Introduction

Primary bile acids are synthesized in the liver and stored in the gall bladder and, following a meal, are secreted into the intestine, where they facilitate lipid absorption , . Virtually all aspects of the enterohepatic circulation involving bile acid synthesis, secretion, conjugation, and resorption in the intestine are dependent on the nuclear receptor farnesoid X receptor FXR encoded by Nr h , which is highly expressed in enterocytes and hepatocytes . Endogenous bile acids such as chenodeoxycholic acid CDCA , deoxycholic acid DCA , and cholic acid CA , are known to activate FXR, while muricholic acid MCA is thought to function as an FXR antagonist . A number of FXR agonists, including bile acid analogs, are currently being tested in clinical trials as potential treatments for steatosis and cholestasis .

Bile acid levels are tightly regulated through a negative feedback loop and end-product feedback inhibition that was irst described almost half a century ago . Such feedback is critical, as elevated levels of bile acids can be toxic and result in severe metabolic complications including cholestasis, diarrhea, lipid malabsorption, inflammation, and cancer . The mechanism for feedback inhibition of bile acid synthesis is highly complex but largely dependent on FXR. In response to elevated bile acid levels, FXR is activated, leading to signaling cascades in both the liver and intestine that result in decreased hepatic mRNA levels of cholesterol .-hydroxylase Cyp a , the rate-limiting enzyme of bile acid synthesis , and Cyp b , which is required for the synthesis of CA . At present, the known mechanisms that lead to decreased Cyp a expression following FXR activation are dependent on transcriptional repression. In one pathway, FXR activates the gene encoding the nuclear receptor SHP Nr b , a classic FXR target gene and transcriptional repressor . In a second pathway, FXR activation in intestinal enterocytes induces the expression and subsequent secretion of FGF FGF in humans into the circulation . FGF / then binds to a heterodimeric receptor on hepatocytes to initiate a signaling cascade leading to transcriptional repression of Cyp a . FGF / signaling was also shown to require SHP . Defects in a number of genes in bile acid metabolism pathways are associated with diverse metabolic disturbances, consistent with the premise that maintaining proper bile acid homeostasis is critical for preventing metabolic diseases.

In the present study, we identify and characterize an FXR-regulated posttranscriptional pathway that controls Cyp a mRNA and bile acid levels. We identi ied ZFP L , also known as TIS b , BRF , or RNF B, as a direct FXR target gene. ZFP L is an RNA-binding protein RBP that has been shown to bind to adenylate-uridylate–rich AU-rich elements in the -UTRs of speci ic cytokine mRNAs in immune cells to promote mRNA degradation . Here, we used synthetic and endogenous FXR agonists to demonstrate that activation of hepatic FXR leads to a rapid induction of hepatic Zfp l mRNA and protein in vivo and in vitro. Our in vitro studies demonstrate that ZFP L can target both human or mouse Cyp a -UTRs. In addition, in vivo ZFP L gain- and loss-of-function studies

Zfp l Zfp l L-KO are partially resistant to diet-induced adiposity and steatosis, due, at least in part, to impaired lipid absorption that results from altered bile acid metabolism.

Results

Pharmacologic FXR activation with the synthetic FXR agonists GSK or GW results in a greater than reduction of Cyp a and Cyp b mRNA levels within hours , . To better determine the kinetics of decay of Cyp a and Cyp b mRNA levels following FXR activation, we treated WT and Fxr–/– mice with a single dose of vehicle or GSK mg/kg body weight mpk and analyzed gene expression minutes, hour, and hours later. Cyp a mRNA levels declined by more than within minutes

The rapid regulation of Cyp a observed following FXR activation Figure A led us to consider the half-life of Cyp a mRNA. A previous study in which HepG cells were used reported the half-life of human CYP A to be . hours . Thus, our observation that Cyp a mRNA levels decreased by approximately within minutes of GSK treatment Figure A suggested that a posttranscriptional mechanism might enhance the degradation of preexisting Cyp a mRNA following FXR activation in mice. To determine the half-life of Cyp a mRNA in vivo is challenging, therefore we irst considered using isolated primary mouse hepatocytes. However, we observed a near-complete loss of Fxr, Cyp a , and Cyp b mRNAs within hours of isolation and culturing of primary mouse hepatocytes Supplemental Figure L , suggesting that these cells are not a suitable system by which to study the regulation of bile acid synthesis genes.

