

Circadian clock in Geneva

Fascinating Rhythm

How circadian expression patterns of certain genes are regulated is crucial for understanding biological clocks. Ueli Schibler *et al.* from Geneva University recently revealed the heat-shock factor HSF1 as a key factor in liver cells.

Rhythms are a key parameter of nature, they return again and again. That's why life has adapted to them. Winter and summer, high tide and low tide, day and night are examples.

Almost every organism organises its activities in a regular way. In mammals many biological processes vary according to a 24 hour period. For example sleep-wake cycles, locomotor activity, blood pressure, body temperature and the secretion of hormones are controlled by circadian clocks. One of these clocks is located in the Suprachiasmatic Nucleus (SCN) of the brain but it is not the only one. Many tissues show SCN-independent circadian behaviour, even fibroblasts on culture plates.

The regular activity of cells is achieved by the expression of genes whose products interact in positive and negative feedback loops. In mice at least four main players are known. Briefly, the transcription factors BMAL1 and CLOCK activate the expression of the proteins PER and CRY that aggregate to multi-subunit complexes. Once these complexes have reached a critical



Group leader Ueli Schibler (right) and Hans Reinke, first author of the latest paper

threshold concentration, they in turn inhibit the activity of BMAL1 and CLOCK and thus prevent their own expression. This again leads to an increase in the activity of BMAL1 and CLOCK and to a new 24 hour cycle of interaction.

The SCN pacemaker plays a special role within the variety of cellular clocks, because its activity is synchronized to the day and night cycle by light. As a "master clock" the SCN imposes its rhythm onto the peripheral clocks in other tissues. The question is which mechanisms synchronize the cells of the liver, kidneys or heart?

The mystery of the early bird

Ueli Schibler from the Department of Molecular Biology at the University of Geneva initially discovered this question by accident. "To begin with I didn't even study circadian mechanisms at all," he says. During his doctoral studies in Bern, his Postdoc in Philadelphia and his time as junior and senior group leader at the Swiss Institute



for Experimental Cancer Research (ISREC) in Lausanne he investigated RNA processing and mechanisms of transcriptional regulation.

In 1990, however, he was suddenly confronted with a mystery. One of his students found the protein DBP, a member of the PAR basic leucine zipper (bzip) family. This protein has a high expression rate in the liver cells of rats. However, when another student wanted to reproduce this finding he failed again and again. Slowly it emerged that the second student had grown

up on a farm and was used to getting up very early in the morning. As a consequence he performed his experiments in the dark morning hours while the original discoverer of the protein worked during daylight hours. The DBP finally turned out to have a circadian expression with a very high peak in the late hours of the day and a depression in the morning. Thus, the farmer's son was not able to detect it.

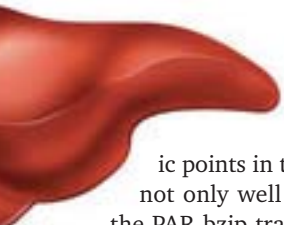
Caught in the act

In the following years, Schibler and his colleagues increasingly focussed on circadian clocks. However, in order to find regulating factors of circadian gene activity they had to solve a problem. Many circadian transcription factors are not expressed in a circadian fashion but constantly. It's their posttranscriptional activation which imposes the rhythm on them. Their fluctuating activity is only detectable while they are binding to the DNA. In order to catch them in the act Schibler and his group eventually invented a method which they called the "differential display of DNA-binding proteins (DDDP)" (*Genes & Dev.* 22, 2008: 331-345).

"In fact, this method is very simple and robust," Schibler explains. "I still wonder why nobody else had the idea." The main principle underlying DDDP is to create a pool of DNA fragments with random sequences and to let them interact with protein extracts from the nuclei of mouse liver cells. Transcription factors often bind to recognition sequences with a length of seven to eight nucleotides. They even tolerate imperfect sequences with one mistake. In a pool of 40,000 randomly arranged nucleotides each possible sequence of eight nucleotides statistically should occur more than once. Thus, when repeating this experiment at successive time points around the clock, circadian gene regulators of liver cells should bind to some of these sequences in a circadian fashion and thereby reveal themselves.

