

## Chapter 2

# Liver Zonation

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### Introduction

Maintenance of liver homeostasis relies on the metabolic function of this organ. To carry out these metabolic functions at a maximal possible efficiency, hepatocytes are both quiescent and highly specialized. They specialize as a function based on their position along the porto-central axis of the liver lobule that determines their fate as either “periportal” (PP), or “perivenous” (PV) hepatocytes. This zonation of function mainly affects ammonia detoxification, glucose/energy metabolism, and xenobiotic metabolism. Over the last 30 years, since the initial discovery of liver zonation, the mechanisms by which this zonation is established and maintained have been widely investigated. The Wnt/ $\beta$ (beta)-catenin developmental pathway has been recently shown to play a key role in this functional heterogeneity of mouse hepatocytes. It is activated in perivenous hepatocytes, partly due to the absence, in the perivenous area, of adenomatous polyposis coli (APC), a tumor suppressor gene product. APC is a negative regulator of Wnt signaling, also described as the “zonation-keeper” of the liver lobule. The Wnt pathway induces the PV genetic program and represses the PP genetic program. The ras/mapk/erk pathway acts in a reciprocal manner to counterbalance Wnt signaling and favors a PP genetic program. More recently, a cross-talk between the transcription factor Hnf4 $\alpha$ (alpha) and Wnt signaling has been proposed as a potential mechanism of liver zonation.

### The Liver Lobule, the Zonated Unit of the Liver

The liver occupies a strategic position for efficient overall metabolic function in the body. As described in Chap. 1, it receives its supply of hydrophilic nutrients through the portal

vein; these nutrients are absorbed by the intestine. It then delivers metabolized products to the other organs through the central vein. The hepatic artery located in the vicinity of the portal vein, within the portal triad (composed of the bile duct, portal vein, and hepatic artery), supplies the liver with blood enriched in oxygen. Blood flow within the liver determines the organization of the anatomical unit of the hepatic parenchyma, the liver cell plate, which is located within the liver lobule or acinus. Here, the blood flows from the portal vein and the hepatic artery (in a 75–25% ratio) to the centrilobular vein, while bile moves from the pericentral area to the periportal one. The liver cell plate consists of 15–25 hepatocytes that extend from the portal triad to the hepatic venule. This structure carries out metabolic functions mostly through specialized hepatocytes, which act in isolation, or together with non parenchymal cells (Fig. 2.1).

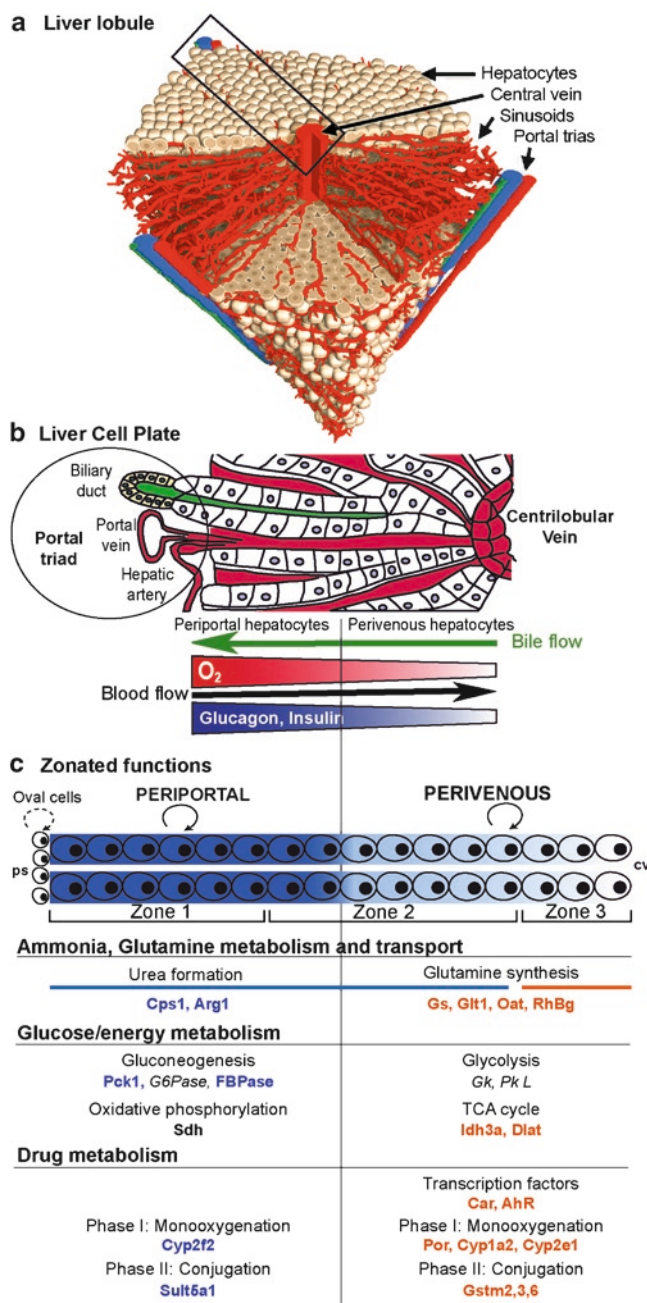
Thirty years ago, K. Jungermann demonstrated that hepatocytes, although being histologically indistinguishable, were specialized, and their function differed depending on their position along the porto-central axis of the liver cell plate [1]. Nearly six to eight PP hepatocytes (zone 1) surround the portal triad and are in close contact with the afferent blood. Nearly two to three PV hepatocytes (zone 3) are found close to the efferent centrilobular vein. A less well defined midlobular population of six to ten hepatocytes (zone 2) has also been described. Jungermann proposed the concept of “zonation.” According to this concept, opposing or complementary metabolic pathways are carried out within distinct non-overlapping regions of the liver lobule to maintain optimal metabolic homeostasis [2].

