KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis during Fasting

Highlights
- A transcription factor library screen reveals a complex between KLF15 and LXR/RXR
- KLF15 interacts with LXR/RXR and recruits the RIP140 corepressor to the Srebf1c promoter
- This enables rapid switching between lipogenesis and gluconeogenesis during fasting
- Hepatic KLF15 augmentation improves the plasma lipid profile

Authors
Yoshinori Takeuchi, Naoya Yahagi, Yuichi Aita, ..., Yuan Lu, Mukesh K. Jain, Hitoshi Shimano

Correspondence
nyahagi-tky@umin.ac.jp

In Brief
Takeuchi et al. find that KLF15 suppresses SREBP-1c transcription through interaction with the LXR/RXR/RIP140 complex. Through this mechanism, KLF15 promotes a fasting adaptation by switching lipogenesis to gluconeogenesis. KLF15 overexpression ameliorates hypertriglyceridemia without affecting LXR-mediated cholesterol metabolism.
KLF15 Enables Rapid Switching between Lipogenesis and Gluconeogenesis during Fasting

Yoshinori Takeuchi,1 Naoya Yahagi,1,4,6* Yuichi Aita,1 Yuki Murayama,1 Yoshikazu Sawada,1 Xiaoying Piao,1 Naoki Toya,1 Yukari Oya,1 Akito Shikama,1 Ayako Takarada,1 Yukari Masuda,1 Makiko Nishi,1 Midori Kubota,1 Yoshihiko Izumida,1 Takashi Yamamoto,2 Motohiro Sekiya,2 Takashi Matsuzaka,2 Yoshimi Nakagawa,2 Osamu Urayama,2 Yasushi Kawakami,2 Yoko Iizuka,3 Takanari Gotoda,3 Keiji Itaka,4 Kazunori Kataoka,4 Ryozo Nagai,3 Takashi Kadowaki,3 Nobuhiro Yamada,2 Yuan Lu,6 Mukesh K. Jain,5 and Hitoshi Shimano2

1Nutrigenomics Research Group, Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan
2Department of Internal Medicine (Endocrinology and Metabolism), Faculty of Medicine, University of Tsukuba, Ibaraki 305-8575, Japan
3Department of Internal Medicine
4Center for Disease Biology and Integrative Medicine
Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan
5Case Cardiovascular Research Institute, Cleveland, OH 44106, USA
6Lead Contact
*Correspondence: nyahagi-tky@umin.ac.jp
http://dx.doi.org/10.1016/j.celrep.2016.07.069

SUMMARY

Hepatic lipogenesis is nutritionally regulated (i.e., downregulated during fasting and upregulated during the postprandial state) as an adaptation to the nutritional environment. While alterations in the expression level of the transcription factor SREBP-1c are known to be critical for nutritionally regulated lipogenesis, upstream mechanisms governing Srebf1 expression remain unclear. Here, we show that the fasting-induced transcription factor KLF15, a key regulator of gluconeogenesis, forms a complex with LXR/RXR, specifically on the Srebf1 promoter. This complex recruits the coactivator RIP140 instead of the coactivator SRC1, resulting in reduced Srebf1 and thus downstream lipogenic enzyme expression during the early and euglycemic period of fasting prior to hypoglycemia and PKA activation. Through this mechanism, KLF15 overexpression specifically ameliorates hypertriglyceridemia without affecting LXR-mediated cholesterol metabolism. These findings reveal a key molecular link between glucose and lipid metabolism and have therapeutic implications for the treatment of hyperlipidemia.

INTRODUCTION

In the postprandial state, the liver takes up nutrients through the portal vein and excess carbohydrates are converted into triglycerides, whereas in a fasting state, this lipogenic pathway shuts down. Previous studies have shown that sterol regulatory element-binding protein-1c (SREBP-1c) plays an important role in controlling hepatic lipogenesis (Shimano et al., 1999; Yahagi et al., 1999, 2002), leading us to examine the mechanism of this nutritional regulation of SREBP-1c expression.

RESULTS

Identification of NuRE on the Srebf1c Promoter by In Vivo Ad-Luc Analyses

mRNA expression of hepatic Srebf1 gene is tightly regulated by nutritional conditions. Using in vivo reporter gene analyses with reporter-transgenic mice, we previously showed that this nutritional regulation of the Srebf1 gene occurs at the transcriptional
level and that a 2.2-kb region of the 5′-flanking sequence on the Srebf1c promoter is sufficient for this regulation (Takeuchi et al., 2007). Moreover, we previously established an intra-organ assay system to directly estimate the activity of the Srebf1c promoter in the livers of living animals using an adenovirus encoding the Srebf1c promoter linked to a luciferase reporter gene (Takeuchi et al., 2010). Using this in vivo Ad-luc promoter analysis technique, we attempted to identify the nutritional regulatory cis element on the Srebf1c promoter; luciferase reporter genes driven by different lengths of the Srebf1c promoter (ranging from 2,200 bp to 150 bp) were adenovirally transduced into mouse liver, and transcriptional activity was assessed by measuring luciferase activity with an IVIS imaging system (Figure 1A). From this experiment, the responsible element was found to be located within −250 to −150 bp upstream of the transcription start site. This result was confirmed by experiments using another series of adenovirus constructs encoding different partial sequences of the Srebf1c promoter to estimate enhancer activity (Figure S1A).

Next, we investigated the −250- to −150-bp region in detail; this region contains two LXR-binding elements (LXREs) (Yoshikawa et al., 2001). Intriguingly, the two LXREs alone were not sufficient for nutritional regulation, although they exhibited a complete response to an LXR ligand (Figure 1B), suggesting that nutritional regulation is not brought about by changes in ligand concentration. Further scrutiny clarified that a cis element flanking the LXRE besides the two LXREs is indispensable for nutritional regulation (Figure 1C). This element, which we designated the nutritional regulatory element (NuRE), was subsequently shown to exert a suppressive effect on the Srebf1c promoter activity in a fasting state (Figures 1D and S1B). Consistent with this finding, an electrophoretic mobility shift assay (EMSA) showed a fasting-specific band (Figure 1E), suggesting that some fasting-acting transcription factor(s) in liver nuclei that binds to NuRE may be involved in the suppression of Srebf1c promoter activity during fasting.

