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Cross-regulation of hepatic glucose metabolism *via* ChREBP and Nuclear Receptors

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The abbreviations used are: acetyl-CoA carboxylase (ACC), ATP-binding cassette transporters C1 (ABCC1) and G1 (ABCG1), Basic-helice-loop-helice leucine zipper (bHLH-ZiP), carbohydrate responsive element binding protein (ChREBP), carbohydrate responsive element (ChoRE), chick embryo hepatocytes (CEH), constitutive androstane receptor (CAR), CREB binding protein (CBP), dominant negative form of Mlx (dn-Mlx), estrogen receptor α (ER α), fatty acid synthase (FAS), farnesoid X receptor (FXR), glucokinase (GK), glucose 6-phosphate (G6P), glucose 6 phosphatase (G6Pase), glucose 6 phosphate dehydrogenase G6PDH), glucose-sensing module (GSM), glucose response conserved element (GRACE), glycogen storage disease type 1 (GSD-1), hepatocyte nuclear factor 4 (HNF4), ligand binding domain (LBD), liver-pyruvate kinase (L-PK), liver X receptor (LXR), liver X receptor element (LXRE), low glucose inhibitory domain (LID), Max-like protein X (Mlx), Mondo Conserved Region (MCR), nuclear export signal (NES), nuclear localization signal (NLS), propylthiouracil (PTU), protein kinase (PKA), peroxisome proliferator-activated receptors (PPARs), phosphatase 2A (PP2A), pregnane X receptor (PXR), real time quantitative polymerase chain reaction (RTQ-PCR), retinoid X receptor (RXR), thyroid hormones (TH), thyroid hormone receptor (TR), sterol regulatory element (SRE), sterol regulatory element binding protein-1c (SREBP-1c), stearyl CoA desaturase 1 (SCD1), streptozotocin (STZ), thyroid hormones (TH), triglycerides (TG), vitamin D receptor (VDR).

Key words: ChREBP, nuclear receptors, glucose metabolism, lipogenesis

Abstract

There is a worldwide epidemic of obesity and type 2 diabetes, two major public health concerns associated with alterations in both insulin and glucose signaling pathways. Glucose is not only an energy source but also controls the expression of key genes involved in energetic metabolism, through the glucose-signaling transcription factor, Carbohydrate Responsive Element Binding Protein (ChREBP). ChREBP has emerged as a central regulator of *de novo* fatty acid synthesis (lipogenesis) in response to glucose under both physiological and physiopathological conditions. Glucose activates ChREBP by regulating its entry from the cytosol to the nucleus, thereby promoting its binding to carbohydrate responsive element (ChoRE) in the promoter regions of glycolytic (L-PK) and lipogenic genes (ACC and FAS). We have previously reported that the inhibition of ChREBP in liver of obese ob/ob mice improves the metabolic alterations linked to obesity, fatty liver and insulin-resistance. Therefore, regulating ChREBP activity could be an attractive target for lipid-lowering therapies in obesity and diabetes. However, before this is possible, a better understanding of the mechanism(s) regulating its activity is needed. In this review, we summarize recent findings on the role and regulation of ChREBP and particularly emphasize on the cross-regulations that may exist between key nuclear receptors (LXR, TR, HNF4 α) and ChREBP for the control of hepatic glucose metabolism. These novel molecular cross-talks may open the way to new pharmacological opportunities.

Research highlights

- ChREBP is a central transcriptional regulator of hepatic *de novo* fatty acid synthesis
- ChREBP inhibition in liver of obese ob/ob mice improves hepatic steatosis and insulin-resistance
- ChREBP represents an attractive target for lipid-lowering therapies in obesity and diabetes
- Cross-regulations exist between ChREBP and liver nuclear receptors for the control of hepatic glucose and lipid metabolism

- Identification of novel molecular cross-talks between ChREBP and nuclear receptors may open the way to new pharmacological opportunities

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Introduction

In mammals, the liver is responsible for the conversion of excess dietary carbohydrates into triglycerides (TG), through *de novo* lipogenesis. Appropriate control of lipogenesis is crucial since excess fatty acid storage leads to hepatic steatosis and other related metabolic diseases [1]. Increased lipogenesis results from transcriptional activation of many genes encoding glycolytic and lipogenic enzymes including glucokinase (GK) [2], liver-pyruvate kinase (L-PK) [3], acetyl CoA carboxylase (ACC) [4], fatty acid synthase (FAS) [5] and stearoyl CoA desaturase (SCD1) [6]. Uptake of glucose by liver is concomitant with increased concentrations of substrates such as glucose but also in the ratio of pancreatic hormones: insulin / glucagon. Until recently, it was thought that insulin and glucagon were the main transcriptional regulators of glycolytic and lipogenic gene expression (respectively up and down regulators).

The transcriptional effect of insulin is mediated by sterol regulatory element binding protein-1c (SREBP-1c) [7], a transcription factor from the basic-helix-loop-helix-leucine zipper (bHLH/LZ) transcription factor family. SREBP-1c induces lipogenic genes by its capacity to bind a sterol response element (SRE) present in the promoter of its target genes [8]. SREBP-1c is regulated by itself but also by liver X receptors (LXRs) [9]. LXRs are ligand-activated transcription factors that belong to the nuclear receptor super-family. LXRs, which activity is controlled by cholesterol metabolites called oxysterols, are important regulators of the lipogenic pathway, since LXRs are central for the transcriptional control of SREBP-1c by insulin [10] and lipogenic genes such as FAS and SCD1 [11]. Transgenic mice that overexpress SREBP-1c in liver or mice gavaged by an agonist of LXRs have an increased expression of most lipogenic genes and develop liver steatosis [12]. Interestingly, mice devoid of SREBP-1c result only in a 50% reduction in fatty acid synthesis [13].

Although insulin is a central regulator of the lipogenic pathway, it is now accepted that glucose also generates an independent signal [14, 15]. Glucose should not be uniquely considered as an energy fuel but also as a signaling molecule necessary for *de novo* lipogenesis, acting in synergy with insulin. L-PK gene expression is stimulated by glucose independently of insulin, in primary cultures of hepatocytes expressing GK [16]. Therefore, metabolism through GK is required to initiate glucose signaling [17]. Glucose-regulated genes share a conserved consensus sequence, named carbohydrate response element (ChoRE), which is required for their glucose-responsiveness [18, 19]. The identification of ChREBP [20], which belongs to the Mondo family of bHLHZiP transcription factors, has shed light on

the mechanism whereby glucose affects gene transcription [21, 22]. ChREBP silencing prevents the glucose-mediated induction of L-PK, ACC and FAS genes in hepatocytes [17]. In a pathophysiological context, liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice [23, 24]. Since ChREBP may represent a potential target for lipid-lowering therapies in obesity and diabetes, an accurate knowledge of the mechanism regulating its expression and activity is needed. ChREBP expression can be regulated by key nuclear receptors of energy homeostasis, namely LXR [25, 26] and the thyroid hormone receptor (TR) [27, 28]. Furthermore, ChREBP interacts with the nuclear receptors HNF4 α [29] and COUP-TF II [30] to modulate the transcription of its target genes such as the L-PK. The aim of this review is to report novel findings on the function and the regulation of ChREBP. In particular, we will describe how, depending on the hormonal and/or nutritional status, specific nuclear receptors establish cross-talks with ChREBP to regulate the signaling pathways that control glucose and lipid homeostasis.

