-synthesis), lowers body temperature and increases the oxygen consumption. All this releases more energy and decreases finally the fat content of the body. Thus leptin serves as an adipostate (Chehab (2008)). If the fat depots of the body shrink, less leptin circulates in the body and the appetite increases. Leptin is thus a hormone, which feeds back negatively from the fat tissue to the brain.

Leptin is important for the control of overweight. Obesity in man and animals does not rest on too high bodyweight, but on too much fat, especially in the fat tissue. Fat tissue can extremely hypertrophe (obesity), but also grossly atrophe (lipodystrophy). The reason are the fat cells, which can store huge amounts of triglycerides, but shrink also to tiny cells under lipid shortcoming. However, leptin does not work in 70% of the cases of heavy overweight, because the blood-brain barrier transporter does not function any more. It is tuned, to work already at low leptin values in the blood serum and to tell the brain, that enough fat reserves abound, to spend energy not only for searching food, but also for other functions such as reproduction, immune system and so on[17]. Low leptin values under hunger interrupts the negative feedback. Simultaneously triglycerides are released from the fat tissue. They inhibit the transport of leptin through the blood-brain barrier, hunger sensation in the brain emerges and at the same time the periphery becomes leptin-resistant. Triglycerides are therefore increased under hunger as well as under overweight (Banks (2008)). In 30% of the obese persons only small amounts of leptin circulates in the blood, and a treatment with leptin would make sense. This is true also for humans with a defect leptin gene, which are constantly hungry.

Leptin is synthesized not only in the fat tissue, but also in the stomach, in the liver, in the ovary and in the placenta. Expensive physiological functions such as reproduction are only possible, if enough leptin is available[18] (Hileman et al. (2000)).

As mentioned already, the hormone leptin and its subordinate signals play an important role in the regulation of the energy balance. Leptin suppresses food uptake and promotes energy-intensive neuroendocrine processes such as reproduction. During these events the steroid hormone estrogen plays an essential role and contributes -like leptin- to the regulation of the energy balance. Estrogen deficit intensifies food uptake and increases body weight, thus reinforcing the effect of leptin. For the interplay of estrogen, leptin and energy-homeostasis see Gao and Horvath (2008). Bodyweight, energy budget and the hormonal control play a role in reproduction (Augustine et al. (2008), Blüher and Mantzoros (2007)Blüher and Mantzoros (2007), Casanueva and Dieguez (1999)). Hill et al. (2008) portrays, how the reproductive axis reacts to the energy-

[16] arcuate, ventromedial and dorsomedial nucleus in the hypothalamus

[17] the same is found in respect to salt. The human body is not adjusted to the high values which can nowadays so easily be reached. Our ancestors had only small amounts of salt at their disposal and the body tries therefore to regain as much as possible from the kidney

[18] leptin initiates menstruation

and metabolic situation of the body and which brain areas are involved. Leptin and insulin operate via classical metabolic pathways in the arcuate nucleus, using NPY/AgRP- and POMC/CART neurons, and the recently found Kisspeptin network. Other hypothalamic nuclei play also a role in this puzzle of metabolic situation and reproductive function.

In this connection it is also interesting, that in the last 30 years puberty of girls began earlier and earlier. The increase in bodyweight of these children, which occurred at the same time, seems to be a main factor. The data suggest, that the increase in weight is the cause for the earlier puberty and not vice verse. Leptin seems to play the decisive role. Mice and women with leptin deficiency do not enter puberty, unless leptin is provided. Already low amounts of leptin stimulate the gonadotropin secretion at the level of the hypothalamus as well as the level of the pituitary. Leptin is here the prerequisite and not the critical metabolic signal for initiating puberty. This assures, that pregnancy and milk production begin only, if enough fat storage is present, which nourishes mother and the growing child (Kaplowitz (2008), Mircea et al. (2007)). The hormonal adaptation in the hypothalamus of the mother during pregnancy makes sure, that in spite of an increasing leptin level food is taken up, which is stored as fat and meets the high metabolic demand during pregnancy and milk production (Augustine et al. (2008)). Antagonist of leptin is ghrelin, which inhibits gonadotropin secretion. It prevents the onset of puberty and inhibits directly gonadal development (Tena-Sempere (2008b)). The secretion of the luteinizing hormone is also arrested (Tena-Sempere (2008a)).

Leptin is needed for the ovarial cycle and interferes at the level of the hypothalamus, the pituitary and the gonads. It is present in the mother milk and required for the neuroendocrine control of secretion of the growth hormone. Leptin informs the hypothalamus and other neuroendocrine loci about the condition of the fat depots, allowing the neuroendocrine system to adjust to the situation of the energy homeostasis (Casanueva and Dieguez (1999)).

Obestatin is a peptide, which in contrast to ghrelin represses appetite (Germain et al. (2008)). It exhibits a circadian rhythm and its concentration is increased in anorexia.

Adiponectin is a peptide hormone, which is produced in the fat cells of humans and animals. It regulates together with leptin, insulin and other hormones hunger sensation and food uptake. It amplifies the effect of insulin on fat cells. If the fat cells contain much fat, small amounts of adiponectin is produced; with little fat the production is increased. Overweight people possess a low adiponectin level. Therefore the insulin concentration is reduced. Together with genetic factors a low adiponectin level increases the danger to catch diabetes, which damages the vessels. A high adiponectin level shields people from diabetes. Adiponectin speeds up the degradation of fat, as shown in experiments with animals.

Resistin is a cysteine-rich protein, which is released in mice and rats by the fat tissue[19]. It could be involved in the development of diabetes mellitus type 2 (T2DM). The resistin content in the blood serum is increased in overweight mice (Lee et al. (2003)). It could represent a

[19] in primates, pigs and dogs by immune cells and epithelium cells

connection between metabolic signals, inflammations and arteriosclerosis.

Visfatin is another adipocytokin, which is released by fat cells of the intestine. It reduces in the same way as insulin the sugar content in the blood by stimulating the glucose uptake of muscles and fat cells. Compared with insulin its concentration is lower and -independant of the food uptake- constant. It affects insulin receptors without competing with insulin.

Fat cells are thus not just fat stores, but dynamic cells, which are responsible for the energy-homeostasis, but also for other tasks (such as the cytokines). The circadian clock allows the fat tissue and other tissues to foresee the changes in glucose-, fatty acid- and triglyceride levels and also the alterations in hormone concentrations (insulin, adrenalin) of the body and to prepare the body for the expected stimuli, permitting fast reactions.

Since mammals possess in almost all tissues autonomous circadian clocks, it was looked for them also in fat tissue (Otway et al. (2008)). 24 hour-rhythms were found in 20% of the fat tissue transcriptoms. But because this tissue is composed of various cell types, cultures of 3T3-L1 adipocytes were studied. Before the measurements they were treated with a serum-pulse, in order to synchronize the expected circadian clocks. Clock-genes were indeed found, the expression of which is circadian. Studies shall now show, whether synthesis and secretion of other adipokines are under circadian control.

7.9 Liver cells

Liver cells (see figure 7.22) are specialized, to remove toxic substances from the blood and to store nutrients and other substances, which are at the moment not needed by the body. Carbohydrates are, for instance, stored as glycogen. Liver cells play also a role in the fat- and protein metabolism.

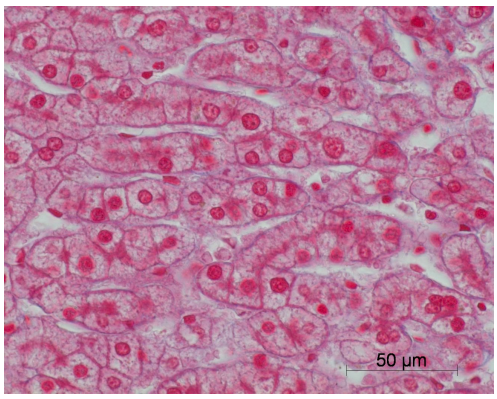


Figure 7.22: Liver cells in a liver lobe of a human. Bright channels are arterial blood capillaries. Image of a preparation of Dr. Klaus Reutter

Numerous proteins[20] in the soluble fraction of the liver are rhythmically expressed, as shown by differential gel-electrophoresis (Akhtar et al. (2002), Reddy et al. (2006)). Micro-array-analysis yielded, however, only 5 to 10% rhythmic expression of all genes. That indicates, that besides transcriptional mechanisms also post-transcriptional and post-translational mechanisms of gene expression are responsible for the circadian rhythm. Therefore not in all protein rhythms the corresponding mRNA-rhythms are observable (Albrecht (2006)). More than 50% of the proteins found were already known, among them a few isoforms. 80% of the proteins were produced during the day (that is, during the resting stage of the mice). At this time

[20] of 642 protein spots 60 exhibit a highly significant and 135 a significant rhythm

the liver detoxifies and regenerates (Turek and Allada (2002)).

Further transcriptome and proteome studies will help to understand the complex and dynamic processes in the liver (Reddy et al. (2006)). Each tissue seems to possess its own set of oscillating genes. In the whole organism it might well be, that each gene is expressed in a circadian way, some in the liver, others in the kidney, again others in the heart. That makes sense, because it allows an optimal function of the corresponding organs for the various tasks and demands of the organism. That is the reason, why it is important to study physiological processes in humans and animal under the aspect of circadian oscillations. This systemic biology will show, how complex systems such as metabolism and brain interact with the circadian system and the environment. It will also lead to an integrated view of the therapy of diseases, which are caused by systemic malfunctions and not by special molecular alterations in a certain process (see chapter 8).

The circadian clock controls also the expression of certain genes in the liver, which are important for cell division (Matsuo et al. (2003), Reddy et al. (2005)).

The clock-genes in den liver cells are controlled by the circadian clocks in the SCN, but the paths and kind of signals to the liver were unknown. With the help of pseudorabies-virus, a transsynaptic marker of nerve tracts, the neural connections between SCN and peripheric tissue could be followed in some physiological systems. Sympathetic and parasympathetic neurons of the autonomous nervous system are involved. Shibata (2004) describes anatomical and physiological experiments concerning the sympathic control of circadian clocks in the liver.

7.10 Keratocytes

According to Brown et al. (2005) in rare cases hair root Keratocytes can be cultivated, which happened to stick to the end of a plucked human hair (figure 7.10). These cells can be cultivated and proliferated like fibroblast. They were made to emit light in a circadian way which was measured. The period lengths are identical with those of fibroblasts of the same person (figure 7.23). Since it is a rare occasion, that the cells stick to the plucked hair, the studies have not been continued.

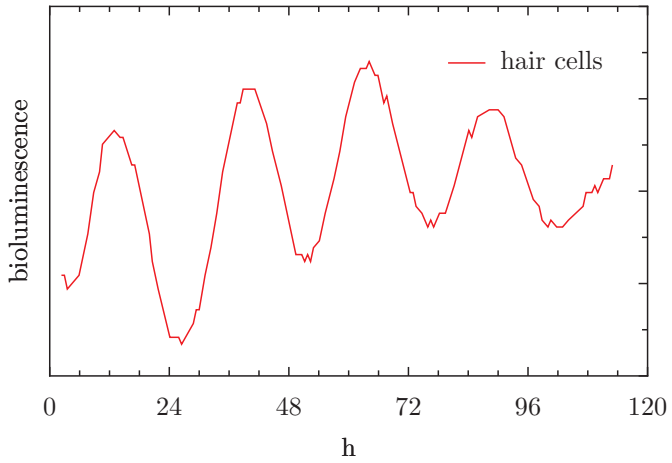
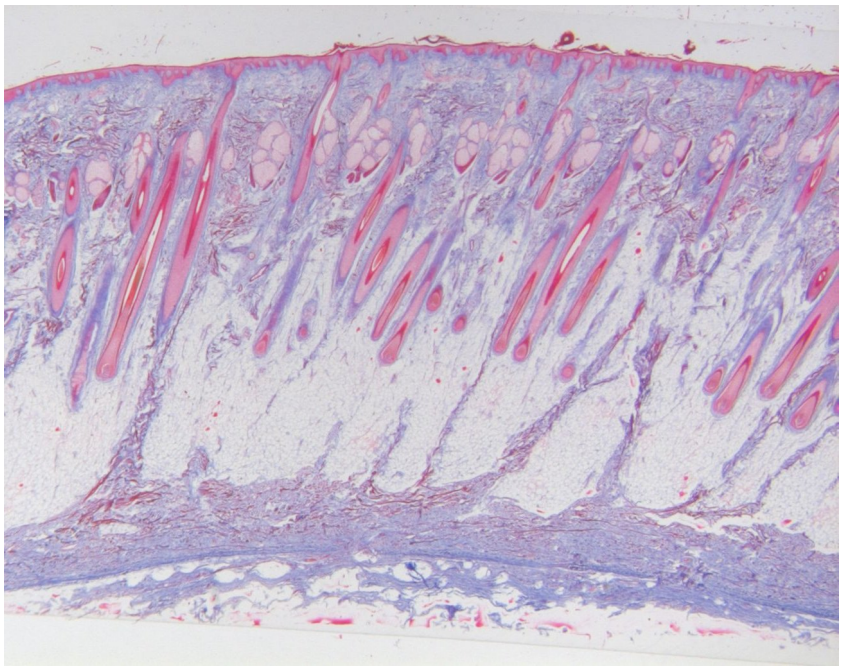
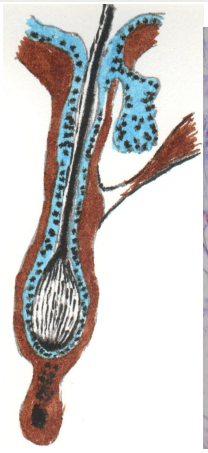
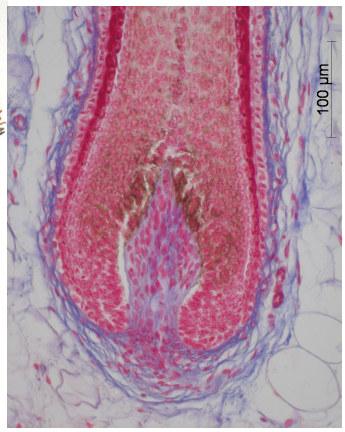


Figure 7.23: *Keratocyte and circadian rhythm of bioluminescence (curve) evoked by a luciferase-gene, attached to a promoter, which was under circadian control. After* Brown et al. (2005)







Top: Skin section with several sectioned hair shafts and hair cells. Bottom left: Epidermis cones grow as hair anlagen inclined in the leather skin. The lower end extends onion-like and is invaded by connective tissue from below (see bottom right image). Keratocyte (hair cell, black, bottom left) in hair bulb with hair (black). At the right the muscle appendage (brown) and above a sebaceous gland (blue). The histological images were kindly supplied by Dr. Klaus Reutter. Sketch after Brown et al. (2005)

8 Diseases due to circadian disturbances

It turned out, that a number of diseases are brought about by disturbances in the circadian system. Klerman (2005) gives an overview on clinical diseases, which are caused or influenced by diurnal or circadian rhythms. He discusses also side effects of a disturbed circadian rhythm such as shiftwork and its consequences for the health system. It is, however, difficult, to perform the required experiments, since the 24-hour fluctuations in pathophysiology or in symptoms of many diseases are causally connected with endogenous circadian rhythms or other diurnal factors, which change during the day like body position, activity, sleep- or wake and metabolism connected with food uptake. As long as the physiological basis is not known in detail, an adequate treatment is not possible.

At the cellular level the circadian clock controls among others cell division and allows it to occur in a certain time window only (Nagoshi et al. (2004)). Mutations in the mechanism of regulation of the cell cycle or in cell division itself can lead to cancer (Fu et al. (2002), Lowrey and Takahashi (2004), section 8.2). Mammals with homozygous Clk show deviations in the freerun and metabolic syndromes such as obesity, hyperlipidemia, hyperglycemia, hypoinsulinemia (Turek et al. (2005), section 8.3). A mutation in the per2-gene of mice changes the phase relationship (Spanagel et al. (2005a)) and leads to a higher alcohol consumption. In humans with clk-mutations alcoholism is more frequently found (section 8.4). In clk-mutations sleep is influenced, likewise in BMal1-mutations (Laposky et al. (2008), section 8.6).

Circadian rhythms and diseases interact not only on the cellular level, but also on another one: Certain diseases and partly fatal events occur more frequently at certain times of the day, for instance those of the heart-blood circulation (section 8.5) and of respiration in the morning hours. This is the case for high blood pressure, heart infarct (heart attacks), *Angina pectoris*, stroke, arrhythmia, sudden heart death, congestive heart failure (White (2007)). Heart artery plaques burst most frequently between 6 and 12 o'clock. apnoe, endocrine, rheumatic and nocturnal hyperglycemia as well as epileptic fits have their special times. On the other hand, therapy is often more effective at certain phases of the daily rhythm, for instance in administering medications.

At a third level behavior can be in conflict with the circadian system, for instance in night-, shift- and rotational work. In shiftworkers exposed to irregular light-dark changes, the sleep-wake-rhythm and the feeding-rhythm is disturbed. This leads often to cardiovascular diseases, obesity, diabetes and other metabolic diseases. The plasmalipid metabolism changes, oxidative stress increases. Increase in weight by shorter sleep and shift work are well documented, although the mechanism is not yet understood.

Since in this field many papers have been published, I restrict myself in the fol-

lowing to a few examples, some of which have been mentioned already in chapter 7.