Previous reports have indicated the involvement of LXR/RXR in the regulation of Srebf1c promoter activity (Rep et al., 2000; Yoshikawa et al., 2001), as supported by our own data demonstrating that the simultaneous knockdown of both LXRα and β markedly reduces the nutritional response of Srebf1c promoter activity (Figures S1C–S1E). Based on these lines of evidence and the fact that mRNA and protein expression levels as well as DNA binding of LXRα/β were not altered by dietary manipulations (Figure 2F), we hypothesized that this unknown transcription factor binding to NuRE conferred a negative regulatory effect on LXR/RXR transcriptional activity.

TFEL Genome-wide Screen of trans-Acting Factors for NuRE
To identify the hypothetical transcription factor(s) that binds to NuRE and represses LXR/RXR on the Srebf1c promoter, we screened 1,588 genome-wide transcription factor genes included in the TFEL (Transcription Factor Expression Library; N.Y. and Y.T., unpublished data), and assessed the suppressive effects of individual transcription factors against the activity of the promoter containing NuRE plus two LXREs in the presence of cotransfected LXRα/RXRα (Figures 1F, S1F, and S1G). After three rounds of screening, two transcription factors (KLF15 and KLF4) were identified as candidates (Figures 1G and S1H). KLF15 and KLF4 suppressed the promoter containing NuRE plus two LXREs in an NuRE-dependent manner, while KLF family members other than KLF15 and KLF4 did not (Figure 2A). In addition, EMSAs demonstrated that recombinant KLF15 and KLF4 proteins bind to the NuRE probe (Figures 2B, S2A, and S2B). This element includes a 5′-CCCCATTC-3′ sequence that resembles the 5′-CCCCACCC-3′ consensus motif of KLF family members (Jiang et al., 2008).

These results were further supported by an in vivo mutation analysis, which demonstrated that the mutation at the KLF-binding site decreased the response of the native Srebf1c promoter activity to dietary manipulations (Figures S2C and S2D). The DNA sequences of this KLF-binding site as well as two LXREs are highly conserved among vertebrates (Figure 2C).

Fasting-Induced KLF15 Binds to NuRE
Next, we examined KLF15 and KLF4 expression levels in the liver. Several KLFs are known to be widely expressed in various organs involved in energy metabolism (Gray et al., 2007; Mori et al., 2005; Teshigawara et al., 2005; Uchida et al., 2000). The expression of KLF15 in the liver was robust and markedly increased with fasting (Figure 2D), consistent with previous reports (Takashima et al., 2010; Teshigawara et al., 2005). By contrast, hepatic KLF4 expression was a thousand-fold lower. Accordingly, the nuclear protein detected using the EMSA to bind to NuRE during fasting was identified as KLF15 based on the disappearance of the band when adding anti-KLF15 antibody (Figure 2E). Furthermore, chromatin immunoprecipitation (ChiP) assays showed that KLF15 binding to the NuRE was markedly increased in the fasting state compared with the re-fed state (Figure 2F). In contrast, the elevation of KLF4 expression to the 2-fold level of the fasting condition using adenovirus had no effects on Srebf1c transcription (Figures S2E–S2H). Based on these data, we excluded KLF4 as a candidate for the NuRE-binding transcription factor and focused on KLF15 in the following studies.

Role of KLF15 in Srebf1 Regulation
To clarify the role of KLF15 in the regulation of Srebf1 gene expression in the in vivo liver, we examined the influences of the forced expression or the knockdown of KLF15 using adenoviruses. Interestingly, forced overexpression of KLF15 in re-fed mouse livers to levels comparable to those observed in the fasted state led to complete repression of Srebf1c promoter activity (Figures 3A, S3A, and S3B), a decrease in mRNA and nuclear protein levels of SREBP-1 (Figures 3B and S3C), a decrease in the promoter activity and mRNA level of Fasn (Figures 3B and 3C), and consequently a decrease in plasma triglyceride levels (Figure 3D). The suppressive effect of KLF15 on Fasn mRNA levels was completely abolished by the enforced expression of SREBP-1c (Figure S3D), demonstrating that the effect of KLF15 on hepatic lipogenesis is mediated via suppression of SREBP-1c. The adenovirally transduced KLF15 also repressed Srebf1 expression while inducing Pck1 (the gene name for phosphoenolpyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis) in primary hepatocytes (Figures S3E and
S3F). In contrast, the mRNA level of Abca1, another representative target gene of LXR, was not influenced by KLF15 overexpression (Figure 3B), demonstrating that this KLF15-mediated suppression of LXR is dependent on NuRE and therefore specific to the Srebf1c promoter.

Conversely, when we evaluated the contribution of KLF15 in the fasting state by knockdown, promoter activity as well as the expression of Srebf1 and, consequently, Fasn genes was markedly elevated despite fasting conditions, whereas Pck1 expression was significantly decreased (Figures 3E, 3F, S3G, and S3H). The two small hairpin RNAs (shRNAs) exerted essentially the same effects on expression levels of SREBP-1c, excluding the possibility of off-target effects (Figures S3I–S3K). In contrast, the mRNA level of Abca1 was not affected by KLF15 knockdown, again showing that the KLF15-mediated inhibition of LXR is specific to the Srebf1c promoter. The effect of KLF15 knockdown was shown to be dependent on NuRE (Figure S3L).

To further check the validity of our model, we analyzed KLF15 knockout mice. KLF15 knockout mice exhibited significantly higher activities of Srebf1c promoter at an early stage of fasting.
(6 hr of fasting) as assessed by luciferase reporter assay and quantified using in vivo imaging system (Figure 3G). In accordance with this, the rapid decrease of Srebf1c, Fasn mRNA, and plasma triglycerides (TGs) as well as the rapid increase in Pck1 mRNA observed in wild-type mice after 6 hr of fasting was not seen in KLF15 knockout mice (Figures 3H, 3I, S4A, and S4B). These data were consistent with those from the knockdown experiments.