### Metabolic Zonal Functions

Not all hepatic processes are strictly zonal. The synthesis of large amounts of serum proteins, such as the transthyretin and transferrin transporters appears to occur in all hepatocytes. Albumin is also synthesized in all hepatocytes, with a higher concentration in the periportal area. The most studied zonated functions currently consist of glucose metabolism,

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**Fig. 2.1** Structure and functions of the zoned liver lobule. (a) Three-dimensional structure of the liver lobule. The liver lobule is centered around a branch of the centrilobular vein, limited at each end by the portal triad consisting of a branch of the portal vein, the hepatic artery, and a bile duct. The sinusoids and the vasculature are depicted in red (Hoehme et. al. [61]) (b) The liver cell plate, with blood circulation indicated in red. Bile is shown in green and circulates in the opposite direction to blood. The concentration of oxygen and hormones decreases along a continuous gradient from the PP area to the PV area. (c) Zonal functions. The proliferation of hepatocytes is achieved mostly by division of the mature hepatocytes themselves (circled arrow), which do not migrate along the portocentral axis, with proliferation from oval cells observed only rarely (dotted circled arrow). The zoned metabolic systems include the ammonia detoxification system, glucose and energy metabolism, and xenobiotic metabolism. The proteins involved in each type of zonal metabolism are indicated.

ammonia detoxification, and the metabolism of drugs and xenobiotics (Fig. 2.1). Other zoned processes include: lipid metabolism, with lipogenesis occurring perivenously and fatty-acid degradation periportally [2, 3]; Cyp7a1-mediated synthesis of bile acids derived from cholesterol, showing clear PV zonation [3, 4]; and the metabolism of several amino acids [3], the catabolism of histidine and serine being mostly periportal, and glutamine synthesis (associated with ammonia detoxification) being perivenous.

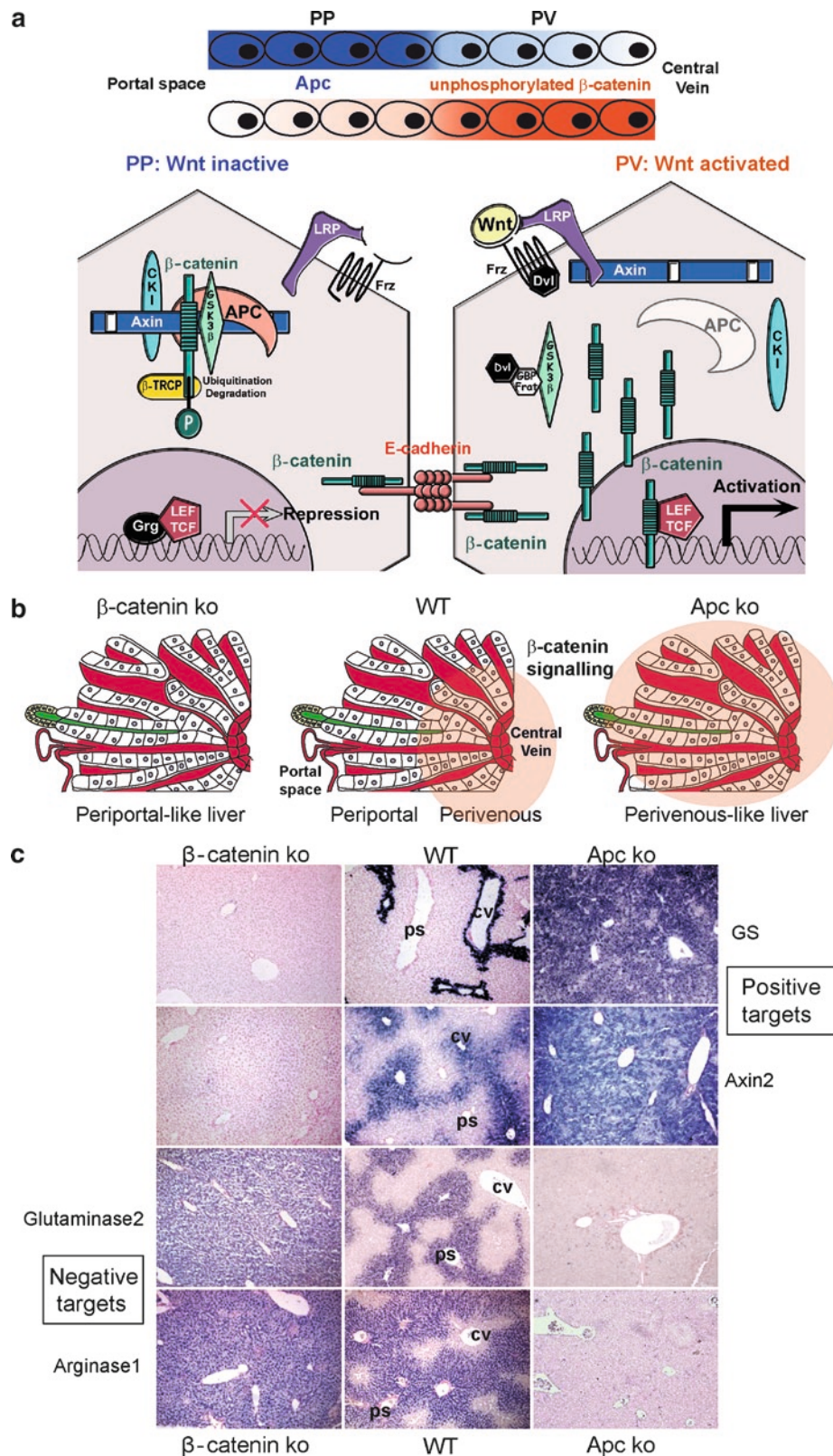
*Glucose metabolism* provides a historical example of compartmentalized metabolism. Jungermann showed that gluconeogenesis was mostly periportal, with gradual accumulation of phosphoenolpyruvate carboxykinase (Pepck1) in this region, whereas glycolysis was mostly perivenous (glucokinase and pyruvate kinase L, but not their respective RNAs, being perivenous) [1]. However, the concentration gradients of these enzymes differ depending on nutritional status, suggesting that nutrients and hormones play a role in the zonation of these components of glucose metabolism. Please see Chap. 8 for a more detailed review of carbohydrate metabolism in the liver.