8.1 Eyes, SCN, blind people

Abnormalities in organs can disturb the circadian system, and vice verse a disturbed circadian system can influence the function of organs (see figure 8.1). For instance, damages in the SCN do not allow to synchronize the peripheral tissue (Lowrey and Takahashi (2004), Czeisler et al. (1989)). If the inputs from the retina to the SCN are lacking, as for instance in some blind persons, the synchronization of the sleep-wake- and the activity rhythm, the body temperature-rhythm and the peripheral oscillators are disturbed. This is, however, not in each blind person the case, since two different photoreceptor systems exist in the retina, one for the normal and the other for the circadian vision (Czeisler et al. (1995)). If the latter is still working, the SCN and the peripheral oscillators controlled by it are still synchronized by the light-dark cycle of the day.

Disturbed outputs of the SCN can also lead to diseases or indispositions. For instance, normally the concentration of the antidiuretic hormone ADH is high during the night. In elder people the rhythm is, however, damped. Therefore more urine is produced during the night and the sleep is disturbed by nocturnal urination. Sleep disturbances are caused also by an abnormal phase position of the SCN-rhythm or by a disturbed synchronization. Thus, a mutation of the CKI-binding-domain of the Per2-gene leads to the non-24-h-sleep syndrome, in which the sleep-wake-rhythm is not synchronized anymore (Hashimoto et al. (1998)).

8.2 Cell divisions and cancer

The circadian clock allows cell division to occur in a certain time window only (Nagoshi et al. (2004)). Mutations in the mechanism of this regulation of the cell cycle including the cell division can cause cancer (Fu et al. (2002), Lowrey and Takahashi (2004)). For instance, polymorphism of the Per3-gene causes an early breast cancer (Zhu et al. (2005)). Likewise, abnormalities in the per1- per2- and per3-gene lead to tumores (Chen et al. (2005) and figure 8.2).

One has to be carefull, however, in interpreting it: Gauger and Sancar (2005) reported, that a disturbance of the circadian clock increases the risk of women to obtain breast cancer and potentiates in mice the tumor rate and mortality rate (induced by ionizing radiation). Cryptochrome 1- and Cryptochrome 2-proteins are clock-components of the circadian clock of mammals. Mice with mutations in both cryptochrome-genes are arrhythmic. To test, whether the hypothesis of Gauger and Sancar (2005) of an increased tumor- and mortality rate due to a disturbed circadian clock is correct, Cry1/Cry2 mice and the fibroblasts obtained from them were checked for radiation-induced cancer, DNA-damages and mortality rate. The Cry1/Cry2-mice did, however, not differ from the wildtype controls. And the fibroblasts of the Cry1/Cry2-mutants had the same susceptibility for ionizing and UV radiation as the wildtype controls. DNA-repair and DNA damages after irradiation are thus in arrhythmic animals not different from the one in the rhythmic control animals.

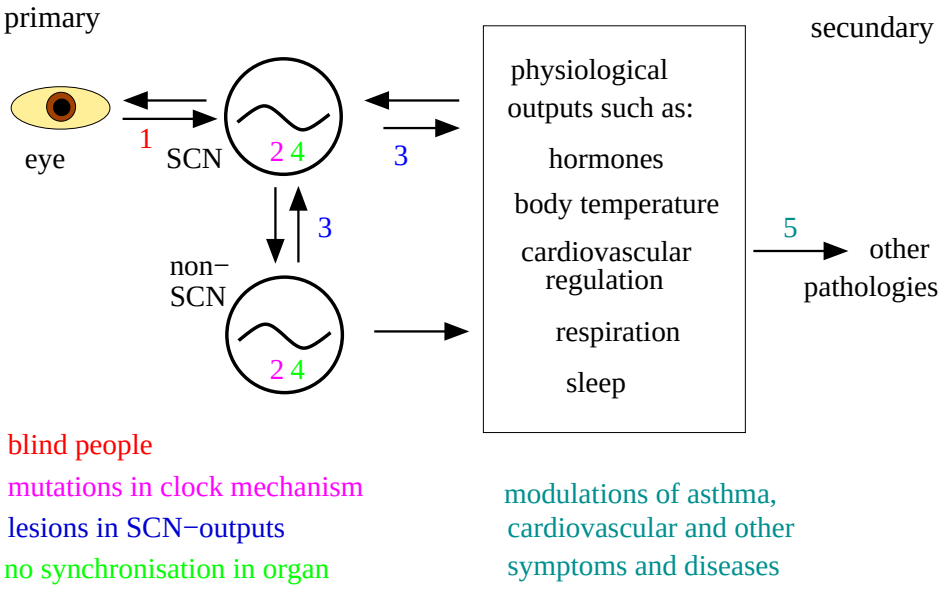


Figure 8.1: *Various possibilities of disturbances in the circadian system, which might lead to diseases. After Klerman (2005)*

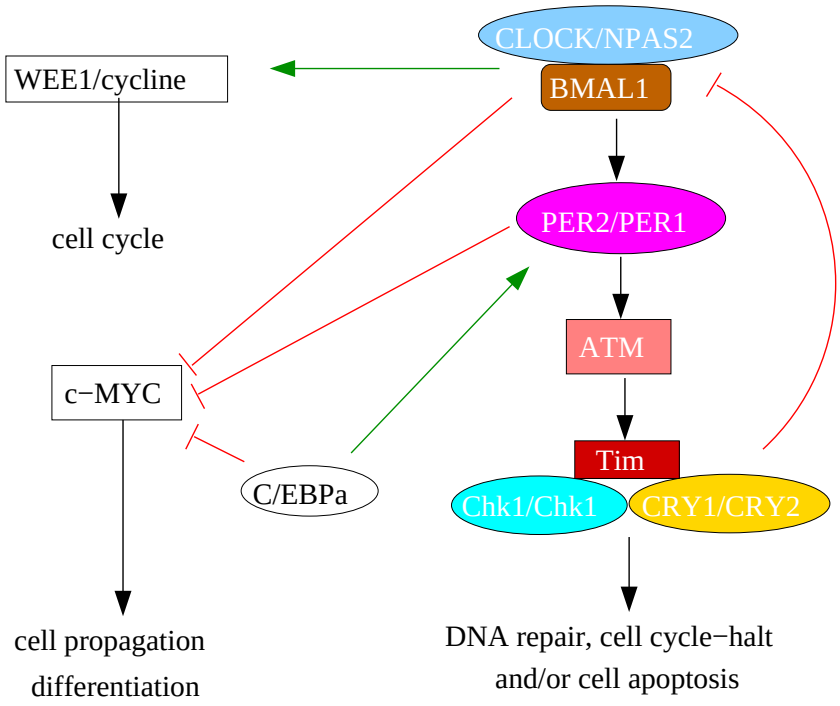


Figure 8.2: Tumors and cell cycle disturbances

8.3 Nutrition, obesity and diabetes

Overweight has become recently in many societies an epidemic. Its cause is eating too much (hyperphagia) and spending too little energy. As a result too much fat accumulates in the body. Fat is a risk factor for diabetes, cancer, cardiovascular and other diseases. In the USA more than half of the population are overweighted and its number is increasing. The fat content of the body depends on the ethnic background, on the gender, the developmental stage and on age. Already in the fetus at the paracrine/autocrine level the development of fat tissue is determined (Kiess et al. (2008)). Nutritional and hormonal conditions during pregnancy and early childhood may irreversibly influence the development of organs, which are involved in food uptake and -processing, and the hypothalamic structures, which are responsible for the behavior of food uptake and control of the energy balance. During this early programing leptin seems to play a critical role (Djiane and Attig (2008)).

Appetite-regulating hormones of the energy balance of fat cells are also involved in constitutional meagerness and anorexia (Anorexia nervosa) (Loucks (2007)). Whereas **ghrelin** (an orexigenic hormone, see Sakurai (2006)) leads to normal eating behavior in constitutional meagerness, an increased ghrelin-level is without effect. Ghrelin regulates the secretion of the growth hormone and the homeostasis of the energy balance. Circulating ghrelin fluctuates in an ultradian pattern. Fat cells can release certain hormones such as leptin in orderly patterns, but others such as adiponectin in unorderly patterns. Ghrelin in the plasma increases in slim people during the night, whereas it does not show a rhythm in overweight persons (Yildiz et al. (2004)).

Another disease caused by overweight is hypoventilation. The mortality rate is high. Leptin shortage or leptin resistance in obese people affects the central respiratory actuator, reduces breathing and leads to hypoxia (Piper and Grunstein (2007)).

Leptin is active also in the periphery. It can modify the insulin sensitivity, the metabolism in tissues, stress reactions and reproductive functions. In addition to a central "leptin-resistance" in obese persons there exists also a peripheral leptin-resistance (Harris (2000)).

Overweight seems to cause asthma, atopic and autoimmune diseases by reducing the immunological tolerance against antigenes. Pregnant women seem to convey this intolerance to the fetus (Hersoug and Linneberg (2007)).

Connections between circadian and metabolic systems and their significance for cardiovascular diseases, obesity and diabetes are pointed out by Green et al. (2008).

The circadian clock controls food utilization and the homeostasis of energy by controlling the expression and/or activity of enzymes, which take part in cholesterol-, amino acid-, fat-, glycogen- and glucose-metabolism (see figure 8.3).

Additionally numerous hormones of the metabolism such as insulin, glucagon, adiponectin, corticosteron, leptin and ghrelin are released in a circadian pattern. Disturbances of the circadian rhythm can lead to cancer, metabolic syndromes and obesity (figure 8.4). On the other hand metabolism and food uptake feed back on the circadian clock. Caloric restrictions synchronize the clocks in the SCN,

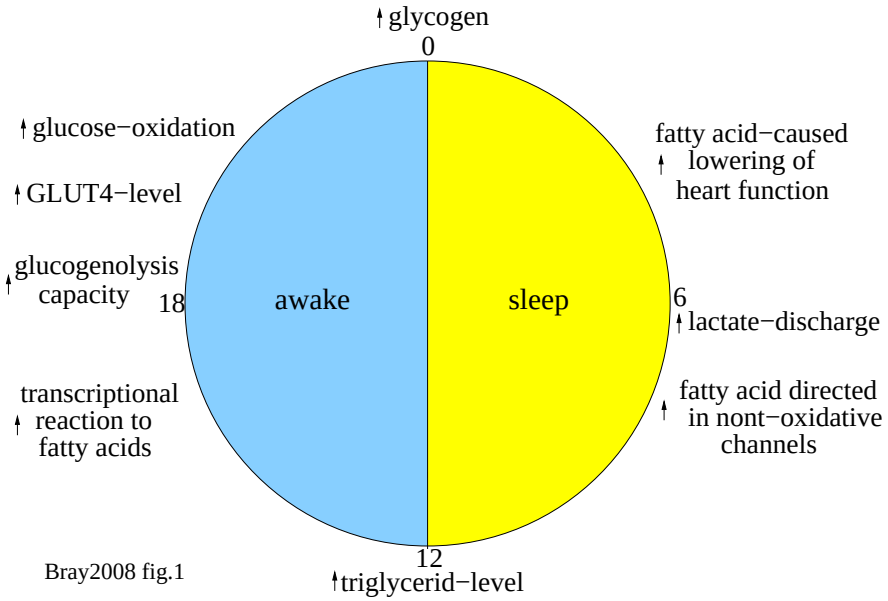


Figure 8.3: *Maxima of various metabolic events in the rat, circadian time 0 at onset of light, 12 at end of light in a 12:12 light-dark cycle and in continuous darkness*

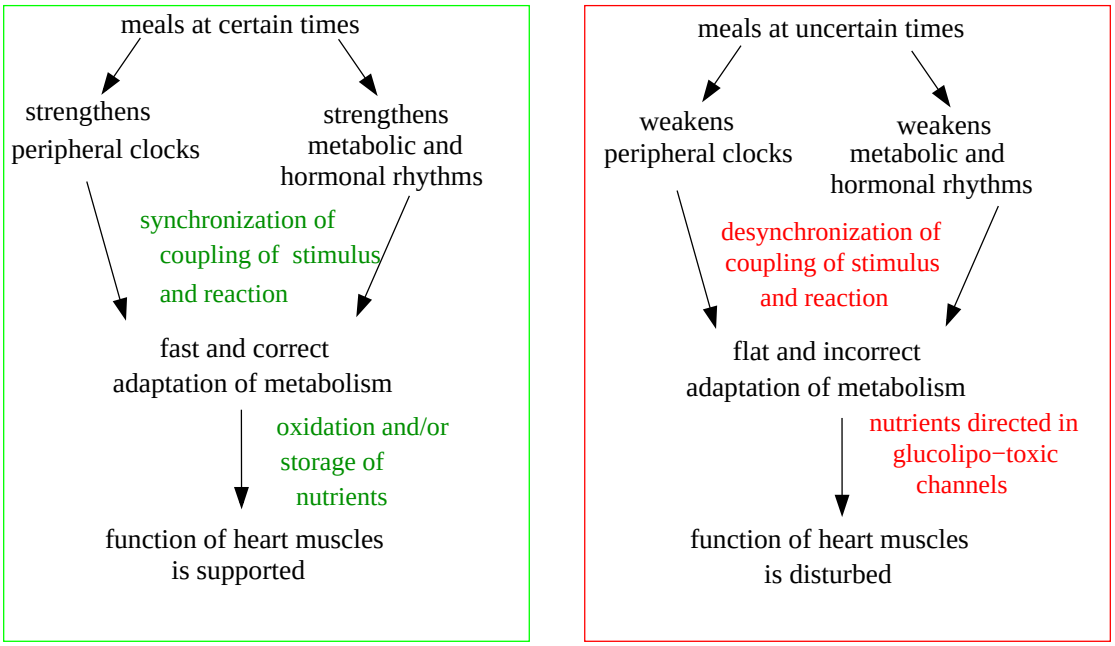


Figure 8.4: Significance of regular meal times for health

whereas food restriction to certain times synchronize peripheral oscillators. Furthermore the redox state of cells, which is determined by the food metabolism, and certain compounds such as glucose, alcohol, adenosine, coffeine, thiamine and retinic acid are able to phase shift circadian rhythms (Froy (2007)).

Peripheric oscillators can be influenced via the SCN and by an altered feeding pattern. In the latter case the right communication between SCN, environment and peripheral target organs/tissues is lacking; they are, however, decisive for the regulation of the body weight. Possible time cues for peripheral clocks are glucocorticoides, retinoine acid and melatonin, which control and mediate the feeding behavior. Satiety factors (leptin, ghrelin) play a role, the rhythm of which occurs independent of food uptake (Anubhuti and Arora (2008), Yildiz et al. (2004)). For the pathogenesis of obesity see Bray and Young (2007), Scott and Grant (2006), Turek et al. (2005). Lipogenesis, storage and lipolysis in the fat tissue are rhythmic (Ando et al. (2005), Alonso-Vale et al. (2008)). The energy metabolism of the whole body and that of specific organs differs. If the SCN is removed in an animal, the leptin-rhythm disappears and the sleep-rhythm changes.

Overweight develops only, if more energy is taken up as is spent. The food composition affects the energy balance. Fat plays thereby a special role, since it is badly regulated at the level of consumption as well as at the level of combustion. Psychological and behavioral profiles of the obese are important, because they influence food selection and eating patterns. It is still unclear, whether the number of meals plays a role for overweight. Eating disorders are involved in obesity, but it is uncertain, whether they are the cause or the result. Diet might lead to excessive eating. At an early stage of fat depositions the existing fat cells are enlarged (hypertrophy) and in a later stage new fat cells develop (hyperplasy). Hereby specific components of the diet such as polyunsaturated fatty acids or proteins may play a role. The critical age for a later overweight is lower in children, which eat much protein, and begins possibly already in early childhood or even in the fetus (Ziegler et al. (2000)).

Fat rich nutrition in rodents leads to metabolic diseases, which resemble the metabolic syndromes of human overweight and insulin resistance. Thereby the time structure of many physiological functions changes. To find out, whether fat-rich food (hypercaloric diet) affects also the clock in the SCN, the synchronization of mice by was studied for three months under conditions of diets rich or poor in fat. As a hand of the clock the running wheel activity and the body temperature was measured. In comparison to the control animals the fat-rich fed animals possessed a higher body weight index, hyperleptinemia, higher glucose concentrations in the blood and an increased insulinemia. At the same time the synchronization of the clock by light was affected in these animals. If the internal clock experienced a jet-lag of 6 hours by changing the light-dark cycle, the rhythm was less rapidly advanced. There were also differences on the molecular level due to light-induced phase shifts (c-FOS and P-ERK[1] reduced).

[1] c-Fos belongs to the 'immediate early gene' family of transcription factors. Transcription is up-regulated by many extracellular signals. Phosphorylation changes activity and stability. Members of the Fos family dimerize with Jun and form the AP-1 transcription factor, which up-

Fat-rich food modifies thus the circadian synchronization by light (Mendoza et al. (2008a)).

The synchronization of the central circadian clock in the SCN by feeding is more effective in rodents, if it is restricted in time and in calories, for instance by a single meal per day at the same time. Since, however, the constant feeding time coincides with the hypocaloric conditions, an ultradian feeding pattern was adopted with six meals per day (6 times 8-minutes, scarce food supply, and 6 times 12-minutes, rich food supply). The daily respectively circadian rhythms of running wheel activity and body temperature were phase shifted under scarce food supply (rhythm is advanced as well as delayed). In the case of the well fed rats without loss of weight the rhythm was hardly or not shifted. This demonstrates, that caloric restriction synchronizes the SCN to the time of feeding in spite of the presence of a light-dark cycle (*Mendoza et al. (2008b)*).