In the absence of primary hepatocytes or the appropriate murine cell lines that express Cyp a , we determined the half-life of CYP A and CYP B mRNA using immortalized human hepatocytes IHHs . IHHs express many of the bile acid synthesis genes, including CYP A . We then treated IHHs with the transcriptional inhibitor actinomycin D for different lengths of time prior to isolation and quanti ication of mRNA levels to determine mRNA half-lives. The half-lives of human CYP A and CYP B mRNAs were approximately and hours, respectively Supplemental Figure , M and N . We then compared the mRNA decay rates observed in IHHs black lines with those from GSK -treated mice blue lines and found that FXR activation with GSK increased the rate of decrease in Cyp a , but not in

Figure 2. Rapid decrease in following FXR activation requires hepatic FXR but is independent of SHP. (A–C) Gene expression of hepatic Cyp7a1 and Bsep and ileal Fgf15 in littermate $Fxr^{fl/fl}$ and Fxr^{L-KO} mice following treatment with either vehicle (n = 13 $Fxr^{fl/fl}$; n = 11 Fxr^{L-KO}) or GSK2324 for 30 minutes (n = 9 $Fxr^{fl/fl}$; n = 8 Fxr^{L-KO}), 1 hour (n = 7 $Fxr^{fl/fl}$; n = 9 Fxr^{L-KO}), 2 hours (n = 8 $Fxr^{fl/fl}$; n = 7 Fxr^{L-KO}), or 4 hours (n = 5 $Fxr^{fl/fl}$; n = 10 Fxr^{L-KO}). (D–F

Cyp b , mRNA levels Figure , E and F . The differences in mRNA half-lives between these conditions are suggestive of the existence of posttranscriptional mechanisms that destabilize Cyp a mRNA.

Two pathways have been identi ied that are involved in feedback regulation of hepatic Cyp a mRNA levels . One involves intestinally derived FGF FGF in humans , an FXR target gene that is secreted into the circulation in response to activation of FXR in intestinal enterocytes . The second involves induction of SHP, a known transcriptional repressor, following activation of hepatic FXR .

To determine whether hepatic FXR is required for the rapid decrease in Cyp a that occurs following FXR activation in vivo, we generated liver-speci ic FXR KO mice Fxr^{L-KO} and littermate control floxed mice $Fxr^{fl/fl}$ by crossing $Fxr^{fl/fl}$ mice with albumin-Cre–expressing mice to delete FXR in hepatocytes. We then treated both $Fxr^{fl/fl}$ and Fxr^{L-KO} animals with either vehicle or GSK for minutes, hour, hours, and hours. Cyp a mRNA levels in the liver were reduced by within minutes, and the levels were almost completely abolished after hours of GSK treatment of $Fxr^{fl/fl}$ mice Figure A . In contrast, no inhibition of Cyp a mRNA levels was observed for up to hours after treatment of Fxr^{L-KO} mice with GSK Figure A . These data demonstrate that the initial and rapid GSK -dependent decrease in Cyp a mRNA requires hepatic FXR. However, hours after GSK treatment of Fxr^{L-KO} mice, there was signi icant inhibition of Cyp a mRNA Figure A , suggesting that this latter inhibition is likely a result of intestinally derived FGF .

Cyp b mRNA levels were consistently reduced at a slower rate than were Cyp a mRNA levels after GSK treatment, and the initial decline in Cyp b levels that occurred within hours of GSK treatment was dependent on hepatic FXR Supplemental Figure A . The FXR target genes Shp and Bsep were induced in the livers of $Fxr^{fl/fl}$, but not Fxr^{L-KO}, mice treated with GSK Figure B and Supplemental Figure B . In contrast, GSK treatment induced Fgf and Fabp iBabp mRNAs in the distal ileum in both $Fxr^{fl/fl}$ and Fxr^{L-KO} mice Figure C and Supplemental Figure C , demonstrating that intestinal FXR and the subsequent induction of intestinal FXR target genes were unaffected by loss of hepatic FXR. These results suggest that a speci ic factor that is controlled by hepatic FXR mediates the rapid decline of Cyp a mRNA levels following FXR activation.