Figure 2. Fasting-Induced KLF15 Binds to NuRE on the Srebf1c Promoter
(A) Inhibitory effects of KLF family members on the Srebf1c promoter in the presence of LXRα/RXRα in HEK293 cells (n = 3 per group).
(B) Electrophoretic mobility shift assay with radiolabeled probe for NuRE and GST-KLF15DBD or GST-KLF4DBD recombinant proteins (+, 100 ng; ++, 500 ng). wt, wild-type; mt, mutant.
(C) Result of a UCSC genome browser query (http://genome.ucsc.edu) showing conservation of the KLF-binding site in the Srebf1c promoter among vertebrate species.
(D) qRT PCR analysis of Klf15 and Klf4 mRNA copy numbers and Srebf1c mRNA levels in various tissues from fasted and re-fed mice (n = 3–4 per group). L, liver; W, white adipose tissue; B, brown adipose tissue; SM, skeletal muscle; CM, cardiac muscle.
(E) Electrophoretic mobility shift assay using radiolabeled probe for the KLF-binding site in the Srebf1c promoter and liver nuclear extracts from fasted and re-fed mice. KLF15-specific binding was shown using an anti-KLF15 antibody (immunoglobulin G [IgG] as a negative control). F, fasted; R, re-fed.
(F) Chromatin immunoprecipitation assay with liver nuclear extracts and anti-KLF15, anti-LXRα/β, anti-RXRα, and anti-SREBP1 antibodies along with IgG as a negative control. PCR was conducted with primers for two LXREs plus NuRE in the Srebf1c promoter or SRE in the Fasn promoter as a control. Results are represented as means ± SEM.
See also Figure S2.
Figure 3. Role of KLF15 in Srebf1 Regulation

(A–D) Effects of KLF15 elevation. KLF15 were elevated to the fasting level in re-fed mouse liver using an adenovirus (Ad-KLF15).

(E and F) Effects of KLF15 knockdown. Knockdown of hepatic KLF15 was performed using adenovirus-mediated RNAi (Ad-KLF15i).

(G–J) Analyses of KLF15 knockout (KO) compared with wild-type (WT) mice.

(legend continued on next page)
By contrast, after prolonged fasting (24 hr of fasting), the Srebf1c promoter activities of KLF15 knockout mice were suppressed to the similar levels as those of the wild-type control mice. To clarify the compensatory mechanism in KLF15 knockout mice that finally suppressed SREBP-1c to the same low levels seen in wild-type mice under the prolonged (24-hr) fasting condition, we examined blood profiles of KLF15 knockout mice. Consistent with a previous report (Gray et al., 2007), KLF15 knockout mice showed significantly lower blood glucose levels (Figure S4C), leading to the marked elevation of plasma glucagon levels (Figure S4D). We found that this in turn activated the hepatic protein kinase A (PKA) pathway in KLF15 knockout mice (Figures 3J and S4E–S4G), which led to the suppression of LXR/RXR transcriptional activity (Yamamoto et al., 2007).

Consistently, the mRNA and protein abundance of Klf15 and Sreb1f1 in the liver showed reciprocal changes according to the time course of fasting or re-feeding (Figures 3K–3M). Moreover, the reverse correlation between KLF15 and SREBP-1 was preserved when animals were re-fed with various amounts of food (Figure 3N).

To further verify if this suppression by KLF15 actually occurs during the physiological time course, we checked the suppressive effect of KLF15 using the Tet-on system, where the rapid induction of KLF15 is driven by doxycycline (Dox) (Figures S4H–S4M). As shown in Figures 3O–3Q (see also Figure S4K), Dox administration quickly suppressed Sreb1f1 promoter activity.

From these multiple lines of evidence, we concluded that fasting-induced KLF15 binds to NuRE and suppresses Sreb1f1 promoter activity by inhibiting LXR/RXR and that KLF15 rapidly controls expression levels of SREBP-1c.

**Mechanism of the KLF15-LXR Interaction**

To elucidate the molecular mechanism of KLF15 suppression against LXR/RXR, the molecular interaction between KLF15 and LXR/RXR was assessed using communoprecipitation experiments. KLF15 and LXR/RXR were shown to form a complex in cultured cell and in vivo liver nuclei (Figure 4A). Notably, the LXR-RXR interaction was not influenced by KLF15 (Figure 4B).

To understand the specificity of this KLF15-LXR/RXR interaction, we investigated the binding of KLF family members, including KLF15 and KLF4, to LXRα and RXRα using glutathione S-transferase (GST) pull-down assays. Among the KLFs that were tested (KLF1, 4, 5, 9, 10, and 15), only KLF15 and KLF4 bound to LXRα (Figure 4C). Essentially the same results were obtained using mammalian two-hybrid experiments (Figure 4D). RXRα showed a broader specificity for KLF5, KLF9, and KLF10, in addition to KLF15 and KLF4. Taken together with the finding that other KLFs such as KLF5, 8, and 10 can bind to the NuRE sequence (Figures S2A and S2B), the specificity of KLF15 and KLF4 on the suppression of Sreb1f1 promoter activity (Figure 2A) is considered to be attributable to the specific binding of LXR to KLF15 and KLF4. Intriguingly, KLF15 also has an affinity for RXRα, and RXRα was interchangeable with LXRα as a partner for KLF15 (Figures S5A and S5B).

Next, we attempted to determine the domain of KLF15 responsible for the interaction with LXRα. KLF15 consists of two domains: an N-terminal repression and activation domain (RAD) and a C-terminal DNA-binding domain (DBD) composed of three zinc-finger domains. S32P-labeled deletion derivatives of KLF15 were subjected to a pull-down assay with GST-LXRα, and the zinc-finger domain of KLF15 was shown to be critical for its interaction with LXRα (Figure 4E). Supporting this finding, a truncated KLF15 lacking the zinc-finger domain had no effect on the inhibition of LXR/RXR activity (Figure 4F). Similarly, to map the domain of LXRα required for this interaction, we performed pull-down assays using GST fusion proteins containing various domains of LXRα, which consists of four domains: a ligand-independent activation function domain (A/B), a DNA-binding domain (C), a ligand-binding domain (DE), and a ligand-dependent transactivation function-2 domain (AF2). These pull-down assays revealed that the C domain of LXRα is critical for its interaction with KLF15 (Figure 4G). A comparable result was observed for the RXRα-KLF15 interaction (Figure S5C).

These results demonstrate that KLF15 and LXRα interact with each other through the DNA-binding domain on each molecule. The DNA-binding domains of transcription factors, including nuclear receptors, can also function as an interacting domain with other proteins (Yin et al., 2002). The EMSA results (Figure S5D) confirmed that KLF15 and LXRα/RXRα actually form a complex on the DNA fragment containing LXRE plus NuRE, whereas the binding of KLF15 or LXRα/RXRα to each corresponding element was not affected by LXRα/RXRα or KLF15, respectively.

