ANNUAL REVIEWS

Annual Review of Pathology: Mechanisms of Disease

Spatiotemporal Metabolic Liver Zonation and Consequences on Pathophysiology

Tomaz Martini,[1] Felix Naef,[1] and Jan S. Tchorz[2]

[1]Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; email: felix.naef@epfl.ch

[2]Novartis Institutes for BioMedical Research, Novartis Pharma AG, Basel, Switzerland; email: jan.tchorz@novartis.com

Annu. Rev. Pathol. Mech. Dis. 2023. 18:439–66

The *Annual Review of Pathology: Mechanisms of Disease* is online at pathol.annualreviews.org

https://doi.org/10.1146/annurev-pathmechdis-031521-024831

Keywords

liver zonation, circadian rhythm, metabolism, WNT signaling, spatial lobular organization, hepatic detoxification, liver monogenic diseases, liver pathophysiology, liver pathology, chronopharmacology

Abstract

Hepatocytes are the main workers in the hepatic factory, managing metabolism of nutrients and xenobiotics, production and recycling of proteins, and glucose and lipid homeostasis. Division of labor between hepatocytes is critical to coordinate complex complementary or opposing multistep processes, similar to distributed tasks at an assembly line. This so-called metabolic zonation has both spatial and temporal components. Spatial distribution of metabolic function in hepatocytes of different lobular zones is necessary to perform complex sequential multistep metabolic processes and to assign metabolic tasks to the right environment. Moreover, temporal control of metabolic processes is critical to align required metabolic processes to the feeding and fasting cycles. Disruption of this complex spatiotemporal hepatic organization impairs key metabolic processes with both local and systemic consequences. Many metabolic diseases, such as nonalcoholic steatohepatitis and diabetes, are associated with impaired metabolic liver zonation. Recent technological advances shed new light on the spatiotemporal gene expression networks controlling liver function and how their deregulation may be involved in a large variety of diseases. We summarize the current knowledge about spatiotemporal metabolic liver zonation and consequences on liver pathobiology.

1. PHYSIOLOGICAL ORGANIZATION OF THE LIVER

1.1. Liver Function

The liver is the largest gland in the human body and is responsible for a diverse set of vital functions. Being the heaviest internal organ that is well perfused, the liver is a large blood reservoir, carrying almost 10% of the body's total blood volume. The liver is best known for its metabolism of nutrients and xenobiotics (1). It controls carbohydrate metabolism by turning glucose into glycogen (glycogenesis) and storing it. When needed, glycogen is converted back into glucose (glycogenolysis) and secreted into the blood. The liver can also synthesize glucose from amino acids (AAs), lactate or glycerol (gluconeogenesis), and glycogen from lactic acid (glyconeogenesis) (2, 3). It is also responsible for protein metabolism, synthesis, and degradation. Except for γ-globulins, all plasma proteins are synthesized in the liver. Examples of liver-derived vital proteins include coagulation factors supporting wound healing and albumin, an important carrier and regulator of the colloid osmotic pressure controlling blood volume. The liver also synthesizes and regulates the blood levels of AAs, controlling supply of building blocks for extrahepatic proteins (4, 5). It also plays an important role in lipogenesis, production of triglycerides (TGs) and lipoproteins, and cholesterol synthesis (6). Production and excretion of bile is required to support fat emulsification in the gut and absorption of vitamins. The liver is also an important recycling station, responsible for breakdown of red blood cells and processing of hemoglobin for storage and use of its iron content (7). Also, plasma proteins are recycled in the liver, and their AAs are used to produce new proteins, supply extrahepatic cells with AAs, or convert them into glucose during gluconeogenesis. Moreover, the liver breaks down insulin and other hormones, and it modifies waste products, enabling their excretion via urine or bile. Examples include the conversion of ammonia into urea that can be excreted via urine and drug metabolism mediated by cytochrome P450 (CYP) enzymes. In addition, the liver also regulates the immune defense during sepsis (1, 3, 6, 8). Given all these vital functions (and many more we cannot cover here in detail) in our body's production, recycling, storage, and waste management, it is not surprising that impaired liver function is associated with many diseases.

1.2. Lobular Architecture of the Liver

The liver is divided into several lobes (right, left, caudate, and quadrate lobes in human). Each lobe consists of repetitive honeycomb-like structures termed lobules, with a central vein in the center and portal triads on the boundary (**Figure 1*a***). The portal triads consist of a portal vein, a hepatic artery, and a bile duct, and the lobular hepatocytes are lined up alongside the porto-central blood flow. The liver receives a dual blood supply from the hepatic portal vein (75%) and hepatic artery (25%). The venous blood arrives from the spleen, pancreas, and gastrointestinal tract, along with its associated organs, making the liver a first-pass organ for most nutrients. Hepatocytes make up 70–85% of the liver volume, whereas nonparenchymal cells account for 6.5% of its volume (but ~40% of the total number of liver cells). Discontinuous layers of fenestrated liver sinusoidal endothelial cells lining the hepatocytes enable bidirectional permeability of nutrients, metabolic products, and other molecules, while phagocytic Kupffer cells (resident hepatic macrophages) and lymphocytes in the sinusoidal space perform immune surveillance. Hepatic stellate cells in the perisinusoidal space store vitamin A and allow for extracellular matrix formation during injury and inflammation. The pericentral veins merge into hepatic veins, which leave the liver via the inferior vena cava. Intrahepatic bile flows in the opposite direction of the lobular blood flow. Bile produced by hepatocytes is collected via bile canaliculi, which radiate toward the portal triads where they merge into the intrahepatic bile ducts (**Figure 1*b***). Bile is temporarily stored in the gallbladder or directly drained into the duodenum (1, 9).

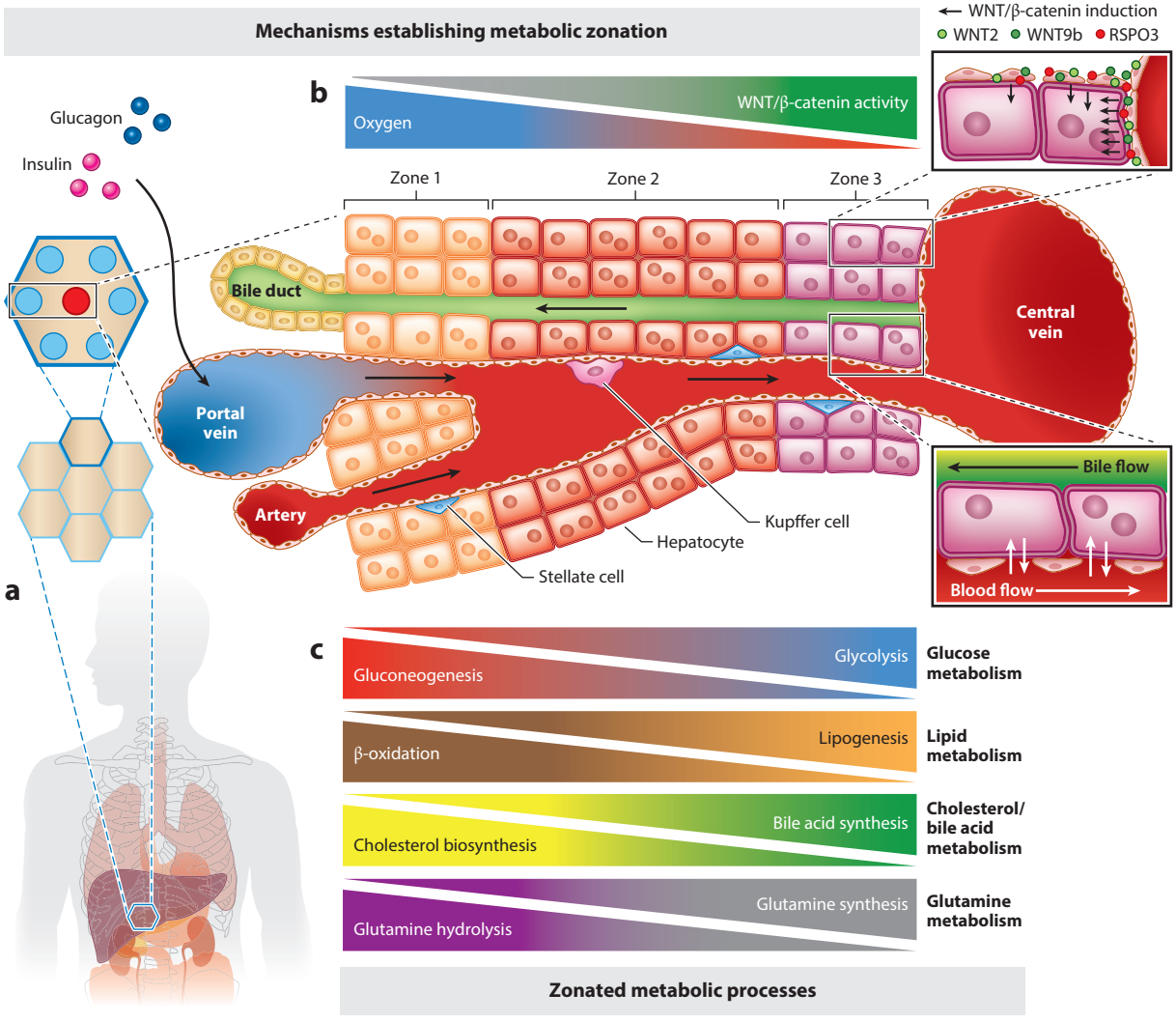

Figure 1

Spatial metabolic liver zonation. (*a*) Architecture of the liver lobule, as described in Section 1 of the main text. (*b*) Spatial physiological organization of the liver, as described in Section 2 of the main text. Higher periportal availability of hormones and gradients of oxygen and WNT/β-catenin activity promote spatial liver zonation. Upper inset illustrates how local availability of RSPO and WNT ligands around the central vein induces spatially confined pathway activity. Lower inset illustrates fenestrated sinusoidal endothelium enabling uptake of substances by hepatocytes and release of metabolites back into the porto-central blood flow. (*c*) Selection of spatially zonated metabolic processes.

2. SPATIAL METABOLIC LIVER ZONATION

2.1. Defining the Hepatic Zones

To manage such an impressive set of divergent metabolic tasks, the liver lobule requires a well-organized microarchitecture. Approximately 100 years ago, Noël (10) divided the liver lobule into three regions: the zone of permanent activity (periportal zone, or zone 1), the intermediary zone

(midlobular zone, or zone 2), and the zone of permanent response (pericentral zone, or zone 3) (**Figure 1*b***). In 1944, Deane (11) was the first to introduce the term zonation when referring to the functional heterogeneity of hepatocytes alongside the lobular axis. In the 1980s and 1990s, Gebhardt, Jungermann, Kietzmann, and others (12–14) uncovered spatial compartmentalization of many metabolic processes in the different hepatic zones (**Figure 1*c***). Using gradient perfusion (15) or laser capture microdissection (16), landmark genes for the different hepatic zones were established. Whereas earlier studies of spatial resolution required labeling of proteins or nucleic acids in the tissue, a novel approach named spatial reconstruction utilized sequencing of RNA from dissociated cells and inference of the spatial context on the basis of landmark zonated genes. Positioning hepatocytes onto a virtual lobular axis allowed in-depth mechanistic studies of spatial (17) and spatiotemporal (18) metabolic liver zonation. These transcriptomics studies provided defined spatial gene expression profiles in the different lobular zones (17–21). Distributed metabolic tasks, which are controlled by spatially confined gene expression, are used as positional markers of the different lobular zones. Among many other markers, periportal hepatocytes express *Pck1*, *Hal*, *Gls2*, *Hsd17b13*, *Ass1*, and *Arg1*, whereas pericentral hepatocytes express *Glul*, *Cyp2e1*, *Cyp1a2*, *Cyp3a4*, and *Oat* (15, 21, 22). While midlobular hepatocytes express *Hamp*, *Hamp2*, and *Igfbp2* (23, 24), the midlobular expression profile is less defined, since many zonal markers reach far into the midlobular zone (e.g., *Cyp2e1* or *Arg1*). Moreover, midlobular hepatocytes are responsible for homeostatic renewal of the hepatocyte pool (21, 25–27), and their increased proliferative capacity correlates with overall lower metabolic function (21). As most of the zonal landmark genes have been defined in rodent studies, and only a subset of them were validated using spatial transcriptomics in human livers (19), more research is required to define hepatic zones in different species.

2.2. Principles of Spatial Metabolic Zonation

Approximately 12–15 hepatocytes are lined up alongside the porto-central blood flow, like workers on an assembly line. Approximately half of the hepatocyte genes are expressed in a zonated spatial gradient in different lobular zones (17), defining the role of each individual worker in the hepatic factory. Decades of research collectively suggest that different principles of spatial liver zonation direct the diversity of hepatic functions (reviewed in 23). Production line patterns are built by sequential metabolic gene expression, enabling stepwise processing via complementary metabolic tasks. One example is the bile acid synthesis cascade, in which the uptake of cholesterol from the blood and its conversion into bile acids (BAs) are orchestrated by sequential spatial expression of the metabolic enzymes driving consecutive steps (17). The liver also shows spatial segregation of opposing metabolic tasks. Examples include gluconeogenesis in periportal hepatocytes and glycolysis in the pericentral zone. Oxidative breakdown of fatty acids (FAs) is mediated by periportal hepatocytes, whereas pericentral hepatocytes control lipogenesis. Other examples with porto-central segregation are cholesterol production and consumption, as well as hydrolysis of glutamine to glutamate versus synthesis of glutamine from glutamate. Some of these opposing metabolic tasks, where metabolites produced in periportal hepatocytes are taken up by pericentral hepatocytes, constitute a spatial recycling loop (23). Importantly, liver zonation allows for the assignment of metabolic tasks to the lobular zone, providing the most suitable work environment. Periportal hepatocytes have better access to oxygenated blood, rendering them more capable of performing energy-demanding tasks such as gluconeogenesis, protein secretion, and β-oxidation (28). Periportal hepatocytes also first receive nutrients, metabolites, and other substances from the digestive tract and are therefore specialized in substance uptake and preprocessing for sequential metabolic tasks in consecutive hepatic zones (29).