Figure 3. is a direct FXR target gene and is induced by both pharmacologic and physiologic FXR agonists. (A) FXR ChIP-seq analysis (21) of the Zfp36l1 genomic loci showing putative FXREs. (B) IHHs were transfected with a plasmid containing the Zfp36l1 promoter region (2 kb) upstream of a luciferase reporter in combination with increasing amounts of a mouse Fxr2 expression plasmid. Cells were treated with vehicle or GW4064 (GW) (1

To determine whether the rapid decrease in Cyp a mRNA observed in response to GSK required the transcriptional repressor SHP, we obtained Shp mice and treated them with vehicle or GSK for minutes, hour, or hours. Notably, Cyp a mRNA levels declined by approximately within minutes of treating Shp$^{-/-}$ mice with GSK , indicating that SHP was not required for the rapid decrease in Cyp a mRNA levels Figure D . Treatment of Shp$^{-/-}$ mice with GSK also resulted in a decrease in Cyp b mRNA after hours Supplemental Figure D . The induction of the known FXR target genes Bsep Figure E and Insig Supplemental Figure E in the liver and induction of Fgf in the distal Ileum Figure F were similar in Shp$^{-/-}$ mice, consistent with normal function of hepatic and intestinal FXR signaling in Shp mice.

Taken together, these data demonstrate that the initial rapid decrease in Cyp a mRNA levels in response to FXR activation in vivo requires hepatic FXR but is independent of both SHP and induction of intestinal Fgf . Furthermore, our data suggested that activation of hepatic FXR utilizes a previously unrecognized posttranscriptional mechanism to control Cyp a mRNA levels.

Various posttranscriptional mechanisms have been described that modulate mRNA stability: for example, the binding of microRNAs miRs to seed sequences within target mRNA, or the interaction of the mRNA with speci ic RBPs , . We previously demonstrated that FXR regulates surprisingly few miRs. Among the direct FXR-regulated miRs we identi ied are the miR /miR cluster and miR . Further, miRs are thought to be ine-tuners of gene expression and are therefore unlikely to be rapid regulators of gene expression . Thus, miRs seemed to be unlikely candidates for mediating the rapid change in Cyp a mRNA. We next considered RBPs, which represent a large class of genes that are reported to be more abundant in the mammalian genome than are transcription factors . RBPs bind RNA targets and either stabilize or destabilize mRNAs, usually in a sequence-speci ic manner . Since FXR activation rapidly decreased Cyp a mRNA levels, we hypothesized that FXR activation might result in the rapid induction of one or more speci ic RBPs that function to destabilize, and therefore enhance, the degradation of preexisting Cyp a mRNA.

To test this hypothesis and to identify potential FXR-regulated RBPs, we searched the literature for RBPs that degrade mRNA targets and manually checked those for potential FXR response elements FXREs using publicly available FXR ChIP-sequencing ChIP-seq data sets , . This approach

decreased CYP A mRNA levels Supplemental Figure , C and D . Taken together, these results show that FXR activation with either synthetic or endogenous agonists results in the induction of ZFP L in both mouse livers and IHHs, consistent with ZFP L as a direct FXR target gene.

We hypothesized that the regulation of ZFP L by FXR at both mRNA and protein levels would have to be rapid if ZFP L were to be involved in the initial min decrease in Cyp a mRNA Figures and . To test this hypothesis, we determined how rapidly ZFP L mRNA and/or protein levels were induced in WT, Fxr$^{-/-}$, Fxr$^{fl/fl}$, Fxr$^{L KO}$, and Shp$^{-/-}$ mice following short-term treatment with GSK . Importantly, we show that the induction of ZFP L mRNA and protein was already observed minutes after GSK treatment of WT Figure , A and B , Fxr $^{fl/fl}$ Figure , C and D , and Shp$^{-/-}$ mice Figure , E and F, and see the complete unedited blots in the supplemental material . In contrast, Zfp l mRNA was not induced following GSK treatment of Fxr$^{-/-}$ and Fxr$^{L KO}$ mice Figure , A and C . We also determined the half-life of ZFP L mRNA to be approximately minutes in IHHs treated with actinomycin D, suggesting that Zfp l