Identification of ChREBP and of its partner Mlx

ChREBP was discovered in 2001 by the group of K. Uyeda [20] on the basis of complementary studies revealing a glucose response element in the promoter of several lipogenic genes (called ChoRE), and composed of two E box (CACGTG) or “E box like” sequences separated by 5 base pairs [19, 31, 32, 33]. Using the ChoRE of the glycolytic enzyme L-PK, Yamashita *et al.* [20] identified ChREBP as a protein enriched in liver nuclear extracts from high carbohydrate diet fed rats. Binding of ChREBP was affected by change of spacing between the two E-box or mutation of the ChoRE. Following these experiments, ChREBP was purified and recognized as the homolog of the human protein named WBSR14 or MondoB, and known to be deleted in the Williams-Beuren syndrome (WBS) [34]. Seventy-five % of WBS patients exhibit impaired glucose tolerance or silent diabetes that may be due to a loss of ChREBP [35]. Several features of ChREBP are consistent with a role as a glucose-regulated transcription factor. Its expression is most abundant in liver, small intestine, kidney, white and brown adipose tissue [36], which are the most active sites of *de novo* lipogenesis in the body. ChREBP mRNA has also been detected in distinct brain regions [37] and in rat pancreatic islets [38]. In addition, ChREBP expression is induced in liver in response to high carbohydrate diet, but not in response to polyunsaturated fatty acids diet fed or fasting [39]. Similarly to SREBP-1c, ChREBP is a member of the bHLHZip (Basic-helix-loop-helix leucine zipper) family of transcription factors, highly conserved among species. ChREBP was first shown to induce the transcriptional activity of the L-PK promoter in

hepatocytes cultured under high glucose concentrations [20]. To address the direct role of ChREBP, we used a siRNA approach to down regulate ChREBP expression in mouse hepatocytes. Our studies revealed, for the first time in a physiological context, that ChREBP mediates the glucose effect on L-PK but also on lipogenic genes (ACC, FAS) and that this transcription factor is a key determinant of lipid synthesis in liver [17]. ChREBP was later shown to directly bind the promoter sequences of L-PK and of lipogenic genes using chromatin immunoprecipitation (ChIP) analysis [22]. These results were confirmed by the characterization of mice lacking the ChREBP gene (ChREBP^{KO} mice) [36]. When maintained on a standard diet, ChREBP^{KO} mice display larger, glycogen-laden livers, smaller adipose depots, and decreased plasma free fatty acid levels. Importantly, ChREBP^{KO} mice show impaired glycolytic and lipogenic pathways in liver and exhibit glucose and insulin intolerance.

Soon after ChREBP discovery, using yeast two-hybrid system, Towle and co-workers identified a bHLHZiP protein, Mlx (Max-like protein X) that interacts with the bHLHZiP domain of ChREBP [21] (Figure 1). Mlx is a member of the Myc/Max/Mad family of transcription factors that can serve as a common interaction partner of a transcription factor network [40]. The evidence that Mlx is the functional partner of ChREBP was demonstrated through the use of an adenovirus expressing a dominant negative form of Mlx (dn-Mlx) [41]. The inhibition of Mlx directly interferes with the endogenous ChREBP/Mlx complex and abrogates the glucose-response of the ACC reporter gene in primary cultures of hepatocytes [41]. The response to glucose can be however partially restored when ChREBP is overexpressed. The regulatory domains of the ChREBP and Mlx proteins have been studied in great details over the last years [21, 41, 42]. According to the model proposed by Ma *et al.* [42], two ChREBP-Mlx heterodimers bind the two E boxes of the ChoRE to provide a transcriptional complex necessary for glucose regulation. Using a structural ChREBP/Mlx structural model followed by specific mutation experiments, three critical residues (F164, I166 and K170) within the Mlx loop that play a crucial role in the binding of the ChREBP/Mlx complex to the ChoRE have been identified [42]. Therefore, it appears that the Mlx loop region, but not the one of ChREBP, is determinant for mediating the response of glucose. Mlx has a significantly longer loop domain than most other bHLHZiP proteins, allowing it to potentially interact across the interface between heterodimer pairs. It is therefore possible that other proteins, *via* interactions involving the Mlx loop, could assist the binding of the ChREBP/Mlx complex to the ChoRE. It was recently reported that adenoviral overexpression of dn-Mlx in 25-week-old male C57BL/6J mice reduces hepatic TG content

and improves glucose intolerance by inhibiting expression of glucose-6-phosphatase (G6Pase) in addition to lipogenic enzymes. A distal promoter region of the G6Pase promoter was previously reported to be glucose responsive in 832/13 cells. The fact that ChREBP binds this region in a glucose-dependent manner [43], supports the fact that G6Pase is most likely a direct target of the ChREBP/Mlx complex.

Regulation of ChREBP activity by glucose

The regulation of ChREBP activity in response to glucose is complex. To date, two mechanisms reporting the glucose-mediated activation of ChREBP have been proposed: one involving on a two-step activation by dephosphorylation on specific residues [44] and one independent involving a dynamic intra-molecular inhibition between two regulatory domains within the ChREBP protein [45] (Figures 1 and 2). ChREBP is a large protein (864 amino acids and $M_r=94,600$) that contains several important domains including a nuclear localization signal (NLS) near the N-terminus, polyproline domains, a basic loop-helix-leucine-zipper (bHLHZip), and a leucine-zipper-like (Zip-like) domain (Figure 1). Deletion of the NLS impairs ChREBP localization in the nucleus and prevents the glucose-induced transcriptional activation of a L-PK luciferase reporter construct in hepatocytes [46]. By studying the importance of ChREBP regulation by phosphorylation, Yamashita *et al.* reported that incubation of the protein ChREBP with the catabolic subunit of protein kinase (PKA) and ATP leads to an increase of its phosphorylation rate and a decrease of both nuclear content and DNA binding activity [20]. This effect could be reversed when a PKA inhibitor and/or the phosphatase 2A (PP2A) was added [20]. From these studies emerged a PP2A-dependent model of ChREBP activation and two important residues (Ser-196 and Thr-666) were identified as potential PKA target sites (Figure 1). Using a phospho-serine antibody, we showed that the glucagon-mediated phosphorylation of Ser-196 was correlated with ChREBP cytosolic localization [47]. Upon glucose stimulation, xylulose 5-phosphate (X5P), a metabolite of the pentose-phosphate pathway, was shown to promote ChREBP nuclear entry based on the specific activation of PP2A which dephosphorylates ChREBP on residue Ser-196, located near the NLS (Figures 1 and 2). Within the nucleus, ChREBP DNA binding and transcriptional activation, would be triggered by a second dephosphorylation on Thr-666, also through a X5P- and PP2A-dependent mechanism [44, 46] (Figure 2). Consistent with this model, Wu *et al* [48] reported that ChREBP global phosphorylation status was decreased when glucose flux through GK, the rate-limiting enzyme of glycolysis was increased.

However, the regulation model of ChREBP by phosphorylation/dephosphorylation raised some controversy, due to questions concerning the involvement of X5P and/or PP2A [45, 49]. Some arguments suggested that glucose 6-phosphate (G6P) produced by GK could be, rather than X5P, the signal metabolite. First, the pentose phosphate pathway is not very active in pancreatic β -cells [50] while ChREBP is expressed and active in these cells [38, 51]. In fact, Li *et al.* [52] recently showed that G6P is sufficient and necessary to activate ChREBP transcriptional activity in INS1 832/13 β cells. Notably, the authors demonstrated that inhibition of the hexokinase by mannoheptulose or overexpression of G6Pase, which reverses hexokinase reaction by dephosphorylating G6P, abolishes glucose responsiveness of a gal4-ChREBP reporter construct. Overexpression of glucose 6-phosphate dehydrogenase (G6PDH), the limiting enzyme of the pentose pathway which converts G6P to 6-phosphogluconolactone, is accompanied by a dramatic decrease in G6P concentrations and leads to an inhibition of ChREBP glucose response in 832/13 INS-1 cells [52]. In contrast, silencing of the enzyme leads to the reverse phenotype, with enhanced G6P concentrations associated with increased ChREBP transcriptional activity [52]. Based on these recent findings in 832/13 INS-1 cells, it would be also important to determine the exact nature of the metabolite signal (G6P vs. X5P) that mediates the effect of glucose in liver cells.