*Ammonia detoxification* is subject to zonation and has been extensively studied by groups led by Gebhardt, Lamers, and Haussinger [5–7]. One of the major roles of the liver is the removal of harmful ammonia arriving from the intestine via the portal vein (Fig. 2.2). Ammonia is first metabolized by PP hepatocytes, through a high-capacity/low-affinity system, to generate urea. This involves the enzymes carbamoyl-phosphate synthetase (Cps1) and arginase 1 (Arg1). Residual ammonia is then converted to glutamine by perivenous hepatocytes, through a low-capacity/high-affinity system involving the perivenous enzyme glutamine synthetase (Gs). Chapter 9 discusses protein metabolism.

*Metabolism of drugs and xenobiotics* also displays well defined zonation, occurring mostly in the PV area. The cytochrome P450 system is responsible for the conversion of xenobiotics into excretable products. This involves monooxygenation followed by conjugation with either glucuronic or sulfuric acid. Monooxygenation mainly occurs in the PV zone, with glucuronidation as the major conjugation reaction in these cells, whereas sulfation is the predominant conjugation reaction in PP cells [2]. Chapter 11 discusses detoxification functions of the liver.

The proteins displaying zonation regulated at the posttranscriptional level are shown in italics. The PV positive Wnt targets are shown in orange, and the PP negative targets are shown in blue. These targets were identified by microarray analysis on PV and PP hepatocytes [3], and on  $\beta$ (beta)-catenin-activated hepatocytes ([12] and unpublished data). *Gk* glucokinase; *PkL* liver-specific pyruvate kinase; *Sdh* succinate dehydrogenase; *Idh3a* isocitrate dehydrogenase 3a; *Dlat* dihydro-lipoamide S-acetyltransferase; *Gstm* glutathione S-transferase mu; *Sult5a1* sulfotransferase family 5A, member 1





**Fig. 2.2** Zonation of Wnt signaling in the liver. **(a)** Localization of Wnt partners. PP hepatocytes are enriched in APC, allowing the accumulation of active unphosphorylated  $\beta$ (beta)-catenin in PV hepatocytes. A schematic diagram of a PP hepatocyte in which Wnt is inactive is shown on the left, and the consequences of Wnt activation in a PV hepatocyte are shown on the right. **(b)** Mouse models of liver-specific  $\beta$ (beta)-catenin inactivation ( $\beta$ (beta)-catenin ko) or activation

(Apc ko). **(c)** In situ hybridization showing the distribution of PV positive target gene expression and of PP negative target gene expression along the portocentral axis of the liver, in  $\beta$ (beta)-catenin-null, wild-type, and APC-null livers. The Axin2 gene is a universal target gene of  $\beta$ (beta)-catenin, and the signal generated for this gene on in situ hybridization is thought to correspond to the area of  $\beta$ (beta)-catenin activation

## Possible Mechanisms for Zonation

Until recently, the mechanisms underlying zonation were poorly understood even if a number of hypotheses have been put forward.

*The developmental hypothesis* suggests that periportal and perivenous hepatocytes are derived from different embryonic origins, and distinct lineages. However, evidence for this is lacking; indeed, the perinatal liver is not zoned and coexpresses perivenous and periportal mRNAs, such as those encoding Gs, and Cps1 [8]. Zonation is only observed in the first week following birth in mice.