**KLF15 Represses LXR by Recruiting the RIP140 Corepressor**

To further explore the detailed mechanism of how KLF15 suppresses LXR/RXR, we attempted to identify additional cofactor(s) that may be operative. Three corepressors (NcoR1, SMRT
[NcoR2], and RIP140) can reportedly be coupled with LXRα or β-mediated transcription (Glass et al., 1997; Hu et al., 2003; Jakobsson et al., 2007; Yamamoto et al., 2007). To test the possible involvement of these corepressors in the KLF15-induced inhibition of LXR activity, mammalian two-hybrid assays were performed. This system detects the binding of a VP16-AD-LXRα fusion protein and GAL4-DBD-corepressor-NiD (a functional domain that interacts with nuclear receptors) in HEK293 cells (Figures 5A and S5E). Unlike NcoR1 and SMRT, the hybridization between RIP140 and LXRα was enhanced by KLF15, indicating that KLF15 promotes the recruitment of the RIP140 corepressor to LXRα. This result was confirmed by immunoprecipitation experiments (Figure 5B). In particular, the amount of LXR-RIP140 complex was much more abundant in the presence of KLF15 in wild-type mice compared with KLF15 knockout mice, although LXR and RIP140 themselves were present at the similar levels in both models of mice (Figure S5F).

We also checked the possible involvement of a major coactivator coupled with LXR, steroid receptor coactivator-1 (SRC1) (Huuskoenen et al., 2004); KLF15 specifically decreased the recruitment of SRC1 to LXRα (Figure 5C), while KLF family members other than KLF15 did not (Figure S5G).

Next, we examined the physiological changes in the amount of LXR-RIP140 and LXR-SRC1 complexes during fasting and refeeding in an in vivo setting. When liver nuclear extracts were immunoprecipitated with anti-LXRα/β antibody and coprecipitated RIP140 and SRC1 were visualized using western blotting, a larger amount of LXR-RIP140 complex was found in the fasting state (Figure 5D). In contrast, SRC1 coupled with LXR was reciprocally decreased in the fasting state, suggesting that RIP140 recruitment assisted by KLF15 competes with SRC1 binding to LXR and thus inhibits transcriptional activity. A ChIP assay using RIP140 and SRC1 antibody also exhibited reciprocal patterns between RIP140 and SRC1 (Figure 5E). When we tested if RIP140 and SRC1 compete against each other when binding to LXRα using a GST pull-down assay, RIP140 inhibited SRC1 binding to LXRα and vice versa (Figure 5F). Knockdown of RIP140 caused a marked elevation in Srebf1 gene promoter activity and, consequently, Srebf1 gene expression in the fasting state (Figures 5G and 5H), but not in the fed state (Figure S5H), consistent with a previous report (Berriel Diaz et al., 2008). Moreover, RIP140 deficiency completely cancelled the effect of KLF15 knockdown on Srebf1c expression in the fasting state (Figure S5I), indicating that the effect of KLF15 on Srebf1c expression is completely dependent on RIP140.

From these results, we concluded that KLF15 suppresses LXR by recruiting a RIP140 corepressor instead of an SRC1 coactivator.

**KLF15 Ameliorates Hypertriglyceridemia**

To explore the possible involvement of KLF15 in the pathogenesis of obesity-related metabolic disorders, we examined the role of KLF15 in the hypertriglyceridemia observed in genetically obese ob/ob mice. As shown in Figure 6A (and also in Figures 6E, 6F, and S6G), the expression level of KLF15 was significantly lower in ob/ob mouse liver compared with control. Forced restoration of KLF15 levels in ob/ob mice to wild-type levels attenuated Srebf1 overexpression and led to an improvement in hypertriglyceridemia (Figures 6B, 6C, and S6C–S6E) without an elevation in blood glucose levels (Figure 6D).

As another animal model of hypertriglyceridemia, we tested the effect of KLF15 activation on mice administered T0901317, a synthetic LXR ligand. Administration of T0901317 induced hepatic SREBP-1c and caused hypertriglyceridemia. When Ad-KLF15 adenovirus was transduced in the livers of T0901317-treated mice, Srebf1 mRNA expression was completely repressed to vehicle-treated control levels (Figures 6E, 6F, and S6G), resulting in a decrease in Fasn mRNA and a concomitant decrease in plasma triglyceride levels with no effect on high-density lipoprotein (HDL) cholesterol levels (Figures 6F–6H). These results suggest that KLF15 is involved in the pathogenesis of obesity-related dyslipidemia and can be a therapeutic target for treatment.

**DISCUSSION**

SREBP-1c is regulated at the transcription level depending upon nutritional states, especially during cycles of fasting and refeeding. Although the Srebf1c promoter is controlled by LXR, the precise molecular mechanism by which SREBP-1c expression is nutritionally regulated was largely unknown. The current study clearly demonstrates, using new nutrigenomic approaches, that fasting-induced KLF15 forms a complex with LXR/RXR on the Srebf1c promoter to repress the expression of SREBP-1c and downstream lipogenic genes during the early and euglycemic phases of fasting (Figure 7). Conversely, in the postprandial...
state, KLF15 levels decrease and the suppressive complex of KLF15-LXR/RXR-RIP140 was replaced by LXR/RXR-SRC1, leading to activation of the Srebf1c promoter.

Before we began this study, it was well known that LXR can be activated by ligands (Forman et al., 1997; Janowski et al., 1996; Lehmann et al., 1997) and that diet-derived LXR ligands might be the key inducer of SREBP-1 and lipogenic gene expression. However, in our series of in vivo Ad-luc experiments elucidating the regulatory mechanism of LXR, we found out that the LXR-binding cis elements alone were not sufficient for fasting-refeeding regulation, although they exhibited a complete response to an LXR ligand (Figure 1B). This finding clearly showed that the fasting-refeeding regulation of SREBP-1c transcription is not brought about by changes in LXR ligand concentration. In accordance with this, LXR downstream genes such as ABCA1, ABCG5, and ABCG8 in the liver show no response to refeeding.

Figure 5. KLF15 Represses LXR by Recruiting the RIP140 Corepressor

(A) Recruitment of corepressors to LXRα by KLF15 as assessed using a mammalian two-hybrid system. HEK293 cells were cotransfected with Gal4-RE-Luc plasmid and expression vectors for VP16-LXRα and GAL4-Corep-NID (Gal4-DBD fused to NID of RIP140, NcoR1 or SMRT) along with KLF15 expression plasmid and incubated with media containing T0901317 (1 μM) (n = 3 per group).

(B) Coimmunoprecipitation of RIP140 with LXRα or KLF15.

(C) Recruitment of coactivator SRC1 to LXRα as assessed using a mammalian two-hybrid system (n = 3 per group).

(D) Coimmunoprecipitation of RIP140 and SRC1 using anti-LXR antibody. F, fasted; R, re-fed.