Another important point against the potential X5P-mediated activation of ChREBP is that 2-deoxyglucose (2-DG), a glucose analog which is phosphorylated by hexokinase but can not be metabolized downstream of the glycolytic metabolic pathway, is able to activate the transcriptional activity of ChREBP in 832/13 INS-1 cells [52]. In addition, the fact that inactivating mutations of ChREBP on PKA phosphorylation sites including Ser-196 and Thr-666 are still responsive to high glucose concentrations, is not in favor of the model proposed reporting that mutated ChREBP (i.e. on both Ser-196 and Thr-666) acts as a constitutive active ChREBP isoform, under low and high glucose concentrations [49]. Since inhibition of PP2A by cantharic acid did not alter the glucose responsiveness of a ChREBP construct fused with a gal4-DNA binding domain, Li and coworkers proposed an alternate model of post-translational regulation for ChREBP in 832/13 INS-1 cells [45] (Figures 1 and 2). By structure function analysis, a glucose-sensing module (GSM) evolutionally conserved in mondo proteins, which contains a low glucose inhibitory domain (LID) and a glucose response conserved element (GRACE) was identified within the ChREBP protein [45] (Figure 1). LID is able to inhibit the ChREBP transactivative activity conferred by GRACE, and this inhibition is lifted under high glucose conditions. A deleted form of the 196 first amino acids of ChREBP lacking LID in Nter is active even in low glucose concentration, despite the

integrity of Thr665 (equivalent to Thr666 in mice) and more importantly despite the loss of the NLS. So far, phosphorylation has been the only post-translational modification described to modulate ChREBP activity, but recent work from our laboratory revealed that ChREBP is also regulated by acetylation on specific lysines in response to glucose [53].

Lastly, ChREBP is exported from the nucleus by a mechanism requiring CRM1 (Chromosome maintenance region 1), a protein that shares sequence similarities with the karyopherin β family of proteins involved in nuclear import pathway, was shown to form a complex with the leucine-rich nuclear export signal (NES) and the ability to bind the 14-3-3 protein [54]. When the NES (NES1, located at residues 5-15 in the rat isoform) (Figure 1) is mutated [55], the binding of ChREBP to 14-3-3 and CRM1 is dramatically decreased [55]. Deletions or mutations of the key ChREBP domains, MCRII (MCR: Mondo Conserved Region) (containing the NES1) [55] [56] or MCRIII (containing the 14-3-3 binding site) [56, 57] (Figure 1) lead to an increase in ChREBP nuclear localization and both mutants keep DNA binding activity [55]. Oddly, these ChREBP mutants display a significant loss in transactivative activity under both low and high glucose conditions, suggesting that ChREBP nuclear localization is not sufficient to mediate its transcriptional effects. In fact, another study casts doubt on the importance of ChREBP nuclear translocation. Davies *et al.* [57] reported that the majority of ChREBP protein is localized in the cytosol under both low and high glucose conditions in 832/13 INS-1 cells. Inhibition of the CRM1-induced nuclear export using leptomycin B treatment trapped ChREBP protein in both glucose conditions, suggesting a continuous shuttle of the protein between cytosol and nucleus [57] rather than an active translocation. Nevertheless, in this study the rate of nuclear entry of ChREBP was greater under high glucose concentrations.

Transcriptional regulation of ChREBP: novel implication of nuclear receptors Liver X receptor (LXR) and thyroid hormone nuclear receptor (TR)

Much research has been directed towards determining the post-translational control of ChREBP of glucose/glucagon (Figures 1 and 2) while only fewer studies have started to document its transcriptional regulation (Figure 3). ChREBP is induced at the mRNA level in liver in response to high carbohydrate diet, but not in response to high fat diet fed or fasting [39]. ChREBP expression clearly depends on the metabolization of glucose *via* GK. We have shown that the induction of ChREBP is markedly reduced in the liver of high carbohydrate diet fed GK deficient mice [17]. Overexpression of GK rescues the expression of glucose-dependent genes in liver of streptozotocin (STZ) treated mice and this independently of

insulin/SREBP-1c, underlining the importance of glucose metabolism in ChREBP activity [58]. However, the molecular mechanism triggering the glucose-mediated regulation of ChREBP expression has not yet been elucidated. Despite the lack of identification of a well-conserved ChoRE on the mouse ChREBP promoter (Figure 3), it has been suggested that ChREBP could regulate its own expression [42]. Indeed, overexpression of dn-Mlx decreases glucose-induced ChREBP expression. The hypothesis of ChREBP/Mlx self-regulation is interesting although it cannot be excluded that other transcription factors/co-receptors be recruited for the transcriptional control of ChREBP in response to glucose. A direct control of ChREBP expression by insulin was even reported. First, Satoh *et al.* showed that SREBP-1c binds a SRE (-101 to -110 bp) motif (Figure 3) within the ChREBP promoter in response to refeeding [60]. However, the fact that SREBP-1c overexpression failed to induce ChREBP in liver of fasted mice does not support the hypothesis of a direct control of ChREBP by SREBP-1c [17]. In addition, ChREBP is adequately induced upon refeeding in liver of LXR deficient (LXR^{KO}) mice, and this in the absence of mature form (*i.e.* transcriptionally active) of SREBP-1c [47]. Interestingly, Sirek *et al.* [61] recently reported that the stimulatory effect of insulin on ChREBP could be independent of SREBP-1c but would rather involve Oct1, another transcription factor. Oct1 would act as a repressor of ChREBP activity: insulin would stimulate ChREBP transcriptional activity by inhibiting the binding of Oct1 on a POU domain. However, it should be noted that the reported effect of Oct1 on ChREBP transcriptional activity was very modest [61].

Recently, two nuclear receptors playing important roles in energy homeostasis, namely LXR and TR, were shown to regulate ChREBP at the transcriptional level in liver [25, 27, 28] (Figure 3). LXRs ($LXR\alpha$ and $LXR\beta$) act as sterol sensors activated by oxidized cholesterol derivatives called oxysterols [62, 63]. In contrast to $LXR\beta$ whose expression is ubiquitous [64, 65, 66, 67], $LXR\alpha$ expression is particularly high in liver, adipose tissue, intestine, but it is also present in spleen kidney and lung. LXR forms a heterodimer with RXR (retinoid X receptor) and binds LXRE sequence(s) within target gene promoter region [64]. LXRs are particularly important for cholesterol metabolism, in which they contribute to excess cholesterol elimination. $LXR\alpha$ controls bile acids synthesis *via* the induction of Cyp7A1 gene in rodents [62, 68], cholesterol excretion in bile acids in liver [69-71] and cholesterol efflux from several cell types *via* the regulation of ABCA1 and ABCG1 transporters [9, 72-74]. The transcriptional activity of the thyroid hormone L-triiodothyronine (T3) has been known since 1960 [75], but the cloning of its responsive receptor, the thyroid hormone nuclear receptor

(TR), occurred only in 1986 [76, 77]. In mammals, two different genes TR α and TR β code for the thyroid receptors TR α 1, TR β 1, TR β 2, and TR β 3 [78]. The expression of TR β 1 is high in liver, in which TR α 1 is less abundant [78]. Interestingly, several studies reported that LXR and TR are closely related. First, LXRs and TR recognize the same consensus sequence: AGGTCA repeated with a 4 base pair gap (DR4) [79-81]. In some cases, both nuclear receptors can bind the same responsive element on a promoter. For example, TR competes with LXR for the binding of the LXRE on the ABC1 promoter and can inhibit the LXR-induced ABC1 expression [82]. Moreover, un-liganded TR β 1, which is known to act as a transcriptional repressor [83], interacts with co-repressors to repress the transactivative activity of LXR α by competing on the LXRE of SREBP-1c promoter *in vitro* [84]. Furthermore, LXR α has been described as a direct TR β 1/T3 target gene in mouse liver, through the recruitment of TR β 1/RXR α (site A : -1300 to -1240 bp) [85]. However, this induction of LXR α and of its target gene ABCA1 by T3 was not confirmed in a follow-up study [28].