*The streaming liver theory* is based on the observation that periportal hepatocytes are more prone to proliferate (see section on proliferation). According to this model, hepatocytes are derived from the periportal area, where putative hepatic stem cells reside. Hepatocytes then migrate further along the portocentral axis to become perivenous, gaining a particular metabolic profile as they mature. However, cell-tracing studies have shown this not to be the case; instead, hepatocyte renewal seems to occur in both parts of the lobule [9].

*The blood hypothesis* offers a more feasible explanation, with aspects still to be explored. Blood entering the sinusoid is a mixture of blood from the hepatic artery and portal vein, and is rich in oxygen. The low oxygen tension in the hepatic venules [10] thus gives rise to a steep oxygen gradient across the sinusoid. Blood then perfuses hepatocytes in the plate sequentially. This means that the composition of blood changes as hepatocytes in the plate are perfused. Thus, hepatocytes located in different parts of the liver cell plate are exposed to different microenvironments. Accordingly, in this model, zonation may be determined by the concentrations of oxygen, hormones, drugs, or metabolites in the blood. However, changes in the hormone or oxygen content of afferent blood reverses the patterns of glycolysis and gluconeogenesis, and such changes do not affect ammonia detoxification [2]. This led to the definition of “dynamic zonal metabolism,” which can be applied to the somewhat plastic processes of glucose and drug metabolism. By contrast, stable zonal metabolic systems, such as ammonia detoxification, cannot easily be reversed or lost.

*Zonation is subjected to transcriptional control.* Nevertheless, functional zonation is mainly controlled by differential expression of genes encoding the enzymes responsible for the functions concerned. This control may involve transcriptional or posttranscriptional regulation along the portocentral axis (Fig. 2.1). Transcriptional mechanisms seem to be crucial, as shown by microarray studies. These studies characterized mRNAs from the PP and PV hepatocytes, and confirmed that zonation of glucose, ammonia, and drug metabolism was mainly under the control of mRNA levels [3].

The identification of the PV glutamine synthetase gene as a direct target of  $\beta$ (beta)-catenin in liver suggested that  $\beta$ (beta)-catenin may be one of the *trans*-acting factors mediating liver zonation [11]. Studies of murine models with  $\beta$ (beta)-catenin signaling activated or inactivated in the liver have shown that the  $\beta$ (beta)-catenin pathway is one critical aspect involved in liver zonation [12–14].

## The Wnt/ $\beta$ (Beta)-Catenin Pathway

The Wnt/ $\beta$ (beta)-catenin pathway, which has been strongly conserved through evolution, plays an important role in development and has been implicated in tumorigenesis in various tissues [15]. A detailed account on this pathway is available in Chap. 20. Wnt signaling is initiated by the binding of secreted ligands (Wnts) to frizzled receptors (fz), leading to the activation of canonical or non-canonical signaling. Canonical signaling activates  $\beta$ (beta)-catenin-mediated gene transcription [16] (Fig. 2.2). In the absence of Wnt signaling,  $\beta$ (beta)-catenin plays a role in cell adhesion, through interaction with cadherins.  $\beta$ (beta)-catenin is kept at low concentrations in the cytosol through its phosphorylation by CK1 and GSK3 kinases, within a degradation complex comprising two tumor suppressors, APC and axin. Phosphorylated  $\beta$ (beta)-catenin is ubiquitinated by  $\beta$ (beta)-TrCP, resulting in it being targeted for degradation by the proteasome. Wnt binding to fz receptors together with LRP5/6 coreceptor activation, causes the dissociation of the  $\beta$ (beta)-catenin degradation complex; unphosphorylated  $\beta$ (beta)-catenin then accumulates and is translocated into the nucleus, where it associates with Lef/Tcf transcription factors to regulate the transcription of its target genes.

The Wnt pathway was initially described for its role in developmental processes, but has also been implicated in the maintenance of stem-cell compartments in adults [17]. These physiological, developmental, and oncogenic effects are mediated by the regulation of different genetic programs, depending on the temporal and spatial contexts.

## $\beta$ (Beta)-Catenin, A Master Signaling Molecule Orchestrating Metabolic Zonation in the Liver

The link between the Wnt pathway and the liver was initially established through the demonstration that  $\beta$ (beta)-catenin-activating mutations frequently occur during liver carcinogenesis [18]. These mutations define a particular carcinogenic route. Indeed, tumors in which  $\beta$ (beta)-catenin is activated display a specific transcriptome, which strongly favors PV

metabolism [13, 19–21]. More recent work has shown this signaling pathway to play a role in liver development and physiology, with a major role in determining the final patterning of the adult liver [12].

Initial observations suggesting a role for  $\beta$ (beta)-catenin in establishing liver zonation was based on the complementary distribution patterns of active  $\beta$ (beta)-catenin in PV hepatocytes and of the negative regulator APC in PP hepatocytes, in wild-type adult mice [12] (Fig. 2.2). Mice were then generated, in which APC was inactivated specifically in the liver. The subsequent activation of  $\beta$ (beta)-catenin signaling in these mice induced a PV genetic program throughout the entire lobule and repressed the PP genetic program. These findings were confirmed in mice producing Dkk1, a Wnt inhibitor, or with inactivation of  $\beta$ (beta)-catenin gene expression in the liver lobule. The PV genetic program was switched off in these mice, whereas the PP genetic program was active throughout the lobule [12, 14, 22] (Fig. 2.2).