(E) Chromatin immunoprecipitation assay with anti-RIP140 and anti-SRC1 antibodies. PCR was performed using primers for two LXREs and an NuRE-containing region in the Srebf1c promoter.

(F) GST pull-down assay with GST-LXRα against RIP140 or SRC1. Unlabeled proteins were added as competitors.

(G and H) Knockdown of hepatic RIP140 using adenovirus-mediated RNAi (Ad-RIP140).

(G) Images captured using IVIS and luciferase activities per transduced adenoviral DNA in the livers of fasted mice are shown (n = 5–12 per group). F, fasted; R, re-fed.

(H) qRT PCR of gene expression in the fasted mouse livers from each group (n = 7–8 per group). Results are presented as means ± SEM. *p < 0.05 and **p < 0.01 versus control of each group. See also Figure S5.
Figure 6. KLF15 Ameliorates Hypertriglyceridemia

(A) Hepatic gene expression in 18-hr-fasted ob/ob mice (n = 6–7 per group).
(B–D) KLF15 was elevated to wild-type levels in ob/ob mice liver using Ad-KLF15. Hepatic gene expression (B), plasma triglyceride levels (C), and blood glucose levels (D) in 18-hr-fasted states are shown (n = 5–6 per group).
(E–H) Effects of KLF15 activation on LXR-stimulated hyperlipidemic mice. 1 day after Ad-KLF15 injection, mice were treated orally with 50 mg/kg T0901317 once a day for 3 days in the ad lib state. (E) Hepatic gene expression at 2 hr after the last T0901317 treatment (n = 5 per group). (F–H) Plasma lipid profiles at 5 hr after the last T0901317 treatment (n = 18–25 per group).

Results are presented as means ± SEM. *p < 0.05 and **p < 0.01 versus control of each group. See also Figure S6.
KLF15 is upregulated in fasting mouse liver and is known to contribute to the regulation of hepatic gluconeogenesis (Gray et al., 2007; Teshigawara et al., 2005). In light of the current work, we propose that the regulation of lipogenesis and gluconeogenesis is coordinated by KLF15 and is reciprocally regulated in response to nutritional conditions. Given that lipogenesis is an adaptive process that facilitates survival during subsequent starvation, it is conceivable that the key factor or factors controlling lipogenesis are negatively regulated by these fasting-induced factors.

In our previous publication, we reported that the PKA phosphorylation of LXR suppresses Srebf1c transcription by recruiting a co-repressor NcoR1 (Yamamoto et al., 2007). To clarify the relationship between KLF15-mediated and PKA-mediated Srebf1c repression, we analyzed KLF15 knockout mice (Gray et al., 2007). As shown in Figure 3G, KLF15 knockout mice exhibited significantly higher Srebf1c promoter activity at an early stage of fasting (6 hr of fasting). In contrast, after prolonged fasting (24 hr of fasting), Srebf1c promoter activity in KLF15 knockout mice was suppressed to levels similar to those of wild-type control mice. A series of experiments revealed that the compensatory mechanism in KLF15 knockout mice that wild-type control mice. A series of experiments revealed that

KLF15 expression to the same low levels presence of LXR/ RXR transcriptional activity that was dependent on the on NcoR1 co-repressor (Figures 3J, S4C–S4E, and S7A–S7E). Supporting this finding, NcoR1 binding to the Srebf1c promoter as assessed by ChIP assay was elevated in KLF15 knockout mice (Figure S7G). Interestingly, PKA-mediated phosphorylation of type mice than in KLF15 knockout mice (Figure S7F). Together with the other important role of KLF15 in gluconeogenesis, the KLF15-mediated pathway can be considered as an adaptive and homeostatic mechanism of energy metabolism working from the euglycemic phase of fasting before hypoglycemia and PKA activation proceeds.

Previously, a detailed mutation analysis of SREBP-1c promoter activity in primary hepatocytes was reported (Chen et al., 2004), with no derepression of promoter activity around the NuRE region. Whereas our analyses were based on intra-organ assays using living animals, Chen et al. used cultured primary hepatocytes in their experiments, which might be the cause of this discrepancy. In fact, the expression of KLF15 severely diminishes soon after primary hepatocytes are prepared (data not shown), demonstrating the differences between in vivo and in vitro settings. It is probable that our strategy of starting from intra-organ assays to screen the cis elements enabled us to successfully identify the KLF15-mediated pathway.

Regarding the upstream regulatory mechanisms by which KLF15 is induced during fasting, the possible involvement of insulin was examined (Figure S7G). We found that STZ-induced insulin deficiency partially increased KLF15 while it decreased SREBP-1c, suggesting some molecular link between insulin and KLF15. At the same time, it has been reported that hormones other than insulin are involved in the regulation of KLF15 expression (Teshigawara et al., 2005). These findings are consistent with a previous report analyzing liver-specific insulin receptor knockout (LIRKO) mice (Haas et al., 2012), which revealed that although insulin signaling is necessary for maximal induction, nutrients (either directly or indirectly, via hormones other than insulin) are sufficient for the induction of the SREBP-1c and lipogenic gene transcripts.

In the present study, we used our original library of transcription factors, TFEL (N.Y. and Y.T, unpublished data), which
covers nearly all the known transcription factors encoded by the mouse genome, to identify the transcription factor binding to NuRE in the presence of LXR/RXR. It is well known that binding site prediction based on binding motif databases suffers from high false-positive and false-negative rates in general (Kim and Park, 2011). In particular, the sequence-based approach has no power to predict transcription factor complexes formed by protein-protein interactions.

Conversely, the search for protein-protein interactions among transcription factors alone may not be sufficient to elucidate the regulatory complexes in certain situations, including the present case, because complexes like KLF15-LXR/RXR also depend on cis elements (i.e., NuRE plus LXRE, in this case). This relatively weak interaction supported by the DNA backbone enables gene-specific regulations and may give more diversity to transcriptional networks. Thus, our combinatorial strategy of searching for a functional cis element and screening for the corresponding transcription factor complex in a context-dependent manner can be a very effective and powerful approach for exploring sophisticated transcriptional networks in detail.