The implication of LXR in the control of the lipogenic pathway was evidenced in LXR α ^{KO} mice, as they present a decrease in SREBP-1c and in several of its target genes in liver [8, 86, 87-89, 90, 91]. Two functional LXREs have been identified in the SREBP-1c promoter [9, 92]. LXR stimulation with its most efficient synthetic agonist, T0901317, allows the increase of SREBP-1c, ACC, FAS and SCD1 expression in liver and increases hepatic and plasma TG in wild type but not in LXR α / β ^{KO}, LXR α ^{KO} mice or SREBP-1c^{KO} mice [11, 13, 93, 94]. The physiological stimulation of SREBP-1c expression occurs in the presence of insulin, but the link between LXR and insulin remains unclear. Tobin *et al.* suggested that insulin stimulates LXR α expression in rat hepatocytes [95]. However, the physiological relevance of this regulation is disputed since supra-physiological concentrations of insulin (up to 500 nM) were used in this study [95]. Nevertheless, SREBP-1c is not induced by insulin in LXR α / β ^{KO} mice and the functional LXREs within the SREBP-1c promoter are necessary for its induction by insulin [10]. The most logical link between insulin and LXR would be that the hormone somehow stimulates the synthesis of a LXR ligand necessary for the action of the nuclear receptor.

Treatment of wild type mice with thyroid hormone (TH) induces lipogenic gene expression (including ACC and FAS) and TR β 1^{KO} mice display decreased expression of these genes [96]. The identification of ChREBP as a novel LXR and TR target gene [25, 27, 28] (Figure 3) is interesting and underlines the role of these two nuclear receptors in the control of

the lipogenic pathway. First, Cha and Repa reported that ChREBP mRNA levels were increased by about 3 fold in livers of fed mice treated with either dietary RXR or LXR agonists, (and by 6 fold when a combination of both agonists was used) [25]. These effects are specific since ChREBP expression was not induced in liver of LXR^{KO} mice treated with the LXR agonist (T0901317) for short (12h) or longer periods of treatment (10 days). This treatment was associated with a loss of induction of ChREBP-target gene, L-PK. In addition, the effect of T0901317 on lipogenic genes (ACC, FAS and SCD1) was attenuated in ChREBP^{KO} mice [25]. The mouse ChREBP promoter contains two functional LXRE able to bind the LXR/RXR complex thereby conferring its receptor/ligand transactivation (Figure 3). It should be noted that LXRE1 showed a greater activity than LXRE2 for LXR binding.

Two recent studies supported the control of ChREBP by thyroid hormones (TH) [27, 28]. In mice turned into a hypothyroid state by a MMI/PTU diet (containing methimazole and propylthiouracil, two inhibitors of TH synthesis), hepatic ChREBP expression (mRNA and protein content) was decreased by about 40% compared to controls. In parallel, hypothyroid mice injected with TH (T3), thus becoming thyrotoxic, present a 3- to 4-fold increase in ChREBP expression [27]. Importantly, in two models of hypothyroid states, pax8^{KO} mice (deprived of thyroid gland) and wild type mice submitted to PTU (PTU: Propylthiouracil used to decrease the synthesis of endogenous TH) treatment, ChREBP expression can be rescued to control levels after TH injection, and this to a greater extent in white adipose than in liver [28]. Modulation of hepatic ChREBP expression in relation to the thyroid state in PTU/MUI mice [27] or in pax8^{KO} mice injected with TH [28] were paralleled with changes in its target genes [28]. In both liver and adipose tissue, the effect of TH on lipogenic gene expression in PTU-treated mice was lost in TR β ^{KO} but not in TR α ^{KO} mice, reinforcing the previously described role of TR β in lipogenesis [96] and revealing its control on ChREBP expression. Interestingly, ChREBP expression was repressed when wild type mice, but not TR β ^{KO} mice, were submitted to a PTU diet, suggesting that un-liganded TR β represses ChREBP expression [28].

Both TR β and TR α were able to activate the mouse ChREBP promoter *in vitro* [28]. Luciferase assays confirmed that T3 increases the activity of the mouse and the human wild type ChREBP promoter but not when the LXRE1 and LXRE2 were deleted [27]. Interestingly, mutation of each or both LXREs on the ChREBP promoter did not lead to a complete loss of LXR or TR response, raising the possibility that other regions mediating the LXR and/or TR response may exist on the ChREBP promoter [28]. The heterodimer TR-

$\beta 1/RXR\alpha$ was able to bind the LXRE2 of the mouse ChREBP promoter, but not the LXRE1, which was shown to be the preferential site for LXR α /RXR α [25] (Figure 3).

This interesting crosstalk between LXR and TR was further explored in LXR^{KO} mice [28]. In PTU-treated LXR^{KO} mice, hepatic ChREBP expression was induced in response to TH, demonstrating that the TH-mediated induction of ChREBP was independent of LXR. Surprisingly, ChREBP expression in response to TH was higher in liver of LXR^{KO} than in wild type mice, suggesting that LXR, when bound to the LXRE1, might limit the access of TRs on the LXRE2. Co-transfection assays of LXR and TR β with one of the two specific ligands (T0901317 or TH) confirmed this hypothesis. Transfected alone TR β or LXR induced the ChREBP promoter activity in the presence of their cognate ligands (TH and T0901317) while co-transfection of both (TR β and LXR) decreased the response of the ChREBP promoter to each ligand (TH and T0901317). Altogether, these data reveal that TH induces ChREBP transcription and protein content in liver *in vivo* and in cells, and that the LXRE2 is central in this regulation (Figure 3). Paradoxically, Hashimoto *et al.* previously reported that SREBP-1c was repressed by T₃ in mouse liver [97] and similar results were reported in human adipocytes [98]. These results are controversial since studies in the chick embryo hepatocytes (CEH) [99] and in HepG2 cells [84] led to opposite results. The understanding of the physiological relevance of such an antagonism between ChREBP and SREBP-1c requires further investigation.

Physiological relevance of the regulation of ChREBP by LXR and/or TR in liver: a role in glucose-sensing ?

An important point that remains to be determined is the physiological relevance of the regulation of ChREBP by LXR and/or TR. Since ChREBP is mostly regulated by glucose/glucose metabolism, studies were first performed to determine the potential implication of LXR and/or TR in the nutritional regulation of ChREBP. Fasting/refeeding experiments in wild type or TR β ^{KO} mice revealed that ChREBP expression is increased to comparable levels in livers of re-fed mice from both genotypes, suggesting that TR is not involved in the nutritional regulation of ChREBP [28]. In fact, addition of TH led to an increase in ChREBP and lipogenic genes (FAS for example) in the fasted state but did not allow further induction in the fed state [28].