Finally it should be pointed out, that obesity and insulin-resistance in hibernating mammals possesses a selective advantage. According to a hypotheses of Neel (1962) this was true also for humans of the past, because for them it was advantageous, if acute, physiological and prognostic events tuned the body to the food shortage during the winter (including T2DM). Nowadays this is, however, pathological and harmful (see Scott and Grant (2006)).

regulates transcription of various genes, which for instance participate in the division and differentiation. c-fos is used as a marker of neural activity, since it occurs during firing of neurons.

P-ERK is an initiation factor of translation in eukaryonts

8.4 Alcohol and other drugs

As mentioned already, a per2-mutation in mice brings about another phase relation (Spanagel et al. (2005a)) and leads at the same time to a higher alcohol consumption. Among humans possessing this mutation alcoholism is frequently found. In clk-mutations sleep is affected, likewise in BMal1-mutations (Laposky et al. (2008)).

Alcohol-metabolism (chronokinetics) and -effect (chronotoxicity) show a circadian pattern. The target organs do also react periodically to alcohol (chronesthesia) (Danel and Touitou (2004)). The driving capability is reduced by alcohol, even at low blood levels. Alcohol increases the danger of collisions and the chances of recovery after accidents. Attentiveness and mental stress are affected. Scarcely studied are the effects of drugs such as marijuana, benzodiazepines and other psychoactive drugs (stimulants and narcotics) (Ogden and Moskowitz (2004)).

Spanagel et al. (2005b) studied, how alcohol and clock-genes interact in mice. In alcohol-favoring mice strains the circadian rhythm of behavior is altered. At the neuronal level alcohol alters the circadian pattern of expression of the per-gene in different regions of the brain including the SCN. The per2-gene activity regulates alcohol uptake by affecting the glutamergic system via the glutamate re-uptake mechanism. This in turn affects a number of physiological events, which are controlled by the circadian clock. Besides neurochemical functions neuroendocrine and immunological functions are disturbed[2].

[2] see however Zghoul et al. (2007)

8.5 Cardiovascular diseases

It is well known, that cardiovascular diseases such as congestive heart failure and coronar insufficiency lead to sleep disturbances. Less well known is, that certain physiological events during longterm sleep deprivation can induce high blood pressure, arteriosclerosis, heart attack and arrhythmia. Chronicle sleep deprivation is a risk factor for overweight and its visceral form, the basis of the metabolic syndrome. Obstructive and central sleep-apnoe are connected with our current overweight epidemic and increase the danger of heart attack or the ischemic attack (Plante (2006)).

Begin and symptomes of diseases such as heart attack, Angina pectoris, heart infarct, ventricular tachycardia depend on the circadian phase. Heart infarct, Angina pectoris and silent ischemia in the case of stable angina occur in most cases between 8 and 12'o clock. ECG-abnormalities and angina-attacks in the case of instable angina occur mostly during the night. Blood pressure and heart rate are high in normotonic and primary high pressure patients during the day, drop during the night and increase again in the morning. 70% of secondary high pressure patients do not show a rhythmic course or exhibit even an increase during the night. Various types of a disease can thus show different circadian patterns of the symptoms. Treatment with drugs has to take this into account and has to occur at the corresponding most favorable time. The pharmacokinetic does also change during the course of a day (shown for various cardiovascular drugs such as propranolole, nifedipine, verapamil, enalapril, isosorbid-5-mononitrate, digoxin and others(Lemmer (1999)).

8.6 Sleep disturbances, overweight, depressions

Sleep depriviation influences endocrine functions and metabolism. The cortisol level increases in the afternoon and early evening. Consequently the glucocorticoid level increases, which could lead to memory shortcomings as found in advanced years. Chronicle sleep deprivation can thus speed up the aging process. Likewise the carbohydrate tolerance is influenced, which increases the risk for diabetes. Finally sleep plays also an important role in the energy equilibrium. Partial sleep deprivation reduces the plasma level of leptin and increases that of ghrelin; hunger and appetite increase (but not for protein rich food). The neuroendocrine regulation of appetite and food uptake is thus influenced by the sleep duration, and sleep deprivation can lead to overweight (Copinschi (2005)).

One reason of obesity can be a changed sleep-wake rhythm . This alters the synchronization of the SCN by the LD-cycle[3]. Lipolysis in the fat tissue occurs rhythmically: It increases during the night, and it decreases again during the afternoon[4]. Sleep deprivation leads to disturbances of the metabolism and obesity, which can lead to diabetes (T2DM). In a study with thousand patients it could be shown, that obese persons had the shortest sleep duration (as an average 15 minutes shorter), were more sleepy during the day and had a more disturbed night sleep as compared to normal weighted persons. Obesity is

[3] in rodents such as the Siberian hamster the body weight is increased under shortday conditions via control of the SCN. In this way the body prepares itself for hibernation

[4] however, in the mutant T1DM lipolysis increases earlier and stays high during the night

according to these studies caused by environmental factors, but has also a genetic component. Both factors increase food uptake and the energy metabolism is compromised. Molecular studies to these questions are from Larkin et al. (2005), Larkin (2006) and Iitaka et al. (2005)).

There is finally also a connection between circadian rhythms, sleep disturbances and depressions (Germain and Kupfer (2008)): It is differentiated between non-seasonal and seasonal depressions. The latter one occur often during the winter and are called Seasonal Affective Disorders (SAD). Winter depressions are partly combined with hypomania during the spring. For an overview see Levitan (2007). Depressives have an increased suicide rate. It fluctuates with a circadian and an annual rhythm (van Houwelingen and Beersma (2001)) and increases with ascending light intensity of the sun (Lambert et al. (2003), Björkstén et al. (2005), Koenigsberg (1984)).

Sleep disturbances are frequently observed during depression: 90% of the depressives encounter difficulties to fall asleep, to sleep through the night, or they wake up too early, 6-30% sleep too much (Roberts et al. (2000)), especially in winter-SAD; sleeplessness, however, occurs more often in summer-begin-SAD. For the control of the sleep-wake-rhythm see Saper et al. (2005).

A number of circadian hypotheses of depression were put forward:

1. Phase shift: The daily rhythm is advanced or delayed, which can be seen also in the SCN rhythm. As a therapy strong light pulses can be given (Lam et al. (1999), Rosenthal et al. (1990)). Morning- and evening light improve the condition (Eastman et al. (1998), whereby morning light seems to be more effective (Lewy et al. (1998), Terman and Terman (2005)). Melatonin administration helps in winter-depressions (Lewy (2007)). According to Terman and Terman (2005) the depression is alleviated by an advanced melatonin-rhythm.

2. Internal phase coincidence: There is a sensitive phase of the circadian rhythm (Borbely (1982)). If the rhythm is shifted, the dissonance between circadian phase and sleep phase increases (Wehr and Goodwin (1975)). Antidepressives such as MAO-inhibitors (MAOI) are also able to shift the rhythm and have a therapeutic effect (Kripke et al. (1983)).

3. Depressions are characterized by a long REM-latency. If the REM-sleep is suppressed by pharmaca or by behavior, mood improves (see however Grözinger et al. (2002))

4. Increased REM at the cost of the *Slow Wave Sleep* (SWS): This disturbs according to Borbely (1982) the S-process (see however Sharpley (1995))

5. Social and accompanying physiological rhythms are disturbed (Ehlers et al. (1988), Frank et al. (1997), Grandin et al. (2006))

6. Clock-gene-polymorphism shall lead to depressions according to newer work (Bunney and Bunney (2000), Benedetti et al. (2003), Serretti et al. (2003), Serretti et al. (2005), Joyce et al. (2005))

Chronotherapeutic measures were proposed by Epperson et al. (2004) and Goel et al. (2003). According to Barbini et al.

(2005) dark therapy shall reduce manic symptoms. Sleep deprivation was used by Pflug (1976) and by Wirz-Justice and van den Hoofdakker (1999). The results are, however, not consistent. Lithium salts are known antidepressives (Schou (2000); see also Colombo et al. (2000), Loving et al. (2002)). A therapy using social rhythms was used by Frank et al. (2005).

The effect of antidepressives on depressions, the circadian rhythm and the sleep-wake-rhythm was discussed by Tsuno et al. (2005), Argyropoulos and Wilson (2005) and Winokur et al. (2001). Tricyclic antidepressives suppress the REM-sleep, MAOI, SSRI (selective serotonin-uptake inhibitors) and SNRI (serotonin-norephinephrine-re-uptake-inhibitors) and newer antidepressives such as agomelatin (acts on melatonin- and serotonin-receptors, San and Arranz (2008)) advance the circadian phase (see Rupprecht et al. (2004)).

Further literature: Wolk et al. (2005), Oishi et al. (2005), Wijnen and Young (2006), Canaple et al. (2006), Fausto et al. (2006), Kornmann et al. (2007), Kornmann (2007), Mendoza (2007), Reddy and Maywood (2007) Maronde et al. (2007), Almon et al. (2008), Bertolucci et al. (2008), Bussone (2008), Downes and Liddle (2008).

Bibliography

Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., and Kyriacou, C. P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cdna microarray, is driven by the suprachiasmatic nucleus. *Current Biology*, 12:540–550. 94

Akimoto, H., Kinumi, T., and Ohmiya, Y. (2005). Circadian rhythm of a TCA cycle enzyme is apparently regulated at the translational level in the dinoflagellate Lingulodinium polyedrum. *Journal of Biological Rhythms*, 20:479–489. 32, 33

Akiyama, S., Nohara, A., Ito, K., and Maéda, Y. (2008). Assembly and disassembly dynamics of the cyanobacterial periodosome. *Molecular Cell*, 29:703–716. 12

Albrecht, U. (2006). Molecular orchestration of the hepatic circadian symphony. *Genome Biol*, 7:234. 94

Albus, H., Bonnefont, X., Chaves, I., Yasui, A., Doczy, J., van der Horst, G. T. J., and Meijer, J. H. (2002). Cryptochrome-deficient mice lack circadian electrical activity in the suprachiasmatic nuclei. *Curr Biol*, 12:1130–1133. 66

Allen, G., Rappe, J., Earnest, D., and Cassone, V. (2001). Oscillating on borrowed time: Diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts. *Journal of Neuroscience*, 21:7937–7943. 86

Almon, R., Yang, E., Lai, W., Androulakis, I., DuBois, D., and Jusko, W. (2008). Circadian variations in rat liver gene expression: Relationships to drug actions. *Journal of Pharmacology and Experimental Therapeutics*, 326:700–716. 108

Alonso-Vale, M. I. C., Andreotti, S., Mukai, P. Y., das Neves Borges-Silva, C., Peres, S. B., Cipolla-Neto, J., and Lima, F. B. (2008). Melatonin and the circadian entrainment of metabolic and hormonal activities in primary isolated adipocytes. *J Pineal Res*, 45:422–429. 104

Ando, H., Yanagihara, H., Hayashi, Y., Obi, Y., Tsuruoka, S., Takamura, T., Kaneko, S., and Fujimura, A. (2005). Rhythmic messenger ribonucleic acid expression of clock genes and adipocytokines in mouse visceral adipose tissue. *Endocrinology*, 146:5631–5636. 104

Antkowiak, B. (1987). Der Einfluss des pH auf die stationäre Glycolyse von Saccharomyces carlsbergensis. Staatsexamensarbeit, University of Bonn. 49

Anubhuti, V. and Arora, S. (2008). Leptin and its metabolic interactions: an update. *Diabetes Obes Metab*, 10:973–993. 91, 104

Aoki, S., Kondo, T., Wada, H., and Ishiura, M. (1997). Circadian rhythm of the cyanobacterium Synechocystis sp. strain PCC 6803 in the dark. *Journal of Bacteriology*, 179:5751–5755. 7, 9, 14

Argyropoulos, S. and Wilson, S. (2005). Sleep disturbances in depression and the effects of antidepressants. *International Review of Psychiatry*, 17:237–245. 108

Aronson, B., Johnson, K., and Dunlap, J. (1994). Circadian clock locus frequency: Protein encoded by a single open reading frame defines period length and temperature compensation. *Proceedings of the National Academy of Sciences of the United States of America*, 91:7683–7687. 54, 55

Aronson, B., Johnson, K., Liu, Q., and Dunlap, J. (1992). Molecular analysis of the Neurospora clock: Cloning and characterization of the frequency and period-4 genes. *Chronobiol. Internat.*, 9:231–239. 54

Arpaia, G., Loros, J., Dunlap, J., Morelli, G., and Macino, G. (1993). The interplay of light and the circadian clock: Independent dual regulation of clock-controlled gene ccg-2(eas). *Plant Physiology*, 102:1299–1305. 55

Augustine, R. A., Ladyman, S. R., and Grattan, D. R. (2008). From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy. *J Physiol*, 586:387–397. 92, 93

Baker, J. and Ranson, R. (1932). Factors affecting the breeding of the field mouse (Microtus agrestis). I. Light. *Proc. Roy. Soc., Series B*, 110:113–332. 82

Banks, W. A. (2008). The blood-brain barrier as a cause of obesity. *Curr Pharm Des*, 14:1606–1614. 92

Barbini, B., Benedetti, F., Colombo, C., Dotoli, D., Bernasconi, A., Cigala-Fulgosi, M., Florita, M., and Smeraldi, E. (2005). Dark therapy for mania: a pilot study. *Bipolar Disord*, 7:98–101. 107

Bartness, T. J., Song, C. K., and Demas, G. E. (2001). SCN efferents to peripheral tissues: implications for biological rhythms. *J Biol Rhythms*, 16:196–204. 68

Benedetti, F., Serretti, A., Colombo, C., Barbini, B., Lorenzi, C., Campori, E., and Smeraldi, E. (2003). Influence of CLOCK gene polymorphism on circadian mood fluctuation and illness recurrence in bipolar depression. *Am J Med Genet B Neuropsychiatr Genet*, 123B:23–26. 107

Benson, J. and Jacklet, J. (1977). Circadian rhythm of output from neurones in the eye of Aplysia. IV. A model of the clock: Differential sensitivity to light and low temperature pulses. *JEBiol*, 70:195–211. 78

Berger, S., Dirk, J., Lindern, L., Wolff, D., and Mergenhagen, D. (1992). Temperature dependency of circadian period in a single cell (Acetabularia). *Bot. Acta*, 105:382–386. 36

Bertolucci, C., Cavallari, N., Colognesi, I., Aguzzi, J., Chen, Z., Caruso, P., Foá, A., Tosini, G., Bernardi, F., and Pinotti, M. (2008). Evidence for an overlapping role of clock and npas2 transcription factors in liver circadian oscillators? *Molecular and Cellular Biology*, 28:3070–3075. 108

Betz, A. and Chance, B. (1965). Phase relationship of glycolytic intermediates in yeast cells withoscillatory metabolic control. *Arch Biochem Biophys*, 109:585–594. 46

Bissonette, T. (1932). Modification of mammalian sexual cycles. I. Reactions of ferrets (Putoris vulgaris) of both sexes to electric light added after dark in November and December. *Proc.Roy.Soc., Series B*, 110:332–336. 82

Björkstén, K. S., Bjerregaard, P., and Kripke, D. F. (2005). Suicides in the midnight sun–a study of seasonality in suicides in west greenland. *Psychiatry Res*, 133:205–213. 107

Block, G., Geusz, M., Khalsa, V., Michel, V., and Whitmore, D. (1995). Cellular analysis of a molluscan retinal biological clock. *Ciba Found Symp*, 183:51–60. 77

Blüher, S. and Mantzoros, C. S. (2007). Leptin in reproduction. *Curr Opin Endocrinol Diabetes Obes*, 14:458–464. 92

Borbely, A. A. (1982). Sleep as a dynamic process. In *CNS Pharmacology, Neuropeptides: Proceedings of the 8th International Congress of Pharmacology, Tokyo, 1981*. Pergamon. 107

Bradley, R. and Reddy, K. (1997). Cloning, sequencing, and regulation of the global nitrogen regulator gene ntcA in the unicellular diazotrophic cyanobacterium Cyanothece sp. strain BH68K. *Journal of Bacteriology*, 179:4407–4410. 16

Brainard, G. C. and Hanifin, J. P. (2005). Photons, clocks, and consciousness. *J Biol Rhythms*, 20:314–325. 79

Bray, G. A. (1965). Rhythmic changes in renal function in the rat. *Am J Physiol*, 209:1187–1192. 89

Bray, M. S. and Young, M. E. (2007). Circadian rhythms in the development of obesity: potential role for the circadian clock within the adipocyte. *Obes Rev*, 8:169–181. 104

Bray, M. S. and Young, M. E. (2008). Diurnal variations in myocardial metabolism. *Cardiovasc Res*, 79:228–237. 87

Broda, H. and Schweiger, H. (1981). Long-term measurement of endogenous diurnal oscillations of the electrical potential in an individual Acetabularia cell. *Eur. J. Cell Biol.*, 26:1–4. 36

Brown, R. L. and Robinson, P. R. (2004). Melanopsin–shedding light on the elusive circadian photopigment. *Chronobiol Int*, 21:189–204. 79