The zonal metabolic pathways affected by changes in  $\beta$ (beta)-catenin signaling include those mediating ammonia metabolism potentially explaining the hyperammonemia observed in mice with aberrant hepatocytic  $\beta$ (beta)-catenin signaling.  $\beta$ (beta)-catenin exerts strict positive control over the PV genes encoding glutamine synthetase (Gs, or glul), Glt-1 (Eaat2 or slc1a2; a transporter of glutamate), and RhBg, a transporter of ammonia. It negatively regulates the glutaminase 2 (Gls2), arginase 1 (Arg1), and carbamoyl-phosphate synthase (Cps1) PP genes with Arg1 and Cps1 being key enzymes of the urea cycle [11, 12] (Figs. 2.1 and 2.2).

Drug metabolism is strongly controlled by the Wnt pathway. A study by Hebrok and colleagues demonstrated that mice with specific deletion of  $\beta$ (beta)-catenin in the liver were insensitive to intoxication with carbon tetrachloride ( $\text{CCl}_4$ ), presumably due to the absence of CypP450-detoxifying enzymes in these mice [14]. Wnt signaling controls the expression of two major CypP450 enzymes, Cyp2e1 which metabolizes ethanol, and Cyp1a2 [13, 14, 23]. The aryl-hydrocarbon receptor (Ahr) and the constitutive androstane receptor (Car) are two PV proteins that act as both xenosensors and as transcription factors. They control the expression of drug-metabolizing enzymes induced by  $\beta$ (beta)-catenin in the liver [13, 14, 23]. Whether  $\beta$ (beta)-catenin signaling has a direct effect on the transcription of detoxifying enzymes or whether it acts indirectly involving Car and Ahr is yet unclear.

Mice with liver-targeted activation of the Wnt pathway have altered glucose metabolism and develop hypoglycemia [24]. This hypoglycemia may be caused by impaired gluconeogenesis, a PP process, through the negative regulation of the Pepck and FBPase genes in  $\beta$ (beta)-catenin-activated livers [12, 24].  $\beta$ (beta)-catenin signaling may also modify the energetic profile of the hepatocyte. In APC null livers, ATP

energy supply seems to be provided through glycolysis rather than through mitochondrial oxidative phosphorylation due to the upregulation of lactate dehydrogenase protein and activity, together with the downregulation of two mitochondrial subunits ATP5 $\alpha$ (alpha)1 and ATP5 $\beta$ (beta) by  $\beta$ (beta)-catenin [24]. The potential zonation of these new  $\beta$ (beta)-catenin targets remains to be investigated.

These data clearly demonstrate that  $\beta$ (beta)-catenin signaling is a key pathway in the control of liver metabolism. APC appears to be the “zonation-keeper” of the liver, consistent with its specific distribution along the portocentral axis of the lobule and the major effects of its inactivation in mice.

## The Consequences of Disrupting Zonation

The effects of activation of  $\beta$ (beta)-catenin signaling differ from the effects of loss of  $\beta$ (beta)-catenin signaling in mice.  $\beta$ (beta)-Catenin-null livers become periportal-like, but with no major effect on phenotype; however, APC-null livers, become perivenous-like, leading to death of the animals, mainly through lethal hyperammonemia arising from the lack of urea-cycle enzymes [12] (Fig. 2.2).

The fact that mice with loss of  $\beta$ (beta)-catenin signaling in the liver are viable implies that the perivenous functions of the hepatocytes are not essential for normal liver homeostasis. These mice are only more sensitive to nutritional dysfunction, with a protein overload leading to mild hyperammonemia, due to defects in perivenous enzymes and ammonia transporters, presumably including GS and RhBg [14]. This suggests that the perivenous genetic program is a scavenger system, which is not required for basal metabolism, but is activated in certain contexts. By contrast, mice with  $\beta$ (beta)-catenin activation in all the hepatocytes of the liver lobule rapidly die. Whether this is due to an essential role for periportal proteins in basal metabolism necessary for life, or to the disruption of liver homeostasis caused by aberrant expression of perivenous proteins throughout the lobule, remains unknown.

## Establishment of Liver Zonation by $\beta$ (Beta)-Catenin and Wnts

The role of  $\beta$ (beta)-catenin during liver development remains a matter of debate.  $\beta$ (beta)-catenin may play a limited role in hepatogenesis. Hepatogenesis occurs as a multistep process during embryonic development, beginning with the emergence of the liver bud from endodermal cells derived from the ventral foregut [25]. Liver bud formation requires



distinct signals emitted by different cell types. The role of  $\beta$ (beta)-catenin in this initial step is controversial, as this molecule seems to antagonize liver bud formation in *Xenopus*, but is required for this process in Zebrafish [26, 27]. At later stages of development, the Wnt pathway affects liver growth, probably in a restricted time-window, controlling the proliferation and differentiation of immature hepatoblasts into the biliary lineage [28, 29]. Wnt signaling also stimulates liver growth after birth [30]. Given that liver zonation appears in early postnatal development, Wnt signaling has to be correlated with the emergence of a compartmentalized liver.