Synthetic LXR ligands have been expected to have atheroprotective effects through the promotion of reverse cholesterol transport and inhibition of intestinal cholesterol absorption (Joseph et al., 2002). However, the unfavorable side effect of elevating plasma triglyceride levels has hampered the therapeutic application of LXR ligands. Our finding that KLF15 can specifically block the lipogenic effect of an LXR ligand provides therapeutic implications for the treatment of dyslipidemia, including obesity-related diseases with SREBP-1c overexpression levels of KLF15 and gluconeogenic genes are very low (data not shown), does not lead to an excess gluconeogenesis (Figures 6D, S3F, and S6D; Table S1). Thus, KLF15 overexpression can improve the plasma lipid profile without exacerbating hyperglycemia. Elucidating the upstream mechanisms of KLF15 regulation will also be important from this therapeutic point of view.

**EXPERIMENTAL PROCEDURES**

**Animals**

6- to 8-week-old ICR male mice were purchased from Central Laboratories for Experimental Animals (CLEA). 6- to 7-week-old ob/ob male mice were purchased from SLC. KLF15 knockout mice were a gift from Prof. M.K. Jain and genotyped as previously described (Fisch et al., 2007), and wild-type littermates were used as controls. All animals were maintained in a temperature-controlled environment with a 12-hr light/dark cycle and given free access to food and water. For the fasting group, animals were starved 24 hr, and for the refeeding group, they were re-fed for 16 hr after a 24-hr starvation. For experiments using ob/ob mice, animals were starved for 18 hr. For LXR ligand experiments of hepatic luciferase activity, T0901317 was administered at a dose of 50 mg/kg after a 24-hr starvation and analyzed 16 hr later. For experiments using insulin-depleted diabetic mice, ICR male mice were administered streptozocin (two intraperitoneal injections of 100 mg/kg body weight with a 1-day interval) as previously described (Takeuchi et al., 2007). Mice were sacrificed in the early light phase in a fasted, re-fed, or nonfasted (ad libitum) state. All animals studied were anesthetized and euthanized according to a protocol approved by the Tsukuba University Animal Care and Use Committee. All experiments were repeated at least twice.

**Preparation of Recombinant Adenoviruses**

Recombinant adenoviruses were constructed using the Gateway system (Invitrogen). Details are provided in Supplemental Experimental Procedures.

**In Vivo Imaging of Luciferase Activity**

In vivo imaging was performed as described previously (Takeuchi et al., 2010). 3–6 days after adenovirus transduction, D-luciferin potassium salt (Wako) was injected intraperitoneally (i.p.) into mice, and luminescence in the liver was captured using an IVIS Imaging System (Xenogen). Relative photon emission over the liver region was quantified using LivingImage software (Xenogen). When hepatic transduction efficiency was determined, quantification of adenoviral DNA in the liver was performed using a previously described qRT-PCR method (Takeuchi et al., 2007), and the result of quantification was used to normalize the in vivo imaging of luciferase activity. Otherwise, paired data from the same animal for the different nutritional conditions (i.e., fasted or re-fed) were continuously obtained, and the ratio between the two quantities was used to cancel the variations in hepatic transduction efficiencies.

**RNA Isolation and Northern Blotting**

Total RNA preparation and blot hybridization with cDNA probes were performed as previously described (Takeuchi et al., 2010). Full-length cDNAs were used for cDNA probes for mouse Klf15 and Klf4. The cDNA probe for mouse Hpd was prepared by RT-PCR using mouse total RNA as a template. The primers were as follows: 5′-CATTTCCACTCGTGACCT-3′ and 5′-TGTTCCGTCACCACCATG-3′. RpL32 was used as a loading control. Blots were exposed to a BAS imaging plate for the BAS2000 Bio Imaging Analyzer (Fuji Photo Film).

**qRT-PCR**

Total RNA (2 µg) was reverse transcribed using the High Capacity cDNA reverse transcription kit (Applied Biosystems). qRT-PCR was performed using SYBR green dye (Kapa Biosystems) on a 7300 real-time PCR system (Applied Biosystems). Primer sets are listed in Table S2.

**Immunoblotting and Immunoprecipitation**

Immunoblotting and immunoprecipitation were performed as described previously (Takeuchi et al., 2010; Yamamoto et al., 2007). Nuclear extract protein from mouse liver and HEK293 cells was prepared as previously described (Sheng et al., 1995; Yahagi et al., 2003, 2004).

**Construction of the Tet-On System**

A Tet-on system to rapidly activate KLF15 expression was constructed as described previously (Uringer et al., 2000). A modified rTetR fragment was amplified by PCR from pHUD172-1 (Gossen et al., 1995). Tet response element (TRE) was cloned from pSIREN-RetroQ-TetP (Clontech). Details are provided in Supplemental Experimental Procedures.
GST Pull-Down Assay
GST and GST fusion proteins were prepared as described previously [Najima et al., 2005] and dialyzed with dialysis buffer (50 mM Tris- HCl [pH 8.0], 50 mM NaCl, 1 mM EDTA, and 1 mM DTT). [35S]Metionine-labeled proteins were prepared using the TNT T7 quick-coupled transcription/translation system (Promega). GST pull-down assays were performed as described previously [Najima et al., 2005; Yamamoto et al., 2007].

Mammalian Two-Hybrid Assay
HEK293 cells were cotransfected with GAL4-RE-Luc plasmids and expression plasmids for GAL4 fusion protein and VP16 fusion protein.

Statistical Analyses
Data are expressed as means ± SEM. Differences between two groups were assessed using an unpaired two-tailed Student’s t test. Datasets involving more than two groups were assessed by ANOVA with Statview Software (BrainPower). Differences were considered statistically significant at p < 0.05 (p < 0.05 and *p < 0.01).

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures, seven figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.069.

AUTHOR CONTRIBUTIONS
N.Y. and Y.T. conceived the experiments. Y.T. performed the experiments and analyzed the data, together with N.Y. Y.L. and M.J. provided the KLF15 knockout mice. N.Y. and Y.T. co-wrote the paper. All authors discussed the results and commented on the manuscript.

ACKNOWLEDGMENTS
This work was supported by grants from MEXT/JSPS KAKENHI (23116006 [Grant-in-Aid for Scientific Research on Innovative Areas: Crosstalk of transcriptional control and energy pathways by hub metabolites], 15H03092 [Grant-in-Aid for Scientific Research (B)], 21591123 and 18590979 [Grant-in-Aid for Scientific Research (C)], 25660392 and 16K13040 [Grant-in-Aid for Challenging Exploratory Research], and 03J10558 [Grant-in-Aid for JSPS Fellows] to N.Y.); by research grants from the Uehara Memorial Foundation, ONO Challenge Exploratory Research, and 03J10558 [Grant-in-Aid for Scientific Research (C)], 26560392 and 16K13040 [Grant-in-Aid for Scientific Research on Innovative Areas: Crosstalk of transcriptional control and energy pathways by hub metabolites], 15H03092 [Grant-in-Aid for Scientific Research (B)], and 18590979 [Grant-in-Aid for Scientific Research (C)] to M.K.J.); and by a grant from the NIH (HL119195l, to M.K.J.).