Brown, S., Fleury-Olela, F., Nagoshi, E., Hauser, C., Juge, C., Meier, C., Chicheportiche, R., Dayer, J., Albrecht, U., and Schibler, U. (2005). The period length of fibroblast circadian gene expression varies widely among human individuals. *PLoS Biology*, 3:1813. 86, 88, 95, 96, 97

Brown, T. M., Colwell, C. S., Waschek, J. A., and Piggins, H. D. (2007). Disrupted neuronal activity rhythms in the suprachiasmatic nuclei of vasoactive intestinal polypeptide-deficient mice. *J Neurophysiol*, 97:2553–2558. 68

Bruce, V. (1970). The biological clock in Chlamydomonas reinhardi. *J. Protozool.*, 17:328–334. 41

Brunner, M. and Diernfellner, A. (2006). How temperature affects the circadian clock of neurospora crassa. *Chronobiology International*, 23:81–90. 42

Brunner, M. and Merrow, M. (2008). The green yeast uses its plant-like clock to

regulate its animal-like tail. *Genes & Development*, 22:825–83. 41

Brunner, M., Simons, M. J. P., and Merrow, M. (2008). Lego clocks: building a clock from parts. *Genes & Development*, 22:1422–1426. 12

Buijs, R. M., la Fleur, S. E., Wortel, J., van Heyningen, C., Zuiddam, L., Mettenleiter, T. C., Kalsbeek, A., Nagai, K., and Niijima, A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. *J Comp Neurol*, 464:36–48. 65

Buijs, R. M., Scheer, F. A., Kreier, F., Yi, C., Bos, N., Goncharuk, X. D., and Kalsbeek, A. (2006). Organization of circadian functions: interaction with the body. *Prog Brain Res*, 153:341–360. 65, 74

Bunney, W. E. and Bunney, B. G. (2000). Molecular clock genes in man and lower animals: possible implications for circadian abnormalities in depression. *Neuropsychopharmacology*, 22:335–345. 107

Bussone, G. (2008). Cluster headache: from treatment to pathophysiology. *Neurol Sci*, 29 Suppl 1:S1–S6. 108

Canaple, L., Rambaud, J., Dkhissi-Benyahya, O., Rayet, B., Tan, N., Michalik, L., Delaunay, F., Wahli, W., and Laudet, V. (2006). Reciprocal regulation of brain and muscle arnt-like protein 1 and peroxisome proliferator-activated receptor {alpha} defines a novel positive feedback loop in the rodent liver circadian clock. *Molecular Endocrinology*, 20:1715–1727. 108

Casanueva, F. F. and Dieguez, C. (1999). Neuroendocrine regulation and actions of leptin. *Front Neuroendocrinol*, 20:317–363. 92, 93

Cassone, V. M. (1991). Melatonin and suprachiasmatic nucleus function. In Cassone, V. M., Klein, D. C., Moore, R. Y., and Reppert, S. M., editors, *Suprachiasmatic nucleus: The mind's clock*, pages 309–323. Oxford University Press, New York. 85

Chance, B., Pye, K., and Higgins, J. (1967). Waveform generation by enzymatic oscillators. *IEEE Spectrum*, 4:79–86. 47

Chehab, F. F. (2008). Obesity and lipodystrophy–where do the circles intersect? *Endocrinology*, 149:925–934. 92

Chen, S.-T., Choo, K.-B., Hou, M.-F., Yeh, K.-T., Kuo, S.-J., and Chang, J.-G. (2005). Deregulated expression of the per1, per2 and per3 genes in breast cancers. *Carcinogenesis*, 26:1241–1246. 100

Chen, T., Chen, T., Hung, L., and Huang, T. (1991). Circadian rhythm in acid uptake by Synechococcus RF-1. *Plant Phys.*, 97:55–59. 5

Chow, T. and Tabita, F. (1994). Reciprocal light-dark transcriptional control of nif and rbc expression and light-dependent posttranslational control of nitrogenase activity in synechococcus sp. strain rf-1. *Journal of Bacteriology*, 176:6281–6285. 15

Colepicolo, P., Camarero, V., and Hastings, J. (1992). A circadian rhythm in the activity of superoxide dismutase in the photosynthetic alga Gonyaulax polyedra. *Chron. Int.*, 9:266–268. 32, 33

Collin, J. P. and Oksche, A. (1981). Structural and functional relationships in the

nonmammalian pineal gland. *The pineal gland*, 1:27–67. 85

Colombo, C., Lucca, A., Benedetti, F., Barbini, B., Campori, E., and Smeraldi, E. (2000). Total sleep deprivation combined with lithium and light therapy in the treatment of bipolar depression: replication of main effects and interaction. *Psychiatry Res*, 95:43–53. 108

Colwell, C. S. (1990). Light and serotonin interact in affecting the circadian system of Aplysia. *Journal of Comparative Physiology A. Sensory Neural and Behavioral Physiology*, 167:841–846. 77

Copinschi, G. (2005). Metabolic and endocrine effects of sleep deprivation. *Essent Psychopharmacol*, 6:341–347. 106

Crosthwaite, S., Dunlap, J., and Loros, J. (1997). Neurospora wc-1 and wc-2: Transciption, photoresponses, and the origin of the circadian rhythmicity. *Science*, 276:763–769. 78

Czeisler, C., Kronauer, R., Allan, J., Duffy, J., Jewett, M., Brown, E., and Ronda, J. (1989). Bright light induction of strong (type 0) resetting of the human circadian pacemaker. *Science*, 244:1328–1333. 100

Czeisler, C. A., Shanahan, T. L., Klerman, E. B., Martens, H., Brotman, D. J., Emens, J. S., Klein, T., and Rizzo, J. F. (1995). Suppression of melatonin secretion in some blind patients by exposure to bright light. *N Engl J Med*, 332:6–11. 100

Danel, T. and Touitou, Y. (2004). Chronobiology of alcohol: from chronokinetics to alcohol-related alterations of the circadian system. *Chronobiol Int*, 21:923–935. 105

Davidson, M. M., Nesti, C., Palenzuela, L., Walker, W. F., Hernandez, E., Protas, L., Hirano, M., and Isaac, N. D. (2005). Novel cell lines derived from adult human ventricular cardiomyocytes. *Journal of Molecular and Cellular Cardiology*, 39:133–147. 87

Delmer, D. and Brody, S. (1975). Circadian rhythms in Neurospora crassa: Oscillation in the level of an adenine nucleotide. *J. Bacteriology*, 121:548–553. 60

Denault, D. L., Loros, J. J., and Dunlap, J. C. (2001). Wc-2 mediates WC-1?FRQ interaction within the PAS protein-linked circadian feedback loop of Neurospora. *EMBO J.*, 20:109–117. 54

Derouiche, A., Anlauf, E., Aumann, G., Mühlstädt, B., and Lavialle, M. (2002). Anatomical aspects of glia–synapse interaction: the perisynaptic glial sheath consists of a specialized astrocyte compartment. *Journal of Physiology-Paris*, 96:177–182. 76

Deutsch, A., Dress, A., and Rensing, L. (1993). Formation of morphological differentiation patterns in the ascomycete Neurospora crassa. *Mechanisms of Development*, 44:17–31. 51

Devlin, P. F. (2002). Signs of the time: environmental input to the circadian clock. *J Exp Bot*, 53:1535–1550. 77

Djiane, j. and Attig, L. (2008). Role of leptin during perinatal metabolic programming and obesity. *J Physiol Pharmacol*, 59 Suppl 1:55–63. 102

Dong, G. and Golden, S. (2008). How a cyanobacterium tells time. *Current Opinion in Microbiology*, 11:541–546. 12, 15

Downes, M. and Liddle, C. (2008). Look who's talking: nuclear receptors in the liver and gastrointestinal tract. *Cell Metabolism*, 7:195–199. 108

Doyle, S. and Menaker, M. (2007). Circadian photoreception in vertebrates. *Cold Spring Harb Symp Quant Biol*, 72:499–508. 79

Duffield, G., Best, J., Meurers, B., Bittner, A., Loros, J., and Dunlap, J. (2002). Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. *Curr Biol*, 12:551–557. 63

Dunlap, J. (1993). Genetic analysis of circadian clocks. *A.R.Physiol.*, 55:683–728. 51

Dunlap, J. (1999). Molecular bases for circadian clocks. *Cell*, 96:271–290. 58

Dunlap, J. (2000). A new slice on an old problem. *Nature Neuroscience*, 3:305–306. 70

Dunlap, J. and Hastings, J. (1981). The biological clock in Gonyaulax controls luciferase activity by regulating turnover. *J Biol Chem*, 256:10509–10518. 25, 34

Dunlap, J., Taylor, J., and Hastings, J. (1981). The control and expression of bioluminescence in dinoflagellates. In Nealson, K., editor, *Bioluminescence: Current perspectives*. Burgess Publ. Co, Minneapolis MN. 26

Dunlap, J. C., Loros, J. J., Crosthwaite, S. K., Liu, Y., Garceau, N., Bell-Pedersen, D., Shinohara, M., Luo, C., Collett, M., Cole, A. B., and Heintzen, C. (1998). The circadian regulatory system in Neurospora. In Chaddick, M., Baumberg, S., Hodgson, D., and Phillips-Jones, M., editors, *Microbial Responses to Light and Time*, pages 279–295. University Press Cambridge. 53, 56

Durgan, D., Hotze, M., Tomlin, T., Egbejimi, O., Graveleau, C., Abel, E., Shaw, C., Bray, M., Hardin, P., and Young, M. (2005). The intrinsic circadian clock within the cardiomyocyte. *American Journal of Physiology- Heart and Circulatory Physiology*, 289:1530–1541. 87

Durgan, D. and Young, M. (2008). Linking the cardiomyocyte circadian clock to myocardial metabolism. *Cardiovascular Drugs and Therapy*, 22:115–124. 87

Duysens, L. and Amesz, J. (1957). Fluorescence spectrophotometry of reduced phosphopyridine nucleotide in intact cells in the near-ultraviolet and visible region. *Biochim. Biophys. Acta*, 24:19–26. 46

Eastman, C., Young, M., Fogg, L., Liu, L., and Meaden, P. (1998). Bright light treatment of winter depression: A placebo-controlled trial. *Archives of General Psychiatry*, 55:883–889. 107

Ehlers, C. L., Frank, E., and Kupfer, D. J. (1988). Social zeitgebers and biological rhythms. a unified approach to understanding the etiology of depression. *Arch Gen Psychiatry*, 45:948–952. 107

Ekström, P. (2003). Evolution of photosensory pineal organs in new light: the fate of neuroendocrine photoreceptors. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 358:1679–1700. 85

Engelmann, W. (2004a). How plants grow and move. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3776. 1

Engelmann, W. (2004b). How to stop a biological clock: Point of singularity. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3xxx. 1

Engelmann, W. (2004c). Rhythms in structures of organisms. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3794. 1

Engelmann, W. (2007). Rhythms of life - an introduction using selected topics and examples. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3798. 1

Engelmann, W. (2008). Flower clocks, time memory and time forgetting. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3801. 1

Engelmann, W. (2009a). Bio-calendar - the year in the life of plants and animals. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3762. 1

Engelmann, W. (2009b). Clocks which run according to the moon - influence of the moon on the earth and its life. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3767. 1

Engelmann, W. (2009c). Flying clocks - the clocks of Drosophila. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3796. 1

Engelmann, W. (2009d). Our internal clocks - biological timing in humans and other mammals. http://tobias-lib.ub.uni-tuebingen.de/volltexte/2009/3774. 1

Engelmann, W. and Schrempf, M. (1979). Membrane models for circadian rhythms. *Photochem. Photobiol. Reviews*, 5:49–86. 38, 39

Epperson, C. N., Terman, M., Terman, J. S., Hanusa, B. H., Oren, D. A., Peindl, K. S., and Wisner, K. L. (2004). Randomized clinical trial of bright light therapy for antepartum depression: preliminary findings. *J Clin Psychiatry*, 65:421–425. 107

Esch, P., Techel, D., Schimmoller, N., and Rensing, L. (1995). Heat shock effects on the circadian rhythm of protein synthesis and phosphorylation of ribosomal proteins in Gonyaulax polyedra. *Chronobiology International*, 12:369–381. 34

Esseveldt, L., Lehman, M., and Boer, G. (2000). The suprachiasmatic nucleus and the circadian time-keeping system revisited. *Brain Research Reviews*, 33:34–77. 68

Fahrenbach, J. P., Mejia-Alvarez, R., and Banach, K. (2007). The relevance of non-excitable cells for cardiac pacemaker function. *The Journal of Physiology*, 585:565–578. 87

Falcon, J. (1999). Cellular circadian clocks in the pineal. *Progress in Neurobiology*, 58:121–162. 85

Falcon, J., Gothilf, Y., Coon, S. L., Boeuf, G., and Klein, D. C. (2003). Genetic, temporal and developmental differences between melatonin rhythm generating systems in the teleost fish pineal organ and retina. *Journal of Neuroendocrinology*, 15:378–382. 85

Fan, Y., Hida, A., Anderson, D., Izumo, M., and Johnson, C. (2007). Cycling of cryptochrome proteins is not necessary for circadian-clock function in mammalian fibroblasts. *Current Biology*, 17:1091–1100. 67

Fausto, N., Campbell, J., and Riehle, K. (2006). Liver regeneration. *Hepatology*, 43(2 Suppl 1):S45–53. 108

Figala, J., Hoffmann, K., and Goldau, G. (1973). Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas. *Oecologia*, 12:89–118. 82

Fogel, M. and Hastings, J. (1971). A substrate binding protein in the Gonyaulax bioluminescence reaction. *Arch. Biochem. Biophys.*, 142:310–321. 25

Förster, C. and Engelmann, W. (1988). Thalassomyxa australis rhythmicity III. Entrainment by combination of different Zeitgeber. *J. Interdisc. Cycle Res.*, 19:275–288. 45, 46, 47

Foster, R., Argamaso, S., Coleman, S., Colwell, C., Lederman, A., and Provencio, I. (1993). Photoreceptors regulating circadian behavior: A mouse model. *Journal of Biological Rhythms Suppl.*, 8:S17–S23. 79

Francis, C. and Sargent, M. (1979). Effects of temperature perturbations on circadian conidiation in Neurospora. *Plant Physiology*, 64:1000–1004. 53

Frank, E., Hlastala, S., Ritenour, A., Houck, P., Tu, X. M., Monk, T. H., Mallinger, A. G., and Kupfer, D. J. (1997). Inducing lifestyle regularity in recovering bipolar disorder patients: results from the maintenance therapies in bipolar disorder protocol. *Biol Psychiatry*, 41:1165–1173. 107

Frank, E., Kupfer, D. J., Thase, M. E., Mallinger, A. G., Swartz, H. A., Fagiolini, A. M., Grochocinski, V., Houck, P., Scott, J., Thompson, W., and Monk, T. (2005). Two-year outcomes for interpersonal and social rhythm therapy in individuals with bipolar i disorder. *Arch Gen Psychiatry*, 62:996–1004. 108

Fritz, L., Morse, D., and Hastings, J. (1990). The circadian bioluminescence rhythm of Gonyaulax is related to the daily variations in the number of light-emitting organelles. *J Cell Sc.*, 95:321–328. 21

Fritz, L., Stringher, C., and Colepicolo, P. (1996). Imaging oscillations in Gonyaulax: A chloroplast rhythm of nitrate reductase visualized by immunocytochemistry. *Brazilian Journal of Medical and Biological Research*, 29:111–117. 32

Froy, O. (2007). The relationship between nutrition and circadian rhythms in mammals. *Front Neuroendocrinol*, 28:61–71. 65, 104

Froy, O., Chapnik, N., and Miskin, R. (2008). The suprachiasmatic nuclei are involved in determining circadian rhythms during restricted feeding. *Neuroscience*, 155:1152–1159. 65

Fu, L., Pelicano, H., Liu, J., Huang, P., and Lee, C. (2002). The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell*, 111:41–50. 99, 100

Gall, A. J., Todd, W. D., Ray, B., Coleman, C. M., and Blumberg, M. S. (2008). The development of day-night differences in sleep and wakefulness in norway rats

and the effect of bilateral enucleation. *J Biol Rhythms*, 23:232–241. 68

Gallon, J. (1981). The oxygen sensitivity of nitrogenase: a problem for biochemists and microorganisms. *TIBS*, 6:19–23. 16

Gallon, J. (1992). Reconciling the incompatible: N2 fixation and O2. *New Phytol.*, 122:571–609. 16

Ganguly, S., Weller, J. L., Ho, A., Chemineau, P., Malpaux, B., and Klein, D. C. (2005). Melatonin synthesis: 14-3-3-dependent activation and inhibition of arylalkylamine N-acetyltransferase mediated by phosphoserine-205. *Proc Natl Acad Sci U S A*, 102:1222–1227. 85

Gao, Q. and Horvath, T. L. (2008). Cross-talk between estrogen and leptin signaling in the hypothalamus. *Am J Physiol Endocrinol Metab*, 294:E817–E826. 92

Gardner, G. and Feldman, J. (1981). Temperature compensation od circadian period length in clock mutants of Neurospora crassa. *Plant Physiology*, 68:1244–1248. 53

Gauger, M. and Sancar, A. (2005). Cryptochrome, circadian cycle, cell cycle checkpoints, and cancer. *Cancer Research*, 65:6828–6834. 100