Indeed. When Schibler's colleague Hans Reinke performed this experiment, a cou-





ple of proteins bound to the random sequences only at specific points in time. Among them were not only well known candidates like the PAR bzip transcription factors but a new one also showed up. This protein had an expression peak at the onset of the dark phase, when mice start becoming behaviourally active. The random sequence it bound to contained an almost perfect heat-shock element (HSE). An antibody test finally revealed it as the heat-shock factor 1 (HSF1).

The rhythm of daily stress

HSF1 is a member of the heat-shock factor family of transcriptional activators. In response to stress conditions that lead to protein denaturation – typically high temperature or oxidative stress – HSF1 becomes hyperphosphorylated and enters the nucleus where it drives the expression of heat-shock genes. In order to gather further confirmation for circadian activity of HSF1 Schibler and his group performed an *in vivo* experiment with chromatin from mouse liver that was prepared at six time points around the clock. Using chromatin-immunoprecipitation they indeed showed that at the onset of the dark phase HSF1 binds to the promoters of the chaperone genes Hsp70 and Hsp105.

Schibler asks, “What role does the circadian activity of HSF1 play? Also, why do mice need to switch on the anti-stress programme when the dark phase starts?” Schibler’s hypothesis is that the main clue lies in the rising activity of mice during the night. He gives two reasons: behaviourally active mice show an increase in overall body temperature by about 3°C which might already mean cytotoxic stress. In addition, mice feed during the night, and therefore accumulate toxic side products of digestion, for example free radicals. To test whether temperature cycles and feeding have an impact on Hsf1-expression, Schibler and his colleagues performed two additional experiments.

The heat of the night

In cultured cells from mice liver they inserted a reporter gene construct which contained the Hsf1 gene and a firefly luciferase gene under the control of the same promoter. Subsequently, they confronted this system with temperature fluctuations between 35°C and 39°C, a range typical for body temperature fluctuations in mice between day and night. The test revealed that each increase in temperature was accompa-

nied by a peak of luciferase glowing – and thus Hsf1-expression.

In a restricted feeding experiment the researchers then shifted the feeding times of mice to the onset of day. As a consequence they found a second peak of Hsf1-expression at this new time point, in addition to the normal one. “These experiments demonstrate one possible way the SCN might synchronize the peripheral oscillators of the liver,” says Schibler. “It imposes cycles of rest and activity onto the organism and through this influences feeding time and body temperature. In turn, this recruits regulators of circadian gene expression like HSF1.”

A central loop

That’s not enough yet, however. Schibler additionally proposes a feedback mechanism by which the peripheral clocks affect the central pacemaker in the brain. Accordingly, HSF1 might play a role in this loop. In another experiment the researchers therefore put wild-type, heterozygous and Hsf1-deficient mice in a wheel and let them run in total darkness for some days. The circadian wheel-running periods of wild-type and heterozygous mice became shorter with time, which is typical for animals without any contact with environmental light input. However, this effect didn’t appear in animals without the Hsf1 gene. Their periods of activity were significantly longer. Apparently, the loss of Hsf1 affected the central control of circadian behaviour.

“For us it becomes more and more likely that feeding and detoxification are the dominant *Zeitgeber* for the peripheral circadian oscillation of the liver,” says Schibler. According to him, it is reasonable to keep chemically incompatible processes like glycogen assembly and disassembly separated in time. However, the circadian expression of proteins like HSF1 might as well help the organism to anticipate different times of the day and thus prepare for digestion and physiological stress. “That’s why some of these proteins might even affect the central clock and the circadian behaviour.”

In the future Schibler and his group would like to find more molecules that regulate the circadian metabolism which may even yield some benefit to medical science. A lot of detoxification enzymes in the liver are expressed in a circadian fashion. “That’s why some drugs kill mice in the morning but not in the evening”, says Schibler. “Hence, the knowledge about circadian processes in the liver might well be crucial for pharmaceuticals.” MATTHIAS NAWRAT