REFERENCES
Amemiya-Kudo, M., Shimano, H., Yoshikawa, T., Yahagi, N., Hasty, A.H., Okazaki, H., Tamura, Y., Shionori, F., Iizuka, Y., Ohashi, K., et al. (2000). Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J. Biol. Chem. 275, 31078–31085.
Berriel Diaz, M., Krones-Herzig, A., Metzger, D., Ziegler, A., Vegiopoulos, A., Klingenspor, M., Muller-Decker, K., and Herzog, S. (2008). Nuclear receptor cofactor receptor interacting protein 140 controls hepatic triglyceride metabolism during wasting in mice. Hepatology 48, 782–791.
Chen, G., Liang, G., Ou, J., Goldstein, J.L., and Brown, M.S. (2004). Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and stimulation of fatty acid synthesis in liver. Proc. Natl. Acad. Sci. USA 101, 11245–11250.
Chen, W., Chen, G., Head, D.L., Mangelsdorff, D.J., and Russell, D.W. (2007). Enzymatic reduction of oxysterols impairs LXR signaling in cultured cells and the livers of mice. Cell Metab. 5, 73–79.
Fisch, S., Gray, S., Heymans, S., Haldar, S.M., Wang, B., Pfister, O., Cui, L., Kumar, A., Lin, Z., Sen-Banerjee, S., et al. (2007). Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. Proc. Natl. Acad. Sci. USA 104, 7074–7079.
Forman, B.M., Ruan, B., Chen, J., Schroeper, G.J., Jr., and Evans, R.M. (1997). The orphan nuclear receptor LXRalpha is positively and negatively regulated by distinct products of mevalonate metabolism. Proc. Natl. Acad. Sci. USA 94, 10588–10593.
Glass, C.K., Rose, D.W., and Rosenfeld, M.G. (1997). Nuclear receptor coactivators. Curr. Opin. Cell Biol. 9, 222–232.
Gossen, M., Freundlieb, S., Bender, G., Muller, G., Hillen, W., and Bujard, H. (1995). Transcriptional activation by tetracyclines in mammalian cells. Science 268, 1766–1769.
Gray, S., Feinberg, M.W., Hull, S., Kuo, C.T., Watanabe, M., Sen-Banerjee, S., DePina, A., Haspel, R., and Jain, M.K. (2002). The Kruppel-like factor KLF15 regulates the insulin-sensitive glucose transporter GLUT4. J. Biol. Chem. 277, 34322–34328.
Gray, S., Wang, B., Ohnueila, Y., Hong, E.G., Fisch, S., Haldar, S., Cline, G.W., Kim, J.K., Peroni, O.D., Kahn, B.B., and Jain, M.K. (2007). Regulation of gluconeogenesis by Kruppel-like factor 15. Cell Metab. 5, 305–312.
Haas, J.T., Miao, J., Chanda, D., Wang, Y., Zhao, E., Haas, M.E., Hirschey, M., Vatheseevan, B., Farese, R.V., Jr., Kurtand, I.J., et al. (2012). Hepatic insulin signaling is required for obesity-dependent expression of SREBP-1c mRNA but not for feeding-dependent expression. Cell Metab. 15, 873–884.
Horton, J.D., Bashmakov, Y., Shimomura, I., and Shireno, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc. Natl. Acad. Sci. USA 95, 5987–5992.
Hu, X., Li, L., Wu, J., Xia, C., and Lai, D.S. (2003). Liver X receptors interact with corepressors to regulate gene expression. Mol. Endocrinol. 17, 1019–1026.
Huuskonen, J., Fielding, P.E., and Fielding, C.J. (2000). Role of p160 coactivator complex in the activation of liver X receptor. Arterioscler. Thromb. Vasc. Biol. 24, 703–708.
Ide, T., Shimano, H., Yahagi, N., Matsuzaka, T., Nakakuki, M., Yamamoto, T., Nakagawa, Y., Takahashi, A., Suzuki, H., Sone, H., et al. (2004). SREBPs suppress IRS-2-mediated insulin signalling in the liver. Nat. Cell Biol. 6, 351–357.
Jakobsson, T., Osman, W., Gustafsson, J.A., Zilliacus, J., and Wärmak, A. (2007). Molecular basis for repression of liver X receptor-mediated gene transcription by receptor-interacting protein 140. Biochem. J. 405, 31–39.
Janowski, B.A., Willy, P.J., Devi, T.R., Falck, J.R., and Mangelsdorf, D.J. (1996). An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature 383, 728–731.
Jiang, J., Chan, Y.-S., Loh, Y.-H., Cai, J., Tong, G.-Q., Lim, C.-A., Robson, P., Zhong, S., and Ng, H.-H. (2008). A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat. Cell Biol. 10, 353–360.
Joseph, S.B., McKilligin, E., Pei, L., Watson, M.A., Collins, A.R., Laffitte, B.A., Chen, M., Noh, G., Goodman, J., Hagger, G.N., et al. (2002). Synthetic LXR ligands inhibit the development of atherosclerosis in mice. Proc. Natl. Acad. Sci. USA 99, 7604–7609.
Kim, T.M., and Park, P.J. (2011). Advances in analysis of transcriptional regulatory networks. Wiley Interdiscip. Rev. Syst. Biol. Med. 3, 21–35.
Kim, J.B., Sarraf, P., Wright, M., Yao, K.M., Mueller, E., Solanes, G., Lowell, B.B., and Spiegelman, B.M. (1998). Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Invest. 101, 1–9.
Lehmann, J.M., Kliewer, S.A., Moore, L.B., Smith-Oliver, T.A., Oliver, B.B., Su, J.L., Sundseth, S.S., Vinafrag, D.A., Blanchard, D.E., Spencer, T.A., and Willson, T.M. (1997). Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. J. Biol. Chem. 272, 3137–3140.
Matsuzaka, T., Shimono, H., Yahagi, N., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Tomita, S., Sekiya, M., et al. (2004). Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin-treated mice. J. Biol. Chem. 279, 560–569.

