

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-