Adenovirus-mediated liver transfer of Dickkopf-1 (Dkk1), a molecule that blocks Wnt signal transduction, also blocks the physiological activation of  $\beta$ (beta)-catenin signaling in the PV area of the liver. Thus,  $\beta$ (beta)-catenin PV signaling requires Wnts [12]. Nineteen Wnt factors have been identified in mice, some of which are expressed in the liver [31]. However, the Wnt factors involved in zonal gene expression and the cells that produce them (presumably in contact with PV hepatocytes) are still unknown. The most likely sources of Wnt are endothelial cells, specifically those surrounding the centrilobular vein, or the PV hepatocytes themselves; this also remains to be resolved.

## **$\beta$ (Beta)-Catenin and Hepatocyte Proliferation**

The PV compartment was long thought to be resistant to proliferative signals and prone to hepatocellular necrosis or apoptosis [32]. Moreover, the oval “stem” cells recruited in very specific conditions for liver regeneration are located in the PP area [33]. The Wnt pathway has been implicated in the proliferation and self-renewal of stem cells; thus, the physiological activation of Wnt signaling in perivenous hepatocytes was unexpected.

This phenomenon must be considered in the context of liver homeostasis, a unique process, which differs, for example, from homeostasis in the intestine. In the intestine, a pool of  $\beta$ (beta)-catenin-activated crypt stem cells is responsible for the constant renewal of the epithelium. This renewal is essential, as enterocytes have a lifespan of 5–7 days. This essential role of the Wnt pathway has been demonstrated in mice showing major consequences of the loss of APC in the small intestine, in particular the enlargement of the crypt compartment [34, 35]. By contrast, the liver cells are quiescent and hepatocytes have a turnover rate estimated between 148 and 400 days. These cells very rarely divide during their lifespan. When such divisions do occur during a physiological process or following injury (as in the experimental model of 2/3 partial hepatectomy), it is

the mature hepatocyte itself that enters the cell cycle. Hepatocytes are renewed independently of their location along the portocentral axis, even if the rate of replication is faster in the PP hepatocytes than in intermediate or PV hepatocytes (Fig. 2.1) [9]. The recruitment of oval “stem” cells for regeneration can be promoted using drugs to block the proliferation of mature hepatocytes [33]. Under such conditions, Wnt/ $\beta$ (beta)-catenin signaling may play an active role in the activation and proliferation of oval cells [36, 37].

Paradoxically, despite the fundamental metabolic role of physiological Wnt signaling in the quiescent liver, aberrant  $\beta$ (beta)-catenin activation throughout the liver leads to marked hepatocellular hyperproliferation [21, 38, 39], and the focal activation of  $\beta$ (beta)-catenin may induce liver cancer formation [21]. The Wnt pathway also plays an important role in liver regeneration, as deletion of the  $\beta$ (beta)-catenin gene in mice delays S-phase by one day after partial hepatectomy. This provides further evidence for the involvement of the Wnt pathway in controlling hepatocyte proliferation.

The set of genes involved in hepatocyte proliferation has yet to be elucidated. Target genes with a potential effect on hepatocyte proliferation, such as those encoding Reg1a and Reg3a/Hip/Pap, have been identified, but functional studies have not yet been performed [40]. In the intestine, the Wnt pathway controls cell proliferation through a cell-autonomous phenomenon, in which *c-myc* is a critical target gene [41]. By contrast, *c-myc* plays no role in liver cell proliferation [21, 38, 42, 43]. Cyclin-D1 has been shown to be a target of the pathway and is critical in G1 to S-phase transition. In many scenarios in liver biology, cyclin-D1 expression and subsequent proliferations are known to be regulated by  $\beta$ (beta)-catenin. Such examples include liver regeneration and development [29, 44]. Moreover,  $\beta$ (beta)-catenin-dependent hepatocyte proliferation is not cell autonomous, and only occurs when Wnt is activated above a critical threshold (activation in more than 70% of hepatocytes) [12, 21]. Future studies will now need to identify  $\beta$ (beta)-catenin target genes mediating this cell non-autonomous and threshold-dependent proliferation. This will provide a better understanding of both  $\beta$ (beta)-catenin-dependent liver carcinogenesis and the control of liver-cell renewal in a zoned-quiescent or regenerating liver.

## **Integration of $\beta$ (Beta)-Catenin Signaling with Other Regulators of Zonal Liver Functions**

Microarray studies comparing the Wnt targetome in various cell types show less than 5% of  $\beta$ (beta)-catenin targets to have a ubiquitous distribution [45]. This tissue-specificity of

$\beta$ (beta)-catenin signaling responses suggest that the molecular mechanisms involved in this signaling are also dependent on cell type. In particular, the mechanisms underlying  $\beta$ (beta)-catenin-induced repression of the PP genetic program need to be elucidated.

