Critical Role for Transcription Coactivator Peroxisome Proliferator-activated Receptor (PPAR)-binding Protein/TRAP220 in Liver Regeneration and PPARα Ligand-induced Liver Tumor Development*

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Disruption of the gene encoding for the transcription coactivator peroxisome proliferator-activated receptor (PPAR)-binding protein (PBP/TRAP220/DRIP205/Med1) in the mouse results in embryonic lethality. Here, we have reported that targeted disruption of the Pbp/Pparbp gene in hepatocytes (Pbp\textsuperscript{\textit{Liv}}) impairs liver regeneration with low survival after partial hepatectomy. Analysis of cell cycle progression suggests a defective exit from quiescence, reduced BrdUrd incorporation, and diminished entry into G\textsubscript{2}/M phase in Pbp\textsuperscript{\textit{Liv}} hepatocytes after partial hepatectomy. Pbp\textsuperscript{\textit{Liv}} hepatocytes failed to respond to hepatocyte growth factor/scatter factor, implying that hepatic PBP deficiency affects c-met signaling. Pbp gene disruption also abolishes primary mitogen-induced liver cell proliferative response. Striking abrogation of CCl\textsubscript{4}-induced hepatocellular proliferation and hepatotoxicity occurred in Pbp\textsuperscript{\textit{Liv}} mice pretreated with phenobarbital due to lack of expression of xenobiotic metabolizing enzymes necessary for CCl\textsubscript{4} activation. Pbp\textsuperscript{\textit{Liv}} mice, chronically exposed to Wy-14,643, a PPARα ligand, revealed a striking proliferative response and clonal expansion of a few Pbp\textsuperscript{\textit{Liv}} hepatocytes that escaped Cre-mediated gene deletion in Pbp\textsuperscript{\textit{Liv}} livers, but no proliferative expansion of PBP null hepatocytes was observed. In these Pbp\textsuperscript{\textit{Liv}} mice, none of the Wy-14,643-induced hepatic adenomas and hepatocellular carcinomas was derived from PBP null hepatocytes; all liver tumors developing in Pbp\textsuperscript{\textit{Liv}} mice maintained non-recombinant Pbp alleles and retained PBP expression. These studies provide direct evidence in support of a critical role of PBP/TRAP220 in liver regeneration, induction of hepatotoxicity, and hepatocarcinogenesis.

Transcription cofactors/coregulators consist of corepressors, coactivators, or coactivator-associated proteins, which participate in nuclear receptor-directed tran-

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2 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PBP, PPAR-binding protein; TRAP, thyroid hormone receptor-associated protein; Pbp\textsuperscript{\textit{Liv}}, PBP liver conditional null; CYP, cytochrome P450; i-PBE, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; CAR, constitutive androstane receptor; TCOBOP, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; ALT, alanine aminotransferase; IGF, insulin-like growth factor; TGF, transforming growth factor; HGF/SF, hepatocyte growth factor/scatter factor.
that conditional PBP mutant mice do not respond to the cell-proliferating response of mice to TCPOBOP, a well known primary mitogen (14, 15). Impairment of the cell-proliferative response of Pbp<sup>ΔLtv</sup> hepatocytes leads to enhanced proliferative expansion of an occasional PBP<sup>+/−</sup> hepatocyte present in these conditional mutant livers, in response to the PPARγ ligand Wy-14,643. Accordingly, none of the liver tumors developing in Pbp<sup>ΔLtv</sup> mice chronically treated with Wy-14,643 was derived from cells with Pbp null genotype. All liver tumors in these conditional null mutant livers exhibited PBP positivity, implying that PBP is essential for hepatocarcinogenesis.

