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REVIEW

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Human low-density lipoprotein receptor gene and its regulation

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Abstract The low-density lipoprotein (LDL) receptor is a transmembrane glycoprotein that mediates the binding and endocytosis of lipoproteins containing apolipoprotein B and E, especially the cholesterol-rich LDL. Mutations in the LDL receptor gene can produce dysfunctional LDL receptors and cause familial hypercholesterolemia. The expression of the LDL receptor gene is under an intriguing regulation by sterol and nonsterol mediators either at the transcriptional level or at the posttranscriptional level, both of which are linked to cell signaling pathways. Upregulation of liver LDL receptor expression is effective in treating hypercholesterolemia. In this review, we focus on the latest progress on the mechanisms and regulation of the LDL receptor gene expression.

Keywords Low-density lipoprotein receptor · Hypercholesterolemia · Transcriptional regulatory elements · mRNA stability · Signal transduction pathways

Abbreviations ARE: AU-rich element · bHLH-Zip: Basic-helix–loop–helix–leucine zipper · bp: Base pair(s) · CDCA: Chenodeoxycholic acid · c/EBP: CCAAT/enhancer binding protein · CHD: Coronary heart disease · EGF: Epidermal growth factor · Egr1: Early growth response gene 1 · ER: Endoplasmic reticulum · ERK: Extracellular signal-regulated kinase · FGF: Fibroblast growth factor · FH: Familial hypercholesterolemia · HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A · IL: Interleukin · Insig: Insulin-induced gene · kb: Kilobases · LDL: Low-density lipoprotein · LDL-c: LDL-cholesterol · MAPK: Mitogen-activated protein kinase · nSREBP: Nuclear SREBP · OM: Oncostatin M · PDGF: Platelet-derived growth factor · PKC: Protein kinase C · PMA: Phorbol-12-myristate-13-acetate · S1P: Site-1 protease · S2P: Site-2 protease · SCAP: SREBP cleavage-activating protein · SIRE: Sterol-independent regulatory element · SRE: Sterol regulatory element · SREBP: Sterol regulatory element-binding protein · SSD: Sterol sensing domain · TNF: Tumor necrosis factor · TPA: 12-*O*-tetradecanoylphorbol-13-acetate · UTR: Untranslated region

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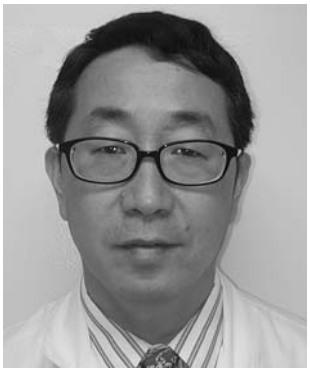

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Introduction

The low-density lipoprotein (LDL) receptor was first discovered by Goldstein and Brown in 1974 on cultured

human skin fibroblasts. Since then, its structure, function, mutation, and physiological as well as pharmacological modulations have been extensively studied. The LDL receptor is a membrane-spanning glycoprotein with a highly conserved structure in human and other animal species [1]. LDL receptor is synthesized in the rough endoplasmic reticulum (ER) as a precursor protein, followed by a maturation process during the transportation to the Golgi apparatus, including elongation of carbohydrate chains, removal of signal peptide, and conformational change [2]. The mature form of the human LDL receptor protein contains 839 amino acid residues with a molecular mass of 160 kDa [3], and has five functional domains: the ligand-binding domain, the epidermal growth factor (EGF) precursor homology domain, the *O*-linked polysaccharide domain, the membrane-spanning domain, and the cytoplasmic domain. Structures and functions of these domains have been reviewed in detail elsewhere [4].