Figure 5. ZFP36L1 gain of function reduces CYP7A1 and bile acid levels in vivo (A) Mouse and (B) human (WT and mutant) Cyp7a1 3′UTR cloned downstream of a luciferase reporter and then transfected in IHHs in combination with increasing amounts of a mouse Zfp36l1 expression plasmid. Luciferase activity was determined after 24 hours and normalized to -gal and is expressed as the fold change (n = 6 wells/condition). (C) ZFP36L1 mRNA and protein, (D) CYP7A1 mRNA and protein, and (E) mRNA levels of bile acid synthesis genes in male C57BL/6 WT mice treated with either adenovirus-control (Ad-Ctr) or Ad-Zfp36l1 (n

human and contains multiple conserved AU-rich elements . To determine whether ZFP L can regulate Cyp a mRNA stability, we generated a reporter plasmid containing the mouse Cyp a -UTR downstream of a constitutively transcribed luciferase reporter. Cotransfection of a plasmid encoding ZFP L , together with the Cyp a ’-UTR reporter gene, resulted in a ZFP L dose–dependent decrease in luciferase activity Figure A . The mouse Cyp a -UTR has a -bp central region that contains several tandem AU-rich elements Figure A . A reporter plasmid containing the mouse Cyp a -UTR that lacked this -bp region was no longer responsive to coexpression of ZFP L Figure A . The human CYP A ’-UTR also contains a similar -bp AU-rich sequence, which, in contrast to the mouse, is found at the end of the CYP A -UTR. Cotransfection of ZFP L with the luciferase reporter gene construct containing the human CYP A -UTR also led to a decrease in luciferase activity Figure B . Furthermore, deletion of the -bp AU-rich sequence from the -terminus of the UTR resulted in a reporter gene that was unresponsive to cotransfected ZFP L Figure B . We conclude that ZFP L targets both human and mouse CYP AmRNA by binding to AU-rich elements in the -UTR, leading to degradation of the mRNA.

To determine whether ZFP L can also target endogenous Cyp a mRNA in vivo, we generated Zfp l -expressing adenovirus particles and infused them into the tail veins of male C BL/ mice. Recombinant adenovirus particles resulted in a modest increase in hepatic ZFP L mRNA and protein levels Figure C . Consistent with our in vitro studies involving hybrid reporter genes, enforced ZFP L expression in WT mice signi icantly

mice were unchanged, although some gene expression levels were
increased Akr d

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Bile acids are both signaling molecules and detergents that facilitate lipid absorption, and mouse models with altered bile acid metabolism have a number of important metabolic phenotypes. For example, transgenic mice that overexpress human CYP A in hepatocytes have increased bile acid synthesis but are also resistant to diet-induced obesity and steatosis . The precise mechanisms by which elevated CYP A levels lead to changes in obesity or steatosis are not well understood. Given our observations that Zfp l L KO mice have elevated CYP A mRNA and protein as well as increased bile acid levels Figure , we hypothesized that loss of hepatic Zfp l would result in metabolic disturbances similar to those reported in CYP A -transgenic mice. We therefore challenged Zfp l L KO and littermate control Zfp l fl/fl

L KO

fl/fl mice. We obtained similar results when we fed Zfp l fl/fl and Zfp l L KO animals a diet only enriched in fat high-fat diet: kCal fat, . cholesterol , and once again, Zfp l L KO mice were resistant to diet-induced weight gain Supplemental Figure , B D . To determine whether the reduced body weight and fat mass were due to differences in energy metabolism, we placed littermate Zfp l fl/fl and Zfp l L KO animals in metabolic chambers and performed comprehensive metabolic analyses after days of Western diet feeding. We observed no signi icant differences in energy expenditure Figure C , oxygen consumption, carbon dioxide production, or activity Supplemental Figure , E G , suggesting that loss of hepatic Zfp l did not result in gross energy metabolism abnormalities. However, despite exhibiting decreased body and fat weights, Zfp l L KO mice consumed more food than did their Zfp l fl/fl littermates Figure D . Moreover, Zfp l L KO mice were markedly protected from diet-induced hepatosteatosis after days on a Western diet Figure E . Lipid extraction from the livers of control Zfp l fl/fl and Zfp l L KO mice con irmed reduced hepatic triglyceride TAG and cholesterol levels in Zfp l L KO mice Figure , F and G . Plasma TAG and cholesterol levels were also lower in the Western diet–fed Zfp l L KO mice as compared with levels in control mice Figure , H and I , as were plasma insulin and ALT levels Figure , J and K . Plasma glucose levels were lower, but not signi icantly, whereas plasma AST levels were not altered Supplemental Figure , H and I . Hepatic expression of the inflammatory cytokines Il b and Tnfa were also signi icantly lower in the livers of Western diet–fed Zfp l L KO mice Supplemental Figure J . Together, these changes are consistent with improved hepatic function and improved metabolic handling in Zfp l L KO mice following challenges with fat-enriched diets.