EXPERIMENTAL PROCEDURES

Generation of PBP Conditional Null Mutation in Liver (Pbp<sup>ΔLtv</sup>), Partial Hepatectomy, and Treatment with CAR and PPARγ Agonists—Homozygous mutant mice lacking PBP in hepatocytes (Pbp<sup>ΔLtv</sup>) were generated as described elsewhere (8). Mice were housed in a pathogen-free animal facility under a 12-h light/12-h dark cycle and maintained on standard rodent chow and water ad libitum. Partial hepatectomy was performed under anesthesia to remove 70% of the hepatic mass (16). TCPOBOP was administered intraperitoneally at a single dose of 3 mg/kg body weight. Wy-14,643 (0.125% weight/weight) was given in powdered diet for 1 week, 4 weeks, and 3 months. ALT assay kit (Sigma). To assess hepatocyte proliferation, BrdUrd (0.5 mg/ml) was injected intraperitoneally either into phenobarbital-pretreated untreated mice (100 mg/kg intraperitoneally daily for 3 days (Sigma). To assess hepatocyte proliferation, BrdUrd (0.5 mg/ml) was administered in drinking water for 3 days and given a single intraperitoneal dose (100 mg/kg body weight) 2 h before killing. Mice were killed by cervical dislocation, and blood collected from the inferior vena cava was used for assaying serum alanine aminotransferase (ALT) activity using an ALT assay kit (Sigma). The Northwestern University Animal Care and Use Committee approved all animal studies.

Liver slices were fixed in 10% formalin or 4% paraformaldehyde, processed for embedding in paraffin, sectioned, and stained with either hematoxylin and eosin or processed for immunohistochemical localization of PBP, BrdUrd, or peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (β–oxidation system (8, 9).

Microarray Approach—Total RNA isolated from liver was glyoxylated, separated on 0.8% agarose gel, transferred to nylon membrane, and probed with selected cDNAs. Quantitative reverse transcription-PCR was performed using the primers (forward primer) 5′-GGTACAAGGGCCCTAGTGAAGTC-3′ and (reverse primer) 5′-CGGTCTTGAATT-GGATACCTTCG-3′ specific for the c-met proto-oncogene (18). Whole liver proteins were subjected to 10% SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted using rabbit polyclonal antibodies against cyclins. The rabbit polyclonal antibody against phospho-retinoblastoma protein (Ser-780) and phospho-c-Jun (Ser-63) were from Cell Signaling Technology.

RESULTS AND DISCUSSION

PBP Is Required for Normal Liver Regeneration—Increased mortality was observed in Pbp<sup>ΔLtv</sup> mice usually between 2 and 20 h following partial hepatectomy. The liver-to-body weight ratio, reflective of the regeneration index after partial hepatectomy, increased progressively in wild-type mice, whereas conditional PBP mutant mice failed to show similar increases (Fig. 1A). Liver cell proliferation, as assessed by BrdUrd incorporation, was minimal in Pbp<sup>ΔLtv</sup> mice at all times after partial hepatectomy, and this inhibition persisted until 120 h after surgery (Fig. 1B). In contrast, in Pbp<sup>fl/fl</sup> wild-type controls, BrdUrd incorporation was prominent, with a marked increase in the number of labeled hepatocyte nuclei, with a peak labeling at 36 and 48 h after partial hepatectomy (Fig. 1, B and C). In these livers, cessation of cell proliferation occurred by 120 h after surgery (Fig. 1B). As expected, all hepatocyte nuclei in Pbp<sup>fl/fl</sup> wild-type livers stained positively for PBP (Fig. 1E). In contrast, PBP expression in Pbp<sup>ΔLtv</sup> mouse livers was absent in hepatocytes, and these PBP-negative hepatocytes, in general, appeared smaller in size as compared with hepatocytes in Pbp<sup>ΔLtv</sup> wild-type littermate controls (Fig. 1, D and F). In the Pbp<sup>ΔLtv</sup> mouse, an occasional hepatocyte in the centrilobular region of the liver that escaped Pbp gene disruption expressed PBP staining in the nucleus (Fig. 1F). These cells with abundant cytoplasm are conspicuous in their appearance as compared with the smaller sized PBP null hepatocytes (Fig. 1, D and E). In these Pbp<sup>ΔLtv</sup> mouse livers, BrdUrd staining was seen only in these rare PBP-expressing hepatocytes (Fig. 1D), which corresponded to cells expressing PBP (Fig. 1F). Oil red O-stained liver sections obtained 24–72 h after partial hepatectomy showed a moderate degree of macrovesicular steatosis in Pbp<sup>ΔLtv</sup> mice as compared with minimally visible microvesicular steatosis associated with early stages of normal regenerative response in wild-type mice (data not shown). The exaggerated hepatic steatosis in the
Gene Expression Changes in Control and PBP-deficient Livers Following Partial Hepatectomy—We examined the changes in the gene expression profile in the liver of the 0-h control and 3 h after partial hepatectomy by using the microarray approach (14, 23). The data revealed that ~25 genes are up-regulated 6-fold or higher 3 h after partial hepatectomy in Pbpfl/fl mice as compared with PbpΔLiv mice (Table 1). Many of the genes up-regulated in the Pbpfl/fl mouse liver in response to partial hepatectomy are immediate early genes known to participate in cell cycle, cell growth, apoptosis, and signal transduction (Table 1; Fig. 2A). These include insulin-like growth factor 1 (Igf1), IGFBP1, E2F transcription factor, metallothionein 1 and 2, follistatin, hepatic-binding epidermal growth factor receptor, nuclear receptors Nurr1 and Nor1, growth arrest and DNA damage-inducible 45 (GADD45y), tumor necrosis factor receptor, interleukin-1 receptor, and suppressor of cytokine signaling 3 (SOCS3), among others (13, 14, 23–26). Northern blot analysis confirmed the elevated levels of expression of SOCS3, IGFBP1, and follistatin in Pbpfl/fl mice 3 h post-hepatectomy (Fig. 2A). For example, follistatin, which is known to be a positive regulator in liver regeneration after partial hepatectomy (26), showed higher levels of expression in Pbpfl/fl mice but not in PbpΔLiv mice (Fig. 2A). Although we did not ascertain the levels of induction of these genes during the later stages of liver regeneration, with IGFBP1, we noted sustained higher levels of expression of this gene up to 48 h in PbpΔLiv mice (Fig. 2C). IGFBPs are important positive regulators of liver regeneration.
growth factor— with wild-type mice (Table 1). These include transforming 6-fold or higher 3 h following partial hepatectomy as compared to known to inhibit cell proliferation. Smad7 induces G0/G1 cell cycle arrest by inhibiting the expression of G1 cyclins (27).