3. TEMPORAL METABOLIC LIVER ZONATION/ COMPARTMENTALIZATION

In addition to spatial zonation, the liver also exhibits rich temporal regulation of physiological functions, orchestrated by the interaction of environmental cues with the circadian clock. Generally, circadian rhythms refer to oscillations in animal behavior and physiology within a period of approximately 24 h that emerged as an evolutionary adaptation to life on Earth. The circadian clock is a hierarchical and distributed timing system in mammals, coordinated by a central pacemaker in the brain, which relays timing cues to clocks in most organs of the body via downstream signaling pathways (**Figure 2*a***). These clocks are genetically encoded and tick autonomously in individual cells (30), with the molecular clockwork thought of as being composed of interlocking transcription-translation feedback loops among core clock genes (*Bmal1*, *Clock*, *Periods*, and *Cryptochromes*). Accordingly, BMAL1:CLOCK drives transcription of PERs and CRYs, which heterodimerize and repress the aforementioned activator complex, shutting down their own transcription (31) (**Figure 2*b***). Despite being autonomous and self-sustained in cells and organs, the molecular clocks need to be constantly readjusted by internal and environmental cues. As a result of these timing cues, liver physiology shows daily fluctuations in virtually all aspects of hepatic functions, with disparate functions reaching maximum effectiveness at different times of the day.

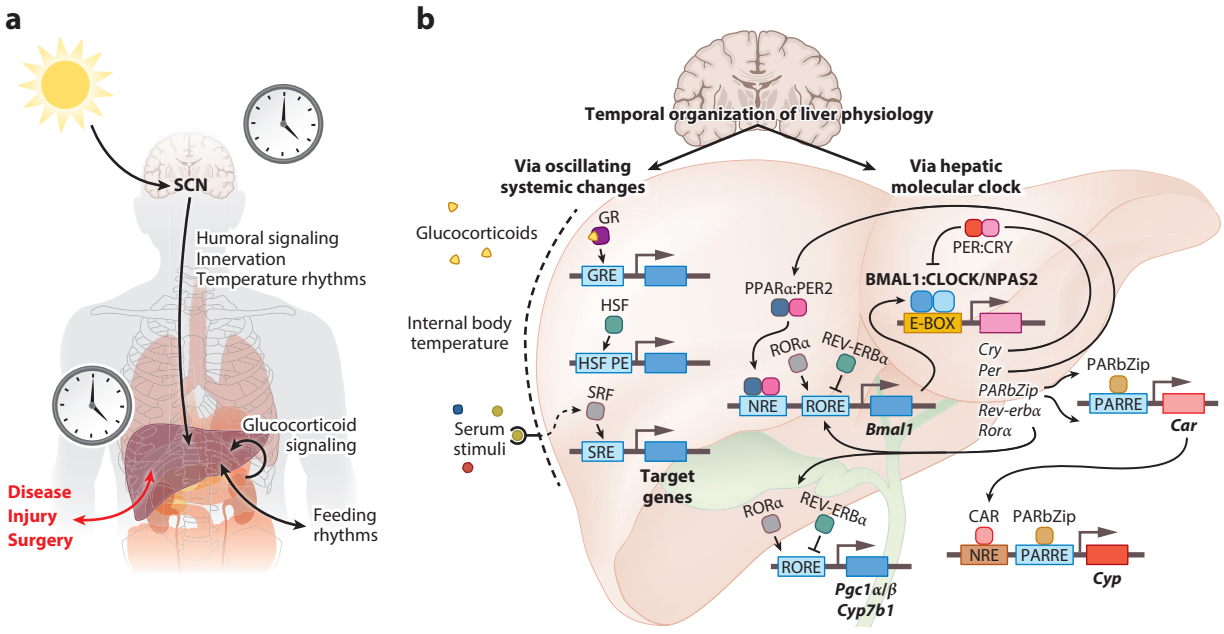

Figure 2

Circadian clocks and liver physiology. (*a*) Temporal resetting of the liver clock is illustrated. Information on the day–night cycle is conveyed from the SCN to the liver by humoral signals and innervation, or via rhythmic systemic changes, such as temperature oscillations and SCN-controlled rhythmic glucocorticoid release from the adrenal gland. The liver also receives timing information from patterns of nutrient influx. Reciprocally, liver rhythms affect feeding patterns. Acute events or chronic diseases can result in the deregulation of the molecular clock, and the clock modulates disease outcome. (*b*) Rhythmic signals either directly activate hepatic target genes (*left*) or influence the hepatic molecular clock (*right*). The molecular clock is thought to use autoregulatory mechanisms in which PER:CRY represses the activational complex of BMAL1:CLOCK. This loop mechanism provides rhythmic outputs via NREs, ROREs, and PARREs. Abbreviations: NRE, nuclear receptor response element; PARRE, PARbZip response element; RORE, retinoic acid receptor–related orphan receptor (ROR) response element; SCN, suprachiasmatic nucleus.

Less common, and conceivably relevant in lipid and endoplasmic reticulum homeostasis, are ultradian 12-h rhythms, affected by the transcriptional factor XBP1 (32, 33). Hence, when studying the liver, both spatial and temporal regulation need to be considered.

3.1. Suprachiasmatic Nucleus

In mammals, the suprachiasmatic nucleus (SCN), located in the hypothalamus and often referred to as the body's master pacemaker, receives direct innervation from the retina via the retinohypothalamic tract, providing the organism with information on the natural light cycle (34). This information is conveyed to central and peripheral tissues by outputs from the SCN, in the form of either innervation or diffusible molecular signals (30). Since the SCN receives input from light, both diurnal and nocturnal animals have temporally aligned rhythms of gene expression in the SCN, and both also secrete pineal melatonin during the night. In contrast, activity, feeding rhythms, and metabolic processes mainly peak at opposing times in nocturnal animals compared with diurnal, despite the dependency of these processes on the SCN (35, 36). This raises the question of how timing information is conveyed to the periphery, which is highly relevant when projecting rodent studies to human subjects.

3.2. Circadian Physiology on an Organismal Level

The SCN sends timing information to the adrenal gland via the hypothalamic-pituitary-adrenal axis, as well as via direct innervation. This, in turn, results in daily oscillations of glucocorticoid release from the gland into the bloodstream. These oscillations, lost upon SCN lesioning of rodents, are important for synchronization of peripheral tissues, including the liver, which expresses high levels of the glucocorticoid receptor (GR) (30, 37). Besides being affected by humoral signals, the liver itself is also complexly innervated (38). This autonomic input, too, affects light-regulated hepatic gene expression (39).

3.3. Temporal Entrainment of the Liver by Feeding Cycles

The liver also receives signals on food intake patterns and the nutrient state. Subjecting nocturnally active mice to food availability for a few hours during the day at a recurring time results in a misalignment of feeding with the light-set cycles of activity and rest, as well as a phase shift of the liver clock and clock-controlled genes (40), suggesting that food is likely the dominating direct timing signal for the liver. Without restrictions, the animals would eat on the basis of SCN-controlled activity-rest rhythms. In fact, feeding rhythms are lost upon SCN lesioning, but anticipation of food availability can be restored by time-restricted feeding, even in the absence of the adrenal gland (41). Without the GR, the response to changed feeding schedules is faster, suggesting that glucocorticoid signaling contributes to the robustness of liver rhythms (42). This robustness likely buffers fluctuations and reduces variability in temporal hepatic physiology despite variation in the time of intake and composition of food. Besides locking its physiology onto recurrent stimuli, the liver needs to respond to isolated events such as the processing of a palatable meal at an unexpected time. Finding the correct balance between robustness of rhythms and the ability to respond acutely may determine the likelihood of pathologies, as chronic disruptions of temporal processes can have devastating consequences for the liver (43). Hence, diurnal variations in gene expression originate either from the oscillatory machinery, which persists in constant conditions, or from repeated responses to stimulation by food or other factors (44). Supporting this concept, mice with a dysfunctional molecular clock show hundreds of rhythmic transcripts under daily recurring feeding, albeit with dampened amplitudes compared with clock-controlled

genes (45, 46). On the contrary, a large proportion of daily rhythmic transcripts cease to show oscillation-like patterns under 24-h fasting in wild-type (WT) mice (45).

In addition, intermittent starvation caused by the aforementioned recurring daytime-restricted feeding results in anticipation of feeding time, which is seen as an activity, and internal body temperature increase in anticipation of the meal, physiological manifestations that clearly point at organ cross talk. These adaptations are absent in mice with a disrupted temporally programmed liver ketone body synthesis or ketone body efflux (47, 48), pointing to the importance of temporally programmed liver functions in extrahepatic signaling.

3.4. Temperature Entrainment of Daily Rhythms

Organisms are also subjected to daily changes in environmental temperature, which can entrain clocks of cultured cells and heterothermic organisms (49). Inverting the usual scenario of warm days and cold nights to warm nights and cold days does not affect the clock in the SCN but results in a phase shift in hepatic gene expression in mice, paralleled by a shifted feeding time and changed patterns of internal body temperature (49), similar to what is observed under daytime-restricted feeding. This perturbation demonstrates that temperature influences resetting of the molecular clock and temporally gated processes. Importantly, mammals show diurnal variation in internal body temperature, controlled by the SCN. These physiological variations are also able to provide a signal for peripheral clock resetting, likely by diurnal regulation of heat shock factor activity (50, 51).

4. SPATIOTEMPORAL ORGANIZATION OF METABOLIC PROCESSES

Spatial and temporal programming of processes in the liver are able to compartmentalize incompatible biochemical processes, such as processes that could compete for the same resources, produce undesired products, or activate opposing signaling pathways. The compartmentalization is often achieved through regulation of rate-limiting enzymes or transporters, as the latter determine the capacity of cross-membrane shuttling of chemical entities (18). The study of the spatiotemporal organization of liver functions revealed either constant, spatially zonated, or rhythmic gene expression, as well as expression that is influenced by both space and time. These effects of space and time can either be independent or interact, for instance, resulting in a temporal phase difference of up to 3 h between periportal and pericentral hepatocytes (18). Interestingly, genes that are either independently or dependently spatially and temporally zonated drive key liver functions and include rate-limiting enzymes, such as *Lipin2* (which converts phosphatidic acid to diacylglycerol), *Dnaja1* (an HSP40 cochaperone), and *Gne* (a rate-limiting enzyme in sialic acid biosynthesis), as well as *Pck1*, *Glul*, *Alas1*, and *Elovl3*, described below in more detail.

4.1. Carbohydrate Metabolism

Glucose metabolism, essential to maintain blood sugar levels, shows clear spatial compartmentalization of opposing tasks, with periportal gluconeogenesis and pericentral glycolysis (52). During feeding, excessive carbohydrates are converted into glycogen (glycogenesis) or FAs (de novo lipogenesis). During fasting conditions, glycogenolysis enables the release of glucose from glycogen stores to maintain blood glucose levels, providing energy to cells across the body (2, 3, 53). Following prolonged fasting or starvation, when the hepatic glycogen stores are depleted, gluconeogenesis promotes de novo glucose synthesis from noncarbohydrate precursors, such as lactate, glycerol (from TGs), and glucogenic AAs, especially alanine (2, 3). Periportal compartmentalization of glycogenesis and gluconeogenesis enables direct access to nutrients provided by the digestive tract as well as an oxygen-rich environment necessary for these energy-demanding

metabolic processes. The expression of enzymes controlling pericentral glycolysis is controlled and spatially confined by HIF1α, a transcription factor induced in hypoxic conditions (2, 54, 55).

The distinct requirements during fasting and feeding call for temporal control of genes involved in glucose metabolism. The involvement of the molecular clock in carbohydrate and lipid metabolism was observed in *Bmal1* knockout (KO) and *Clock* mutant mice (56), which exhibit attenuated diurnal variation in plasma glucose and TGs, as well as impaired gluconeogenesis. Circadian expression of glucose transporters, glucagon receptor, and genes involved in glycogenesis may enable temporal control in glucose uptake, storage, and insulin response during feeding (57). On the contrary, mechanisms of gluconeogenesis get activated during the period of reduced food intake. Among others, a key and rate-limiting enzyme in gluconeogenesis, phosphoenolpyruvate carboxykinase (*Pck1*), with higher periportal expression, peaks daily at the end of the fasting period (58). *Pck1* spatiotemporal expression is aligned with that of *Glut2*, which is required for glucose efflux into the blood. Such temporal programming of gluconeogenesis allows temporal separation from glycolysis.