Work by Schwarz and colleagues provided some initial insight into the mechanisms of  $\beta$ (beta)-catenin signaling in the liver. This group identified a Ras/Mapk/Erk signal in the periportal hepatocyte, which is activated by blood-borne molecules. This pathway favors the expression of a PP genetic program and blocks the PV program [46]. In this model, the Ras and  $\beta$ (beta)-catenin signaling pathways are antagonistic. However, in addition to this apparent physiological antagonism, cooperation between  $\beta$ (beta)-catenin and the Ras pathway appears to accelerate liver tumorigenesis [47]. Further characterization of the interaction between the  $\beta$ (beta)-catenin and Ras pathways could therefore help our understanding of liver physiopathology.

## Hnf4 $\alpha$ (Alpha) Is a Liver-Enriched Factor Specializing $\beta$ (Beta)-Catenin Transcriptome

Recent studies have suggested that Hnf4 $\alpha$ (alpha) is a master player in establishing the molecular network that drives liver zonation. Hnf4 $\alpha$ (alpha) had been previously described as a major transactivator of genes associated with liver differentiation and metabolism [48–52]. A pioneering genome-wide study revealed 12% of the promoters in liver bound Hnf4 $\alpha$ (alpha), of which 80% were transcriptionally active [51]. However, Hnf4 $\alpha$ (alpha) displays a relatively homogenous distribution throughout the liver lobule and controls transcription of both zoned and not zoned genes. Thus, despite its fundamental roles in the liver, Hnf4 $\alpha$ (alpha) seemed an unlikely candidate for controlling liver zonation [53].

Two recent studies have demonstrated that Hnf4 $\alpha$ (alpha) is a modulator of the zonal expression of genes [54, 55], acting through cross-talk with the Wnt pathway [54].

The first of these studies involved the analysis of zonal gene expression in Hnf4 $\alpha$ (alpha)-null livers. Stanulovic et al. showed reexpression of some (but not all) of the PV genes, such as *Gs* and *Oat* in the PP hepatocytes of these mice [55]. This concept was further tested in fetal rodent hepatocytes, which can be differentiated in culture to display a hepatocyte phenotype [54]. In this model, only PP genes were transcribed, and the activation of Wnt pathway by the GSK3 $\beta$ (beta) inhibitor 6-bromoindirubin-3'-oxime (BIO) redirected the expression profile towards the PV program. The authors showed that one member of the Tcf/Lef family of transcription factors, Lef1, can interact with

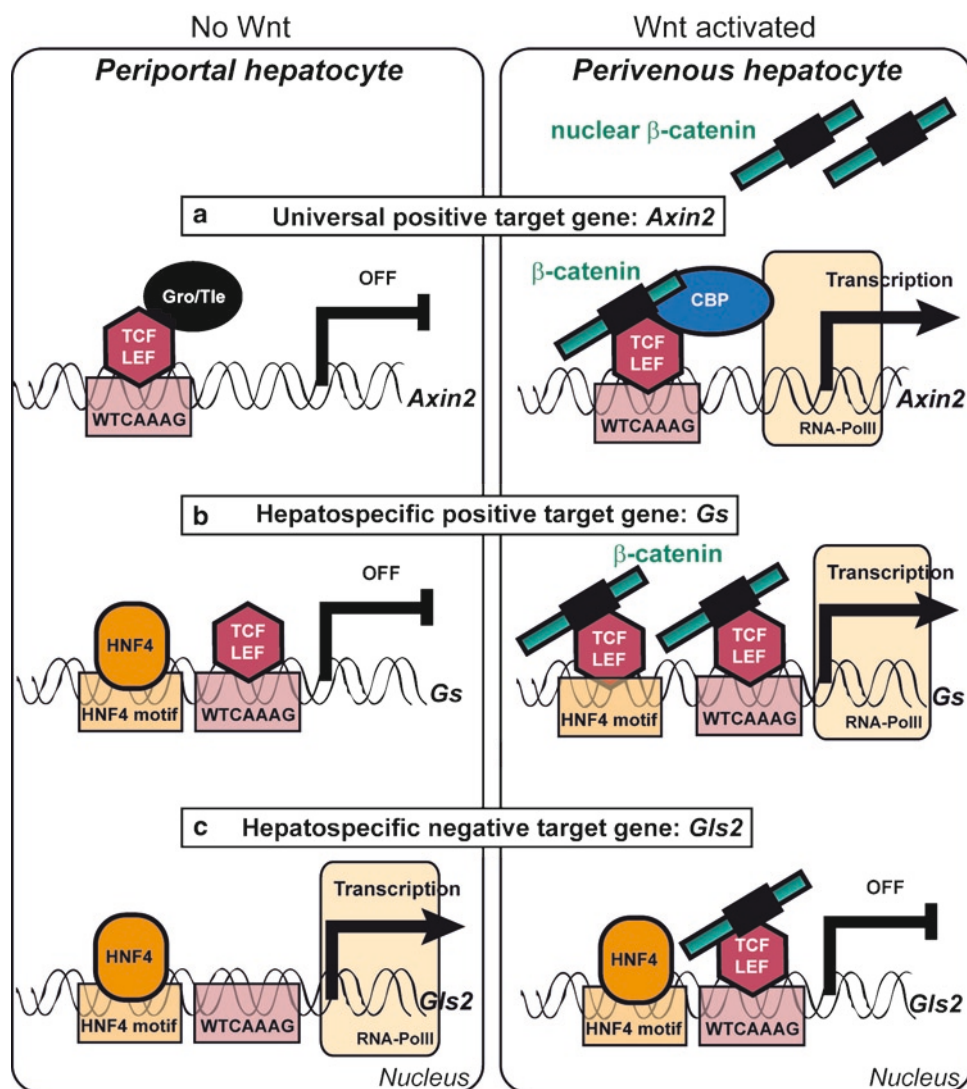
Hnf4 $\alpha$ (alpha). They used chromatin immunoprecipitation assays to evaluate binding to regulatory elements in vivo and found that Hnf4 $\alpha$ (alpha) and Lef1 interaction was required for *Gs* expression, whereas Hnf4 $\alpha$ (alpha) binding alone seemed to repress the expression of this gene. Conversely, on three periportal promoters, the authors show that Hnf4 $\alpha$ (alpha) alone activates these genes in the PP area, while the presence of  $\beta$ (beta)-catenin in the PV zone leads to the replacement of Hnf4 $\alpha$ (alpha) by Lef1, silencing their expression (Fig. 2.3).