We also measured bile acid parameters in Zfp l L KO mice fed a Western diet. Total biliary bile acid levels were elevated in Western diet–fed Zfp l L KO mice, and the change was largely restricted to elevated T- -MCA levels Figure L . Moreover, Western diet–fed Zfp l L KO mice had increased hepatic CYP A mRNA and protein levels and altered expression of a number of bile acid synthesis genes Figure , M and N, and see the complete unedited blots in the supplemental material . These changes are consistent with our analysis of mice on a standard rodent diet Figure .

Given the central role of bile acids as detergents, we hypothesized that loss of hepatic Zfp l might affect intestinal lipid absorption, particularly under Western diet–fed conditions, as a consequence of the altered bile acid metabolism. To determine differences in lipid absorption, we irst subjected Western diet–fed littermate Zfp l fl/fl and Zfp l L KO mice to an intragastric fat challenge and measured circulating triglycerides over a -hour period. Zfp l L KO mice had a reduced excursion of plasma TAG levels after the fat challenge, suggesting that loss of hepatic Zfp l resulted in defective lipid absorption Figure O .

Reduced lipid absorption should also result in increased caloric content in the feces, and, indeed, the total energy in the form of calories that was recovered in the feces from Zfp l L KO mice was almost double that in feces from control mice Figure P . Fecal calories were elevated even when expressed per gram, but the increased fecal calorie content was compounded by an increased fecal output by Zfp l L KO mice Supplemental Figure , K and L . Taken together, these data suggest that loss of hepatic Zfp l results in pronounced whole-body metabolic changes that are explained, at least in part, by decreased lipid absorption resulting from altered bile acid metabolism.

Discussion

Here, we identify Zfp l as an FXR target gene that functions to enhance the rate of degradation of Cyp a mRNA. We used gain- and loss-of-function studies to demonstrate that hepatic ZFP L expression is inversely proportional to bile acid levels and, in addition, affects the composition of the bile acid pool. Thus, the current indings identify and characterize a previously unrecognized pathway by which activated FXR rapidly regulates bile acid synthesis. We show that FXR activation in vivo results in an intensely rapid decrease in Cyp a mRNA by a posttranscriptional mechanism that requires hepatic FXR but is independent of the transcriptional repressor SHP. To determine the molecular mechanism of the degradation of Cyp a mRNA, we identi ied the RBP Zfp l . The Zfp l gene locus contains multiple putative FXR response elements, consistent with its induction in response to natural CA or synthetic GW , GSK FXR agonists in vivo. Further, reporter gene assays indicated the presence of a functional FXRE in the proximal promoter of the Zfp l gene. Importantly, we show that in mice, induction of Zfp l mRNA and protein in response to FXR activation with GSK occurs within minutes. Taken together, these data suggest that rapid FXR-dependent induction of Zfp l leads to a reduction of Cyp a mRNA via a posttranscriptional mechanism, a hypothesis supported by our in vitro reporter gene studies.

We show that luciferase reporter genes linked to either the mouse or human Cyp a -UTRs are repressed following overexpression of ZFP L . Additional reporter gene assays indicate that ZFP L -dependent regulation requires a region containing conserved AU-rich elements in the -UTR of Cyp a mRNA. Indeed, previous studies have shown that Zfp l functions to repress cytokine and cell-cycle mRNAs by binding to AU-rich sequences in their '-UTRs , , . However, a role for Zfp l in regulating hepatic mRNAs in vivo, including those involved in lipid and bile acid metabolism, has not been reported. Notably, cytokine mRNAs were unaffected in the liver following treatment of mice with Ad-Zfp l or in Zfp l L KO mice data not shown .

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Our gain- and loss-of-function studies show that there are signi icant metabolic consequences to modulation of Zfp l . Hepatic overexpression of Zfp l in mice resulted in a decrease in both Cyp a and Cyp b mRNA levels. Complementary loss-of-function studies demonstrated that loss of Zfp l in the livers of mice results in an elevated expression of a number of mRNAs, including Cyp a and Cyp b , in both normal and Western diet–fed mice. Consistent with these indings, Zfp l LKO mice have increased biliary bile acid levels and an altered biliary bile acid composition. These results are consistent with the hypothesis that Zfp l normally functions to repress Cyp a mRNA and reduce bile acid synthesis. The reduction in plasma cholesterol levels that accompanied the increased bile acid levels is also consistent with increased bile acid synthesis in Zfp l LKO mice.