4.2. Lipid and Bile Acid Metabolism

FA uptake from the blood is mostly handled by periportal hepatocytes, whereas pericentral hepatocytes drive FA elongation and export into extrahepatic fat depots. Tight spatiotemporal control of lipogenesis and lipid catabolism in pericentral hepatocytes is necessary to avoid TG accumulation in the liver resulting in steatosis and nonalcoholic fatty liver disease (NAFLD). FA β-oxidation is a multistep catabolic process promoting the breakdown of FAs to produce energy. It primarily takes place in the periportal zone where the majority of energy- and oxygen-demanding metabolic processes reside (54, 59).

De novo cholesterol synthesis is mainly located in the periportal hepatocytes, in line with higher expression of ATP citrate lyase (*Acly*) [generating acetyl coenzyme A (acetyl-CoA) for cholesterol synthesis] in this zone. BAs are synthesized from cholesterol in a CYP-mediated multistep process and transported in the opposite direction to the porto-central blood flow, namely via bile canaliculi toward the bile ducts in the portal tract, where they are collected and drained into the intestinal tract. BAs not only support fat digestion but also are important regulators of gene expression in lipid and glucose metabolism via the activation of FXR (60). CYP7A1 is the rate-limiting enzyme of the neutral pathway of BA synthesis and is predominantly expressed in pericentral hepatocytes, resulting in spatial compartmentalization of BA synthesis. Zonation of BA synthesis may be explained by feedback inhibition of BA synthesis in periportal hepatocytes, which show higher BA uptake compared with pericentral hepatocytes (61).

The influence of the molecular clock and feeding rhythms on hepatic lipid metabolism is striking. TGs, in mice, predominantly peak during the second half of the fasting phase (62). HMGCR, the rate-limiting enzyme of cholesterol biosynthesis, peaks on the mRNA and protein level during the active-to-rest phase transition, resulting in cholesterol biosynthesis in mice mainly during the resting period (18, 57). In humans, cholesterol biosynthesis mostly occurs during the night, which is why some statins (inhibitors of HMGCR) with shorter half-lives should be taken before bedtime for higher efficacy and reduced adverse effects (63, 64). The pericentrally expressed *Cyp7a1* peaks during the early active phase in mice, allowing for BA synthesis during times of food consumption (18, 57). In this way, the synthesis and degradation of cholesterol (which is biotransformed into BAs) are elegantly temporally compartmentalized. Interestingly, *Cyp7a1* expression shows interacting effects of space and time, meaning that the amplitude of its expression is different within different lobular zones. Similarly, *Acly* (involved in acetyl-CoA production) is also affected by codependencies of space and time, with a lower amplitude periportally, where it shows higher expression (18).

Lipid absorption and lipase activity also peak at the beginning of the active period (65). In addition, roughly half of all nuclear receptors (NRs) oscillate in expression, and their activity may depend on temporally programmed coactivation, as discussed below (66–68). The degradation of lipophilic molecules is also temporally compartmentalized. As an example, rate-limiting steps of FA oxidation are affected by the clock via diurnal expression of enzymes, such as *Cpt1* (69). Normally, *Cpt1* peaks during the active phase (night) in mice but is shifted toward the feeding time under daytime-restricted food availability (47). Furthermore, global *Clock* mutant mice exhibit metabolic syndrome, including hepatic steatosis, highlighting the importance of circadian control of lipid metabolism (70).

4.3. Detoxification and Xenobiotic Metabolism

The liver is also vital for detoxification and xenobiotic clearance. An essential hepatic function is the removal of toxic ammonia arriving via the portal vein. First, ammonia is metabolized by periportal hepatocytes into urea involving the spatially confined enzymes CPS1 and ARG1. Residual ammonia is then converted into glutamine in pericentral hepatocytes, involving glutamine synthetase (GS), encoded by *Glul*. Pharmacologically active compounds are also primarily metabolized in the liver, where they undergo chemical transformation—mostly oxidation but also reduction or hydrolysis in phase I metabolism. This increases their polarity and enables either direct excretion or conjugation in phase II metabolism, which involves binding of highly hydrophilic molecules to convert active compounds to secretable products (4, 71).

The majority of the CYP enzymes are expressed in the pericentral zone, driving monooxygenation in phase I metabolism and enabling predominantly pericentral glucuronidation in phase II metabolism, whereas sulfonation is more prominent in periportal hepatocytes (14). Many of the enzymes crucial for phase I metabolism, such as cytochromes (*Cyp*), flavin-containing monooxygenases (*Fmo*), and the CYP oxidoreductase (*Por*), to name a few, show distinct diurnal profiles (46). Several enzymes of phase II metabolism also show diurnal expression rhythms. These include glucuronosyltransferases (*Ugt*), sulfotransferases (*Sult*), and glutathione S-transferases (*Gst*), which covalently add glucuronic acid, sulfate, or glutathione, respectively, to xenobiotics, enabling their excretion (65, 72) (**Figure 3*a***). Many of these metabolic genes are regulated by rhythmic NRs. As an example, the predominantly pericentral constitutive androstane receptor (CAR) drives transcription of *Cyp* enzymes, likely partially explaining their spatial zonation. In addition, the clock regulates PARbZip transcription factors, and PARbZip-deficient mice exhibit a general downregulation of genes involved in xenobiotic detoxification, either through lack of direct binding of PARbZip factors to promoters of detoxifiers or through attenuated regulation of *Car* (73) (**Figure 2*b***). These mice suffer from dramatically increased mortality, morbidity, and accelerated aging (74). The downregulation of *Cyp* enzymes in these mice is noteworthy, as approximately three-quarters of small molecule drugs are biotransformed by CYPs (71). Importantly, there are considerable differences in *Cyp* enzymes between species; hence, results from mouse models need to be carefully interpreted. That aside, globally, CYPs require heme as their prosthetic group, and *Alas1*, peaking in mice at the resting-to-activity transition, encodes the rate-limiting enzyme in heme biosynthesis. Since heme availability is diurnal, it is likely that it affects CYP activity. In parallel, the pericentrally expressed *Por*, required for electron transfer to CYPs and their activation, also peaks during the beginning of the active period. One could, therefore, speculate that the highest capacity for cytochrome-mediated oxidation in mice is temporally programmed to coincide with the active and feeding period. Interestingly, both *Alas1* and *Por* are regulated by CAR (75). With respect to phase II metabolism, generally, *Ugt* expression is highest during the daytime in nocturnal mice. *Gst* enzymes peak during the early light phase, and *Sult* enzymes peak during the light-to-dark transition. Importantly, many membrane proteins, crucial for transport

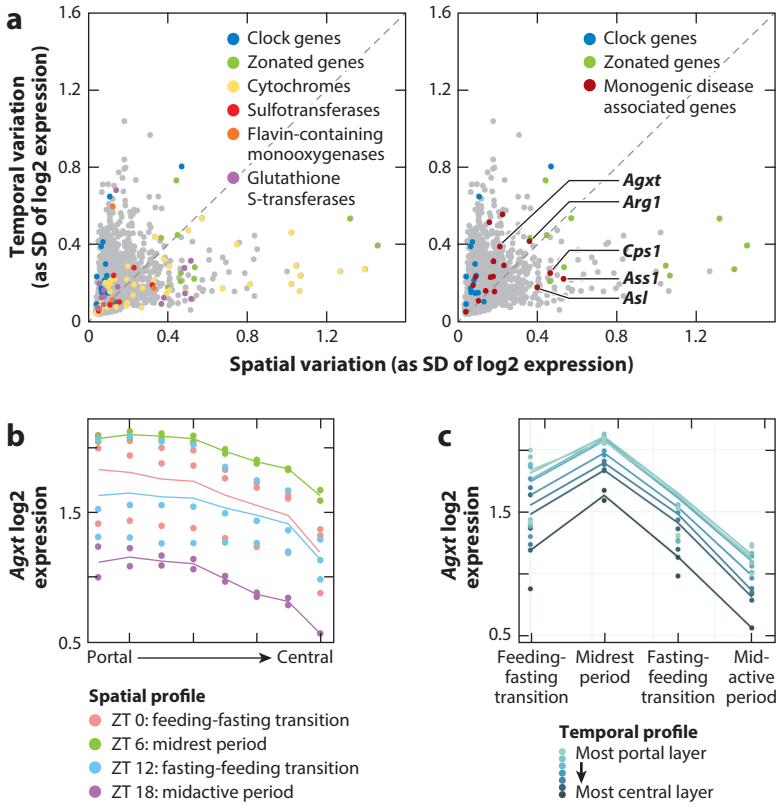

Figure 3

Spatiotemporal hepatic gene expression in mice. (*a*) Changes in gene expression in lobular space (x-axis) and time (y-axis) shown as the standard deviation of log2 mRNA expression. Examples of detoxification genes (*left panel*) and monogenic disease genes (*right panel*) are shown. The distance from zero corresponds to higher expression changes along the lobular axis or along the duration of one day, respectively. (*b*,*c*) *Agxt* shown as an example gene where mRNA expression changes with both lobular space (*b*) and circadian time (*c*). Abbreviations: SD, standard deviation; ZT, Zeitgeber time. Figure adapted from Reference 18.

of xenobiotics in and out of the liver, also show diurnal rhythms (65). Undoubtedly, this simplified description of metabolism already demonstrates the importance of an intact rhythm for drug chemical transformation and excretion.

4.4. Energy Metabolism

We have now established that detoxification and processing of nutrients strongly rely on redox reactions, and these firmly depend on NADH and NADPH. The rate-limiting enzyme in biosynthesis of NAD^+, *Nampt*, peaks in the liver near the resting-to-active phase transition in mice, resulting in rhythms of NAD^+, a crucial metabolic coenzyme. SIRT1 is an important NAD^+-dependent deacetylase, which interacts with BMAL1, CLOCK, and PER2 (76, 77). Inhibition of *Nampt* prevents SIRT1-mediated suppression of the activity of the BMAL1:CLOCK complex (78). Both *Nampt* and *Sirt1* are also influenced by glucose or caloric restriction, potentially linking metabolism and timekeeping (65, 79, 80). In addition to being a pivotal metabolic coenzyme, NAD^+ is the precursor of $NADP^+$, crucial for nucleotide and FA synthesis. Its reduced form, NADPH, also acts as a cofactor of FMOs, which show temporally and spatially

zonated expression (**Figure 3*a***) and which are required for POR-mediated activation of CYPs (81, 82). Redox reactions are also the basis for mitochondrial function, and hepatocytes are among the most mitochondria-rich cell types. In addition, circadian biology of oxidative states, determined by daily variation of NAD^+ and FAD, affects cellular respiration, the mitochondrial process leading to energy production in the form of ATP. Mitochondrial function is dynamically influenced by oxygen and hormone supply, and periportal hepatocytes show a significantly higher cytosolic ATP/ADP ratio (83, 84). Additionally, the mitochondrial lipidome, proteome, and CLOCK-influenced acetylome show daily rhythms, as do mitochondrial shape and volume (85).

4.5. Protein and Amino Acid Metabolism

Serum proteins are recycled in the liver via breakdown into AAs and synthesis of new proteins. In addition, AAs from digested nutrient proteins reach the portal vein during feeding cycles. AAs not only are building blocks for protein synthesis but also serve as fuel for gluconeogenesis. The periportal zone has the highest capacity for uptake and catabolism of AAs (except for glutamate and aspartate) (4, 5). Glutamine and glutamate metabolism are spatially segregated processes. Periportal hepatocytes nonrhythmically express phosphate-activated glutaminase (*Gls2*) (18), which hydrolyzes glutamine into glutamate and ammonia. Periportal confinement of this process is important to enable subsequent ammonia conversion into urea in this zone. The hepatic glutamine–glutamate cycle is closed when pericentral hepatocytes take up glutamate, convert it back into glutamine via GS, and secrete it into circulation. This cycle is important to scavenge ammonia, maintain low ammonia concentrations in the blood, and adjust ammonia flux into either urea or glutamine to regulate acid–base balance. Maintaining glutamine levels is essential, as glutamine participates in many vital physiological processes, such as the formation of UDP-*N*-acetylglucosamine, which is a precursor for all macromolecules containing amino sugars (including membrane hormone receptors and heparin) (5, 86).

ASS1 is involved in the synthesis of the urea cycle intermediate, argininosuccinic acid, which is also the precursor of biosynthesized arginine. *Ass1*, a textbook example of periportal expression, does not exhibit robust daily oscillation, but it is acetylated by CLOCK (87). Ureagenesis and arginine synthesis are rhythmically controlled by acetylation of ASS1, causing its inactivation (87). Hence, *Ass1* is an example of a spatially zonated gene, with no temporal zonation on the level of mRNA. Its temporal activity is gated via posttranslational modifications rather than transcription or translation itself.

5. MECHANISMS CONTROLLING SPATIAL ZONATION

5.1. Oxygen Gradient

The liver with its many energy-demanding metabolic tasks requires substantial amounts of oxygen. Hence, there is a porto-central oxygen gradient ranging from 60–65 mm Hg in periportal blood to ~30–35 mm Hg in pericentral blood, paralleled by differences in intracellular pO_2 as well as in numbers and structure of mitochondria in periportal and pericentral hepatocytes. Given the influence of O_2 on carbohydrate metabolism, hypoxia-inducible factors (HIFs), and generation of reactive oxygen species (ROS), it is considered to be a key regulator of liver zonation. HIFs are transcription factors, which are predominantly activated in pericentral hepatocytes and regulate many metabolic processes (e.g., HNF1α promotes glycolysis and HIF2α suppresses gluconeogenesis). Regulation of spatial zonation by the oxygen gradient and the interplay with other pathways driving lobular compartmentalization are reviewed by Kietzmann (28).