Germain, A. and Kupfer, D. J. (2008). Circadian rhythm disturbances in depression. *Hum Psychopharmacol*, 23:571–585. 107

Germain, N., Galusca, B., Grouselle, D., Frere, D., Tolle, V., Zizzari, P., Lang, F., Epelbaum, J., and Estour, B. (2008). Ghrelin/obestatin ratio in two populations with low bodyweight: Constitutional thinness and anorexia nervosa. *Psychoneuroendocrinology*, 34:413–419. 93

Gibor, A. (1966). Acetabularia: A useful giant cell. *Scientific American*, 215:118–124. 35

Goel, N., Terman, M., and Terman, J. S. (2003). Dimensions of temperament and bright light response in seasonal affective disorder. *Psychiatry Res*, 119:89–97. 107

Golden, S. S. (2007). Integrating the circadian oscillator into the life of the cyanobacterial cell. *Cold Spring Harb Symp Quant Biol*, 72:331–338. 7, 12, 15

Golden, S. S. and Canales, S. R. (2003). Cyanobacterial circadian clocks -timing is everything. *Nature Reviews*, 1:191–199. 12, 13

Golden, S. S., Tsinoremas, N. F., Liu, Y., Kutsuna, S., Lebedeva, N., Andersson, C., Aoki, S., Johnson, C. H., Ishiura, M., and Kondo, T. (1995). The quest for the cyanobacterial circadian clock. *Plant Physiology*, 108 (2 Suppl.):15. 15

Goodwin, B. (1965). Oscillatory behavior in enzymatic control processes. In Weber, G., editor, *Advances in enzyme regulation*. Pergamon Press, Oxford. 55

Grandin, L. D., Alloy, L. B., and Abramson, L. Y. (2006). The social zeitgeber theory, circadian rhythms, and mood disorders: review and evaluation. *Clin Psychol Rev*, 26:679–694. 107

Green, C. B. and Besharse, J. C. (2004). Retinal circadian clocks and control of retinal physiology. *J Biol Rhythms*, 19(2):91–102. 79

Green, C. B., Takahashi, J. S., and Bass, J. (2008). The meter of metabolism. *Cell*, 134:728–742. 102

Grell, K. (1987). Der Formwechsel von Thalassomyxa australis (Promycetozoidae)". Movie. 45

Grell, K. G. (1985). Der Formwechsel des plasmodialen Rhizopoden Thalassomyxa australis n. G., n. Sp. *Protistologica*, 21:215–233. 45

Gross, I., Hardeland, R., and Wolf, R. (1994). Circadian rhythm of tyrosine aminotransferase activity in Gonyaulax polyedra. *Biological Rhythm Research*, 25:51–58. 34

Grossman, A. R., Croft, M., Gladyshev, V. N., Merchant, S. S., Posewitz, M. C., Prochnik, S., and Spalding, M. H. (2007). Novel metabolism in Chlamydomonas through the lens of genomics. *Curr Opin Plant Biol*, 10:190–198. 41

Grözinger, M., Beersma, D. G. M., Fell, J., and Röschke, J. (2002). Is the nonREM-REM sleep cycle reset by forced awakenings from REM sleep? *Physiol Behav*, 77:341–347. 107

Grundschober, C., Delaunay, F., Puhlhofer, A., Triqueneaux, G., Laudet, V., Bartfai, T., and Nef, P. (2001). Circadian regulation of diverse gene products revealed by mrna expression profiling of synchronized fibroblasts. *Journal of Biological Chemistry*, 276:46751–46758. 65

Guo, H., Brewer, J. M. K., Champhekar, A., Harris, R. B. S., and Bittman, E. L. (2005). Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proceedings of the National Academy of Sciences*, 102:3111–3116. 65

Guo, H., Brewer, J. M. K., Lehman, M. N., and Bittman, E. L. (2006). Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: effects of transplanting the pacemaker. *J Neurosci*, 26:6406–6412. 65

Hämmerling, J. (1963). Nucleo-cytoplasmatic interactions in Acetabularia and other cells. *Annual Rev. Plant Physiol.*, 14:65–92. 36

Hannibal, J. (2006). Roles of pacap-containing retinal ganglion cells in circadian timing. *Int Rev Cytol*, 251:1–39. 79

Hardeland, R. (1980). Effects of catecholamines on bioluminescence in Gonyaulax polyedra (Dinoflagellata). *Comp. Biochem. Physiol.*, 66C:53–58. 34

Harris, E. (1998). *Introduction to Chlamydomonas*. Springer. 42

Harris, R. B. (2000). Leptin–much more than a satiety signal. *Annu Rev Nutr*, 20:45–75. 102

Hartwig and Schweiger (1986). Cellular aspects of circadian rhythms. *J Cell Sciences*, 4 Suppl.:181–200. 38

Hashimoto, S., Nakamura, K., Honma, S., and Honma, K. I. (1998). Free-running of plasma-melatonin rhythm prior to full manifestation of a non-24 hour sleep-wake syndrome. *Psychiatr. Clin. Neurosc.*, 52:264–265. 100

Hastings, J. (1959). Unicellular clocks. *Ann. Rev. Microbiol.*, 13:297–312. 21

Hastings, J. (1960). Biochemical aspects of rhythms: Phase shifting by chemicals. *Cold Spring Harbor Sympos. Quant. Biology*, 25:131–140. 20

Hastings, J. (1994). Biological clocks. *Mitteilungen der Alexander von Humboldt-Stiftung*, 63:17–23. 19

Hastings, J., Astrachan, L., and Sweeney, B. (1961). A persistent daily rhythm in photosynthesis. *J.gen.Physiol.*, 45:69–76. 30, 31

Hastings, J. and Sweeney, B. (1957). On the mechanism of temperature independence in a biological clock. *Proc. Natl. Acad. Sci. U.S.A.*, 43:804–811. 21, 22

Hastings, J. W. (2006). http://www.mcb.harvard.edu/hastings/dino.html. 19

Hastings, M. and Maywood, E. (2000). Circadian clocks in the mammalian brain. *BioEssays*, 22:23–31. 65, 71

Hastings, M., O'Neill, J. S., and Maywood, E. S. (2007). Circadian clocks: Regulators of endocrine and metabolic rhythms. *Journal of Endocrinology*, 195:187–198. 66

Hasunuma, K., Funadera, K., Shinohara, Y., Furukawa, K., and Watanabe, M. (1987). Circadian oscillation and light-induced changes in the concentration of cyclic nucleotides in Neurospora. *Current Genetics*, 12:127–133. 53

Haus, E. (2007). Chronobiology in the endocrine system. *Adv Drug Deliv Rev*, 59:985–1014. 66

Heintzen, C., Loros, J., and Dunlap, J. (2000). VIVID, light adaptation and the circadian clock: The PAS protein VVD defines a feedback loop that represses light input pathways and regulates clock resetting. *Cell*, 3:453–464. 54

Hellebust, J., Terborgh, J., and Mcleod, G. (1967). The photosynthesis rhythm of Acetabularia c. II. Measurements of photoassimilation of carbon dioxid and the activities of enzymes of the reductive pentose cycle. *Biol. Bull.*, 130:670–678. 35

Herman, E. and Sweeney, B. (1975). Circadian rhythm of chloroplast ultrastructure in Gonyaulax polyedra, concentric organization around a central cluster of ribosomes. *J. Ultrastr. Research*, 50:347–354. 28, 29

Hersoug, L.-G. and Linneberg, A. (2007). The link between the epidemics of obesity and allergic diseases: Does obesity induce decreased immune tolerance? *Allergy*, 62:1205–1213. 102

Herzog, E. D., Geusz, M. E., Khalsa, S. B., Straume, M., and Block, G. D. (1997). Circadian rhythms in mouse suprachiasmatic nucleus explants on multimicroelectrode plates. *Brain Res*, 757:285–290. 67

Hess, B. and Boiteux, A. (1971). Oscillatory phenomena in biochemistry. *Ann.Rev. Biochem.*, 40:237–258. 48

Heyde, F., Wilkens, A., and Rensing, L. (1992). The effects of temperature on the circadian rhythms of flashing and glow in Gonyaulax polyedra: Are the two rhythms controlled by two oscillators? *J. Biological Rhythms*, 7:115–123. 26, 27

Hileman, S. M., Pierroz, D. D., and Flier, J. S. (2000). Leptin, nutrition, and reproduction: Timing is everything. *J Clin Endocrinol Metab*, 85:804–807. 92

Hill, J. W., Elmquist, J. K., and Elias, C. F. (2008). Hypothalamic pathways linking energy balance and reproduction. *Am J Physiol Endocrinol Metab*, 294:E827–E832. 92

Hitomi, K., Okamoto, K., Daiyasu, H., Miyashita, H., Iwai, S., Toh, H., Ishiura, M., and Todo, T. (2000). Bacterial cryptochrome and photolyase: Characterization of two photolyase-like genes of Synechocystis sp. PCC6803. *Nucleic Acids Research*, 28:2353–2362. 15

Hochberg, M. and Sargent, M. (1974). Rhythms of enzyme activity associated with circadian conidiation in Neurospora crassa. *J. Bacteriol.*, 120:1164–1176. 53

Hoffmann, K. (1973). Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas. *Oecologia*, 12:89–118. 82

Hoffmann, K. (1981). The role of the pineal gland in the photoperiodic control of seasonal cycles in hamsters. In Follett, B. and Follett, D., editors, *Biological clocks in seasonal reproductive cycles*, pages 237–250. Wright, Bristol. 82

Holtman, C., Chen, Y., Sandoval, P., Gonzales, A., Nalty, M., Thomas, T., Youderian, P., and Golden, S. (2005). High-throughput functional analysis of the Synechococcus elongatus PCC 7942 genome. *DNA Research*, 12:103–115. 15, 16

Holzberg, D. and Albrecht, U. (2003). The circadian clock: a manager of biochemical processes within the organism. *J Neuroendocrinol*, 15:339–343. 63

Homma, K., Haas, E., and Hastings, J. (1990). Phase of the circadian clock is accurately transferred from mother to daughter cells in the dinoflagellate Gonyaulax polyedra. *Cell Biophys.*, 16:85–97. 31

Homma, K. and Hastings, J. (1988). Cell cycle synchronization of Gonyaulax polyedra by filtration: Quantized generation time. *JBR*, 3:49–58. 31

Homma, K. and Hastings, J. (1989). Cell growth kinetics, division asymmetry and volume control at division in the marine dinoflagellata Gonyaulax polyedra: A model of circadian clock control of the cell cycle. *J Cell Sc.*, 92:303–318. 31

Huang, T.-C. and Pen, S.-Y. (1994). Induction of a circadian rhythm in Synechococcus RF-1 while the cells are in a 'suspended state'. *Planta*, 194:436–438. 16

Ibata, Y., Okamura, H., Tanaka, M., Tamada, Y., Hayashi, S., Iijima, N., Matsuda, T., Munekawa, K., Takamatsu, T., Hisa, Y., Shigeyoshi, Y., and Amaya, F. (1999). Functional morphology of the suprachiasmatic nucleus. *Frontiers in Neuroendocrinology*, 20:241–268. 69

Iitaka, C., Miyazaki, K., Akaike, T., and Ishida, N. (2005). A role for glycogen synthase kinase-3 {beta} in the mammalian circadian clock. *Journal of Biological Chemistry*, 280:29397—29402. 107

Ikonomov, O. C., Stoynev, A. G., and Shisheva, A. C. (1998). Integrative coordination of circadian mammalian diversity: Neuronal networks and peripheral clocks. *Prog Neurobiol*, 54:87–97. 88

Illnerova, H. and Vanecek, J. (1982). Two-oscillator structure of the pacemaker controlling the circadian rhythm of N-acetyltransferase in the rat pineal gland. *Journal of Comparative Physiology*, A145:539–548. 68

Inagaki, N., Honma, S., Ono, D., Tanahashi, Y., and Honma, K. I. (2007). Separate oscillating cell groups in mouse suprachiasmatic nucleus couple photoperiodically to the onset and end of daily activity. *Proc Natl Acad Sci U S A*, 104:7664–7669. 67, 69

Inouye, C., Okamoto, K., Ishiura, M., and Kondo, T. (1998). The action spectrum of phase shift by light signal in the circadian rhythm in cyanobacterium. *Plant Cell Physiol*, 39:82. 14

Ishiura, M., Kutsuna, S., Aoki, S., Iwasaki, H., Andersson, C., Tanabe, A., Golden, S., Johnson, C., and Kondo, T. (1998). Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria. *Science*, 281:1519–1523. 12, 15

Iuvone, P., Tosini, G., Pozdeyev, N., Haque, R., Klein, D., and Chaurasia, S. (2005). Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. *Progress in Retinal and Eye Research*, 24:433–456. 85

Ivleva, N. B., Bramlett, M. R., Lindahl, P. A., and Golden, S. S. (2005). LdpA: A component of the circadian clock senses redox state of the cell. *The EMBO Journal*, 24:1202–1210. 12, 14

Iwasaki, H. and Dunlap, J. (2000). Microbial circadian oscillatory system in Neurospora crassa and Synechococcus: Models for cell clocks. *Current Opinion in Microbiol.*, 3:189–196. 57

Iwasaki, H. and Kondo, T. (2004). Circadian timing mechanism in the prokaryotic clock system of cyanobacteria. *Journal of Biological Rhythms*, 19:436–444. 12

Iwasaki, H., Williams, S., Kitayama, Y., Ishiura, M., Golden, S., and Kondo, T. (2000). A KaiC-interacting sensory histidine kinase, SasA, necessary to sustain robust circadian oscillation in Cyanobacteria. *Cell*, 101:223–233. 12

Jacklet, J. (1981). Circadian timing by endogenous oscillators in the nervous system: Toward cellular mechanisms. *Biol. Bull.*, 160:199–227. 25

Jacklet, J. W. (1969). Electrophysiological organization of the eye of Aplysia. *J General Physiology*, 53:21–42. 77

Jagota, A., de la Iglesia, H. O., and Schwartz, W. J. (2000). Morning and evening circadian oscillations in the suprachiasmatic nucleus in vitro. *Nature Neuroscience*, 3:372–376. 68

James, F. (2007). Expression of clock genes in human peripheral blood mononuclear cells throughout the sleep/wake and circadian cycles. *Chronobiology International*, 24(6):1009–1034. 88

Jean, Y., Bagayogo, I., and Dreyfus, C. (2008). *Release of Trophic Factors and Immune Molecules from Astrocytes*. Springer. 75

Johnson, C., Golden, S., and Kondo, T. (1998). Adaptive significance of circadian programs in cyanobacteria. *Trends in Microbiology*, 6:407–410. 16, 17

Johnson, C., Inoue, S., Flint, A., and Hastings, J. (1985). Compartmentalization of algal bioluminescence: Autofluorescence of bioluminescent particles in the dinoflagellate G. as studied with image-intensified video microscopy and flow cytometry. *J.Cell Biol.*, 100:1435–1446. 21

Johnson, C., Roeber, J., and Hastings, J. (1984). Circadian changes in enzyme concentration account for rhythm of enzyme activity in Gonyaulax. *Science*, 233:1428–1430. 25, 34

Joyce, P. R., Porter, R. J., Mulder, R. T., Luty, S. E., McKenzie, J. M., Miller, A. L., and Kennedy, M. A. (2005). Reversed diurnal variation in depression: Associations with a differential antidepressant response, tryptophan: Large neutral amino acid ratio and serotonin transporter polymorphisms. *Psychol Med*, 35:511–517. 107

Kallies, A., Gebauer, G., and Rensing, L. (1998). Heat shock effects on second messenger systems of Neurospora crassa. *Arch. Microbiol.*, 170:191–200. 60

Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Miyajima, N., Hirosawa, M., Sazuka, T., Sugiura, M., and Tabata, S. (1996). Genome sequencing project of a cyanobacterium Synechocystis sp. strain PCC6803. *Plant Cell Physiol*, 37:1–153. 3, 14

Kaplowitz, P. B. (2008). Link between body fat and the timing of puberty. *Pediatrics*, 121 Suppl 3:S208–S217. 93

Karakashian, M. W. and Schweiger, H. G. (1976). Circadian properties of the rhythmic system in individual nucleated and enucleated cells of Acetabularia mediterranea. *Experimental. Cell Research*, 97:366–377. 36

Kateriya, S., Nagel, G., Bamberg, E., and Hegemann, P. (2004). 'vision' in single-celled algae. *News Physiol Sci*, 19:133–137. 41

Kiess, W., Petzold, S., Töpfer, M., Garten, A., Blüher, S., Kapellen, T., Körner, A., and Kratzsch, J. (2008). Adipocytes and adipose tissue. *Best Pract Res Clin Endocrinol Metab*, 22:135–153. 102

Kiessig, R., Herz, J., and Sweeney, B. (1979). Shifting the phase of the circadian rhythm in bioluminescence in Gonyaulax with vanillic acid. *Plant Physiol.*, 63:324–327. 34

Kim, Y., Dong, G., Carruthers Jr, C., Golden, S., and Liwang, A. (2008). The day/night switch in KaiC, a central oscillator component of the circadian clock of Cyanobacteria. *Proc Natl Acad Sci US A*, 105:12825–30. 12

Kippert, F. and Lloyd, D. (1995). Rhythmic CO_{2} formation in fermentizing and in respiring Saccharomyces pombe cultures. *Microbiol.*, 141:S888. 5, 7, 49, 50