One of the major functions of bile acids is to facilitate lipid absorption, and it is clear that not all bile acids act in the same manner , . For example, Cyp b / mice have increased T--MCA levels and no detectable tauro-CA. These same Cyp b mice have increased CYP A enzymatic activity and an increased bile acid pool, yet decreased cholesterol absorption . Interestingly, Cyp b / mice were recently shown to be resistant to steatosis and weight gain . The improved steatosis and decreased adiposity are similar to our indings in Zfp l LKO mice, which also had increased bile acid levels and, in particular, increased levels of T–MCA. Thus, the different bile acid composition of 37

was unable to reduce Cyp a mRNA levels in Shp mice . We recently described an FXR-dependent pathway that functions through another transcriptional repressor, MAFG, which does not directly repressCyp a . MAFG functions to repress numerous genes in the bile acid synthesis pathway, including Cyp b , the regulatory gene of CA synthesis . The relative contribution of each of these pathways to feedback regulation of bile acid synthesis has not been comprehensively established. Previous studies using Shp/ mice showed that treatment with GW failed to repressCyp a mRNA levels , , supporting the idea that SHP has a role in FXR-dependent repression of this gene. In contrast, we and others have reported that Cyp a mRNA levels decline following treatment of Shp/ mice with either GW , CA , , or GSK , as we have shown here. At present, it is unclear whether these divergent results are due to the different genetic backgrounds of the animal models or to differences in the modes of administration, tissue uptake, or pharmacokinetics of the FXR agonists. Consequently, the exact role and importance of SHP in the repression of Cyp a remains to be clearly elucidated, although our data suggest that SHP is not essential for feedback regulation of bile acid synthesis.

The inding that treatment of Zfp l LKO mice with GSK results in reduced Cyp a expression, albeit at a slower rate than in their WT littermates, suggests that Zfp l is also not essential for feedback regulation of bile acid synthesis. Nonetheless, the indings that hepatic loss of ZFP L results in elevated levels of Cyp a and bile acids, reduced adiposity, and steatosis indicate that this protein functions in controlling Cyp a mRNA. Importantly, here we show that GSK treatment of WT, Shp / , and Zfp l LKO mice reduces Cyp a mRNA levels. In contrast, Cyp a mRNA levels were unaltered in Fxr mice treated with GSK . Taken together, we conclude that FXR utilizes multiple redundant pathways to mediate feedback inhibition of bile acid synthesis. Redundancy in feedback inhibition of bile acid synthesis was also proposed in the original reports characterizing Shp mice , .

Our identi ication of ZFP L as a regulator of Cyp a mRNA and bile acid composition provides a pathway that complements the known transcriptional repression pathways that decrease bile acid synthesis. ZFP L may function to rapidly decrease preexisting Cyp a mRNA levels, and subsequent transcriptional repression mechanisms in response to FGF and/or SHP then maintain reduced levels of Cyp a mRNA under conditions of elevated bile acids and/or FXR activation. In addition to ZFP L , we have identi ied at least other RBP that appears to be FXR regulated and can target and degrade Cyp a mRNA data not shown . However, we have not yet characterized this pathway in detail. It remains to be determined whether the different RBPs that are responsive to FXR activation target Cyp a mRNA function in concert.

Studies conducted over a decade ago by Davis and colleagues had proposed that Cyp a might be regulated by posttranscriptional mechanisms . However, no molecular mechanism was identi ied at that time, although the AU-rich elements present in the Cyp a UTR were found to be required for the proposed posttranscriptional regulation . Since ZFP L requires these AU-rich elements to repress Cyp a mRNA levels, we believe we have identi ied a factor involved in the posttranscriptional regulation of Cyp a .

ground. All mice were maintained on a -hour light/ -hour dark cycle. In general, animals were used at days of age, fasted at am for to hours, and then sacri iced between pm and pm. For treatments with FXR agonists, GSK was dissolved in water and administered once to mice via i.p. injection at mpk for minutes, hour, hours, or hours before sacri ice. Vehicle-treated mice were injected with water for randomly assigned time points between min and h . All mice were fasted for hours and sacri iced at the same time of day pm to ensure a consistent circadian time point. Studies with GW and GSK at mpk for days were described previously .

Adenovirus production. All adenoviruses were prepared in bio safety level –category BSL –category facilities. Briefly, cDNAs for mouse *Zfp l* were cloned from whole-liver cDNA into a pAdTrack CMV plasmid and prepared as described previously . For animal experiments,

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