5.2. WNT/β-Catenin Signaling

WNT/β-catenin signaling is a master regulator of spatial liver zonation. A centro-portal WNT/β-catenin activity gradient orchestrates the expression of many pericentral metabolic enzymes that are transcriptionally regulated by β-catenin (e.g., GS, CYP1A2, and CYP2E1) (88, 89). Different β-catenin target genes seem to have different thresholds for expression. GS expression is restricted to the first one to two layers of pericentral hepatocytes, which also show the highest WNT/β-catenin activity indicated by *Lgr5* expression (a β-catenin target gene that is only expressed in cells with very high WNT/β-catenin activity). CYP2E1 expression extends far into the midlobular zone, matching the reach of the general WNT/β-catenin target *Axin2* (90). The regulatory mechanism setting different thresholds for β-catenin-controlled metabolic genes remains to be identified. The WNT/β-catenin activity gradient is promoted by the confined expression of WNT and RSPO ligands in central vein and sinusoidal endothelial cells (91–93) (**Figure 1*b***), as well as by zonated expression of regulatory pathway components (e.g., periportal expression of negative regulator APC) (94). While the mechanisms enabling zonated expression of these ligands have not been identified, it is possible that pericentral hypoxia is involved in this process, in a similar manner as in adipogenic cells (95), although definitive studies are lacking. Since WNT/β-catenin signaling promotes hepatocyte proliferation, and activating mutations in the pathway are major drivers of liver cancer, the liver requires tight control mechanisms enabling spatial zonation while preventing unnecessary proliferation. The RSPO-LGR4/5-ZNRF3/RNF43 module is essential for regulating hepatic WNT/β-catenin activity and spatial metabolic zonation (96). In addition to directly controlling pericentral metabolic gene expression, WNT/β-catenin signaling indirectly directs periportal genes. Ectopic activation of WNT/β-catenin signaling in various mutant mice reprogrammed periportal hepatocytes into pericentral hepatocytes, whereas blockage of WNT/β-catenin signaling induced widespread activation of periportal metabolic enzymes (21, 89, 92, 94, 96). This indirect regulation may be explained by the competition of HNF4α and β-catenin for binding to T cell factor (TCF). In the absence of WNT/β-catenin signaling, the HNF4α:TCF complex promotes periportal gene expression, whereas nuclear translocation of β-catenin favors a β-catenin/TCF complex driving pericentral gene expression (22). Several WNT ligands expressed in pericentral endothelial cells exhibited diurnal mRNA fluctuations, suggesting that WNT signaling may fluctuate along the day (18).

5.3. Other Pathways

Hedgehog (Hh) signaling, despite showing only low baseline activity in hepatocytes, is involved in metabolic zonation. Hh signaling is higher in periportal hepatocytes, and studies with mutant mice inhibiting the pathway suggest a role in partially counteracting WNT/β-catenin signaling and regulation of the IGF1 axis (involved in glucose homeostasis) (97). The RAS/RAF/ERK pathway attenuates pericentral metabolic gene expression in periportal hepatocytes (98). Hormones, such as insulin and glucagon, which are more abundant in the periportal zone and have opposing regulatory functions in glucose metabolism, play important roles in spatial metabolic zonation. For example, glucagon was found to counteract WNT/β-catenin signaling (99). Moreover, *Dicer* is essential for liver zonation since its deletion caused broader expression of GS and diffuse extension of periportal enzymes throughout the liver, suggesting that microRNAs may influence spatial gene expression in the liver (100). The role of YAP/HIPPO signaling in metabolic zonation is unclear. While one study found that YAP is essential to regulate GS expression in zebrafish and mice (101), other studies suggest intact GS expression (102, 103) or even increased GS expression (104) in mice with YAP deletion. It is to be hoped that future research will shed more light on the role and interplay of hepatic signaling pathways in regulating metabolic liver zonation.

6. MECHANISMS CONTROLLING TEMPORAL HEPATIC PROCESSES

6.1. Transcriptional Mechanisms

Temporal expression patterns are based on a handful of identified mechanisms. One of the first reported outputs of the mammalian molecular clock was the E-box-mediated regulation of the *Avp* gene (105). Such regulation depends on the binding of BMAL1:CLOCK to an extra- or intragenic hexamer enhancer motif or similar noncanonical motifs (106–108) (**Figure 2*b***). BMAL1 and CLOCK bind to regulatory sites of thousands of transcripts, suggesting that regulation via E-box motifs accounts for a large fraction of rhythmically expressed genes in the liver (109, 110).

The NRs RORα/β/γ and REV-ERBα/β, which compete for binding to the retinoic acid receptor–related orphan receptor (ROR) response element (RORE), are also controlled by BMAL1:CLOCK (111) (**Figure 2*b***). In fact, ROR and REV-ERB are important regulators of metabolic processes. Mutation of RORα, a transcriptional activator, disrupts cholesterol, lipoprotein, and TG metabolism, whereas REV-ERBα, a repressor, regulates the pericentrally expressed *Elovl3*, involved in FA elongation (112, 113). Moreover, ROREs are also present on the *Bmal1* promoter, thus contributing an additional feedback loop in the core clock (114, 115).

The above-described PARbZip factors, consisting of (mammalian) members *Dbp*, *Hlf*, and *Tef*, are prominent direct clock targets (75), and *Dbp* is regulated via multiple E-box motifs (107).

6.2. Glucocorticoid Signaling

Outputs from the clock are also regulated via the previously mentioned glucocorticoid signaling (**Figure 2*b***). Glucocorticoids peak during the rest-to-active phase transition (116, 117) and activate the hepatic GR, allowing it to regulate its downstream targets by binding to glucocorticoid response elements. Important GR targets are *Per* genes (118), and activation of target genes via GR binding is attenuated by binding of CRY1/2 to GR (119). Glucocorticoids also play an important role in regulating spatial compartmentalization by regulating periportal metabolic genes (120).

6.3. Nuclear Receptor Coactivation

NRs are important transcriptional regulators that sense small diffusible molecules, known as ligands. Notably, the previously mentioned GR, CAR, REV-ERB, and ROR are all NRs, and so are peroxisome proliferator-activated receptors that regulate FA metabolism and glucose homeostasis (121). Rhythmic expression of coactivators seems to temporally compartmentalize NR activation potential. Unsurprisingly, many coactivators are important regulators of hepatic metabolism, and many are rhythmically expressed, such as *Pgc1a* (122). Interestingly, one well-characterized NR coactivator is also the core clock protein PER2 (66, 67). Supporting the importance of this mechanism, core clock proteins are enriched at both E-boxes and NR binding sites of target genes (109).

6.4. Other Systemic Cues

Clock-controlled mechanisms regulate rhythmic patterns of internal body temperature (40, 49). The heat shock factor HSF1 shows diurnal rhythms of binding to the liver chromatin, primarily driving gene expression in mice at the onset of the active phase, when the animals have the highest body temperature (48, 51). The serum response factor senses oscillating bloodborne signaling proteins (123) (**Figure 2*b***). A further systemic signal is oxygen concentration. Whereas hypoxia affects processes along the lobular axis, it can also influence temporal patterns of gene expression,

as PER2 interacts with HIF1α, increasing HIF1α's activity (124). The time-dependent susceptibility of the transcriptome to hypoxia is abolished in *Per1/2* double mutants (125). It is therefore not surprising that hypoxia's impact on tissues depends on time of day (125). Pathological conditions perturbing the supply of oxygen can also influence the clock or even cause temporal misalignment between organs (125).

6.5. Posttranscriptional Mechanisms

Several estimates suggest that up to a third of rhythmic mRNAs could be attributed to posttranscriptional mechanisms, predominantly daily rhythms of mRNA degradation resulting from stabilizing or destabilizing effects of mRNA-binding proteins (109, 126). The temporal regulation of alternative splicing is comparatively less studied but thought to be important also (127). As an example, in *Per1*, a light-inducible splicing switch results in an unstable protein (128).

6.6. Translation, Proteome, and Posttranslational Modifications

Frequently, rhythmicity in mRNA results in rhythms in corresponding proteins, with the peak of protein abundance exhibiting an approximately 6-h shift compared with that of mRNA, together with dampened amplitudes (129). However, proteomics studies of temporal hepatic expression have revealed that rhythmicity in mRNA does not necessarily result in rhythmicity of protein abundance. On the other hand, many proteins that show rhythmicity in abundance have no corresponding oscillations on the mRNA level, suggesting temporal regulation of translation and posttranslational events (129). Partially, this could be explained by circadian degradation of mRNA and proteins, which determines the half-lives of both mRNA and proteins (130). However, there are also subsets of genes, especially those involved in mitochondrial activity and the translation machinery, that exhibit daily rhythms in translation, which is transcription independent. The first group of genes is translated at the late rest phase and the latter during the midactive phase in mice. These genes have 5′TOP and TISU regulatory elements, and their translation is regulated by the interplay of the molecular clock and feeding rhythms (131). The ability to functionally annotate these proteins points at a specific hepatic physiological role.

Peaks in protein abundance in mouse liver show enrichments at two times, either the midactive or midrest phase (129). Since proteins are effector molecules, this observation potentially points at bimodality of liver functions. Most secreted proteins accumulate rhythmically in liver tissue with a peak in the midactive phase, despite showing flat mRNA levels (129). Another layer of temporal regulation concerns posttranslational modifications, such as phosphorylation. Posttranslational modifications are indispensable for regulation of the stability, degradation, and nuclear-cytoplasmic distribution of proteins (132, 133). Analysis of total versus nuclear hepatic proteomes showed that oscillations in protein abundance are not necessarily mirrored in nuclear protein shuttling, supporting the notion that protein complex formation is vital for temporal programs of nuclear translocation (126).

6.7. Chromatin and Transcription Factor Binding

Work conducted in the late 2000s led to detailed knowledge of time-dependent core clock protein binding to promoters of target genes. BMAL1 and CLOCK bind to thousands of genomic targets, with DNA binding occurring during the resting phase under constant conditions in the mouse liver (109, 110). On the contrary, PER1/2 and CRY2 binding corresponds to the first part of the active phase, whereas CRY1 peaks at the active-to-resting phase transition. Clock proteins bind to promoter, intergenic, and intronic regions (109). REV-ERBα/β, too, have thousands of binding sites, with considerable overlap with BMAL1 (134).

6.8. Epigenetic Marks

The molecular clock affects all levels of temporal organization of hepatic processes, including daily recurring epigenetic changes, such as repeated histone modifications (135), changes in the accessibility of chromatin (136), and oscillations in chromatin topology (137), all of which modulate circadian transcriptional patterns. In addition, DNA occupancy of the RNA polymerase II changes biphasically in the mouse liver, with peak activities in the active and resting phase (135).

7. PATHOPHYSIOLOGY ASSOCIATED WITH IMPAIRED SPATIOTEMPORAL METABOLIC ZONATION

7.1. Monogenic Liver Disease

Important lessons can be learned from hereditary monogenic diseases caused by mutations in spatially compartmentalized metabolic enzymes. Urea cycle disorders (UCDs) are a group of diseases in which key enzymes involved in ammonia detoxification are mutated. Ornithine transcarbamylase deficiency (*Otc* mutations) and citrullinemia (*Ass1* mutations) are examples of urea cycle disorders with mutated genes expressed in periportal hepatocytes. Ornithine aminotransferase deficiency (*Oat* mutations) and ultrarare glutamine synthetase deficiency (*Glul* mutations) are examples of mutations in pericentral metabolic enzymes. UCDs cause severe chronic hyperammonemia, resulting in devastating neurological defects and related signs of lethargy, anorexia, hyper- or hypoventilation, hypothermia, seizures, neurologic posturing, and coma (18, 138, 139). In primary hyperoxaluria type 1 (PH1) (*Agxt* mutations), a dysfunctional periportal alanine-glyoxylate aminotransferase impairs the conversion of glyoxylate to glycine, resulting in excessive accumulation of glyoxylate and its metabolite oxalate, which causes severe systemic symptoms, including kidney stones (18, 140). PH1 is only one out of many examples of rare inborn errors of AA metabolism. Glycogen storage disease type 2 (Pompe disease; *Gaa* mutations) leads to glycogen accumulation in various tissues due to defective periportal acid alpha-glucosidase (an enzyme involved in glycogen degradation), causing myopathy, cardiomyopathy, cardiomegaly, and respiratory distress (141). Together, monogenic diseases highlight not only the importance of the respective enzymes for the spatially compartmentalized metabolic processes they mediate but also how important these vital hepatic processes are to prevent systemic complications. Many patients with chronic liver injury suffer from similar systemic complications (142), as their disease impairs key metabolic processes or causes severe zonated hepatocyte damage.

