and CFLAR-ASK1 might be more advantageous than targeting either one alone.

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# Liver Size: Waning by Day, Waxing by Night

The liver has to cope with various metabolic and physiological changes that normally recur every day and result primarily from rest-activity and feeding-fasting cycles. Periods of high nutrient availability allow storage of energy as glycogen, triglycerides, and proteins to sustain anabolism and cell growth, whereas during the resting phase low energy levels require the funnelling of these reservoirs into adenosine triphosphate–generating processes. Similarly, detoxification pathways are scheduled to be active when needed, usually upon ingestion of xenobiotic compounds present in the diet.

Several interdependent mechanisms work in concert to drive rhythmic liver physiology. Hepatocytes, like most cells in mammals, harbor an endogenous circadian clock, which regulates their daily physiology through rhythmic gene expression. In addition, the central circadian oscillator in the suprachiasmatic nuclei (SCN) of the hypothalamus is aligned to the geophysical time upon daily exposure to light. This master clock generates rhythmic neuronal and humoral output signals that play a central role in the control of liver physiology. In fact, signals emanating from feeding-fasting cycles appear to be the most dominant rhythmic cues for peripheral organs in general, and especially for the liver. Hence, diurnal control of physiology is driven by a conjuncture of signaling pathways that are cell autonomous or systemically driven by the SCN and serve to synchronize liver function with the time of day. Consequently, it is of paramount importance that light exposure and feeding-fasting cycles are correctly aligned. Misalignments, as in the case of binge eating at night or shift work in humans, are associated with various liver pathologies, such as metabolic diseases and malignancies.[(1)]

Circadian changes in liver mass, hepatocyte morphology and size, were reported already one quarter of a century ago.[(2)] This intriguing observation has since been scarcely studied, and the underlying mechanisms as well as the physiological relevance remained largely unknown. A recent publication from the lab of Ueli Schibler showed that daily changes in actin dynamics in liver of mice are accompanied by pronounced size oscillations of mouse hepatocytes.[(3)] In a follow-up publication from the same lab published recently in *Cell*, Sinturel et al.[(4)] demonstrated that these size changes are primarily regulated by feeding time.

Remarkably, daily rhythms in liver size require the feeding-fasting rhythm of animals to be properly aligned with the light-dark cycle. In their study, animals were subjected to three different feeding regimes. Mice were fed either *ad libitum* or exclusively during the night or the light phase. As expected from nocturnal animals, mice ingest most of their food during the dark phase (~80%) when food is provided *ad libitum*. Accordingly, the researchers observed rhythmic changes in hepatocyte size upon nighttime feeding and, to a lesser extent, in mice fed *ad libitum*, with nadir levels during the beginning of the dark phase and zenith levels early in the light phase (Fig. 1). If animals eat at the "wrong" time of day, during rest (i.e., in the light phase), the cell size oscillations vanish. This effect was independent of the absolute amount of food consumed and was liver specific because it was not observed in kidney or other tissues.

The researchers also attempted to identify molecular mechanisms underlying the oscillations in hepatocyte size. They were able to provide several pieces of evidence pointing toward rhythmic translation efficiency that is triggered by imbalanced synthesis of ribosomal components as the driver of rhythmic cell size. Initially, they observed circadian cycles of the hepatic RNA/DNA ratio, which indicated oscillating ribosomal RNA (rRNA) levels, given that rRNA constitutes the majority of the cellular RNA pool. Along this line, polysome profiling showed a global increase in translation efficiency and, consequently, protein accumulation at the same time as the cells reach their maximal size. Unexpectedly, it turned out that transcription of the 47S/45S precursor, rRNA, is not rhythmic, and that cycling rRNA levels are rather regulated posttranscriptionally. More specifically, nuclear polyadenylation of 18S- and 28S-rRNA is rhythmic, with high levels in the light phase upon night feeding and nonrhythmic upon daytime feeding. Notably, the deadenylase, Nocturnin, despite its pronounced oscillation, does not appear to be involved in the daily rRNA polyadenylation cycle. In experiments with cultured cells, the researchers identified PAPD5 as the enzyme that polyadenylates those rRNA molecules that are incompletely packaged into ribosomes, and EXOSC10 as the exonuclease activity required to degrade the polyadenylated rRNA. However, none of these enzymes showed rhythmic protein levels. Ribosome profiling experiments indicated that oscillating translation efficiency leads to rhythmic translation of ribosomal proteins and, as a consequence, superfluous rRNA is degraded at times of reduced ribosomal protein synthesis. In