The implication of LXR remains, to date, somehow controversial since some set of studies reported that LXR might be implicated in the glucose signaling pathway [26, 100], while some were against this hypothesis [47, 101]. A lot of emphasis came from the study of

Mitro *et al.* [26], which placed LXR at the center of the glucose-mediated regulation of hepatic lipogenesis. It was reported that a gal4 reporter construct of the ligand binding domain (LBD) of LXR fused to a DNA binding domain (DBD) of yeast gal4 was highly responsive to D-glucose in HepG2 cells. In addition, the activity of a LXRE-luciferase reporter construct was found to be activated in the presence of D-glucose and/or G6P in HepG2 cells co-transfected with a LXR expression plasmid. Interestingly, this stimulatory effect of D-glucose/D-G6P on LXR reporter activity was comparable to the one of T0901317. FRET assay revealed that D-glucose and D-G6P induced the recruitment of LXRs co-activators and this to similar levels than T0901317. Surprisingly, L-glucose was also presented as a direct agonist in FRET assay. Moreover, Mitro *et al.* reported that addition of glucose to a maximal dose of synthetic ligand further increased the recruitment of LXR co-activators [26]. Since labeled D-glucose and D-G6P were able to compete with synthetic LXR ligands, the authors suggested that glucose was able to bind on specific LXR sites. Altogether, this study suggested that LXR α could serve as a *glucose-sensor* detecting glucose excess and converting it into TG by inducing lipogenic genes directly or indirectly *via* ChREBP and SREBP-1c [26]. Several objections were raised against this model. First, we [47] and Oosterveer *et al.* [102] reported that ChREBP expression, its nuclear translocation and the induction of its target gene, L-PK, were not altered in response to high carbohydrate diet in liver of LXR α / β ^{KO} mice, suggesting that effect of glucose on ChREBP is independent of LXR [47]. Secondly, the expression of LXR target genes ABCG1, ABCA1, ABCG5 and ABCG8 was not affected by high carbohydrate diet feeding in liver of wild type or LXR α / β ^{KO} mice, suggesting that direct LXR targets are not nutritionally regulated [47, 102]. The relevance of a direct binding of glucose/G6P on LXR was also challenged because of the hydrophilic nature of glucose, its weak affinity (in mM) compared to other nuclear receptor ligands and more importantly due to the inability of G6P to properly enter the cells. Finally, L-glucose was presented as very rarely active in biological process [101]. Along these lines, we were not able to reproduce in a cell-free assay the ability of either D-glucose or G6P to influence the interaction between LXR α or LXR β , and their known co-activator peptides [47]. Interestingly, the respective roles of ChREBP and LXR in controlling lipogenic genes were further addressed in a mouse model of glycogen storage disease type 1 (GSD-1) [103]. Treatment of mice with S4048, a pharmacological inhibitor of the glucose 6-phosphatase transporter, which is part of G6Pase, recapitulated the metabolic defects associated with GSD-1: reduction in blood glucose levels, increased G6P, glycogen and TG concentrations. S4048-

treatment was also associated with a marked increase in lipogenic gene expression, through a ChREBP-dependent mechanism. More importantly, this study reveals that G6P is not able to activate LXR α *in vivo* and therefore does not support the hypothesis that G6P could be a direct ligand for LXR as previously reported [26].

Taken together, a direct link between glucose/G6P and LXR seems uncertain. However, could another kind of regulation by glucose exist for LXR? A study reported that LXRs could be modified by *O*-linked β -N-acetylglucosamine (*O*-GlcNAcylation or *O*-GlcNAc) in Huh7 cells [100]. *O*-GlcNAcylation plays an important role in the modulation of protein stability, cellular localization, activity and partner interactions [106] and key transcription factors were recently shown to be modified by *O*-GlcNAcylation in liver [100, 104, 105]. *O*-GlcNAcylation is the end product of the hexosamine biosynthetic pathway (HBP), a metabolic pathway which has recently emerged as a major determinant of metabolic disorders associated with insulin resistance and/or type 2-diabetes. Anthonisen *et al.* [100] demonstrated that *O*-GlcNAcylation of LXR is elevated in liver of fed mice and in Huh7 cells incubated in high glucose concentrations. In liver of mice rendered diabetic with a STZ treatment, (*i.e.* characterized by hyperglycemia in nearly absence of insulin), LXR *O*-GlcNAcylation was higher than in non-diabetic controls and was correlated with a greater induction of LXR-target gene, SREBP-1c [100]. In this study, it would have been interesting to study the effects of LXR *O*-GlcNAcylation on ChREBP activity in order to establish a potential link between glucose, LXR and ChREBP. Nevertheless, it would be interesting to determine whether enhanced LXR *O*-GlcNAcylation levels in liver of diabetic mice can explain the induction of ChREBP under hyperglycemic states.

One interesting physiological situation in which a cross-regulation between LXR and TR could occur is the suckling-weaning transition. Bobard *et al.* [107] reported that LXR expression and binding activity is high during the suckling period (a period during which mice consume milk rich in cholesterol and lipids but poor in carbohydrates), leading in turn to an increase in SREBP-1c precursor protein levels. The SREBP-1c protein is not cleaved during this period because insulin concentrations are low, and as a consequence the expression of its target genes (GK and FAS) remains low. This suggests that the increase in SREBP-1c precursor forms during the suckling period, in response to activated LXR α , could prepare the liver to rapidly convert in fat the increase of carbohydrates that occurs at weaning due to the switch of diet. Following the same model, and given that rodents display an increase of T3 concentrations during the last days of the suckling period [108], it could be

envisioned that LXR α and/or TR β increase ChREBP expression in a carbohydrate-independent manner during this period of development.

Lastly, a potential link between polyunsaturated fatty acids (PUFAs), LXR and/or TR and ChREBP could be envisioned. Through mechanisms only partially understood, long-chain n-3 PUFAs have the ability to control the transcriptional activity of nuclear receptors and, thereby, the transcription rate of specific genes related to lipid and carbohydrate metabolism [109]. n-3 long-chain PUFAs bind to peroxisome proliferator-activated receptors (PPARs) α , β , γ 1 and γ 2 [110] and the actions of PUFAs are, in great part, mediated by PPARs. However, PUFAs also bind to other nuclear receptors such as LXR α [111], hepatocyte nuclear factor-4 (HNF4 α and γ) [112, 113] and RXR [114]. Another mechanism involved in the actions of PUFAs is the decrease in the nuclear abundance of SREBP-1c, ChREBP [39] and Mlx [115]. Considering the transcriptional control exerted by LXR on ChREBP [25], it would be interesting to determine whether some of beneficial effects of PUFA on hepatic lipogenesis and TG synthesis, are triggered, at least in part by ChREBP through a LXR dependent pathway. A similar molecular mechanism for TR does not seem conceivable since it was reported that long-term consumption of n-3 PUFAs enhances thyroid hormone action in mouse liver [116]. Further studies will be required to better understand a potential contribution of TR to the TG-lowering effect of PUFA.

The glucose-regulated L-PK gene model: an interplay between ChREBP and key nuclear receptors

With the discovery of ChREBP, our understanding of the transcriptional effect of glucose in liver has made considerable progress. The glycolytic enzyme L-PK, whose expression is strictly dependent on glucose/glucose metabolism is now widely considered as a ChREBP-dependent gene. Its expression has been studied for many years as it represents a model gene for other glucose-regulated genes. A 200 bp of the 5' region of the L-PK promoter was shown to be glucose-responsive in rat hepatocytes [18]. Within this region four response elements, including L3 and L4 were identified [117]. The L3 and L4 are closely located from nucleotides -145 to -125 for the L3 and from -168 to -144 for the L4 (Figure 4). In 1992, Bergot *et al.* demonstrated that the maximal induction of the L-PK promoter by glucose requires a necessary interaction between the sequence responsive for HNF4 α , the L3 site and the L4 site, now known as containing the ChoRE [19] (Figure 4). HNF4 α , also called NR2A1, is a nuclear receptor that has recently be reported to bind in a reversible way linoleic