Expression of cyclin D1 (which correlates with exit from G0) and of cyclin E (which controls S-phase entry) peaked between 30 and 48 h after partial hepatectomy in Pbpfl/fl mice, but these changes were diminished and delayed in their onset in PbpALiv mice (Fig. 2D). Because cyclin D1 is thought to stimulate entry into S-phase by phosphorylating phospho-Rb (23), we examined the changes in the phosphorylation state of phospho-Rb and its family member p107 (Fig. 2D). In the Pbpfl/fl mouse liver, the levels of phospho-Rb phosphorylation and p107 were higher than in the PbpALiv mouse. The expression of cyclins D and E is generally under the control of immediate early genes, such as c-jun, and their expression reflects exit from the G0 phase (28). Cyclins A and B regulate S/G2 transition and M phase progression, respectively, of the cell cycle (28). In Pbpfl/fl livers, the levels of these two proteins increased, and such increases were not readily apparent in PbpALiv mouse liver after partial hepatectomy (Fig. 2D). The phosphorylated c-jun reached peak levels 6 h after partial hepatectomy in Pbpfl/fl mouse liver, and this increase was not prominent in the PbpALiv mouse liver. Finally, flow cytometric analysis revealed ~86% liver cells in G0/G1 phase and ~8% in G2/M in PbpALiv mouse livers 36 h after partial hepatectomy. This was in contrast to 54% G0/G1 and 40% G2/M in the wild-type control (data not shown). These results, together with changes in immediate early gene expression and differences in BrdUrd incorporation, establish that disruption of Pbp gene in liver results in reduced G0/G1 transition and a block in entry to the G2/M phase of the cell cycle.

We also noted that primary hepatocytes obtained from PbpALiv mice failed to migrate in response to HGF/SF in a standard wound-healing assay (Fig. 1G). HGF/SF exerts its effects through its receptor c-met, a proto-oncogene, and defective c-met signaling has been implicated in impaired liver regeneration and in the etiology and progression of certain human cancers (18, 20, 29, 30). Quantitative PCR data revealed a reduction in c-met mRNA level in PbpALiv hepatocytes, suggesting a possible defect in HGF/c-met signaling (Fig. 1H). The HGF/c-met signaling pathway is important for liver regeneration, and it appears that PBP deletion affects this signaling mechanism.