7.2. NAFLD, NASH, and Metabolic Syndrome

NAFLD is characterized by the accumulation of TGs due to zonal imbalances in FA metabolism, providing a prototype example of impaired production line patterns. Steatosis can result from increased periportal FA uptake or reduced pericentral FA efflux (**Figure 4*a***). Induction of β-oxidation-related genes and increased circulating ketone bodies in NAFLD suggest that reduced β-oxidation is not the major cause for reduced FA efflux. Instead, a limited capacity to secrete very low density lipoproteins may account for TG accumulation in pericentral hepatocytes (54, 59). Consistently, steatosis in adult NAFLD patients mostly occurs in the pericentral zone and then extends into other lobular zones during disease progression (143). Lipotoxicity and resulting inflammation during NAFLD to nonalcoholic steatohepatitis (NASH) progression may be exacerbated in pericentral hepatocytes due to higher oxidative stress in this zone. The detailed underlying mechanisms resulting in the imbalance causing NAFLD, as well as a potential impact of different stages of the disease on spatial liver zonation, remain to be studied.

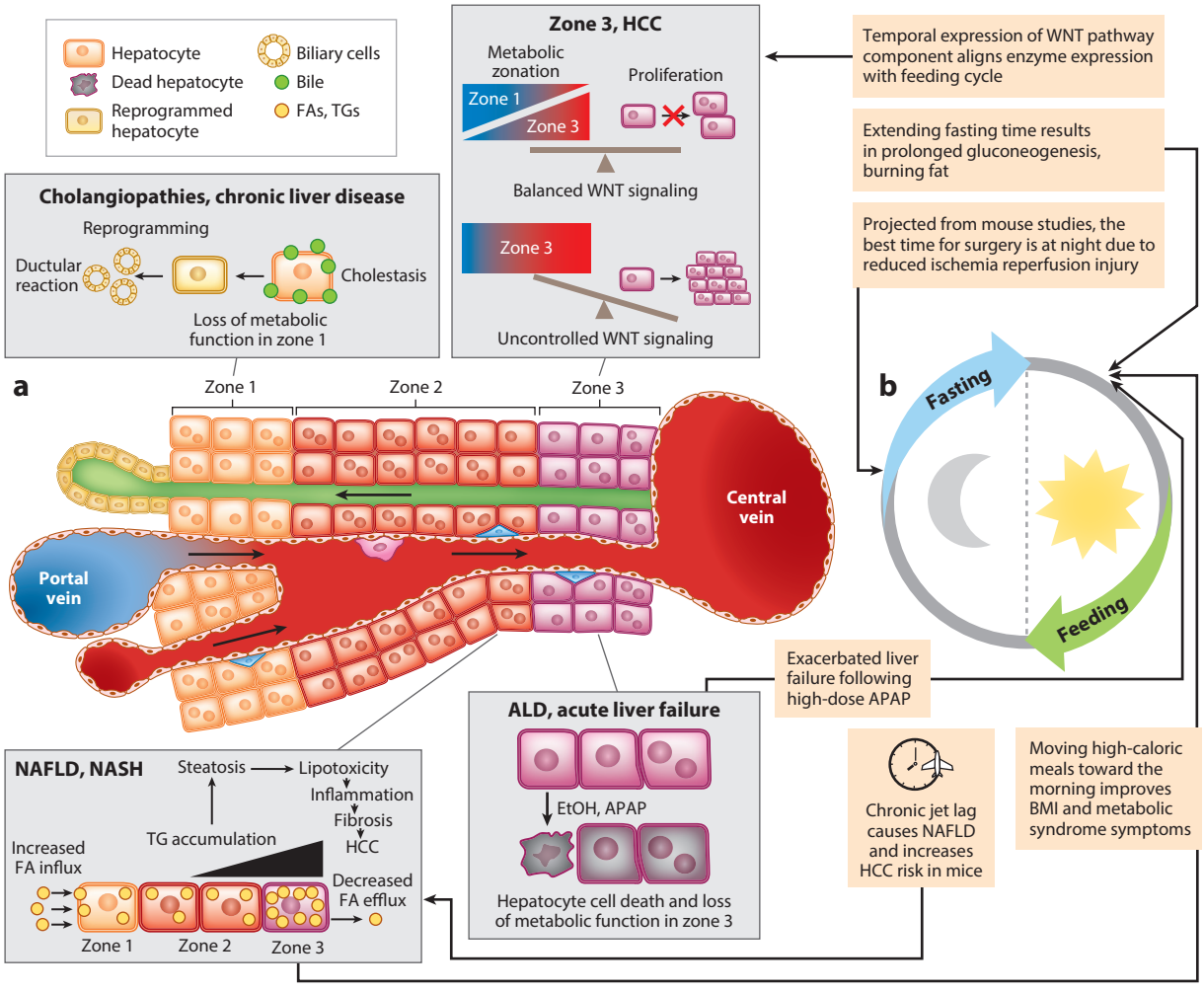

Figure 4

Spatiotemporal zonation and pathophysiology. (*a*) Examples of liver pathologies (*gray*) and their assignment to the lobular zones to which they are related. (*b*) Influence of the circadian rhythm on liver pathology. (*a*,*b*) Cholestasis promotes reprogramming of hepatocytes causing loss of metabolic function. Balancing WNT/β-catenin activity in zone 3 is critical to enable metabolic zonation while preventing unwanted proliferation and HCC formation. Temporal control of WNT/β-catenin signaling aligns pathway activity with the time when the β-catenin-regulated enzymes are required. Spatial imbalances in lipid metabolism, chronic jet lag, and eating high-caloric meals in the evening promote NAFLD/NASH. Extending fasting time prolongs fat burning. ALD and acute toxicities damage pericentral hepatocytes, causing cell death and loss of pericentral metabolic function. APAP toxicity is exacerbated in the beginning of the feeding cycle. Mouse studies suggested lower ischemic injury in the resting period. Abbreviations: ALD, alcoholic liver disease; APAP, acetaminophen; EtOH, ethyl alcohol; FA, fatty acid; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TG, triglyceride.

Moreover, NAFLD is considered to be the hepatic component of metabolic syndrome, in which periportal gluconeogenesis is insufficiently suppressed by insulin, causing hyperinsulinemia and eventually hyperglycemia and type 2 diabetes. Interestingly, pericentral de novo lipogenesis remains sensitive to insulin induction. The resulting coexistence of gluconeogenesis and de novo lipogenesis, causing both hyperglycemia as well as hyperlipidemia in patients with metabolic syndrome, is an interesting example of dysregulation of opposing metabolic tasks (54, 144).

Concerning the metabolic syndrome, there are considerable temporal differences in response to food quality and quantity. In individuals with high body mass, a high-caloric breakfast and reduced intake at dinner appear beneficial in the management of obesity and metabolic syndrome, which is likely intrinsically tied to temporal programs of hepatic metabolism (145). We discussed above that genes involved in processing of food, at least in mouse models, peak at the beginning of the feeding phase, partially explaining this phenomenon (**Figure 4*b***).

We further explained that temporal zonation is vital for normal liver function, therefore disruptions of the diurnal patterns of liver physiology unsurprisingly lead to pathologies (146). Chronic jet lag, with mice aged from 4 to 90 weeks transferred weekly between two rooms, in which the only difference is an 8-h change in the light onset, results in NAFLD at 90 weeks in 96% of WT animals compared with 20% in the control nonjetlagged group. Similarly, the incidence of hepatocellular carcinoma (HCC) also rose upon chronic jet lag (43). These results became even more dramatic in molecular clock KOs or mutants, with substantially increased mortality for WT as well as clock mutants under chronic jet lag, starting before the end of the first year of life (43) (**Figure 4*b***). Deletion of the previously mentioned XBP1 also leads to NAFLD, due to either the absence of the transcription factor or disturbed temporal programs (147).

The aforementioned autonomic innervation, which affects circadian hepatic gene expression, is cut during orthotropic liver transplantation. The procedure can, in the long term, lead to dysregulation of metabolism (38), opening the question of whether this dysregulation happens due to partial desynchrony of temporal processes between different organs. In this case, a strict temporal feeding pattern may help alleviate the condition. In addition, a mouse model of NAFLD shows degeneration of sympathetic innervation in early stages of NAFLD and a collapse during steatohepatitis (38).

7.3. WNT/β-Catenin Signaling and Disease

Given the importance of WNT/β-catenin signaling in controlling spatial liver zonation, deregulation of the pathway in several disease settings disrupts key metabolic processes. The role of the RSPO-LGR4/5-ZNRF3/RNF43 module and WNT/β-catenin signaling in general in NAFLD and NASH remains unclear, with diametrically opposite findings related to the role of the pathway regulating steatosis (reviewed in 148). Both hepatitis B virus (HBV) and hepatitis C virus (HCV) impair spatial metabolic zonation by deregulation of WNT/β-catenin signaling. Hepatocyte transduction and viral replication are higher in pericentral hepatocytes and seem to require WNT/β-catenin activity. HBV and HCV infections further correlate with HCC formation driven by activating mutations in β-catenin (reviewed in 149). WNT/β-catenin signaling promotes hepatocyte proliferation, and activating mutations in the WNT/β-catenin pathway (*CTNNB1* and *AXIN1* mutations in one-quarter to one-third of all HCC cases) are also major drivers in nonviral-associated HCC (150, 151). HCCs with aberrant β-catenin activation are often well differentiated, low proliferative, and associated with cholestasis. Less tumor-associated immune cell abundance also has implications for treatment, since it renders β-catenin-active HCC resistant to immune checkpoint inhibitors (152, 153) and sensitive to mTOR inhibition (154). Especially the *CTNNB1*-mutated HCCs show a pericentral expression pattern with tumor-wide GS expression (154). One possible explanation is the dilemma of the pericentral hepatic niche, which requires constant WNT/β-catenin signaling to maintain metabolic zonation while not requiring extensive hepatocyte proliferation due to their low homeostatic turnover. The liver therefore requires tight control mechanisms enabling pericentral gene expression while preventing unnecessary proliferation (**Figure 4*a***). ZNRF3 and RNF43 negatively regulate WNT/β-catenin signaling, thereby balancing metabolic gene expression and proliferation (21). Panzonal upregulation of RNF43, which is

usually restricted to pericentral hepatocytes, is critical to maintain metabolic zonation and prevent uncontrolled proliferation and tumor formation in response to WNT/β-catenin pathway activation. Combined deletion of ZNRF3/RNF43, or their inhibition via injections of RSPO ligand, reprogrammed periportal hepatocytes into pericentral hepatocytes, resulting in impaired metabolic liver zonation (21). Interestingly, only a subset of hepatocytes proliferate upon ZNRF3/RNF43 deletion, and despite upregulation of WNT/β-catenin signaling in hepatocytes across the lobule in different liver injury models, the expression of GS remained restricted to pericentral hepatocytes (90). However, the mechanisms enabling the WNT/β-catenin pathway to drive hepatocyte proliferation during regeneration while maintaining metabolic zonation remain to be elucidated.

7.4. Alcoholic Liver Disease

Pericentral hepatocytes metabolize alcohol via alcohol dehydrogenase and CYP2E1. Both enzymes convert ethanol into acetaldehyde, but CYP2E1 activity also results in ROS production. The toxicity of ethanol and its metabolites, combined with the increased oxidative stress, results in damage of pericentral hepatocytes (**Figure 4*a***). During chronic progression of the disease, impaired lipid metabolism due to impaired metabolic zonation and function of the pericentral zone results in alcoholic steatohepatitis (155, 156). Similar to other chronic liver disease patients, alcoholic liver disease (ALD) patients suffer from a range of systemic complications including severe cognitive symptoms that already appear in noncirrhotic patients (142) and may be attributed to impaired ammonia detoxification and other processes controlled by pericentral hepatocytes. Persistent liver injury and oxidative stress exacerbate inflammation and fibrosis in pericentral hepatocytes and, together with the carcinogenic action of alcohol metabolites, ultimately promote HCC development. In chronic stages, the disease also extends toward periportal hepatocytes, increasing the range of systemic symptoms by coagulopathy, cachexia, hypoalbuminemia, jaundice, ascites, and other complications, which are related to metabolic processes in the periportal zone (142, 155). In contrast to NAFLD and NASH, which usually follow a linear progression path, ALD can cause often fatal acute-on-chronic liver failure or acute alcoholic hepatitis, depending on the drinking behavior of the patient and other complications.

7.5. Cholangiopathies

Although considered to be mostly a disease of the biliary system, cholangiopathies also impact metabolic liver zonation. Patients with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) frequently suffer from cholestasis, which is the accumulation of cytotoxic bile causing periportal hepatocyte damage (157, 158). As shown in mouse models of cholestatic liver injury, YAP signaling reprograms periportal hepatocytes either into biliary epithelial cells supporting the formation of an auxiliary ductular network draining bile or into progenitor-like cells (102, 159). Either way, reprogrammed hepatocytes largely lose metabolic function, thereby impacting periportal metabolic programs (159) (**Figure 4*a***). PBC and PSC patients frequently suffer from fatigue and associated autonomic dysfunction, as well as vitamin D deficiency (157, 158). How cholangiopathies affect metabolic zonation in patients, and to what extent loss of metabolic function in hepatocytes contributes to disease, remains to be addressed.