acid in liver of fed mice [118]. HNF4 α is expressed in adult liver but also in the gastrointestinal tract, kidney and pancreas [119]. This transcription factor, conserved in evolution, plays an important role in embryonic development in xenopus [120] and in mammals. Liver specific HNF4 α knockout mice die at about the eighth week of life with alterations of gene expression involved in lipid and bile acid metabolism [121]. HNF4 α mutations are also implicated in rare case of Maturations Onset Diabetes of the Young (MODY) [122, 123]. Diaz-Guerra *et al.* demonstrated that the principal factor binding to the L3 sequence of the L-PK promoter in rat liver nuclear extracts was HNF4 α , despite the presence of other several transcription factors including nuclear receptor COUP-TFII as we will discussed below [124]. Overexpression of HNF4 α alone is able to induce the activity of the L-PK promoter in fibroblastic NIH 3T6 cell lines devoid of endogenous HNF4. Interestingly, transfection of the HNF4 α isobinder COUP-TFII in primary hepatocytes inhibits in a L3-dependent manner the activity of a L-PK promoter, probably due to a competition between these two nuclear receptors. While the implication of HNF4 α on the transcriptional control of the L-PK gene is well admitted, its regulation in response to glucose is controversial and remains to be clearly determined. On one hand, Xu *et al.* reported that the nuclear abundance of HNF4 α was not modified in liver of refeed rats, nor its binding to the L-PK promoter in primary rat hepatocytes in response to glucose [115]. Secondly, Eckert *et al.* showed that HNF4 α was constitutively bound to the L-PK promoter under low glucose concentrations, and that this binding was increased by high glucose concentrations [125]. Recently, Burke and co-workers presented an elegant model in which a protein complex containing ChREBP, HNF4 α and the co-activator CBP is necessary for the glucose-mediated induction of the L-PK gene (Figure 4) [29]. SeqChIP experiments in 832/13 INS-1 cells revealed the recruitment of ChREBP, HNF4 α and CBP in response to high glucose concentrations on the L-PK promoter, a recruitment that is decreased by forskolin, demonstrating that the cAMP pathway alters their occupancy on the L-PK promoter despite the presence of glucose. Previous studies evidenced an association between HNF4 α and the transcription co-activator CBP [126, 127] (Figure 4). Decreased CBP expression by siRNA led to the loss of glucose-induced L-PK expression [29] and in contrast, CBP overexpression was sufficient to overcome the repression by forskolin of the wild type L-PK promoter activity but not of the L-PK promoter construct mutated on the ChoRE. Furthermore, when the responsive element of HNF4 α , L3, was mutated, the glucose induction of L-PK promoter activity was blocked, in the presence or the absence of CBP overexpression. Interestingly,

overexpression of wild type ChREBP or a mutant of the three known PKA phosphorylation sites (mutants S196A, S626A, T666A), did not overcome the repression induced by forskolin on the L-PK promoter [29].

These results demonstrate that CBP is limiting for the formation or the disruption of the CHREBP/HNF4 α /CBP complex in response to glucose or cAMP signal (Figure 4). Lastly, Perilhou *et al.* established a potential link between COUP-TFII and ChREBP. COUP-TFII was first identified as a homodimer that binds the direct repeat site (DR-1) on the chicken ovalbumin promoter [128]. Later it was shown that this nuclear receptor acts mostly as a transcriptional repressor through its interaction with co-repressors [129] or through a titration of the nuclear receptor RXR, a transcriptional partner of several other nuclear receptors. COUP-TFII is expressed, among others, in metabolic tissues such as liver, adipose tissue, skeletal muscle and endocrine pancreas [130]. COUP-TFII represses insulin genes and insulin secretion in 832/13 INS-1 cells [30]. Specific deletion of COUP-TFII in β -cells leads to an impairment of glucose tolerance and of the glucose-induced insulin secretion in mice [131].

COUPTF-II was first cloned in rat liver nuclear extracts using one-hybrid selection approach in yeast. While COUP-TF II was previously shown to bind the L3 sequence and compete with HNF4 α as we discussed above, it was reported that COUPTF-II was also able to bind on two direct repeat sequences (DR-1 and DR-7 sites) within the L4 region of L-PK promoter [132]. These DRs sites overlap with the first E-box of the ChoRE. Interestingly, COUP-TFII was shown to induce and to be induced by HNF4 α , and COUP-TFII can repress its own expression by competition with HNF4 α for binding on its own promoter [30]. COUP-TFII expression is repressed by glucose and insulin signals in liver *in vivo* and in primary cultures of hepatocytes suggesting that glucose metabolism is essential for COUP-TFII inhibition [30]. In agreement with this hypothesis, the glucose-mediated repression of COUP-TFII is lost in liver of GK^{KO} mice. In addition, in liver of obese and diabetic ob/ob mice in which ChREBP expression is elevated [23], a parallel decrease in COUP-TFII expression was observed. This decrease can be rescued when ChREBP is down-regulated using a shARN strategy [30]. Altogether, these results support the idea that ChREBP may indirectly or directly represses COUP-TFII expression and suggests that an active ChREBP may prevent the mediated repression of COUP-TFII on the L-PK promoter in hepatocytes within the L4 site under fed or high glucose conditions (Figure 4).

FXR, another important nuclear receptor was suggested to prevent the glucose/ChREBP-mediated induction of the L-PK gene [133]. FXR is a nuclear receptor controlling bile acid levels, notably by decreasing Cyp7A1 expression [134]. Interestingly, FXR^{KO} mice display increased hepatic TG levels in the fed state along with an accelerated induction of glycolytic (LPK) and lipogenic (ACC) gene in response to high carbohydrate feeding [133]. Treatment of primary hepatocytes with GW4064, a FXR agonist decreases the glucose-induced expression of these genes. Therefore, FXR could be a direct mediator of the repression of glycolytic/lipogenic genes as it binds as a homodimer to the L3 region but not the L4 region on a L4-L3 luciferase L-PK promoter construct [133] (Figure 4). Although ChREBP expression was not reported to be modulated in liver of FXR^{KO} mice, analysis of ChREBP nuclear localization and/or activity upon FXR activation would be useful in order to further understand the mechanism(s) by which FXR represses glucose-regulated gene expression.

Concluding remarks

The molecular control of hepatic glucose metabolism is complex as it requires an interplay of transcription factors and/or nuclear receptors in response to hormonal stimuli and/or nutrients [135]. Among them, we focused our attention on the action and regulation of ChREBP, which has emerged over the recent years, as a major determinant of glycolysis and lipogenesis control in response to glucose. Future studies will help identify other nuclear receptors that can either regulate and/or interact with ChREBP for the molecular regulation of the lipogenic pathway. For example, a recent study reported that agonists of PPAR γ , VDR (vitamin D receptor), PXR (pregnane X receptor) or antagonists of FXR, CAR (constitutive androstane receptor), and ER α (estrogen receptor) are able to stimulate lipid accumulation in human hepatocytes and/or hepatoma cell lines [136]. For instance, the activation of PXR, a nuclear receptor mostly implicated in sensing and elimination of xenobiotic and drugs [137], leads to an increase in FAS and SCD1 expression, that is independent of a variation in SREBP-1c expression or nuclear abundance [138]. Although Moreau *et al.* [139] reported that the PXR-mediated activation of *de novo* lipogenesis in human hepatocytes was also independent of a variation in ChREBP expression, additional studies will be required to determine whether PXR agonists affect somehow ChREBP transcriptional activity and/or nuclear abundance. Interestingly, a study also recently reported that ChREBP acts as a critical and direct mediator of glucose repression of the PPAR α gene in pancreatic β -cells suggesting that ChREBP may be important for glucose-suppression of β -oxidation in β -cells [140].

Altogether, these potential novel molecular cross-talks may help identify new molecular targets for control of fatty acid synthesis and for the prevention of hepatic steatosis and related metabolic alterations.