Impaired Hepatocellular Proliferation in PbpALiv Mouse in Response to a Primary Mitogen—Hepatomigen TCPOBOP, a ligand and activator for the nuclear receptor CAR, induces robust hepatocellular proliferation (14, 15). We undertook a detailed examination of hepatocellular proliferation in Pbpfl/fl and PbpALiv mice 24, 30, 36, 48, and 96 h after a single intraperitoneal injection of TCPOBOP. Increases in liver to body weight ratio as well as BrdUrd labeling indices were evident in Pbpfl/fl mice commencing at ~24 h after TCPOBOP injection, but PbpALiv mice showed almost no such alterations (Fig. 3, A and B). We also examined the effect of TCPOBOP on the inducibility of mRNA expression of certain CAR target genes in liver between 0 and 96 h after injection (Fig. 3C). Deletion of Pbp gene significantly attenuated CAR-mediated induction of its
target genes after TCPOBOP treatment, whereas TCPOBOP treatment resulted in the induction of hepatic CYP3A11, CYP2B10, CYP1A2, and GSTπ mRNAs that occurred in Pbp^fl/fl mice (31–33). Reduction in Mrp3 (multi drug resistance protein 3) and CAR mRNA levels was noted in TCPOBOP-treated Pbp^fl/fl mice (Fig. 3C). To further understand the deficit in liver proliferation after TCPOBOP injection, we analyzed the cell cycle-associated proteins. In Pbp^fl/fl mice, both cyclin A and cyclin D1 appeared to be up-regulated in liver at 48 and 96 h after TCPOBOP injection but not in Pbp^fl/fl mice. E2F, which controls cell growth, showed a slightly higher level of expression at 30, 36, and 48 h after TCPOBOP treatment in Pbp^fl/fl mice than in Pbp^fl/fl mice. In the livers of PBP^+/+ mice, the level of phospho-Rb phosphorylation was higher than Pbp^fl/fl mice (Fig. 3D). These observations suggest that the absence of PBP in hepatocytes affects the function of CAR and that CAR ligand TCPOBOP does not elicit hepatocellular proliferative response and fails to induce xenobiotic metabolizing enzymes.

**Pbp^fl/fl Mice Exhibit Resistance to CCl₄-induced Hepatotoxicity**—To further address the issue of hepatic regeneration and injury in Pbp^fl/fl mice, we used a single injection of CCl₄ to induce hepatic necrosis to evaluate the regenerative response. CCl₄ is metabolized by CYP3A, CYP2B, and possibly by CYP1A2 to form a toxic and highly reactive trichloromethyl radical, CCl₃ (31). In Pbp^fl/fl mice not pretreated with phenobarbital, CCl₄ induced liver cell proliferation and only a mild degree of hepatic necrosis in the centrilobular regions at 2 days with resultant increases in serum ALT levels (Fig. 4, A–C). Pretreatment with phenobarbital resulted in profound amplification of CCl₄-induced necrosis in Pbp^fl/fl livers with significant elevation of serum ALT levels (Fig. D–F). Hepatocellular proliferation in these Pbp^fl/fl mice was higher starting at 1 day after CCl₄ injection, and this increase persisted for 7 days (Fig. 4D). Complete repair of CCl₄-induced liver injury was apparent at 7 days in these wild-type mice (Fig. 4, C and F). In contrast, increases in hepatocellular proliferation were not evident in Pbp^fl/fl mice given CCl₄ without or with phenobarbital pretreatment (Fig. 4, A and D). Also of interest is that CCl₄ failed to induce hepatocellular necrosis in Pbp^fl/fl mice even with phenobarbital pretreatment (Fig. 4F). These results indicate that, in the absence of PBP, phenobarbital apparently fails to increase CCl₄ metabolism, resulting in the abrogation of hepatotoxicity. We previously reported that PBP is involved in the regulation of hepatic CAR function and the induction of drug-metabolizing enzymes and that PBP deficiency in liver abrogates acetaminophen hepatotoxicity (9, 10). Previously, we reported that the absence of PBP in liver cells prevents translocation of the xenobiotic receptor CAR into the hepatocyte nucleus under *in vivo* and *in vitro* conditions, even in the presence of excess exogenous CAR (9, 10). CAR target gene transcription requires the presence of CAR in the hepatocyte nucleus, and coactivator PBP, by virtue of its pivotal role as an anchor for TRAP/DRIP/ARC/Mediator complex, is required
response to Wy-14,643, are indeed the residual hepatocytes that escaped PBP gene deletion (Fig. 5A). Of interest is that these PBP-positive cells exhibit a profound increase in their ability to proliferate in a milieu where the majority of hepatocytes do not express PBP and are thus refractory to the mitogenic stimulus (Fig. 5A).