A genome-wide analysis of targets bound by Tcf4 in colon cancer cell lines identified several Hnf4 $\alpha$ (alpha)-binding sites present at a high frequency in the vicinity of Tcf4-binding sites, and this reinforces the link between Hnf4 $\alpha$ (alpha) and Wnt dependent transcriptions in epithelial tissues [56].

The cross-talk between Hnf4 $\alpha$ (alpha) and Wnt signaling to ensure proper liver zonation should be extended on other hepato-specific genes. But, it opens up new perspectives in this domain, raising several new questions: What are the mechanisms determining whether Hnf4 $\alpha$ (alpha) activates or silences target genes? Why is Hnf4 $\alpha$ (alpha) not sufficient to activate the transcription of PV genes, such as the gene encoding glutamine synthetase, despite the recognition of its binding motif? How does  $\beta$ (beta)-catenin control the equilibrium between Lef1 and Hnf4 $\alpha$ (alpha) binding to chromatin?

## Conclusion

Studies over the last three years have provided considerable insight into the molecular mechanisms involved in liver zonation. In particular, these studies have established the involvement of  $\beta$ (beta)-catenin pathway and Hnf4 $\alpha$ (alpha) transcription factor. The complex zonal organization of the liver is of particular interest in the study of hepatocarcinogenesis. The overall patient prognosis and disease course of HCC in  $\beta$ (beta)-catenin mutated versus non-mutated HCC remains unsettled. Two routes of hepatocarcinogenesis have been described, based on analyses of tumor transcriptome profiles [20, 57, 58]. The first of these is linked to a high level of genomic instability, with frequent LOH and loss of p53 [59]. The second carcinogenic pathway defines a different type of tumor progression, with maintenance of a stable chromosome profile during HCC development. Tumors undergoing this second route of progression are enriched in  $\beta$ (beta)-catenin mutations, and are well-differentiated tumors with a predominant activation of PV gene transcription. Immunostaining of these tumors with GS antibodies is now used as a marker of these  $\beta$ (beta)-catenin-mutated well-differentiated HCCs [19, 60]. The effect of this particular metabolic PV transcriptome on  $\beta$ (beta)-catenin-depen-



**Fig. 2.3** Trans-acting partners of  $\beta$ (beta)-catenin and Lef/Tcf factors in the nuclei of PV versus PP hepatocytes. (a) Conventional interaction of  $\beta$ (beta)-catenin with Lef/Tcf factors on an example of universal target gene (*Axin2*). In the absence of  $\beta$ (beta)-catenin in PP nuclei, Lef/Tcf factors, bound to its recognition motif (WTCAAAG) recruit Groucho/Tle cofactors to repress the transcription of genes. In the presence of  $\beta$ (beta)-catenin, Lef/Tcf factors recruit CBP coactivator that establish a link to the preinitiation complex and the RNA Polymerase II

to activate the transcription of genes. (b) For the transcription of the hepatospecific  $\beta$ (beta)-catenin target gene *Gs*, Lef1 factor is recruited to both Lef/Tcf and Hnf4 motifs in presence of  $\beta$ (beta)-catenin. In the absence of  $\beta$ (beta)-catenin, Hnf4 and Lef1 bind to their respective motif, and this has a repressive impact on transcription. (c) The transcription of the hepatospecific negative  $\beta$ (beta)-catenin target *Gls2* is mediated by Hnf4 in the PP nuclei. The binding of Lef1 on its recognition motif in PV chromatin inhibits this Hnf4-dependent transcription

dent hepatocarcinogenesis is yet to be determined. This metabolic profile may offer new perspectives for targeted therapeutic strategies for the treatment of this subset of  $\beta$ (beta)-catenin-activated HCCs. This would represent a novel and unanticipated use of our current knowledge of liver zonation.

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