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Figure legends

Figure 1. ChREBP protein structure. The ChREBP protein (864 amino acids; Mr= 94,600) contains several domains: a nuclear localization signal (NLS) (residues 158-173 in the rat isoform); two nuclear export signals (NES1 and NES2) located near the N-terminus (residues 5-15 and 85-95 in the rat isoform) [55]; a polyproline domain and a leucine-zipper-like (Zip-like) domain (residues 382-585 and 795-835, respectively in the human isoform,) [34]; and a basic loop-helix-leucine-zipper (bHLH/Zip) (residues 660-736 in the rat isoform) [45]. The MCRII (MCR: Mondo Conserved Region) (containing the NES1) [55] [56] and the MCRIII (containing the 14-3-3 binding site, localized between residues 125 and 135 in the rat isoform) [55, 56, 57] are also indicated. ChREBP contains at least three phosphorylation sites by protein kinase (PKA) (Ser196, Ser626 and Thr666 (mouse)/ Thr665 (rat)). Several other phosphorylation sites have been identified but are not indicated on this figure [141]. The functional partner of ChREBP, Mlx, interacts with ChREBP bHLHZiP domain as indicated. By structure function analysis, a glucose-sensing module (GSM) containing a low glucose inhibitory domain (LID, residues 37-192) and a glucose response conserved element (GRACE, residues 197-298) was identified in the rat ChREBP protein [45]. LID inhibits the ChREBP transactivative activity conferred by GRACE, and this inhibition is lifted under high glucose conditions. A deleted form of ChREBP 196 first amino acids lacking LID in the N-terminus is active even under low glucose concentration, despite the integrity of Thr666 and more surprisingly despite the loss of the NLS. In addition, although not indicated on this figure, several acetylation sites on specific lysines have been identified on the mouse ChREBP protein [53].

Figure 2. Transcriptional activation of lipogenic genes by ChREBP/Mlx, SREBP-1c and LXR α in liver. Insulin activates the transcription of the SREBP-1c gene leading to the synthesis of a precursor form of SREBP-1c (pSREBP-1c), which will be anchored in the membranes of the endoplasmic reticulum. Insulin activates the proteolytic cleavage of the precursor form and the processed mature SREBP-1c (mSREBP-1c) translocates to the nucleus where it activates lipogenic genes after binding to its regulatory binding elements (SRE) [7]. The phosphorylation of glucose in G6P, by hepatic GK, is an essential step for glucose metabolism as well as for the induction of lipogenic genes. To date, two mechanisms

reporting the glucose-mediated activation of ChREBP have been proposed: one involving on a two-step activation by dephosphorylation on specific residues (Ser-196 and Thr-666) two target sites of protein kinase A (PKA) [44] and one independent involving a dynamic intramolecular inhibition between two regulatory domains (LID and GRACE) within the ChREBP protein [45]. One and/or both of these mechanisms lead to ChREBP translocation into the nucleus under both high glucose and insulin concentrations. Within the nucleus, ChREBP binds the ChoRE and activates its target genes. ChREBP heterodimerizes with Mlx to mediate the glucose response: two ChREBP-Mlx heterodimers bind to the two E boxes of the ChoRE to provide a transcriptional complex necessary for glucose regulation. It remains to be determined where (cytosol *vs.* nucleus) the association between ChREBP and Mlx occurs and whether Mlx is regulated by glucose metabolism like ChREBP. LXR α directly binds to its regulatory binding site (LXRE) and contributes to the induction of lipogenic genes. However, the activation of LXR α in response to insulin and/or glucose remains unclear as discussed. Location of the ChoRE, LXR α and SRE motifs on the rat FAS promoter are indicated.

Figure 3. Transcriptional regulation of ChREBP. The heterodimers LXR α /RXR α and TR β 1/RXR α transactivate the ChREBP promoter and induce its expression in the liver in response to their synthetic (LXR α) or natural (TR β 1) ligands. However, neither LXR α nor TR β 1 seem to be implicated in the regulation of ChREBP by glucose. Although it has been suggested that ChREBP could regulate its own expression [42], a functional ChoRE still awaits to be identified and characterized on the ChREBP promoter. However, a SRE motif was also identified suggesting a potential cross-regulation between SREBP-1c and ChREBP.

Figure 4. Nutritional and/or hormonal regulation of the L-PK gene. In the fed state (in response to glucose metabolism), the ChREBP/Mlx heterotetramer, the nuclear receptor homodimer HNF4 α and the co-activator CBP form a complex that binds to the glucose sensing domain L3/L4 on the L-PK promoter. As a result, L-PK expression is induced, allowing the last step of glycolysis to be activated. In response to glucose/glucose metabolism, ChREBP would repress COUP-TFII expression/activity by a mechanism not yet elucidated, thereby preventing COUP-TFII binding to the L3/L4 domain. Under fasting conditions (in response to the glucagon/camp signal), the ChREBP/Mlx/HNF4 α /CBP complex is disrupted, does not bind to the ChoRE, thereby preventing the stimulation of the

L-PK gene. In addition, COUP-TFII and/or FXR, acting as transcriptional repressors on the L3/L4, would maintain L-PK gene silent.

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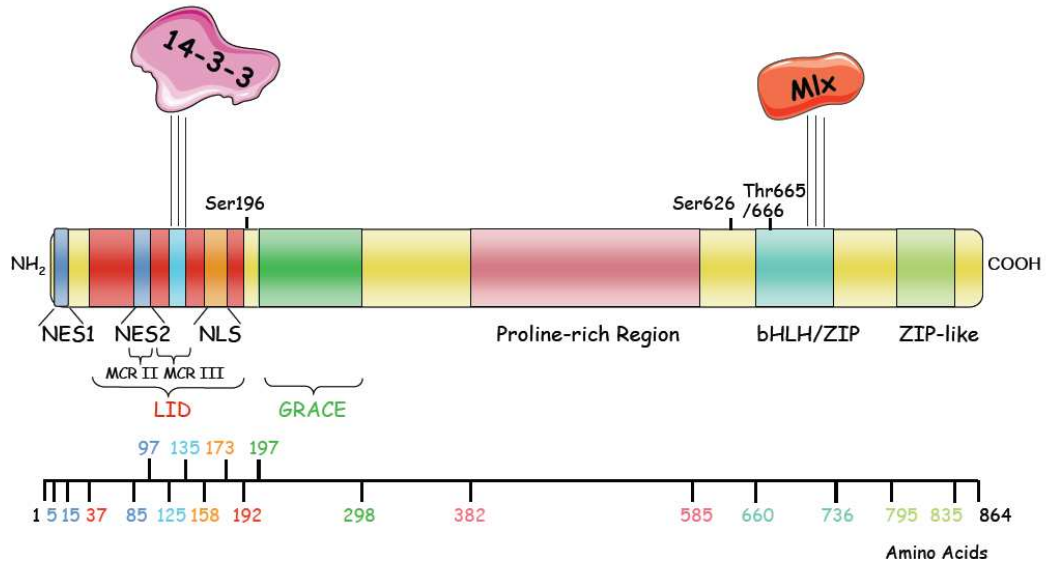