These results suggest that few PBP-expressing hepatocytes present in Pbp\textsuperscript{Δliv} mouse exhibit profound growth advantage, whereas the PBP-negative hepatocyte population fail to show cell proliferation and induction of peroxisomal β-oxidation enzymes (35). Immunohistochemical staining for \( \iota \)-PBE, the second enzyme of the fatty acid oxidation system (35), demonstrates that \( \iota \)-PBE induction occurs only in PBP-expressing hepatocytes and not in PBP-negative liver cells. These data clearly show that PBP is required for Wy-14,643-induced proliferative expansion of hepatocytes and for the induction of the \( \iota \)-PBE enzyme. Next, we estimated the relative area occupied by large Pbp-positive hepatocytes using hematoxylin- and eosin-stained sections of Pbp\textsuperscript{Δliv} mouse liver at 1 week, 4 week, and 3 months of treatment with Wy-14,643 by using Scion Image software (Fig. 5C). The large hepatocytes in the Pbp\textsuperscript{Δliv} mouse without treatment showed for the retention and concentration of CAR in the nucleus to elicit target gene transcription (10). Regulation of CAR activity by the transcription coactivator PBP may be an important clinical strategy for preventing or minimizing drug-induced liver injury. Activators and inhibitors of nuclear receptor coactivator PBP function may serve as useful tools to modulate drug-induced liver injury.

**PBP Is Necessary for Wy-14,643-induced Hepatocyte Proliferation and Tumorigenesis**—Wy-14,643, a potent peroxisome proliferator and a rodent liver carcinogen, exerts its effects by activating the nuclear receptor PPAR\( \alpha \) (34–36). In view of the refractoriness of Pbp\textsuperscript{Δliv} hepatocytes to liver regeneration induced by partial hepatectomy and TCP0BOP treatment, we tested whether PBP is required for Wy-14,643-induced hepatocyte proliferation and more importantly hepatic tumorigenesis. Administration of Wy-14,643 to Pbp\textsuperscript{Δliv} mice resulted in the proliferation of liver cells with large cytoplasms, and these cells were positive for PBP. BrdUrd labeling as well as PBP and \( \iota \)-PBE immunostaining established that these proliferating cell clusters, which appeared to expand rapidly and progressively in only 0.58 ± 0.06% but at the 3-month treatment period increased to 58 ± 17%. In view of this dramatic differential response in Pbp\textsuperscript{Δliv} mouse livers, we wanted to determine whether PBP is required for mouse liver tumor development in response to Wy-14,643, a non-genotoxic hepatocarcinogen (35). After 52 weeks of Wy-14,643 (0.05% w/w) treatment, 15 Pbp\textsuperscript{Δliv} mice and 15 Pbp\textsuperscript{fl/fl} were killed, and their livers were analyzed for the presence of tumors. The livers of both Pbp\textsuperscript{Δliv} and Pbp\textsuperscript{fl/fl} mice revealed multiple, grossly visible tumors that were randomly distributed among all liver lobes (Fig. 5B). The liver tumor load was similar in both groups (8.63 ± 2.63 in Pbp\textsuperscript{fl/fl} versus 7.8 ± 2.68 in Pbp\textsuperscript{Δliv} mice). We examined ~50 tumors from each group (Pbp\textsuperscript{fl/fl} and Pbp\textsuperscript{Δliv} mice) for the expression of PBP to ascertain whether any of the tumors developing in Pbp\textsuperscript{Δliv} mice were derived from PBP-negative hepatocytes (Fig. 5B, a and b). Interestingly, all adenomas and hepatocellular carcinomas that developed in Pbp\textsuperscript{Δliv} mice were PBP-positive. The surrounding non-tumor portions of liver in Pbp\textsuperscript{Δliv} mice did not express PBP (Fig. 5B, c–f). None of the tumors developing in Pbp\textsuperscript{Δliv} mice were PBP-negative, imply-
ing that Pbp\textsuperscript{\textalpha Liv} hepatocytes are resistant to malignant change. All tumors in the Pbp\textalpha Liv mouse liver stained positive for PBP, and in these livers, hepatocytes in all non-tumor areas also expressed PBP (Fig. 5B, e). From these results, we conclude that long term stimulation with Wy-14,643 induces rapid and sustained proliferation of Pbp\textalpha Liv (Pbp-positive) hepatocytes in Pbp\textalpha Liv mice, and because all of these Pbp-positive hepatocytes respond to the inductive effects of this PPAR\alpha ligand on fatty acid oxidation systems, there is an additional burden of oxidative stress. These two features impart the potential to acquire neoplastic changes. In contrast, dramatic increases in Pbp-positive hepatocytes were not seen in Pbp\textalpha Liv mouse livers due to the relatively short treatment period (5 days after partial hepatectomy; 4 days treatment with TCPBOBOP). It should be noted that only a few cells in the Pbp\textalpha Liv mouse liver are Pbp-positive. This paucity further underscores the importance of the magnitude of cell proliferation of these Pbp\textalpha Liv cells to overwhelm the liver. Of note is that PBP-positive hepatocytes in Pbp\textalpha Liv mouse liver are very rare, accounting for <1.0% of the hepatocyte population. Nonetheless, these cells seem to be overtly sensitive to long term stimulation with Wy-14,643.