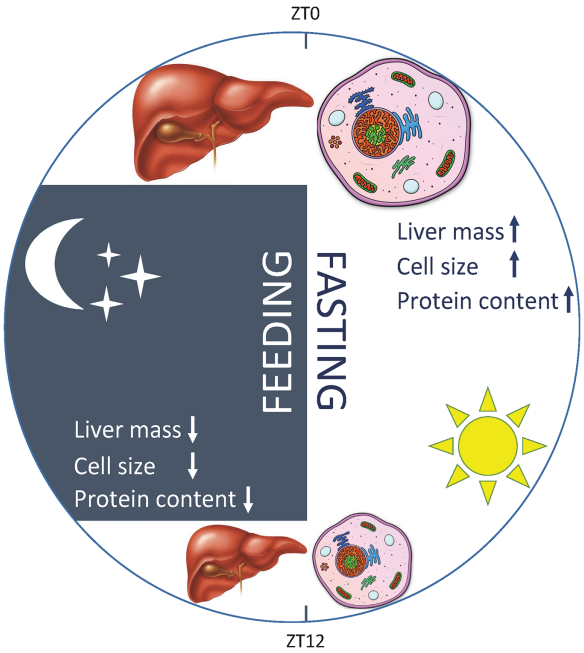


**FIG. 1.** A schematic depiction of the diurnal changes in liver mass, hepatocyte size, and protein content in response to daily feeding fasting cycles. Zeitgeber Time (ZT), ZT0-light is turned on, ZT12-light is turned off.

synopsis, rate-limiting production of complete ribosomes attributed to cyclic translation efficiency of ribosomal proteins causes rhythmic global protein levels to oscillate in accord with cell size changes. Furthermore, the study provides novel insight on the complex mechanism of ribosome assembly and suggests that precursor rRNA is constitutively synthesized in excess throughout the day, whereas ribosomal proteins are translated rhythmically from nearly constant numbers of ribosomal protein mRNAs. The excess of rRNAs not incorporated into ribosomes is polyadenylated and degraded.

The current study specified changes in protein synthesis as the primary determinant for cell size oscillations. The varying amounts of total cellular protein content, which constitutes around 25% of eukaryotic cell mass, provides a conceivable explanation for these changes. Furthermore, a large body of work shows that hepatic cell size changes induced by hormones or osmotic gradients can have many pleiotropic effects on organ function, among others an increase in cell volume which inhibits proteolysis and stimulates global protein translation in liver cells,[5] effectively resulting in the same positive correlation between cell size and protein mass as in the present study.[4] At this point,

however, we cannot exclude the possible involvement of other cellular mechanisms or molecular constituents in the process of hepatic cell size oscillations. It was recently reported that hepatic lipid composition exhibits feeding-dependent daily rhythms.[6] In addition, levels of osmotically active molecules, such as amino acids, vary in the liver depending on food intake.[5]

Like any important advance, this work raises exciting questions for future studies. What are the direct physiological consequences of the daily changes in liver size? Are these oscillations altered and/or do they participate in liver pathologies such as fatty liver and cirrhosis? Is it possible that, in addition to the timing, also the composition of the food plays a role? It has been shown previously that scheduling meals to the right time of the activity cycle can revert adverse metabolic changes caused by a high-fat diet.[7] Why are these changes exclusive to the liver and are not detected in other organs? Earlier reports indicated that constraints from extracellular tissue might limit cell swelling,[5] whereas the present study moves the focus toward cell-specific differences in global translation efficiency.[4] Are the changes in cell size accompanied by alterations in the size and/or morphology of intracellular organelles? In this context, it was recently shown that the lipid composition of the nucleus and mitochondria exhibit daily rhythms.[8] The current study and the emerging open questions carry wide implications on our basic understanding of liver function in health and disease.

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