Kitayama, Y., Nishiwaki, T., Terauchi, K., and Kondo, T. (2008). Dual KaiC-based oscillations constitute the circadian system of Cyanobacteria. *Genes & Development*, 22:1513. 12

Klein, D. (2004). The 2004 Aschoff/Pittendrigh lecture: theory of the origin of the pineal gland–a tale of conflict and resolution. *Journal of Biological Rhythms*, 19:264. 85

Klein, D. (2006). Evolution of the vertebrate pineal gland: the AANAT hypothesis. *Chronobiology International*, 23:5–20. 85, 86

Klein, D. C. (1985). Photoneural regulation of the mammalian pineal gland. *Ciba Found Symp*, 117:38–56. 85

Klein, D. C., Baler, R., Roseboom, P. H., Weller, J. L., Bernard, M., Gastel, J. A., Zatz, M., Iuvone, P. M., Begáy, V., and Falcón, J. (1998). The molecular basis

of the pineal melatonin rhythm: Regulation of serotonin N-acetylation. In *Handbook of behavioral state control: cellular and molecular mechanisms*. Boca Raton: CRC Press. 85

Klein, D. C. and Krieger, D. T., editors (1979). *Circadian rhythms in the pineal gland*. Raven Press, New York. 85

Klerman, E. B. (2005). Clinical aspects of human circadian rhythms. *J Biol Rhythms*, 20:375–386. 99, 101

Klisch, C., Mahr, S., and Meissl, H. (2006). Circadian activity rhythms and phase-shifting of cultured neurons of the rat suprachiasmatic nucleus. *Chronobiol Int*, 23:181–190. 67

Knoetzel, J. and Rensing, L. (1990). Characterization of the photosynthetic apparatus from the marine dinoflagellate Gonyaulax polyedra. I Pigment and polypeptide composition of the pigment protein complexes. *J.Pl.Ph.*, 136:271–279. 30

Koenigsberg, H. W. (1984). Indications for hospitalization in the treatment of borderline patients. *Psychiatr Q*, 56:247–258. 107

Kohsaka, A. and Bass, J. (2007). A sense of time: how molecular clocks organize metabolism. *Trends Endocrinol Metab*, 18:4–11. 2, 67

Kondo, T. and Ishiura, M. (1994). Circadian rhythms of Cyanobacteria: Monitoring the biological clocks of individual colonies by bioluminescence. *Journal of Bacteriology*, 176:1881–1885. 7, 9

Kondo, T., Mori, T., Lebedeva, N., Aoki, S., Ishiura, M., and Golden, S. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science*, 275:224–227. 12

Kondo, T., Strayer, C., Kulkarni, R., Taylor, W., Ishiura, M., Golden, S., and Johnson, C. (1993). Circadian rhythms in prokaryotes: Luciferase as a reporter of circadian gene expression in cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 90:5672–5676. 7, 8, 9, 10

Kondo, T., Tsinoremas, N., Golden, S., Johnson, C., Kutsuna, S., and Ishiura, M. (1994). Circadian clock mutants of cyanobacteria. *Science*, 266:1233–1236. 11

Koop, H.-U., Schmid, R., Heunert, H.-H., and Milthaler, B. (1978). Chloroplast migration: A new rhythm in Acetabularia. *Protoplasma*, 97:301–310. 36

Kornmann, B. (2007). The central circadian clock and peripheral clocks: Decentralization and hierarchical control. *Med Sci (Paris)*, 23:349–350. 108

Kornmann, B., Schaad, O., Bujard, H., Takahashi, J., and Schibler, U. (2007). System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. *PLoS Biol*, 5:e34. 108

Kriegsfeld, L. and Silver, R. (2006). The regulation of neuroendocrine function: Timing is everything. *Hormones and Behavior*, 49:557–574. 66

Kripke, D. F., Risch, S. C., and Janowsky, D. (1983). Bright white light alleviates depression. *Psychiatry Res*, 10:105–12. 107

Kumar, A., Munehiko, A., and Makoto, S. (1999). Light-dependent and rhythmic psbA transcripts in homologous/heterologous cyanobacterial cells. *Biochemical & Biophysical Research Communications*, 255:47–53. 15

Kumar, V., Juss, T. S., and Follett, B. K. (1993). Melatonin secretion in quail provides a seasonal calendar but not one used for photoperiodic time measurement. In Touitou, Y., Arendt, J., and Pévet, P., editors, *Melatonin and the Pineal Gland-From Basic Science to Clinical Application*, pages 163–168. Elsevier, Amsterdam, The Netherlands. 84

Kurumiya, S. and Kawamura, H. (1988). Circadian oscillation of the multiple unit activity in the guinea pig suprachiasmatic nucleus. *J Comp Physiol [A]*, 162:301–308. 65

Kurumiya, S. and Kawamura, H. (1991). Damped oscillation of the lateral hypothalamic multineuronal activity synchronized to daily feeding schedules in rats with suprachiasmatic nucleus lesions. *J Biol Rhythms*, 6:115–127. 65

Lakin-Thomas, P. (2000). Circadian rhythms: new functions for old clock genes. *Trends in Genetics*, 16:135–142. 57

Lakin-Thomas, P. L. (1998). Choline depletion, frq mutations, and temperature compensation of the circadian rhythm in Neurospora crassa. *Journal of Biological Rhythms*, 13:268–277. 59

Lakin-Thomas, P. L., Gooch, V. D., and Ramsdale, M. (2001). Rhythms of differentiation and diacylglycerol in Neurospora. *Philos Trans R Soc Lond B Biol Sci*, 356:1711–1715. 52

Lam, R. W., Carter, D., Misri, S., Kuan, A. J., Yatham, L. N., and Zis, A. P. (1999). A controlled study of light therapy in women with late luteal phase dysphoric disorder. *Psychiatry Res*, 86:185–192. 107

Lambert, G., Reid, C., Kaye, D., Jennings, G., and Esler, M. (2003). Increased suicide rate in the middle-aged and its association with hours of sunlight. *Am J Psychiatry*, 160:793–795. 107

Land, M. and Fernald, R. (1992). The evolution of eyes. *Annual Review of Neuroscience*, 15:1–29. 79

Lapointe, M. and Morse, D. (2008). Reassessing the role of a 3'-UTR-binding translational inhibitor in regulation of circadian bioluminescence rhythm in the dinoflagellate Gonyaulax. *Biological Chemistry*, 389:13–19. 21

Laposky, A. D., Bass, J., Kohsaka, A., and Turek, F. W. (2008). Sleep and circadian rhythms: Key components in the regulation of energy metabolism. *FEBS Lett*, 582:142–151. 99, 105

Larkin, E. K. (2006). 108 candidate genes for sleep disordered breathing. *Sleep Medicine*, 7:3–3. 107

Larkin, E. K., Elston, R. C., Patel, S. R., Tishler, P. V., Palmer, L. J., Jenny, N. S., and Redline, S. (2005). Linkage of serum leptin levels in families with sleep apnea. *International Journal of Obesity*, 29:260–267. 107

Lee, J. H., Chan, J. L., Yiannakouris, N., Kontogianni, M., Estrada, E., Seip, R., Orlova, C., and Mantzoros, C. S. (2003). Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by

fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *Journal of Clinical Endocrinology & Metabolism*, 88:4848–4856. 93

Lee, K., Loros, J., and Dunlap, J. (2000). Interconnected feedback loops in the Neurospora circadian system. *Science*, 289:107–110. 55

Lehman, M., Silver, R., Gladstone, W., Kahn, R., Gibson, M., and Bittman, E. (1987). Circadian rhythmicity restored by neural transplant. Immunocytochemical characterization of the graft and its integration with the host brain. *Journal of Neurosciences*, 7:1626–1638. 66

Lemmer, B. (1999). Chronopharmacology and its impact on antihypertensive treatment. *Acta Physiol Pharmacol Bulg*, 24(3):71–80. 106

Lengeler, J. W. and Drews, G. (1998). *Biology of the Procaryotes*. Georg Thieme. 3

Levitan, R. D. (2007). The chronobiology and neurobiology of winter seasonal affective disorder. *Dialogues Clin Neurosci*, 9:315–324. 107

Lewy, A., Ahmed, S., Jackson, J., and Sack, R. (1992). Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International*, 9:380–392. 84

Lewy, A., Bauer, V., Cutler, N., Sack, R., Ahmed, S., Thomas, K., Blood, M., and Latham-Jackson, J. (1998). Morning vs evening light treatment of patients with winter depression. *Archives of General Psychiatry*, 55:890–896. 107

Lewy, A. J. (2007). Melatonin and human chronobiology. *Cold Spring Harb Symp Quant Biol*, 72:623–636. 107

Lin, R., Chou, H., and Huang, T. (1999). Priority of light-dark entrainment over temperature in setting the circadian rhythms of the prokaryot Synechococcus RF-1. *Planta*, 209:202–206. 14

Linden, H., Ballario, P., Arpaia, G., and Macino, G. (1999). Seeing the light: news in neurospora blue light signal transduction. *Adv Genet*, 41:35–54. 57

Lindgren, K. M. (1994). *Characterization of ccg-1, a clock-controlled gene in Neurospora crassa*. PhD thesis, Dartmouth College, USA. 53

Liu, Y., Garceau, N. Y., Loros, J. J., and Dunlap, J. C. (1997). Thermally regulated translational control of FRQ mediates aspects of temperature responses in the neurospora circadian clock. *Cell*, 89:477–486. 53, 57

Liu, Y., Merrow, M., Loros, J. J., and Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science*, 281:825–829. 53, 55

Lloyd, D. and Gilbert, D. (1998). Temporal organization of the cell division cycle in eukaryotic microbes. In Caddick, M., Baumberg, S., Hodgson, D., and Phillips-Jones, M., editors, *Microbial responses to light and time*, volume 56 of *Soc. Gen. Microbiol. Symp.*, pages 251–278. Cambridge University Press. 32

Lloyd, D. and Kippert, F. (1987). Temperature-compensated ultradian clocks time protozoan cell cycles. *Chronobiologia*, 14:197–198. 48

Lloyd, D. and Rossi, E. (1992). *Ultradian rhythms in life processes*. Springer London, Berlin, Heidelberg, New York, Paris, Tokyo, Hong Kong, Barcelona, Budapest. 48

Lloyd, D. and Volkov, E. (1990). Quantized cell cycle times: Interaction between a relaxation oscillator and ultradian clock pulses. *BioSystems*, 23:305–310. 32

Locket, N. A. (1999). *Vertebrate photoreceptors*. Kluwer Academic Pub. 79

Loros, J. (1995). The molecular basis of the Neurospora clock. *Semin. Neurosci.*, 7:3–13. 55

Loros, J. and Dunlap, J. (2001). Genetic and molecular analysis of circadian rhythms in Neurospora. *Ann. Rev. Physiol.*, 63:757–794. 60, 61

Loros, J. and Feldman, J. (1986). Loss of temperature compensation of circadian period length in the frq-9 mutant of Neurospora crassa. *Journal of Biological Rhythms*, 1:187–198. 57

Loros, J., Richman, A., and Feldman, J. (1986). A recessive circadian clock mutant at the frq locus in Neurospora crassa. *Genetics*, 114:1095–1110. 57

Loucks, A. B. (2007). Energy availability and infertility. *Curr Opin Endocrinol Diabetes Obes*, 14:470–474. 102

Loving, R. T., Kripke, D. F., and Shuchter, S. R. (2002). Bright light augments antidepressant effects of medication and wake therapy. *Depress Anxiety*, 16:1–3. 108

Lowrey, P. L. and Takahashi, J. S. (2004). Mammalian circadian biology: Elucidating genome-wide levels of temporal organization. *Annual Review of Genomics and Human Genetics*, 5:407–441. 99, 100

Lu, J., Zhang, Y. H., Chou, T. C., Gaus, S. E., Elmquist, J. K., Shiromani, P., and Saper, C. B. (2001). Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. *J Neurosci*, 21:4864–4874. 72

Mackey, S. R. and Golden, S. S. (2007). Winding up the cyanobacterial circadian clock. *Trends Microbiol*, 15(9):381–388. 3

Marcovic, P., Roenneberg, T., and Morse, D. (1996). Phased protein synthesis at several circadian times does not change protein levels in Gonyaulax. *J. Biol. Rhythms*, 11:57–67. 34

Maronde, E., Pfeffer, M., Glass, Y., and Stehle, J. (2007). Transcription factor dynamics in pineal gland and liver of the Syrian hamster (mesocricetus auratus) adapts to prevailing photoperiod. *Journal of Pineal Research*, 43:16–24. 108

Matsuo, T., Okamoto, K., Onai, K., Niwa, Y., Shimogawara, K., and Ishiura, M. (2008). A systematic forward genetic analysis identified components of the Chlamydomonas circadian system. *Genes & Development*, 22:918–930. 41

Matsuo, T., Onai, K., Okamoto, K., Minagawa, J., and Ishiura, M. (2006). Real-time monitoring of chloroplast gene expression by a luciferase reporter: Evidence for nuclear regulation of chloroplast circadian period. *Molecular and Cellular Biology*, 26:863–870. 41

Matsuo, T., Onai, K., Onai, K., Okamoto, K., Okamoto, K., Okamoto, K., Minagawa, J. U. N., Ishiura, M., Ishiura, M.,

and Ishiura, M. (2005). Nuclear regulation of circadian gene expression in the chloroplast. *Genes & Genet Syst*, 80:487. 41

Matsuo, T., Yamaguchi, S., Mitsui, S.; Emi, A., Shimoda, F., and Okamura, H. (2003). Control mechanism of the circadian clock for timing of cell division in vivo. *Science*, 302:255–259. 95

Maywood, E. S., O'Neill, J. S., Chesham, J. E., and Hastings, M. H. (2007a). Minireview: The circadian clockwork of the suprachiasmatic nuclei analysis of a cellular oscillator that drives endocrine rhythms. *Endocrinology*, 148:5624. 65

Maywood, E. S., O'Neill, J. S., Reddy, A. B., Chesham, J. E., Prosser, H. M., Kyriacou, C. P., Godinho, S. I. H., Nolan, P. M., and Hastings, M. H. (2007b). Genetic and molecular analysis of the central and peripheral circadian clockwork of mice. *Cold Spring Harbor Symposia on Quantitative Biology*, 72:85–94. 65

Meijer, J. and Schwartz, W. (2003). In search of the pathways for light-induced pacemaker resetting in the suprachiasmatic nucleus. *Journal of Biological Rhythms*, 18:235–249. 68

Menaker, M., Moreira, L., and Tosini, G. (1997). Evolution of circadian organization in vertebrates. *Brazilian Journal of Medical and Biological Research*, 30:305–313. 79

Mendoza, J. (2007). Circadian clocks: setting time by food. *Journal of Neuroendocrinology*, 19:127–137. 108

Mendoza, J., Clesse, D., Pévet, P., and Challet, E. (2008a). High-fat feeding alters the clock synchronization to light. *J Physiol*, 586:5901–5910. 105

Mendoza, J., Drevet, K., Pévet, P., and Challet, E. (2008b). Daily meal timing is not necessary for resetting the main circadian clock by calorie restriction. *J Neuroendocrinol*, 20:251–260. 105

Mergenhagen, D. and Schweiger, H. (1974). Circadian rhythmicity: Does intercellular synchronization occur in Acetabularia? *Plant Science letters*, 3:387–389. 40

Mergenhagen, D. and Schweiger, H. (1975). The effect of different inhibitors of transcription and translation of the expression and control of circadian rhythm in individual cells of Acetabularia. *Exp. Cell Res.*, 92:127–. 36

Merrow, M., Boesl, C., Ricken, J., Messerschmitt, M., Goedel, M., and Roenneberg, T. (2006). Entrainment of the Neurospora circadian clock. *Chronobiol Int*, 23:71–80. 41

Michel, S., Geusz, M., Zaritsky, J., and Block, G. (1993). Circadian rhythm in membrane conductance expressed in isolated neurons. *Science*, 259:239–241. 77

Mihalcescu, I., Hsing, W., and Leibler, S. (2004). Resilient circadian oscillator revealed in individual Cyanobacteria. *Nature*, 430:81–85. 7

Min, H., Liu, Y., Johnson, C. H., and Golden, S. S. (2004). Phase determination of circadian gene expression in Synechococcus elongatus PCC 7942. *Journal of Biological Rhythms*, 19:103–112. 14

Mircea, C. N., Lujan, M. E., and Pierson, R. A. (2007). Metabolic fuel and clinical implications for female reproduction. *J Obstet Gynaecol Can*, 29:887–902. 93

Mitsui, A., Kumazawa, S., Takahashi, A., Ikemoto, H., Cao, S., and Arai, T. (1986). Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nat.*, 323:720–722. 3, 5

Mittag, M. (1998). Molecular mechanisms of clock-controlled proteins in phytoflagellates. *Protist*, 149:101–107. 24

Mittag, M., Kiaulehn, S., and Johnson, C. (2005). The circadian clock in Chlamydomonas reinhardtii. What is it for? What is it similar to? *Plant Physiology*, 137:399–409. 41

Mittag, M. and Wagner, V. (2003). The circadian clock of the unicellular eukaryotic model organism Chlamydomonas reinhardtii. *Biological chemistry*, 384:689–695. 41

Moore-Ede, M., Sulzman, F., and Fuller, C. (1982). *The clocks that time us. Physiology of the circadian timing system*. Harvard University Press, Cambridge, London. 82, 85