7.6. Drug-Induced Liver Injury

Many drugs and xenobiotics are metabolized by CYP450 enzymes, often forming toxic metabolites that damage hepatocytes (71). Therefore, spatial compartmentalization often results in zonated injury, restricted to the hepatocytes expressing the CYP450 enzyme responsible for the toxic metabolite. A well-studied example is acetaminophen-induced toxicity in pericentral hepatocytes,

which accounts for almost half of all acute liver failure incidents. The reason hepatocyte damage is mostly confined to pericentral hepatocytes is that the toxic metabolite is formed by CYP2E1 and CYP3A4. This toxic effect is further aggravated by alcohol-induced expression of CYP2E1, contraindicating the use of acetaminophen while drinking (160). Interestingly, glutathione synthetase shows periportal expression (18), and glutathione is crucial for successful acetaminophen detoxification. Compartmentalization of injury allows hepatocytes in other zones to support regeneration via compensatory proliferation, migration into the injury zone, and metabolic reprogramming in the new compartment (90). When injury is excessive or when metabolic zonation is already perturbed, drug-induced liver injury (DILI) can cause acute liver failure, which is often fatal unless liver transplantation is possible (149). Together, this suggests that metabolic liver zonation combined with high plasticity and regenerative capacity of hepatocytes across all zones protects the liver from DILI.

Interestingly, besides causing primarily pericentral toxicity, the toxic effects of acetaminophen also depend on the time of administration. In fact, due to toxic metabolites, acetaminophen toxicity is highest at times when metabolized at the highest rate. In rodents, this occurs early in the nighttime and corresponds to their active phase; however, this time-dependent toxicity can be dramatically affected by patterns of food intake (161) (**Figure 4*b***).

7.7. Circadian Clock Impact on Liver Regeneration

Partial hepatectomy is a common approach for removal of resectable liver tumors, and it can also be used for live donation of liver. However, it can be accompanied by major complications, and postresectional liver failure often yields fatal complications (162). Meanwhile, SIRT1, controlled by the clock via *Nampt*, displays a crucial role in regeneration and hepatocellular proliferation. In a mouse study of partial hepatectomy, *Sirt1* KO not only resulted in a delay in cell proliferation by interfering with the G0 to G1 cell cycle transition but also transiently enhanced liver steatosis (163), a risk factor for postoperative complications in human patients (162). In addition, absence of CREM, a regulator of outputs from the clock, delays proliferation and results in delayed S-phase entry. However, this gene also seems to be induced upon hepatectomy, potentially compensating the clock-mediated effects on regeneration (164, 165). Thus, the role of the clock in processes following partial hepatectomy is interesting, but questions remain on how to utilize these insights. Partial hepatectomy is a discrete event, which is followed by regeneration occurring on a timescale that allows for multiple repetitive clock-controlled regulation. To our knowledge, there is a lack of studies that take the time of hepatectomy into account.

7.8. Ischemia-Reperfusion Injury

In the mouse liver, *Rev-Erba* KO increases the severity of ischemia-reperfusion injury, accompanied by increased inflammatory responses and elevated serum liver enzymes compared with controls. Surgery at the midactive phase in WT mice, corresponding to low *Rev-Erba* levels, causes more severe liver damage than when performed with half a day shift (166). Since hepatic rhythms in humans are mostly inverted, a beneficial time for performing procedures might speculatively be during the night, which is impractical (**Figure 4*b***). However, human studies in cardiac surgery have already demonstrated dramatic differences in outcomes between morning and afternoon surgeries (167).

7.9. Implications for Treatment

The questions on how to leverage these novel insights into spatiotemporal separation of processes remain. Spatial zonation may have to be considered for gene replacement therapies, since

both viral and lipid-nanoparticle-based gene delivery may promote ectopic enzyme activity in hepatocytes outside of the physiologically assigned zone. While gene correction could maintain spatiotemporal control of the gene locus, episomal promoter-gene constructs may be disconnected from this regulatory process. In addition, maintenance of functional liver zonation needs to be considered for therapies increasing the regenerative potential of hepatocytes and thereby possibly deregulating metabolism. The increased resolution of spatiotemporal metabolic processes in the liver could further highlight avenues for novel treatment concepts, restoring liver zonation and function in patients with chronic liver disease.

Chronobiologists often put forward the notion of chronopharmacology, where gene expression rhythms are taken into consideration when planning the time of administration of a drug to maximize efficacy and minimize unwanted effects. This to some extent challenges the principles of modern drug design, which aims at developing drugs that are robust in terms of pharmacodynamics and pharmacokinetics across a wide array of populations, resulting in a huge buffering capacity of most drugs, which often surpasses the diurnal fluctuations of target abundance. Biological differences, patient noncompliance, and the dysregulation of biological targets in disease add complexity. However, treatment of complex diseases such as cancer could benefit from the time of administration, and chronomodulated therapy was shown to notably improve tolerability of multiple anticancer agents, albeit with limited improvement in efficacy and overall survival (72, 168, 169). However, novel insights point at a very significant extrahepatic success in progression-free and overall survival in chronotherapy with checkpoint inhibitors (170). Concomitantly, circadian disruption in tumors is associated with poor outcomes (169). The lessons learned may be, at least to a certain degree, applied to managing HCC or liver metastases. On the other hand, with the rise in use of biological targeted therapies, such as antibodies, with half-lives often exceeding multiple circadian cycles, the benefit of timed administration seems less obvious.

An alternative route would be to directly target the circadian system, either to affect synchronization of systems or cells, or to affect the amplitude of rhythms. Indeed, multiple molecules that target the molecular clock have been developed (171, 172). Increasing the amplitude of players involved in hepatic regeneration, such as *Sirt1*, could have strong positive effects. Restoring temporal zonation of processes to promote normal physiology seems beneficial. Mitigating hepatic temporal dysregulation in NAFLD and NASH also seems to offer avenues for improved outcomes. There are indications that restoring rhythmicity in neoplastic tissues could be beneficial, especially since the circadian system affects both cell cycle as well as apoptosis (169, 173).

8. CONCLUSIONS AND FUTURE CHALLENGES

Meticulous dissection of spatiotemporal metabolic processes in the liver over the past decades has significantly improved our understanding of many hepatic and systemic diseases. Since most of these studies were performed in rodents, translation of key findings in patient samples will be important. Novel profiling techniques with increasing spatial resolution will also enable us to study how metabolic liver zonation is impacted in different disease settings. Combined with our knowledge from mouse mutants but also monogenic liver disease in patients, we may be able to derive novel treatment concepts aiming at restoring liver function. It is intriguing to see how systemic manifestations in patients with monogenic liver disease, induced by mutations in individual zonated genes, are similar to what patients with chronic liver disease experience, in which hepatocytes in a specific zone are damaged. Future replacement therapies for monogenic diseases might need to take temporal gene expression patterns into consideration to allow for segregation of incompatible processes, as these might lead to unwanted effects. A better understanding of the mechanisms promoting reprogramming of hepatocytes, ultimately resulting in loss of their zonal metabolic assignment, will help to design concepts to restore function of the hepatic factory.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Alan Abrams for providing illustrations and graphic design and Annual Reviews' team of Illustration Editors for editing the figures.

LITERATURE CITED

1. Trefts E, Gannon M, Wasserman DH. 2017. The liver. *Curr. Biol.* 27:R1147–51
2. Han HS, Kang G, Kim JS, Choi BH, Koo SH. 2016. Regulation of glucose metabolism from a liver-centric perspective. *Exp. Mol. Med.* 48:e218
3. Petersen MC, Vatner DF, Shulman GI. 2017. Regulation of hepatic glucose metabolism in health and disease. *Nat. Rev. Endocrinol.* 13:572–87
4. Häussinger D, Lamers WH, Moorman AF. 1992. Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. *Enzyme* 46:72–93
5. Hou Y, Hu S, Li X, He W, Wu G. 2020. Amino acid metabolism in the liver: nutritional and physiological significance. *Adv. Exp. Med. Biol.* 1265:21–37
6. Nguyen P, Leray V, Diez M, Serisier S, Le Bloc'h J, et al. 2008. Liver lipid metabolism. *J. Anim. Physiol. Anim. Nutr.* 92:272–83
7. Meynard D, Babitt JL, Lin HY. 2014. The liver: conductor of systemic iron balance. *Blood* 123:168–76
8. Strnad P, Tacke F, Koch A, Trautwein C. 2017. Liver—guardian, modifier and target of sepsis. *Nat. Rev. Gastroenterol. Hepatol.* 14:55–66
9. Saxena R, Theise ND, Crawford JM. 1999. Microanatomy of the human liver—exploring the hidden interfaces. *Hepatology* 30:1339–46
10. Noël R. 1922. *Recherches histo-physiologiques sur la cellule hépatique des mammifères*. Diss., Univ. de Paris, Masson, Paris
11. Deane HW. 1944. A cytological study of the diurnal cycle of the liver of the mouse in relation to storage and secretion. *Anat. Rec.* 88:39–65
12. Gebhardt R. 1992. Metabolic zonation of the liver: regulation and implications for liver function. *Pharmacol. Ther.* 53:275–354
13. Jungermann K, Katz N. 1982. Functional hepatocellular heterogeneity. *Hepatology* 2:385S–95S
14. Jungermann K, Keitzmann T. 1996. Zonation of parenchymal and nonparenchymal metabolism in liver. *Annu. Rev. Nutr.* 16:179–203
15. Braeuning A, Ittrich C, Köhle C, Hailfinger S, Bonin M, et al. 2006. Differential gene expression in periportal and perivenous mouse hepatocytes. *FEBS J.* 273:5051–61
16. Brosch M, Kattler K, Herrmann A, von Schönfels W, Nordström K, et al. 2018. Epigenomic map of human liver reveals principles of zonated morphogenic and metabolic control. *Nat. Commun.* 9:4150
17. Halpern KB, Shenhav R, Matcovitch-Natan O, Toth B, Lemze D, et al. 2017. Single-cell spatial reconstruction reveals global division of labour in the mammalian liver. *Nature* 542:352–56
18. Droin C, Kholtei JE, Bahar Halpern K, Hurni C, Rozenberg M, et al. 2021. Space-time logic of liver gene expression at sub-lobular scale. *Nat. Metab.* 3:43–58
19. Andrews TS, Atif J, Liu JC, Perciani CT, Ma XZ, et al. 2021. Single-cell, single-nucleus, and spatial RNA sequencing of the human liver identifies cholangiocyte and mesenchymal heterogeneity. *Hepatol. Commun.* 6(4):821–40
20. Hildebrandt F, Andersson A, Saarenpää S, Larsson L, Van Hul N, et al. 2021. Spatial transcriptomics to define transcriptional patterns of zonation and structural components in the mouse liver. *Nat. Commun.* 12:7046
21. Sun T, Annunziato S, Bergling S, Sheng C, Orsini V, et al. 2021. ZNRF3 and RNF43 cooperate to safeguard metabolic liver zonation and hepatocyte proliferation. *Cell Stem Cell* 28:1822–37.e10

22. Gougelet A, Torre C, Veber P, Sartor C, Bachelot L, et al. 2014. T-cell factor 4 and β-catenin chromatin occupancies pattern zonal liver metabolism in mice. *Hepatology* 59:2344–57
23. Ben-Moshe S, Itzkovitz S. 2019. Spatial heterogeneity in the mammalian liver. *Nat. Rev. Gastroenterol. Hepatol.* 16:395–410
24. Truksa J, Lee P, Beutler E. 2007. The role of STAT, AP-1, E-box and TIEG motifs in the regulation of hepcidin by IL-6 and BMP-9: lessons from human HAMP and murine *Hamp1* and *Hamp2* gene promoters. *Blood Cells Mol. Dis.* 39:255–62
25. Chen F, Jimenez RJ, Sharma K, Luu HY, Hsu BY, et al. 2020. Broad distribution of hepatocyte proliferation in liver homeostasis and regeneration. *Cell Stem Cell* 26:27–33.e4
26. He L, Pu W, Liu X, Zhang Z, Han M, et al. 2021. Proliferation tracing reveals regional hepatocyte generation in liver homeostasis and repair. *Science* 371:eabc4346
27. Wei Y, Wang YG, Jia Y, Li L, Yoon J, et al. 2021. Liver homeostasis is maintained by midlobular zone 2 hepatocytes. *Science* 371:eabb1625
28. Kietzmann T. 2017. Metabolic zonation of the liver: the oxygen gradient revisited. *Redox. Biol.* 11:622–30
29. Colnot S, Perret C. 2011. Liver zonation. In *Molecular Pathology of Liver Diseases*, ed. SPS Monga, pp. 7–16. Boston: Springer
30. Dibner C, Schibler U, Albrecht U. 2010. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* 72:517–49
31. Mermet J, Yeung J, Naef F. 2017. Systems chronobiology: global analysis of gene regulation in a 24-hour periodic world. *Cold Spring Harb. Perspect. Biol.* 9(3):a028720
32. Hughes ME, DiTacchio L, Hayes KR, Vollmers C, Pulivarthy S, et al. 2009. Harmonics of circadian gene transcription in mammals. *PLOS Genet.* 5:e1000442
33. Zhu B, Zhang Q, Pan Y, Mace EM, York B, et al. 2017. A cell-autonomous mammalian 12 hr clock coordinates metabolic and stress rhythms. *Cell Metab.* 25:1305–19.e9
34. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. 2001. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat. Neurosci.* 4:1165
35. Kumar Jha P, Challet E, Kalsbeek A. 2015. Circadian rhythms in glucose and lipid metabolism in nocturnal and diurnal mammals. *Mol. Cell. Endocrinol.* 418(Part 1):74–88
36. Stephan FK, Zucker I. 1972. Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *PNAS* 69:1583–86
37. Moore RY, Eichler VB. 1972. Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res.* 42:201–6
38. Adori C, Daraio T, Kuiper R, Barde S, Horvathova L, et al. 2021. Disorganization and degeneration of liver sympathetic innervations in nonalcoholic fatty liver disease revealed by 3D imaging. *Sci. Adv.* 7(30):eabg5733
39. Terazono H, Mutoh T, Yamaguchi S, Kobayashi M, Akiyama M, et al. 2003. Adrenergic regulation of clock gene expression in mouse liver. *PNAS* 100:6795–800
40. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U. 2000. Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 14:2950–61
41. Stephan FK, Swann JM, Sisk CL. 1979. Anticipation of 24-hr feeding schedules in rats with lesions of the suprachiasmatic nucleus. *Behav. Neural Biol.* 25:346–63
42. Le Minh N, Damiola F, Tronche F, Schutz G, Schibler U. 2001. Glucocorticoid hormones inhibit food-induced phase-shifting of peripheral circadian oscillators. *EMBO J.* 20:7128–36
43. Kettner NM, Voicu H, Finegold MJ, Coarfa C, Sreekumar A, et al. 2016. Circadian homeostasis of liver metabolism suppresses hepatocarcinogenesis. *Cancer Cell* 30:909–24
44. Kornmann B, Schaad O, Bujard H, Takahashi JS, Schibler U. 2007. System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. *PLOS Biol.* 5:e34
45. Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S. 2009. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *PNAS* 106:21453–58