Mori, T., Sakaue, H., Iuchi, H., Gomi, H., Okada, Y., Takahashi, Y., Nakamura, K., Nakamura, T., Yamanauchi, Y., Kubota, N., et al. (2005). Role of Kruppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J. Biol. Chem. 280, 12867–12875.

Najima, Y., Yahagi, N., Takeuchi, Y., Matsuzaka, T., Sekiya, M., Nakagawa, Y., Amemiya-Kudo, M., Okazaki, H., Okazaki, S., Tamura, Y., et al. (2005). High mobility group protein-B1 interacts with sterol regulatory element-binding proteins to enhance their DNA binding. J. Biol. Chem. 280, 27523–27532.

Nakagawa, Y., Shimano, H., Yoshikawa, T., Ide, T., Tamura, M., Furusawa, M., Yamamoto, T., Inoue, N., Matsuzaka, T., Takahashi, A., et al. (2006). TFE3 transcriptionally activates hepatic IRS-2, participates in insulin signaling and ameliorates diabetes. Nat. Med. 12, 107–113.

Oosterveer, M.H., van Dijk, T.H., Greffhorst, A., Bloks, V.W., Havinga, R., Kuipers, F., and Reijngoud, D.J. (2008). Lxra deficiency hampers the hepatic adaptive response to fasting in mice. J. Biol. Chem. 283, 25437–25445.

Repah, J.J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J.-M.A., Shimomura, I., Shan, B., Brown, M.S., Goldstein, J.L., and Mangelsdorf, D.J. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. Genes Dev. 14, 2819–2830.

Sekiya, M., Yahagi, N., Matsuzaka, T., Takeuchi, Y., Nakagawa, Y., Takahashi, H., Okazaki, H., Iizuka, Y., Ohashi, K., Gotoda, T., et al. (2007). SREBP-1-independent regulation of lipogenic gene expression in adipocytes. J. Lipid Res. 48, 1581–1591.

Sheng, Z., Otani, H., Brown, M.S., and Goldstein, J.L. (1995). Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. Proc. Natl. Acad. Sci. USA 92, 935–938.

Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., et al. (1999). Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J. Biol. Chem. 274, 35832–35839.

Shimomura, I., Bashmakov, Y., Ikemoto, S., Horton, J.D., Brown, M.S., and Goldstein, J.L. (1999). Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA 96, 13656–13661.

Sun, X., Haas, M.E., Miao, J., Mehta, A., Graham, M.J., Crooke, R.M., Pais de Barros, J.P., Wang, J.G., Aikawa, M., Masson, D., and Biddinger, S.B. (2016). Insulin dissociates the effects of liver X receptor on lipogenesis, endoplasmic reticulum stress, and inflammation. J. Biol. Chem. 291, 1115–1122.

Takahashi, M., Ogawa, W., Hayashi, K., Inoue, H., Kinoshita, S., Okamoto, Y., Sakaue, H., Wataoka, Y., Emi, A., Senga, Y., et al. (2010). Role of KLF15 in regulation of hepatic gluconeogenesis and metformin action. Diabetes 59, 1608–1615.

Takeuchi, Y., Yahagi, N., Nakagawa, Y., Matsuzaka, T., Shimizu, R., Sekiya, M., Iizuka, Y., Ohashi, K., Gotoda, T., Yamamoto, M., et al. (2007). In vivo promoter analysis on refeeding response of hepatic sterol regulatory element-binding protein-1c expression. Biochem. Biophys. Res. Commun. 363, 329–335.

Takeuchi, Y., Yahagi, N., Izumida, Y., Nishi, M., Kubota, M., Teraoka, Y., Yamamoto, T., Matsuzaka, T., Nakagawa, Y., Sekiya, M., et al. (2010). Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autolopous regulatory circuit. J. Biol. Chem. 285, 11681–11691.

Teshigawara, K., Ogawa, W., Mori, T., Matsuki, Y., Watanabe, E., Hiramatsu, R., Inoue, H., Miyake, K., Sakaue, H., and Kasuga, M. (2005). Role of Kruppel-like factor 15 in PEPCK gene expression in the liver. Biochem. Biophys. Res. Commun. 327, 920–926.

Uchida, S., Tanaka, Y., Ito, H., Saitoh-Ohara, F., Inazawa, J., Yokoyama, K.K., Sasaki, S., and Marumo, F. (2000). Transcriptional regulation of the CLC-K1 promoter by myc-associated zinc finger protein and kidney-enriched Kruppel-like factor 1, a novel zinc finger repressor. Mol. Cell. Biol. 20, 7319–7331.

Urlinger, S., Baron, U., Thellmann, M., Hasan, M.T., Bujard, H., and Hillen, W. (2000). Exploring the sequence space for tetracycline-dependent transcriptional activators: novel mutations yield expanded range and sensitivity. Proc. Natl. Acad. Sci. USA 97, 7963–7968.

Yahagi, N., Shimano, H., Hasty, A.H., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Shionoiri, F., Ohashi, K., Osuga, J., et al. (1999). A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J. Biol. Chem. 274, 35840–35844.

Yahagi, N., Shimano, H., Hasty, A.H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., et al. (2002). Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. J. Biol. Chem. 277, 19353–19357.

Yahagi, N., Shimano, H., Matsuzaka, T., Najima, Y., Sekiya, M., Nakagawa, Y., Ide, T., Tomita, S., Ohashi, K., Tamura, Y., et al. (2003). p53 Activation in adipocytes of obese mice. J. Biol. Chem. 278, 25395–25400.

Yahagi, N., Shimano, H., Matsuzaka, T., Sekiya, M., Najima, Y., Ohashi, K., Sekiya, M., Okazaki, K., Takahashi, A., et al. (2004). p53 involvement in the pathogenesis of fatty liver disease. J. Biol. Chem. 279, 20571–20575.

Yamamoto, T., Shimano, H., Inoue, N., Nakagawa, Y., Matsuzaka, T., Takahashi, A., Yahagi, N., Sone, H., Suzuki, H., Toyoshima, H., and Yamada, N. (2007). Protein kinase A suppresses sterol regulatory element-binding protein-1c expression via phosphorylation of liver X receptor in the liver. J. Biol. Chem. 282, 11687–11695.

Yin, L., Zhang, Y., and Hillgartner, F.B. (2002). Sterol regulatory element-binding protein-1 interacts with the nuclear thyroid hormone receptor to enhance acetyl-CoA carboxylase-α transcription in hepatocytes. J. Biol. Chem. 277, 19554–19565.

Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A.H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., et al. (2001). Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. Mol. Cell. Biol. 21, 2991–3000.