Figure 1

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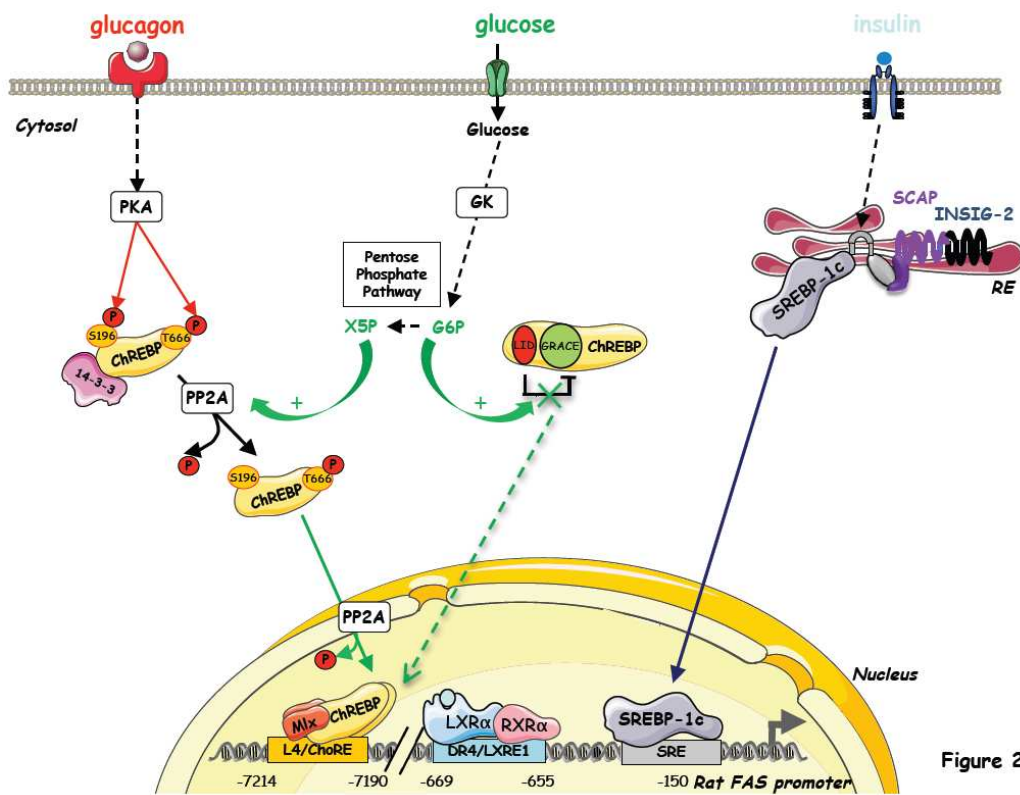


Figure 2

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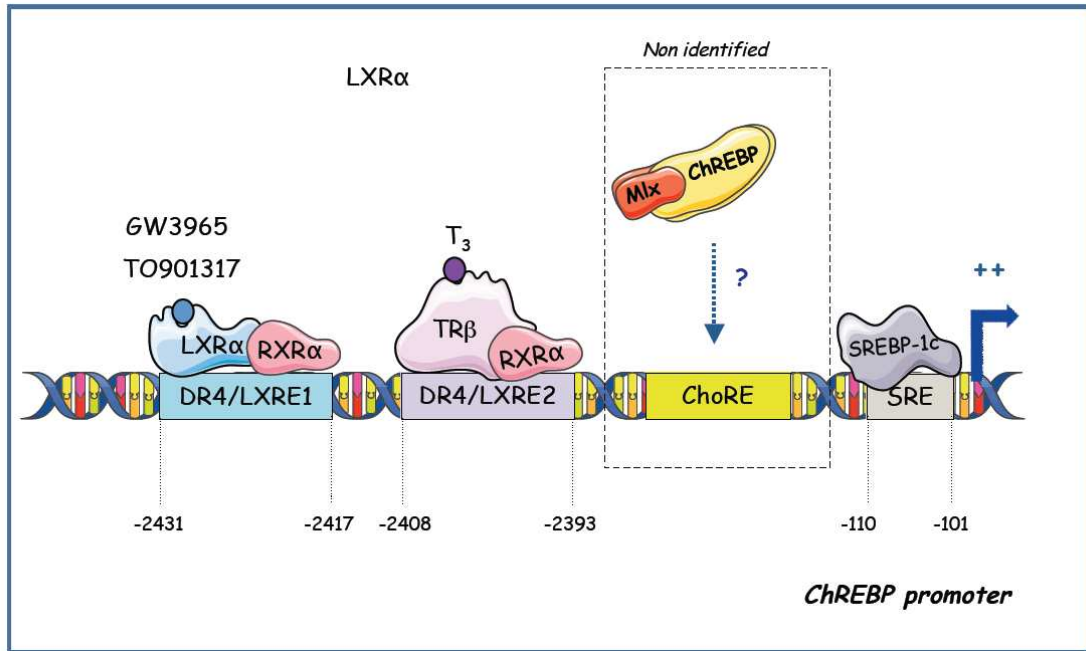


Figure 3

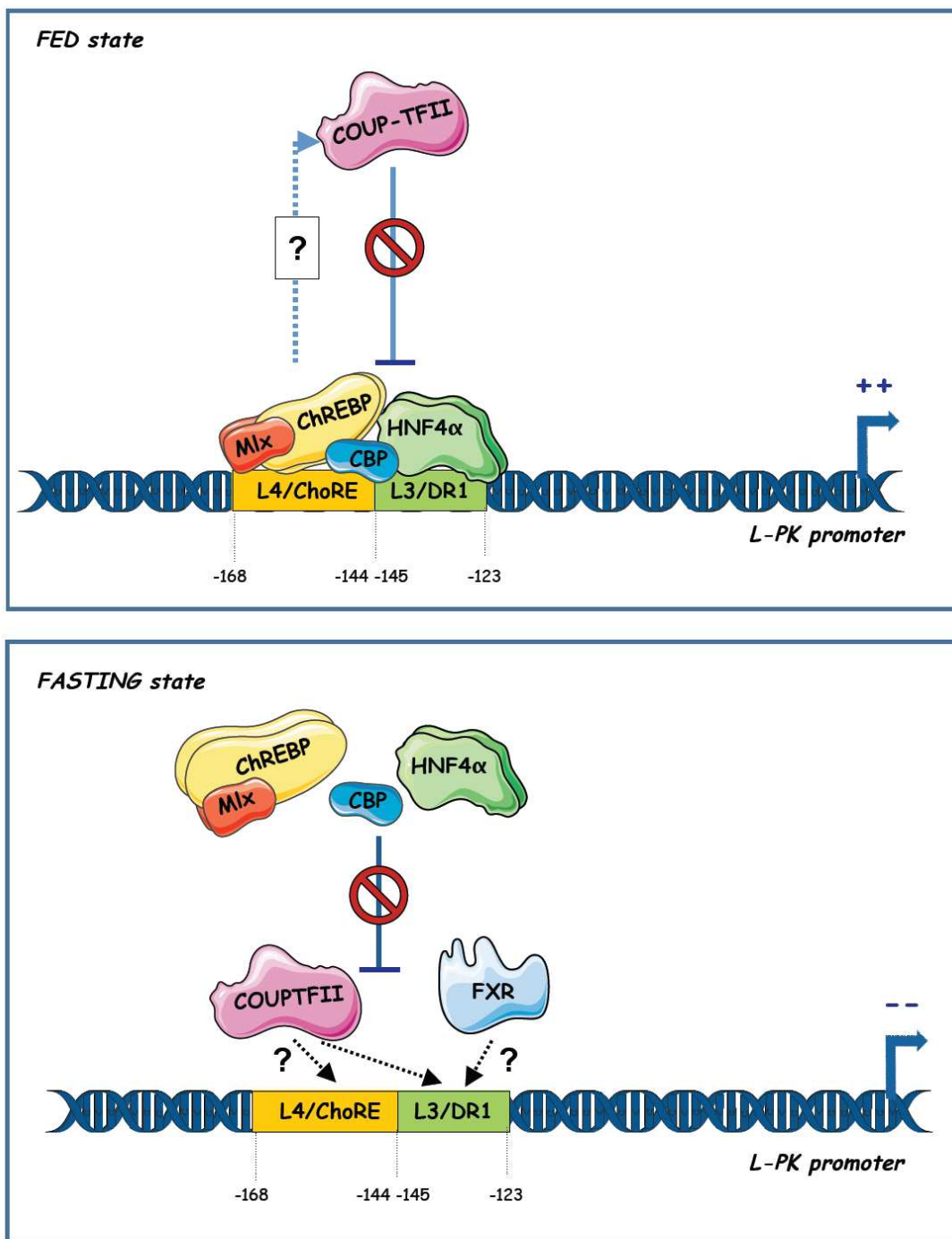


Figure 4