Mori, T., Binder, B., and Johnson, C. (1996). Circadian gating of cell division in cyanobacteria growing with average doubling times of less than 24 hours. *Proceedings of the National Academy of Sciences of the United States of America*, 93:10183–10188. 6, 14

Mori, T., Saveliev, S., Xu, Y., Stafford, W., Cox, M., Inman, R., and Johnson, C. (2002). Circadian clock protein KaiC forms ATP-dependent hexameric rings and binds DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 99:17203–17208. 12

Mörike, K. and Mergenthaler, W. (1959). *Biologie des Menschen*. Quelle und Meyer, Heidelberg. 80, 81

Morin, L. P. and Allen, C. N. (2006). The circadian visual system, 2005. *Brain Res Rev*, 51:1–60. 79

Morse, D., Fritz, L., and Hastings, J. (1990). What is the clock? Translational regulation of circadian bioluminescence. *TIBS*, 15:262–265. 21, 23, 27

Morse, D., Hastings, J., and Roenneberg, T. (1994). Different phase responses of two circadian oscillators in Gonyaulax. *J. Biol. Rhythms*, 9:263–274. 26, 28, 29

Morse, D., Markovic, P., and Roenneberg, T. (1996). Several clocks may simplify the circadian system of Gonyaulax. *Brazilian Journal of Medical and Biological Research*, 29:101–103. 28

Morse, D., Milos, P., Roux, E., and Hastings, J. (1989). Circadian regulation of the synthesis of substrate binding protein in the Gonyaulax bioluminescent system involves translational control. *PNAS*, 86:172–176. 24

Murray, J. D. (1993). *Mathematical Biology, vol. 18*. Springer Biomathematics Texts. 55

Nagel, G., Szellas, T., Kateriya, S., Adeishvili, N., Hegemann, P., and Bamberg, E. (2005). Channelrhodopsins: Directly light-gated cation channels. *Biochem Soc Trans*, 33:863–866. 41

Nagoshi, E., Saini, C., Bauer, C., Laroche, T., Naef, F., and Schibler, U. (2004). Circadian gene expression in individual fibroblasts cell-autonomous and self-sustained oscillators pass time to daughter cells. *Cell*, 119:693–705. 75, 87, 99, 100

Nakahira, Y., Katayama, M., Miyashita, H., Kutsuna, S., Iwasaki, H., Oyama, T.,

and Kondo, T. (2004). Global gene repression by kaic as a master process of prokaryotic circadian system. *Proceedings of the National Academy of Sciences*, 101:881–885. 14

Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., and Oyama, T. ans Kondo, T. (2005). Reconstitution of circadian oscillation of cyanobacterial kaiC phosphorylation in vitro. *Science*, 308:414–415. 12

Nakamura, H., Kishi, Y., Shimomura, O., Morse, D., and Hastings, J. (1989). Structure of dinoflagellate luciferin and its enzymatic and non-enzymatic air-oxidation products. *J. Am. Chem. Soc.*, 111:7607–7611. 24

Nayak, S. K., Jegla, T., and Panda, S. (2007). Role of a novel photopigment, melanopsin, in behavioral adaptation to light. *Cell Mol Life Sci*, 64:144–154. 82

Neel, J. V. (1962). Diabetes mellitus: a thrift genotype rendered detrimental by progress? *Am J Hum Genet*, 14:353–362. 105

Newman, G. and Hospod, F. (1986). Rhythm of suprachiasmatic nucleus 2-deoxyglucose uptake in vitro. *Brain Res.*, 381:345–350. 68

Nikaido, S. S. and Johnson, C. H. (2000). Daily and circadian variation in survival from ultraviolet radiation in Chlamydomonas reinhardtii. *Photochemistry and Photobiology*, 71:758–765. 41

Nishiwaki, T., Iwasaki, H., Ishiura, M., and Kondo, T. (2000). Nucleotide binding and autophosphorylation of the clock protein KaiC as a circadian timing process of cyanobacteria. *Proc Natl Acad Sci U S A*, 97:495–499. 12

Njus, D., Gooch, D., and Hastings, J. (1981). Precision of the Gonyaulax circadian clock. *Cell Biophys.*, 3:223–231. 21

Nomura, K., Castanon-Cervantes, O., Davidson, A., and Fukuhara, C. (2008). Selective serotonin reuptake inhibitors and raft inhibitors shorten the period of period1-driven circadian bioluminescence rhythms in rat-1 fibroblasts. *Life Sciences*, 82:1169–1174. 86

Ogden, E. J. D. and Moskowitz, H. (2004). Effects of alcohol and other drugs on driver performance. *Traffic Inj Prev*, 5:185–198. 105

Oishi, K., Amagai, N., Shirai, H., Kadota, K., Ohkura, N., and Ishida, N. (2005). Genome-wide expression analysis reveals 100 adrenal gland-dependent circadian genes in the mouse liver. *DNA Research*, 12:191–202. 108

Oldenhof, H., Zachleder, V., and Ende, H. (2006). Blue-and red-light regulation of the cell cycle in Chlamydomonas reinhardtii (Chlorophyta). *European Journal of Phycology*, 41:313–320. 41

Ortega-Calvo, J. J. and Stal, L. J. (1991). Diazotrophic growth of the unicellular cyanobacterium Gloeothece sp. PCC 6909 in continuous culture. *Journal of general microbiology*, 137:1789–1797. 16

Oster, H., Maronde, E., and Albrecht, U. (2002). The circadian clock as a molecular calendar. *Chronobiology International*, 19:507–516. 77

Otway, D., Frost, G., and Johnston, J. (2008). Identification of a circadian clock within adipocyte cells. *Endocrine Abstracts*, 15:P126. 94

Page, T. L. (2001). *Circadian systems of invertebrates*, volume 1. Kluwer Academic/Plenum Publishers. 77

Peters, J. L. (2005). *Astrocytes and the circadian clock: Roles for calcium, light, and melatonin*. PhD thesis, Texas University. 76

Peters, J. L., Earnest, B. J., Tjalkens, R. B., Cassone, V. M., and Zoran, M. J. (2005). Modulation of intercellular calcium signaling by melatonin in avian and mammalian astrocytes is brain region-specific. *J Comp Neurol*, 493:370–380. 77

Pflug, B. (1976). The effect of sleep deprivation on depressed patients. *Acta Psychiatr Scand*, 53:148–158. 108

Piper, A., J. and Grunstein, R. R. (2007). Current perspectives on the obesity hypoventilation syndrome. *Curr Opin Pulm Med*, 13:490–496. 102

Pittendrigh, C. S. and Daan, S. A. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. V. Pacemaker structure: A clock for all seasons. *Journal of Comparative Physiology*, 106:333–355. 68

Plante, G. E. (2006). Sleep and vascular disorders. *Metabolism*, 55(10 Suppl 2):S45–S49. 106

Prolo, L. M., Takahashi, J. S., and Herzog, E. D. (2005). Circadian rhythm generation and entrainment in astrocytes. *Journal of Neuroscience*, 25:404–408. 76

Provencio, I., Cooper, H., and Foster, R. (1998). Retinal projection in mice with inherited retinal degeneration: Implication for circadian photoentrainment. *J. Neurosc.*, 395:417–439. 79

Ralph, M., Joysner, A., and Lehman, M. (1993). Culture and transplantation of the mammalian circadian pacemaker. *Journal of Biological Rhythms*, 8:S83–S87. 66

Ramalho, C., Hastings, J., and Colepicolo, P. (1995). Circadian oscillation of nitrate reductase activity in Gonyaulax polyedra is due to changes in cellular protein levels. *Plant Physiology*, 107:225–231. 32, 33

Reddy, A., Karp, N., Maywood, E., Sage, E., Deery, M., O'Neill, J., Wong, G., Chesham, J., Odell, M., and Lilley, K. (2006). Circadian orchestration of the hepatic proteome. *Current Biology*, 16:1107–1115. 94, 95

Reddy, A. and Maywood, E. (2007). Circadian rhythms: Per2bations in the liver clock. *Current Biology*, 17:292–294. 108

Reddy, A., Wong, G., O'Neill, J., Maywood, E., and Hastings, M. (2005). Circadian clocks: neural and peripheral pacemakers that impact upon the cell division cycle. *Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis*, 574:76–91. 95

Remè, C. E., Wirz-Justice, A., and Terman, M. (1991). The visual input stage of the mammalian circadian pacemaking system: I. Is there a clock in the mammalian eye? *J Biol Rhythms*, 6:5–29. 79

Rensing, L. (1993). *Oscillations and morphogenesis*. Marcel Dekker New York, Basel, Hong Kong. 52

Reuss, S. (2003). The clock in the brain: Anatomy of the mammalian circadian timing system. In Peschke, E., editor, *Endokrinologie*, pages 9–48. Abhandlungen der Saechsischen Akademie

der Wissenschaften zu Leipzig, Mathematisch-naturwissenschaftliche Klasse. 68

Roberts, M. and Moore, R. (1987). Localization of neuropeptides in efferent terminals of the eye in the marine snail, Bulla gouldiana. *Cell. Tissue. Res.*, 248:67–73. 78

Roberts, R. E., Shema, S. J., Kaplan, G. A., and Strawbridge, W. J. (2000). Sleep complaints and depression in an aging cohort: A prospective perspective. *Am J Psychiatry*, 157:81–88. 107

Roenneberg, T. and Carpenter, E. (1993). Daily rhythm of O2-evolution in the cyanobacterium Trichodesmium thiebautii under natural and constant conditions. *Marine Biol.*, 117:693–697. 16

Roenneberg, T., Colfax, G., and Hastings, J. (1989). A circadian rhythm of population behavior in Gonyaulax polyedra. *JBR*, 4:201–216. 28

Roenneberg, T. and Merrow, M. (2001). Seasonality and photoperiodism in fungi. *Journal of Biological Rhythms*, 16:403–414. 59

Roenneberg, T. and Morse, D. (1993). Two circadian oscillators in one cell. *Nature*, 362:362–364. 26, 28

Roenneberg, T., Nakamura, H., Cranmer, L., Ryan, K., Kishi, Y., and Hastings, J. (1991). Synthesis of (+)-Gonyauline, an endogenous substance shortening the period of the circadian rhythm in the unicellular alga Gonyaulax polyedra. *Experientia*, 47:103–106. 34

Roenneberg, T., Nakamura, H., and Hastings, J. (1988). Creatine accelerates the circadian clock in a unicellular alga. *Nat.*, 334:432–434. 28, 34

Roenneberg, T. and Rehman, J. (1998). Survival in a temporal world - the circadian program of the marine unicell Gonyaulax. In Chaddick, M. X., Baumberg, S., Hodgson, D. A., and Phillips-Jones, M. K., editors, *Microbial Responses to Light and Time*, pages 237–250. University Press Cambridge. 21, 57

Roenneberg, T. and Taylor, W. (1994). Light-induced phase responses in Gonyaulax are drastically altered by creatine. *Journal of Biological Rhythms*, 9:1–12. 34

Rosenthal, N. E., Joseph-Vanderpool, J. R., Levendosky, A. A., Johnston, S. H., Allen, R., Kelly, K. A., Souetre, E., Schultz, P. M., and Starz, K. E. (1990). Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep*, 13:354–361. 107

Rougemont, J. and Naef, F. (2007). Stochastic phase oscillators and circadian bioluminescence recordings. *Cold Spring Harb Symp Quant Biol*, 72:405–411. 75

Ruan, G. X., Zhang, D. Q., Zhou, T., Yamazaki, S., and McMahon, D. G. (2006). Circadian organization of the mammalian retina. *Proc Natl Acad Sci U S A*, 20:9703–9708. 79

Ruby, N. F. (2003). Hibernation: when good clocks go cold. *J Biol Rhythms*, 18:275–286. 66

Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *J Neurosci*, 22:357–364. 65

Ruoff, P., Vinsjevik, M., Monnerjahn, C., and Rensing, L. (1999). The Goodwin oscillator: On the importance of degradation reactions in the circadian clock. *JBR*, 14:469–479. 55

Rupprecht, R., Baghai, T., and Möller, H. (2004). Neuentwicklungen in der Pharmakotherapie der Depression. *Der Nervenarzt*, 75:273–280. 108

Sakurai, T. (2006). Roles of orexins and orexin receptors in central regulation of feeding behavior and energy homeostasis. *Current Drug Targets-CNS and Neurological Disorders*, 5:313. 102

Samuelsson, G., Sweeney, B., Matlick, H., and Prezelin, B. (1983). Changes in photosystem II account for the circadian rhythm in photosynthesis in Gonyaulax polyedra. *Pl.Phys.*, 73:329–331. 29, 30

San, L. and Arranz, B. (2008). Agomelatine: a novel mechanism of antidepressant action involving the melatonergic and the serotonergic system. *Eur Psychiatry*, 23:396–402. 108

Saper, C. B., Cano, G., and Scammell, T. E. (2005). Homeostatic, circadian, and emotional regulation of sleep. *J Comp Neurol*, 493:92–98. 107

Scemes, E. and Giaume, C. (2006). Astrocyte calcium waves: What they are and what they do. *Glia*, 54:716. 76

Schibler, U., Ripperger, J., and Brown, S. (2003). Peripheral circadian oscillators in mammals: time and food. *J Biol Rhythms*, 18:250–260. 63

Schmid, R. and Koop, H. (1983). Properties of the chloroplast movement during the circadian chloroplast migration in Acetabularia mediterranea. *Zeitschrift für Pflanzenphysiologie*, 112:351–357. 38

Schmidt, M., Gessner, G., Luff, M., Heiland, I., Wagner, V., Kaminski, M., Geimer, S., Eitzinger, N., Reissenweber, T., Voytsekh, O., et al. (2006). Proteomic analysis of the eyespot of Chlamydomonas reinhardtii provides novel insights into its components and tactic movements. *The Plant Cell*, 18:1908. 42

Schmitz, O., Katayama, M., Williams, S., Kondo, T., Golden, S., and xx, x. (2000). CikA, a bacteriophytochrome that resets the cyanobacterial circadian clock. *Science*, 289:765–768. 15

Scholübbers, H., Taylor, W., and Rensing, L. (1984). Are membrane properties essential for the circadian rhythm of Gonyaulax? *Am.J.Physiol.*, 247:R250–256. 34

Schou, M. (2000). [50 years lithium treatment]. *Encephale*, 26:1–6. 108

Schröder-Lorenz, A. and Rensing, L. (1987). Circadian changes in protein-synthesis rate and protein phosphorylation in cell-free extracts of Gonyaulax polyedra. *Planta*, 170:7–13. 25

Schulz, R., Pilatus, U., and Rensing, L. (1986). On the role of energy metabolism in Neurospora circadian clock function. *Chronobiol. Intern.*, 2:223–233. 60

Schussnig, B. (1954). *Grundriss der Protophytologie*. VEB Gustav Fischer, Jena. 19

Schwartz, W. and Gainer, H. (1977). Suprachiasmatic nucleus: Use of 14C-labeled deoxyglucose uptake as a functional marker. *Science*, 197:1089–1091. 68

Schweiger (1984). Auf der Suche nach dem molekularen Mechanismus der circadianen Uhr. *Mannheimer Forum, Boehringer, Mannheim*, 84/85:115–172. 37, 39

Schweiger, E., Wallraff, H., and Schweiger, H. (1964). Endogenous circadian rhythm in cytoplasm of Acetabularia: Influence of the nucleus. *Science*, 146:658–659. 36, 37, 38

Schweiger, H. (1977). Circadian rhythms in unicellular organisms: An endeavor to explain the molecular mechanism. *Int. Rev. Cytol.*, 51:315–342. 25, 36

Schweiger, H., Broda, H., Wolff, D., and Schweiger, G. (1983). A method for the simultaneous long-term recording of oxygen evolution and chloroplast migration in an individual cell of Acetabularia. In Gnaiger and Forstner, editors, *Polarographic oxygen sensors*, chapter II.7, pages 190–194. Springer Berlin, Heidelberg. 39

Schweiger, H. and Schweiger, M. (1977). Circadian rhythms in unicellular organisms: an endeavor to explain the molecular mechanism. *Int Rev Cytol*, 51:315–342. 36, 38

Schweiger, H. G., Hartwig, R., and Schweiger, M. (1986). Cellular aspects of circadian rhythms. *Journal of Cell Science*, 20:181–200. 39

Scott, E. M. and Grant, P. J. (2006). Neel revisited: The adipocyte, seasonality and type 2 diabetes. *Diabetologia*, 49:1462–1466. 104, 105

Sernagor, E. (2005). Retinal development: second sight comes first. *Curr Biol*, 15:R556–R559. 82

Serretti, A., Benedetti, F., Mandelli, L., Lorenzi, C., Pirovano, A., Colombo, C., and Smeraldi, E. (2003). Genetic dissection of psychopathological symptoms: insomnia in mood disorders and clock gene polymorphism. *Am J Med Genet B Neuropsychiatr Genet*, 121B:35–38. 107

Serretti, A., Cusin, C., Benedetti, F., Mandelli, L., Pirovano, A., Zanardi, R., Colombo, C., and Smeraldi, E. (2005). Insomnia improvement during antidepressant treatment and clock gene polymorphism. *Am J Med Genet B Neuropsychiatr Genet*, 137B:36–39. 107