46. Weger BD, Gobet C, David FPA, Atger F, Martin E, et al. 2021. Systematic analysis of differential rhythmic liver gene expression mediated by the circadian clock and feeding rhythms. *PNAS* 118(3):e2015803118
47. Chavan R, Feillet C, Costa SS, Delorme JE, Okabe T, et al. 2016. Liver-derived ketone bodies are necessary for food anticipation. *Nat. Commun.* 7:10580
48. Martini T, Ripperger JA, Chavan R, Stumpe M, Netzahualcoyotzi C, et al. 2021. The hepatic monocarboxylate transporter 1 (MCT1) contributes to the regulation of food anticipation in mice. *Front. Physiol.* 12:665476
49. Brown SA, Zumbrunn G, Fleury-Olela F, Preitner N, Schibler U. 2002. Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr. Biol.* 12:1574–83
50. Buhr ED, Yoo SH, Takahashi JS. 2010. Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330:379–85
51. Reinke H, Saini C, Fleury-Olela F, Dibner C, Benjamin IJ, Schibler U. 2008. Differential display of DNA-binding proteins reveals heat-shock factor 1 as a circadian transcription factor. *Genes Dev.* 22:331–45
52. Katz N, Teutsch HF, Jungermann K, Sasse D. 1977. Heterogeneous reciprocal localization of fructose-1,6-bisphosphatase and of glucokinase in microdissected periportal and perivenous rat liver tissue. *FEBS Lett.* 83:272–76
53. Sasse D. 1975. Dynamics of liver glycogen: the topochemistry of glycogen synthesis, glycogen content and glycogenolysis under the experimental conditions of glycogen accumulation and depletion. *Histochemistry* 45:237–54
54. Hijmans BS, Grefhorst A, Oosterveer MH, Groen AK. 2014. Zonation of glucose and fatty acid metabolism in the liver: mechanism and metabolic consequences. *Biochimie* 96:121–29
55. Jungermann K, Kietzmann T. 1997. Role of oxygen in the zonation of carbohydrate metabolism and gene expression in liver. *Kidney Int.* 51:402–12
56. Rudic RD, McNamara P, Curtis AM, Boston RC, Panda S, et al. 2004. BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLOS Biol.* 2:e377
57. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, et al. 2002. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109:307–20
58. Lamia KA, Storch KF, Weitz CJ. 2008. Physiological significance of a peripheral tissue circadian clock. *PNAS* 105:15172–77
59. Schleicher J, Tokarski C, Marbach E, Matz-Soja M, Zellmer S, et al. 2015. Zonation of hepatic fatty acid metabolism—the diversity of its regulation and the benefit of modeling. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1851:641–56
60. Trauner M, Claudel T, Fickert P, Moustafa T, Wagner M. 2010. Bile acids as regulators of hepatic lipid and glucose metabolism. *Digestive Dis.* 28:220–24
61. Ugele B, Kempen H, Gebhardt R, Meijer P, Burger H, Princen H. 1991. Heterogeneity of rat liver parenchyma in cholesterol 7α-hydroxylase and bile acid synthesis. *Biochem. J.* 276:73–77
62. Adamovich Y, Rousso-Noori L, Zwighaft Z, Neufeld-Cohen A, Golik M, et al. 2014. Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. *Cell Metab.* 19:319–30
63. Plakogiannis R, Cohen H. 2007. Optimal low-density lipoprotein cholesterol lowering—morning versus evening statin administration. *Ann. Pharmacother.* 41:106–10
64. Saito Y, Yoshida S, Nakaya N, Hata Y, Goto Y. 1991. Comparison between morning and evening doses of simvastatin in hyperlipidemic subjects. A double-blind comparative study. *Arterioscler. Thromb.* 11:816–26
65. Zmrzljak UP, Rozman D. 2012. Circadian regulation of the hepatic endobiotic and xenobiotic detoxification pathways: the time matters. *Chem. Res. Toxicol.* 25:811–24
66. Martini T, Stojan J, Rozman D, Prosenc Zmrzljak U. 2017. Interaction of PER2 with the constitutive androstane receptor possibly links circadian rhythms to metabolism. *Acta Chim. Slov.* 64:571–76
67. Schmutz I, Ripperger JA, Baeriswyl-Aebischer S, Albrecht U. 2010. The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. *Genes Dev.* 24:345–57
68. Yang X, Downes M, Yu RT, Bookout AL, He W, et al. 2006. Nuclear receptor expression links the circadian clock to metabolism. *Cell* 126:801–10

69. Gooley JJ, Chua EC. 2014. Diurnal regulation of lipid metabolism and applications of circadian lipidomics. *J. Genet. Genom.* 41:231–50
70. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, et al. 2005. Obesity and metabolic syndrome in circadian *Clock* mutant mice. *Science* 308:1043–45
71. Zanger UM, Schwab M. 2013. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacol. Ther.* 138:103–41
72. Cederroth CR, Albrecht U, Bass J, Brown SA, Dyhrfjeld-Johnsen J, et al. 2019. Medicine in the fourth dimension. *Cell Metab.* 30:238–50
73. Gachon F, Firsov D. 2011. The role of circadian timing system on drug metabolism and detoxification. *Expert Opin. Drug Metab. Toxicol.* 7:147–58
74. Gachon F. 2007. Physiological function of PARbZip circadian clock-controlled transcription factors. *Ann. Med.* 39:562–71
75. Gachon F, Olela FF, Schaad O, Descombes P, Schibler U. 2006. The circadian PAR-domain basic leucine zipper transcription factors DBP, TEF, and HLF modulate basal and inducible xenobiotic detoxification. *Cell Metab.* 4:25–36
76. Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, et al. 2008. SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134:317–28
77. Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, et al. 2008. The NAD^+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134:329–40
78. Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, et al. 2009. Circadian clock feedback cycle through NAMPT-mediated NAD^+ biosynthesis. *Science* 324:651–54
79. Chen D, Bruno J, Easlon E, Lin SJ, Cheng HL, et al. 2008. Tissue-specific regulation of SIRT1 by calorie restriction. *Genes Dev.* 22:1753–57
80. Imai S. 2010. "Clocks" in the NAD World: NAD as a metabolic oscillator for the regulation of metabolism and aging. *Biochim. Biophys. Acta Proteins Proteom.* 1804:1584–90
81. Alfieri A, Malito E, Orru R, Fraaije MW, Mattevi A. 2008. Revealing the moonlighting role of NADP in the structure of a flavin-containing monooxygenase. *PNAS* 105:6572–77
82. Eswaramoorthy S, Bonanno JB, Burley SK, Swaminathan S. 2006. Mechanism of action of a flavin-containing monooxygenase. *PNAS* 103:9832–37
83. Quistorff B, Grunnet N. 1987. Dual-digitonin-pulse perfusion. Concurrent sampling of periportal and perivenous cytosol of rat liver for determination of metabolites and enzyme activities. *Biochem. J.* 243:87–95
84. Tosh D, Alberti GM, Agius L. 1988. Glucagon regulation of gluconeogenesis and ketogenesis in periportal and perivenous rat hepatocytes. Heterogeneity of hormone action and of the mitochondrial redox state. *Biochem. J.* 256:197–204
85. Manella G, Asher G. 2016. The circadian nature of mitochondrial biology. *Front. Endocrinol.* 7:162
86. Häussinger D, Schliess F. 2007. Glutamine metabolism and signaling in the liver. *Front. Biosci.* 12:371–91
87. Lin R, Mo Y, Zha H, Qu Z, Xie P, et al. 2017. CLOCK acetylates ASS1 to drive circadian rhythm of ureagenesis. *Mol. Cell* 68:198–209.e6
88. Russell JO, Monga SP. 2018. Wnt/β-catenin signaling in liver development, homeostasis, and pathobiology. *Annu. Rev. Pathol. Mech. Dis.* 13:351–78
89. Yang J, Mowry LE, Nejak-Bowen KN, Okabe H, Diegel CR, et al. 2014. β-Catenin signaling in murine liver zonation and regeneration: a Wnt-Wnt situation! *Hepatology* 60:964–76
90. Sun T, Pikiolek M, Orsini V, Bergling S, Holwerda S, et al. 2020. $AXIN2^+$ pericentral hepatocytes have limited contributions to liver homeostasis and regeneration. *Cell Stem Cell* 26:97–107.e6
91. Ma R, Martinez-Ramirez AS, Borders TL, Gao F, Sosa-Pineda B. 2020. Metabolic and non-metabolic liver zonation is established non-synchronously and requires sinusoidal Wnts. *eLife* 9:e46206
92. Preziosi M, Okabe H, Poddar M, Singh S, Monga SP. 2018. Endothelial Wnts regulate β-catenin signaling in murine liver zonation and regeneration: a sequel to the Wnt-Wnt situation. *Hepatol. Commun.* 2:845–60
93. Rocha AS, Vidal V, Mertz M, Kendall TJ, Charlet A, et al. 2015. The angiocrine factor Rspondin3 is a key determinant of liver zonation. *Cell Rep.* 13:1757–64

94. Benhamouche S, Decaens T, Godard C, Chambrey R, Rickman DS, et al. 2006. Apc tumor suppressor gene is the "zonation-keeper" of mouse liver. *Dev. Cell* 10:759–70
95. Park YK, Park B, Lee S, Choi K, Moon Y, Park H. 2013. Hypoxia-inducible factor-2α-dependent hypoxic induction of Wnt10b expression in adipogenic cells. *J. Biol. Chem.* 288:26311–22
96. Planas-Paz L, Orsini V, Boulter L, Calabrese D, Pikiolek M, et al. 2016. The RSPO-LGR4/5-ZNRF3/RNF43 module controls liver zonation and size. *Nat. Cell Biol.* 18:467–79
97. Matz-Soja M, Aleithe S, Marbach E, Bottger J, Arnold K, et al. 2014. Hepatic Hedgehog signaling contributes to the regulation of IGF1 and IGFBP1 serum levels. *Cell Commun. Signal.* 12:11
98. Braeuning A, Menzel M, Kleinschnitz EM, Harada N, Tamai Y, et al. 2007. Serum components and activated Ha-ras antagonize expression of perivenous marker genes stimulated by β-catenin signaling in mouse hepatocytes. *FEBS J.* 274:4766–77
99. Cheng X, Kim SY, Okamoto H, Xin Y, Yancopoulos GD, et al. 2018. Glucagon contributes to liver zonation. *PNAS* 115:E4111–19
100. Sekine S, Ogawa R, McManus MT, Kanai Y, Hebrok M. 2009. Dicer is required for proper liver zonation. *J. Pathol.* 219:365–72
101. Cox AG, Hwang KL, Brown KK, Evason K, Beltz S, et al. 2016. Yap reprograms glutamine metabolism to increase nucleotide biosynthesis and enable liver growth. *Nat. Cell Biol.* 18:886–96
102. Planas-Paz L, Sun T, Pikiolek M, Cochran NR, Bergling S, et al. 2019. YAP, but not RSPO-LGR4/5, signaling in biliary epithelial cells promotes a ductular reaction in response to liver injury. *Cell Stem Cell* 25:39–53.e10
103. Verboven E, Moya IM, Sansores-Garcia L, Xie J, Hillen H, et al. 2021. Regeneration defects in *Yap* and *Taz* mutant mouse livers are caused by bile duct disruption and cholestasis. *Gastroenterology* 160:847–62
104. Fitamant J, Kottakis F, Benhamouche S, Tian HS, Chuvin N, et al. 2015. YAP inhibition restores hepatocyte differentiation in advanced HCC, leading to tumor regression. *Cell Rep.* 10:1692–707
105. Jin X, Shearman LP, Weaver DR, Zylka MJ, de Vries GJ, Reppert SM. 1999. A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell* 96:57–68
106. Menet JS, Pescatore S, Rosbash M. 2014. CLOCK:BMAL1 is a pioneer-like transcription factor. *Genes Dev.* 28:8–13
107. Ripperger JA, Schibler U. 2006. Rhythmic CLOCK-BMAL1 binding to multiple E-box motifs drives circadian *Dbp* transcription and chromatin transitions. *Nat. Genet.* 38:369–74
108. Ueshima T, Kawamoto T, Honda KK, Noshiro M, Fujimoto K, et al. 2012. Identification of a new clock-related element EL-box involved in circadian regulation by BMAL1/CLOCK and HES1. *Gene* 510:118–25
109. Koike N, Yoo SH, Huang HC, Kumar V, Lee C, et al. 2012. Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* 338:349–54
110. Rey G, Cesbron F, Rougemont J, Reinke H, Brunner M, Naef F. 2011. Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver. *PLOS Biol.* 9:e1000595
111. Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, et al. 2002. Extensive and divergent circadian gene expression in liver and heart. *Nature* 417:78–83
112. Anzulovich A, Mir A, Brewer M, Ferreyra G, Vinson C, Baler R. 2006. *Elovl3*: a model gene to dissect homeostatic links between the circadian clock and nutritional status. *J. Lipid Res.* 47:2690–700
113. Solt LA, Kojetin DJ, Burris TP. 2011. The REV-ERBs and RORs: molecular links between circadian rhythms and lipid homeostasis. *Future Med. Chem.* 3:623–38
114. Guillaumond F, Dardente H, Giguere V, Cermakian N. 2005. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. *J. Biol. Rhythms* 20:391–403
115. Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, et al. 2002. The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110:251–60
116. Chung S, Son GH, Kim K. 2011. Circadian rhythm of adrenal glucocorticoid: its regulation and clinical implications. *Biochim. Biophys. Acta Mol. Basis Dis.* 1812:581–91

117. Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, et al. 2006. The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab.* 4:163–73
118. Oki S, Ohta T, Shioi G, Hatanaka H, Ogasawara O, et al. 2018. ChIP-Atlas: a data-mining suite powered by full integration of public ChIP-seq data. *EMBO Rep.* 19(12):e46255
119. Lamia KA, Papp SJ, Yu RT, Barish GD, Uhlenhaut NH, et al. 2011. Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* 480:552–56
120. Christoffels VM, Grange T, Kaestner KH, Cole TJ, Darlington GJ, et al. 1998. Glucocorticoid receptor, C/EBP, HNF3, and protein kinase A coordinately activate the glucocorticoid response unit of the carbamoylphosphate synthetase I gene. *Mol. Cell. Biol.* 18:6305–15
121. Grygiel-Gorniak B. 2014. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications—a review. *Nutr. J.* 13:17
122. Liu C, Li S, Liu T, Borjigin J, Lin JD. 2007. Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. *Nature* 447:477–81
123. Gerber A, Esnault C, Aubert G, Treisman R, Pralong F, Schibler U. 2013. Blood-borne circadian signal stimulates daily oscillations in actin dynamics and SRF activity. *Cell* 152:492–503
124. Kobayashi M, Morinibu A, Koyasu S, Goto Y, Hiraoka M, Harada H. 2017. A circadian clock gene, PER2, activates HIF-1 as an effector molecule for recruitment of HIF-1α to promoter regions of its downstream genes. *FEBS J.* 284:3804–16
125. Manella G, Aviram R, Bolshette N, Muvkadi S, Golik M, et al. 2020. Hypoxia induces a time- and tissue-specific response that elicits intertissue circadian clock misalignment. *PNAS* 117:779–86
126. Wang J, Symul L, Yeung J, Gobet C, Sobel J, et al. 2018. Circadian clock-dependent and -independent posttranscriptional regulation underlies temporal mRNA accumulation in mouse liver. *PNAS* 115:E1916–25
127. El-Athman R, Fuhr L, Relogio A. 2018. A systems-level analysis reveals circadian regulation of splicing in colorectal cancer. *EBioMedicine* 33:68–81
128. Preussner M, Wilhelmi I, Schultz AS, Finkernagel F, Michel M, et al. 2014. Rhythmic *U2af26* alternative splicing controls PERIOD1 stability and the circadian clock in mice. *Mol. Cell* 54:651–62
129. Mauvoisin D, Wang J, Jouffe C, Martin E, Atger F, et al. 2014. Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. *PNAS* 111:167–72
130. Luck S, Thurley K, Thaben PF, Westermark PO. 2014. Rhythmic degradation explains and unifies circadian transcriptome and proteome data. *Cell Rep.* 9:741–51
131. Atger F, Gobet C, Marquis J, Martin E, Wang J, et al. 2015. Circadian and feeding rhythms differentially affect rhythmic mRNA transcription and translation in mouse liver. *PNAS* 112:E6579–88
132. Gallego M, Virshup DM. 2007. Post-translational modifications regulate the ticking of the circadian clock. *Nat. Rev. Mol. Cell Biol.* 8:139–48
133. Robles MS, Humphrey SJ, Mann M. 2017. Phosphorylation is a central mechanism for circadian control of metabolism and physiology. *Cell Metab.* 25:118–27
134. Cho H, Zhao X, Hatori M, Yu RT, Barish GD, et al. 2012. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. *Nature* 485:123–27
135. Le Martelot G, Canella D, Symul L, Migliavacca E, Gilardi F, et al. 2012. Genome-wide RNA polymerase II profiles and RNA accumulation reveal kinetics of transcription and associated epigenetic changes during diurnal cycles. *PLOS Biol.* 10:e1001442
136. Sobel JA, Krier I, Andersin T, Raghav S, Canella D, et al. 2017. Transcriptional regulatory logic of the diurnal cycle in the mouse liver. *PLOS Biol.* 15:e2001069
137. Mermet J, Yeung J, Hurni C, Mauvoisin D, Gustafson K, et al. 2018. Clock-dependent chromatin topology modulates circadian transcription and behavior. *Genes Dev.* 32:347–58
138. Ah Mew N, Simpson KL, Gropman AL, Lanpher BC, Chapman KA, Summar ML. 2003. Urea cycle disorders overview. In *GeneReviews*, ed. MP Adam, HH Ardinger, RA Pagon, SE Wallace, LJH Bean, et al. Seattle, WA: University of Washington (updated June 22, 2017)
139. Spodenkiewicz M, Diez-Fernandez C, Rufenacht V, Gemperle-Britschgi C, Haberle J. 2016. Minireview on glutamine synthetase deficiency, an ultra-rare inborn error of amino acid biosynthesis. *Biology* 5(4):40

140. Wang X, Danese D, Brown T, Baldwin J, Sajeev G, et al. 2021. Primary hyperoxaluria type 1 disease manifestations and healthcare utilization: a multi-country, online, chart review study. *Front. Med.* 8:703305
141. van der Ploeg AT, Reuser AJ. 2008. Pompe's disease. *Lancet* 372:1342–53
142. Newton JL, Jones DEJ. 2012. Managing systemic symptoms in chronic liver disease. *J. Hepatol.* 56:S46–55
143. Brunt EM. 2010. Pathology of nonalcoholic fatty liver disease. *Nat. Rev. Gastroenterol. Hepatol.* 7:195–203
144. Schwarz J-M, Linfoot P, Dare D, Aghajanian K. 2003. Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. *Am. J. Clin. Nutr.* 77:43–50
145. Jakubowicz D, Barnea M, Wainstein J, Froy O. 2013. High caloric intake at breakfast versus dinner differentially influences weight loss of overweight and obese women. *Obesity* 21:2504–12
146. Maury E, Ramsey KM, Bass J. 2010. Circadian rhythms and metabolic syndrome: from experimental genetics to human disease. *Circ. Res.* 106:447–62
147. Meng H, Gonzales NM, Lonard DM, Putluri N, Zhu B, et al. 2020. XBP1 links the 12-hour clock to NAFLD and regulation of membrane fluidity and lipid homeostasis. *Nat. Commun.* 11:6215
148. Annunziato S, Sun T, Tchorz JS. 2022. The RSPO-LGR4/5-ZNRF3/RNF43 module in liver homeostasis, regeneration, and disease. *Hepatology* 76(3):888–99
149. Panday R, Monckton CP, Khetani SR. 2022. The role of liver zonation in physiology, regeneration, and disease. *Semin. Liver Dis.* 42:1–16
150. Boyault S, Rickman DS, de Reynies A, Balabaud C, Rebouissou S, et al. 2007. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. *Hepatology* 45:42–52
151. Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, et al. 2009. Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular carcinoma. *Cancer Res.* 69:7385–92
152. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, et al. 2019. Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. *Clin. Cancer Res.* 25:2116–26
153. Ruiz de Galarreta M, Bresnahan E, Molina-Sanchez P, Lindblad KE, Maier B, et al. 2019. β-Catenin activation promotes immune escape and resistance to anti-PD-1 therapy in hepatocellular carcinoma. *Cancer Discov.* 9:1124–41
154. Adebayo Michael AO, Ko S, Tao J, Moghe A, Yang H, et al. 2019. Inhibiting glutamine-dependent mTORC1 activation ameliorates liver cancers driven by β-catenin mutations. *Cell Metab.* 29:1135–50.e6
155. Avila MA, Dufour JF, Gerbes AL, Zoulim F, Bataller R, et al. 2020. Recent advances in alcohol-related liver disease (ALD): summary of a *Gut* round table meeting. *Gut* 69:764–80
156. Doody EE, Groebner JL, Walker JR, Frizol BM, Tuma DJ, et al. 2017. Ethanol metabolism by alcohol dehydrogenase or cytochrome P450 2E1 differentially impairs hepatic protein trafficking and growth hormone signaling. *Am. J. Physiol. Gastrointest. Liver Physiol.* 313:G558–69
157. Karlsen TH, Folseraas T, Thorburn D, Vesterhus M. 2017. Primary sclerosing cholangitis—a comprehensive review. *J. Hepatol.* 67:1298–323
158. Trauner M, Meier PJ, Boyer JL. 1998. Molecular pathogenesis of cholestasis. *N. Engl. J. Med.* 339:1217–27
159. Li W, Yang L, He Q, Hu C, Zhu L, et al. 2019. A homeostatic Arid1a-dependent permissive chromatin state licenses hepatocyte responsiveness to liver-injury-associated YAP signaling. *Cell Stem Cell* 25:54–68.e5
160. Anundi I, Lahteenmaki T, Rundgren M, Moldeus P, Lindros KO. 1993. Zonation of acetaminophen metabolism and cytochrome P450 2E1-mediated toxicity studied in isolated periportal and perivenous hepatocytes. *Biochem. Pharmacol.* 45:1251–59
161. DeBruyne JP, Weaver DR, Dallmann R. 2014. The hepatic circadian clock modulates xenobiotic metabolism in mice. *J. Biol. Rhythms* 29:277–87
162. van den Broek MA, Olde Damink SW, Dejong CH, Lang H, Malago M, et al. 2008. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int.* 28:767–80

163. Bellet MM, Masri S, Astarita G, Sassone-Corsi P, Della Fazia MA, Servillo G. 2016. Histone deacetylase SIRT1 controls proliferation, circadian rhythm, and lipid metabolism during liver regeneration in mice. *J. Biol. Chem.* 291:23318–29
164. Foulkes NS, Borjigin J, Snyder SH, Sassone-Corsi P. 1996. Transcriptional control of circadian hormone synthesis via the CREM feedback loop. *PNAS* 93:14140–45
165. Servillo G, Della Fazia MA, Sassone-Corsi P. 1998. Transcription factor CREM coordinates the timing of hepatocyte proliferation in the regenerating liver. *Genes Dev.* 12:3639–43
166. Lin Y, Lin L, Gao L, Wang S, Wu B. 2020. Rev-erbα regulates hepatic ischemia-reperfusion injury in mice. *Biochem. Biophys. Res. Commun.* 529:916–21
167. Montaigne D, Marechal X, Modine T, Coisne A, Mouton S, et al. 2018. Daytime variation of perioperative myocardial injury in cardiac surgery and its prevention by Rev-Erbα antagonism: a single-centre propensity-matched cohort study and a randomised study. *Lancet* 391:59–69
168. Innominato PF, Roche VP, Palesh OG, Ulusakarya A, Spiegel D, Levi FA. 2014. The circadian timing system in clinical oncology. *Ann. Med.* 46:191–207
169. Levi F, Okyar A, Dulong S, Innominato PF, Clairambault J. 2010. Circadian timing in cancer treatments. *Annu. Rev. Pharmacol. Toxicol.* 50:377–421
170. Karaboue A, Collon T, Pavese I, Bodiguel V, Cucherousset J, et al. 2022. Time-dependent efficacy of checkpoint inhibitor nivolumab: results from a pilot study in patients with metastatic non-small-cell lung cancer. *Cancers* 14(4):896
171. Miller S, Hirota T. 2020. Pharmacological interventions to circadian clocks and their molecular bases. *J. Mol. Biol.* 432:3498–514
172. Rahman S, Wittine K, Sedic M, Markova-Car EP. 2020. Small molecules targeting biological clock; a novel prospective for anti-cancer drugs. *Molecules* 25(21):4937
173. Iurisci I, Filipski E, Reinhardt J, Bach S, Gianella-Borradori A, et al. 2006. Improved tumor control through circadian clock induction by Seliciclib, a cyclin-dependent kinase inhibitor. *Cancer Res.* 66:10720–28