The results described here provide unequivocal evidence for the essential role of PBP in liver regeneration induced by partial heptectomy. Mice lacking PBP in liver cells exhibited no DNA synthesis and failed to exit the G\textsubscript{0}/G\textsubscript{1} phase of the cell cycle. Hepatocellular regeneration was also not seen in these PBP null livers when exposed to CAR ligand TCPBOBOP. Of considerable interest is that, although Pbp\textalpha Liv mice developed liver tumors when chronically treated with PPAR\alpha ligand Wy-14,643 (a non-genotoxic hepatocarcinogen (34–36)), none of the tumors originated from PBP null hepatocytes. All liver tumors expressed PBP, and their rapid growth and sensitivity was attributed, in part, to the predominantly PBP-negative milieu in these Pbp\textalpha Liv livers. These observations implicate, for the first time, the involvement of a transcription coactivator in hepatocellular regenerative response and in hepatocarcinogenesis. These Pbp\textalpha Liv mice should provide an opportunity to explore the role of this coactivator in controlling receptor-specific target gene expression in a cell-specific need-based demand.

**CONCLUSION**

Transcription coactivator PBP/TRAP220/MED1 functions as an anchor for TRAP-DRIP-Mediator complex, and disruption of this gene in the mouse results in embryonic lethality. Cre/loxP-mediated gene targeting showed that PBP is essential for the function of nuclear receptors PPAR\alpha and CAR in liver. A critical role for PBP in the xenobiotically induced transcriptional activation of certain nuclear receptors in liver is exemplified by its requirement in PPAR\alpha- and CAR-regulated gene transcription (8, 9). Responses of Pbp\textalpha Liv mice to PPAR\alpha and CAR ligands are similar to mice lacking the respective receptor (32, 36). The results described here provide unequivocal evidence for the essential role of PBP in liver regeneration induced by partial heptectomy. Mice lacking PBP in liver cells exhibited no DNA synthesis and failed to exit the G\textsubscript{0}/G\textsubscript{1} phase of the cell cycle. Hepatocellular regeneration was also not seen in these PBP null livers when exposed to CAR ligand TCPBOBOP. Of considerable interest is that, although Pbp\textalpha Liv mice developed liver tumors when chronically treated with PPAR\alpha ligand Wy-14,643 (a non-genotoxic hepatocarcinogen (34–36)), none of the tumors originated from PBP null hepatocytes. All liver tumors expressed PBP, and their rapid growth and sensitivity was attributed, in part, to the predominantly PBP-negative milieu in these Pbp\textalpha Liv livers. These observations implicate, for the first time, the involvement of a transcription coactivator in hepatocellular regenerative response and in hepatocarcinogenesis. These Pbp\textalpha Liv mice should provide an opportunity to explore the role of this coactivator in controlling receptor-specific target gene expression in a cell-specific need-based demand.

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