Sgarbossa, A., Checcucci, G., and Lenci, F. (2002). Photoreception and photomovements of microorganisms. *Photochemical & Photobiological Sciences*, 1:459–467. 41

Sharpley, A. L. and. Cowen, P. J. (1995). Effect of pharmacologic treatments on the sleep of depressed patients. *Biol Psychiatry*, 37:85–98. 107

Shibata, S. (2004). Neural regulation of the hepatic circadian rhythm. *Anat Rec A Discov Mol Cell Evol Biol*, 280:901–909. 95

Shibata, S. and Moore, R. Y. (1993). Neuropeptide y and optic chiasm stimulation affect suprachiasmatic nucleus circadian function in vitro. *Brain Res*, 615:95–100. 69

Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takekida, S., Yan, L., Tei, H., Moriya, T., Shibata, S., Loros, J., Dunlap, J., and Okamura, H. (1997). Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. *Cell*, 91:1043–1053. 70

Silver, R., Lehman, M., Gibson, M., Gladstone, W., and Bittman, E. (1990). Dispersed cell suspensions of fetal SCN

restore circadian rhythmicity in SCN-lesioned adult hamsters. *Brain Research*, 525:45–58. 66

Silyn-Roberts, H. and Engelmann, W. (1986). Thalassomyxa australis: A model organism for the evolution of circadian rhythms? *Endocyt. C.Res.*, 3:239–242. 45

Silyn-Roberts, H., Engelmann, W., and Grell, K. (1986). Thalassomyxa australis rhythmicity I. Temperature dependence. *Interdisc. Cycle Res.*, 17:181–187. 45, 48

Simonneaux, V. and Ribelayga, C. (2003). Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacological Reviews*, 55:325–395. 72

Smietanko, A., Stader, L., Förster, C., and Engelmann, W. (1988). Thalassomyxa australis rhythmicity II. No entrainment by light-dark-cycles and temperature cycles? *J. Interdisc. Cycle Res.*, 19:235–240. 45, 46

Smith, R. and Williams, S. (2006). Circadian rhythms in gene transcription imparted by chromosome compaction in the cyanobacterium Synechococcus elongatus. *Proceedings of the National Academy of Sciences*, 103(22):8564–8569. 14

Sollars, P., Kimble, D., and Pickard, G. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *J. Neurosciences*, 15:2109–2122. 66

Spanagel, R., Pendyala, G., Abarca, C., Zghoul, T., Sanchis-Segura, C., Magnone, M., Lascorz, J., Depner, M., Holzberg, D., Soyka, M., Schreiber, S., Matsuda, F., Lathrop, M., Schumann, G., and Albrecht, U. (2005a). The clock gene per2 influences the glutamatergic system and modulates alcohol consumption. *Nat Med*, 11:35–42. 99, 105

Spanagel, R., Rosenwasser, A. M., Schumann, G., and Sarkar, D. K. (2005b). Alcohol consumption and the body's biological clock. *Alcoholism: Clinical and Experimental Research*, 29:1550. 105

Springer, M. (1993). Genetic control of fungul differenciation: The three sporulation pathways of Neurospora crassa. *BioEssays*, 15:365–374. 51

Stal, L. and Krumbein, M. (1985a). Nitrogenase activity in the non-heterocystous cyanobacterium Oscillatoria sp. grown under alternating light-dark cycles. *Arch. Microbiol.*, 143:67–71. 3

Stal, L. and Krumbein, M. (1985b). Oxygen protection of nitrogenase in the aerobically nitrogen fixing non-heterocystous cyanobacterium Oscillatoria sp. *Arch. Microbiol.*, 143:72–76. 4

Steinlechner, R. and Heldmaier, G. (1989). Photoperiodic and thermal regulation in vertebrate body temperature rhythms and thermogenetic acclimation. *JBR*, 4:251–265. 82

Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., and Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science*, 291:490–493. 65

Storch, K., Paz, C., Signorovitch, J., Raviola, E., Pawlyk, B., Li, T., and Weitz, C. (2007). Intrinsic circadian clock of the mammalian retina: Importance for retinal processing of visual information. *Cell*, 130:730–741. 63

Sulzman, F., Gooch, D., Homma, K., and Hastings, J. (1982). Cellular autonomy of the Gonyaulax circadian clock. *Cell Biophysics*, 4:97–103. 21

Suzuki, L. and Johnson, C. (2002). Photoperiodic control of germination in the unicell Chlamydomonas. *Naturwissenschaften*, 89:214–220. 42, 43

Sweeney, B. (1960). The photosynthetic rhythm in single cells of Gonyaulax polyedra. *Cold Spring Harbor Sympos. Quant. Biology*, 25:140–148. 30, 31

Sweeney, B. (1974). The potassium content of Gonyaulax polyedra and phase changes in the circadian rhythm of stimulated bioluminescence by short exposures to ethanol and valinomycin. *Plant Physiol.*, 53:337–342. 34

Sweeney, B. (1981). The circadian rhythms in bioluminescence, photosynthesis and organellar movements in the large dinoflagellate, Pyrocystis fusiformis. In Schweiger, H., editor, *International Cell Biology 1980-1981*, pages 807–814. Springer, Berlin, Heidelberg. 30, 34

Sweeney, B. (1984). Circadian rhythmicity in dinoflagellates. In Spector, editor, *Dinoflagellates*, pages 343–364. Ac. Press NY. 21, 31

Sweeney, B. and Borgese, A. (1989). A circadian rhythm in cell division in a prokaryote, the cyanobacterium Synechococcus WH7803. *J.Physiol*, 25:183–186. 9

Sweeney, B. and Hastings, J. (1958). Rhythmic cell division in populations of Gonyaulax polyedra. *J.Protozoology*, 5:217–224. 31

Sweeney, B. and Hastings, J. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harbor Symp.*, 25:87–104. 21

Sweeney, B. and Haxo, F. (1961). Persistence of a photosynthetic rhythm in enucleated acetabularia. *Science*, 134:1361–1363. 36

Sweeney, B., Prezelin, B., Wong, D., and Govindjee (1979). In vivo chlorophyll a fluorescence transient and the circadian rhythm of photosynthesis in Gonyaulax polyedra. *Photochem. Photobiol.*, 30:309–311. 30

Sweeney, B., Tuffli, C., and Rubin, R. (1967). The circadian rhythm in photosynthesis in Acetabularia in the presence of actinomycin D, Puromycin, and chloramphenicol. *J. gen. Physiol.*, 50:647–659. 39

Sweeney, B. M. and Herz, J. M. (1977). Evidence that membranes play an important role in circadian rhythms. In *Proc. XII Int. Conf. Int. Soc. Chronobiol.*, pages 751–761. 34

Taylor, W., Gooch, V., and Hastings, J. (1979). Period shortening and phase shifting effects of ethanol in the Gonyaulax. glow rhythm. *J. Comp. Physiol.*, 130:355–358. 34

Taylor, W. and Hastings, J. (1979). Aldehydes phase shift the Gonyaulax polyedra clock. *J. comp. Physiol.*, 130:359–362. 34

Taylor, W., Krasnow, R., Dunlap, J. C., Broda, H., and Hastings, J. W. (1982). Critical pulses of anisomycin drive the circadian oscillator in Gonyaulax towards its singularity. *JCP*, 148:11–25. 25

Teikari, P. (2006). *Biological effects of light*. PhD thesis, Helsinki University of Technology. 67, 73

Tena-Sempere, M. (2008a). Ghrelin and reproduction: Ghrelin as novel regulator of the gonadotropic axis. *Vitam Horm*, 77:285–300. 93

Tena-Sempere, M. (2008b). Ghrelin as a pleotrophic modulator of gonadal function and reproduction. *Nat Clin Pract Endocrinol Metab*, 4:666–674. 93

Terazono, H., Mutoh, T., Yamaguchi, S., Kobayashi, M., Akiyama, M., Udo, M., Ohdo, S., Okamura, H., and Shibata, S. (2003). Adrenergic regulation of clock gene expression in mouse liver. *Proc Natl Acad Sci U S A*, 100:6795–6800. 74

Terborgh, J. and McLeod, G. (1967). The photosynthetic rhythm of Acetabularia crenulata. I. Continuous measurements of oxygen exchange in alternating light-dark regimes and in constant light of different intensities. *Biol. Bull.*, 133:659–669. 35, 36

Terman, M. and Terman, J. S. (2005). Light therapy for seasonal and nonseasonal depression: Efficacy, protocol, safety, and side effects. *CNS Spectr*, 10:647–63. 107

Thanbichler, M., Viollier, P. H., and Shapiro, L. (2005). The structure and function of the bacterial chromosome. *Curr Opin Genet Dev*, 15:153–162. 14

Theodosis, D., Poulain, D., and Oliet, S. (2008). Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. *Physiological Reviews*, 88:983. 77

Thorey, I., Rode, I., Harnau, G., and Hardeland, R. (1987). Conditionality of phase resetting by inhibitors of 80S translation in Gonyaulax polyedra. *JCP*, 157B:85–89. 25

Tosini, G., Davidson, A., Fukuhara, C., Kasamatsu, M., and Castanon-Cervantes, O. (2007). Localization of a circadian clock in mammalian photoreceptors. *The FASEB Journal*, 21:3866. 79

Tosini, G. and Fukuhara, C. (2003). Photic and circadian regulation of retinal melatonin in mammals. *J Neuroendocrinol*, 15:364–369. 79

Tosini, G., Pozdeyev, X., Sakamoto, K., and Iuvone, P. M. (2008). The circadian clock system in the mammalian retina. *Bioessays*, 30:624–633. 79

Tousson, E. and Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *J Neurosci*, 24:2983–2988. 72

Tsinoremas, N., Ishiura, M., Kondo, T., Andersson, C., Tanaka, K., Takahashi, H., Johnson, C., and Golden, S. (1996). A sigma factor that modifies the circadian expression of a subset of genes in cyanobacteria. *EMBO Journal*, 15:2488–2495. 16

Tsuno, N., Besset, A., and Ritchie, K. (2005). Sleep and depression. *J Clin Psychiatry*, 66:1254–69. 108

Turek, F. W. and Allada, R. (2002). Liver has rhythm. *Hepatology*, 35:743–745. 95

Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D. R., Eckel, R. H., Takahashi,

J. S., and Bass, J. (2005). Obesity and metabolic syndrome in circadian clock mutant mice. *Science*, 308:1043–1045. 99, 104

Tyson, J. J. (1987). Size control of cell division. *Journal of theoretical biology*, 126:381–391. 32

van Houwelingen, C. A. and Beersma, D. G. (2001). Seasonal changes in 24-h patterns of suicide rates: A study on train suicides in The Netherlands. *J Affect Disord*, 66:215–223. 107

van Reeth, O., Olivares, E., Zhang, Y., Zee, P. C., Mocaer, E., Defrance, R., and Turek, F. W. (1997). Comparative effects of a melatonin agonist on the circadian system in mice and syrian hamsters. *Brain Research*, 762(1-2):185–194. 79, 84

vanden Driessche, T. (1966). Circadian rhythms in Acetabularia: Photosynthetic capacity and chloroplast shape. *Exp. Cell Research*, 42:18–30. 36

vanden Driessche, T. (1967). The nuclear control of the chloroplasts' circadian rhythms. *Sci. Progr., Oxford*, 55:293–303. 36

vanden Driessche, T. (1970). Inability of rifampicin to inhibit circadian rhythmicity in Acetabularia in spite of RNA synthesis. *Biochim. Biophys. Acta*, 224:631–634. 36, 39

vanden Driessche, T. and Bonotto, S. (1969). The circadian rhythm in RNA synthesis in Acetabularia mediterranea. *Biochimica and Biophysica Acta*, 179:58–66. 36

vanden Driessche, T., Dujardin, E., Magnusson, A., and Sironval, C. (1976). Acetabularia mediterranea: Circadian rhythms of photosynthesis and associated changes in molecular structure of the thylakoid membranes. *Int.J.Chronobiol.*, 4:111–124. 36

Vicker, M., Becker, J., Gebauer, G., Schill, W., and Rensing, L. (1988). Circadian rhythms of cell cycle processes in the marine dinoflagellate Gonyaulax polyedra. *Chronobiol. Internat.*, 5:5–17. 31, 32

Vollrath, L. (2002). Chrono-endocrinology–quo vadis? *Ann Anat*, 184:583–593. 66

Wagner, V., Gessner, G., Heiland, I., Kaminski, M., Hawat, S., Scheffler, K., and Mittag, M. (2006). Analysis of the phosphoproteome of Chlamydomonas reinhardtii provides new insights into various cellular pathways. *Eukaryot Cell*, 5:457–468. 42

Wagner, V., Gessner, G., and Mittag, M. (2005). Functional proteomics: A promising approach to find novel components of the circadian system. *Chronobiol Int*, 22:403–415. 42

Wainright, P., Hinkle, G., Sogin, M., and Stickel, S. (1993). Monophyletic origins of the metazoa: An evolutionary link with fungi. *Science*, 260:340–342. 51

Warren, W. S. and Cassone, V. M. (1995). The pineal gland: Photoreception and coupling of behavioral, metabolic, and cardiovascular circadian outputs. *J Biol Rhythms*, 10:64–79. 84

Wehr, T. and Goodwin, F. K. (1975). Biorhythms and manic-depressive illness. In *128th annual meeting of the American Psychiatric Association, Anaheim, May*. 107

Welsh, D., Logothetis, D., Meister, M., and Reppert, S. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron*, 14:697–706. 67

Welsh, D. K., Yoo, S. H., Liu, A. C., Takahashi, J. S., and Kay, S. A. (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Current Biology*, 14:2289–2295. 74, 86

White, W. B. (2007). Importance of blood pressure control over a 24-hour period. *J Manag Care Pharm*, 13(8 Suppl B):34–39. 99

Wijnen, H. and Young, M. (2006). Interplay of circadian clocks and metabolic rhythms. *Annual Review of Genetics*, 40:409. 108

Winokur, A., Gary, K., Rodner, S., Rae-Red, C., Fernando, A., and Szuba, M. (2001). Depression, sleep physiology, and antidepressant drugs. *Depression and Anxiety*, 14:19–28. 108

Wirz-Justice, A. and van den Hoofdakker, R. H. (1999). Sleep deprivation in depression: What do we know, where do we go? *Biol Psychiatry*, 46:445–453. 108

Wolk, R., Gami, A. S., Garcia-Touchard, A., and Somers, V. K. (2005). Sleep and cardiovascular disease. *Curr Probl Cardiol*, 30:625–662. 108

Wollnik, F. (1995). Die innere Uhr der Säugetiere. *Biologie in unserer Zeit*, 25:37–43. 66, 83

Woolum, J. (1991). A re-examination of the role of the nucleus in generating the circadian rhythm in Acetabularia. *Journal of Biological Rhythms*, 6:129–136. 38

Xu, Y., Mori, T., and Johnson, C. H. (2003). Cyanobacterial circadian clockwork: Roles of KaiA, KaiB and the kaiBC promoter in regulating KaiC. *EMBO J*, 22:2117–2126. 12

Yagita, K. (2004). Signal transduction pathways resetting the mammalian circadian clock in cultured fibroblast cell lines (entrainment of biological clocks, Symposium, Proceeding of the annual Meeting of the Zoological Society of Japan). *Zoological science*, 21:1235–1236. 87

Yagita, K., Tamanini, F., van der Horst, G., and Okamura, H. (2001). Molecular mechanisms of the biological clock in cultured fibroblasts. *Science*, 292:278–281. 87

Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M., and Okamura, H. (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science*, 302:1408–1412. 68

Yamanaka, I., Koinuma, S., Shigeyoshi, Y., Uchiyama, Y., and Yagita, K. (2007). Presence of robust circadian clock oscillation under constitutive over-expression of mcry1 in rat-1 fibroblasts. *FEBS Letters*, 581:4098–4102. 67

Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D., and Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus in vivo. *J Neurosci*, 18:10709–10723. 72, 86

Yang, Y. H., Cheng, P., Zhi, G., and Liu, Y. (2001). Identification of a calcium/calmodulin-dependent protein kinase that phosphorylates the Neurospora circadian clock protein FREQUENCY. *J. Biol. Chemistry*, 276:41064–41072. 59

Yildiz, B. O., Suchard, M. A., Wong, M.-L., McCann, S. M., and Licinio, J. (2004). Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci U S A*, 101:10434–10439. 102, 104

Yoshikawa, T. and Oishi, T. (1998). Extraretinal photoreception and circadian systems in nonmammalian vertebrates. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, 119:65–72. 86

Young, M. E. (2006). The circadian clock within the heart: potential influence on myocardial gene expression, metabolism, and function. *Am J Physiol Heart Circ Physiol*, 290:H1–16. 87

Zghoul, T., Abarca, C., Sanchis-Segura, C., Albrecht, U., Schumann, G., and Spanagel, R. (2007). Ethanol self-administration and reinstatement of ethanol-seeking behavior in Per1(Brdm1) mutant mice. *Psychopharmacology (Berl)*, 190:13–19. 105

Zhu, Y., Brown, H. N., Zhang, Y., Stevens, R. G., and Zheng, T. (2005). Period3 structural variation: a circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol Biomarkers Prev*, 14:268–270. 100

Ziegler, O., Quilliot, D., and Guerci, B. (2000). Physiopathology of obesity. Dietary factors, and regulation of the energy balance. *Ann Endocrinol (Paris)*, 61 Suppl 6:12–23. 104