The matured LDL receptor proteins are guided to the cell surface, where they cluster into the coated pits on the cell membrane [5]. LDL receptors on the cell surface bind and uptake apolipoprotein B- and apolipoprotein E-containing lipoproteins (especially LDL) from the circulation. After endocytosis, the LDL receptor uncouples from its ligand and returns to the cell surface for recycling, while the LDL undergoes further metabolism [6]. The LDL is the most cholesterol-rich lipoprotein in the plasma, and elevation of plasma LDL cholesterol (LDL-c) is a major risk factor for atherosclerosis and coronary heart disease (CHD). LDL receptor plays a pivotal role in the clearance and metabolism of LDL. In the human body, the liver is the most LDL-receptor-abundant organ and accounts for more than 70% of the total LDL clearance in plasma [7]. Thus, LDL receptor gene mutations often result in familial hypercholesterolemia (FH), either heterozygous or homozygous, depending on the genetic background of the parents. The heterozygote of FH is one of the most common autosomal dominant genetic diseases in humans, whereas the homozygote is rare but more severe [8, 9]. Numerous LDL receptor gene mutations have been identified, and the mutations have been divided into several classes based on their phenotypic effects on the protein [4, 10].

At present, upregulation of liver LDL receptor expression has been proven to be one of the most effective means to lower plasma cholesterol level [11]. In this review, we focus on the latest progression on the regulation of the LDL receptor gene expression.

Human LDL receptor gene

The human LDL receptor structural gene is located in the short arm of chromosome 19. It spans approximately 45 kb and consists of 18 exons, each coding for a different protein domain and 17 introns [12]. The promoter is located on the 5′-flanking region, within which the majority of *cis*-acting DNA elements are found between base pairs (bp) −58 and −234, with the A of the initiator methionine codon as +1. The promoter region spans 177 bp, including three imperfect direct repeats with 16 bp of each, two TATA-like sequences, and several transcription initiation sites, all of which are essential for gene expression and regulation (Fig. 1, [13]). Among the three direct repeats, repeats 1 and 3 contain sequences that can be recognized by the general transcription factor Sp1. Their role is to maintain the basic transcription level of the LDL receptor gene regardless of the presence or absence of sterols [13, 14]. However, they are not sufficient for a high-level expression of the LDL receptor gene in the absence of sterols, in which the contribution from repeat 2 is needed. Repeat 2 contains a 10-bp DNA element termed sterol regulatory element (SRE) (Fig. 1, [15]), and its function will be discussed in detail.

The human LDL receptor mRNA has a 5.3-kb sequence in length, which contains an unusually 2.5-kb-long 3′ untranslated region (UTR) [3]. There are three AU-rich elements (AREs) in the 5′ proximal region and three copies of *Alu*-like repeat in the 3′ distal region of the 3′ UTR. These structures play a key role for the stability of the LDL receptor mRNA and serve as *cis*-acting elements for the posttranscriptional regulation of the LDL receptor gene expression [3, 16].

Like other eukaryotic genes, the expression of the LDL receptor gene is under a complex regulation, either at the transcriptional or posttranscriptional level, mediated through intriguing signaling pathways. Cholesterol and derivatives, and nonsterol mediators, like cytokines, growth factors, and some hormones, are able to regulate LDL receptor expression, many of which are of important significance in the clinic [17, 18].

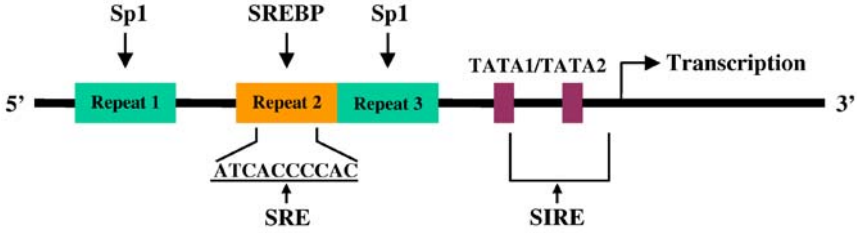

Fig. 1 Promoter region of the LDL receptor gene. Three direct repeats (*Repeat 1–3*) and two TATA-like sequences are identified within the promoter region. The *cis*-acting element of sterols is located on repeat 2, whereas the regulatory element for cytokine OM (SIRE) overlaps the TATA-like sequences

Transcriptional regulation of the LDL receptor gene

Sterols

The sterol regulatory element-binding protein (SREBP) pathway is crucial in the transcriptional regulation of the LDL receptor gene expression by cholesterol and its derivatives [19]. The SREBPs are transcription factors belonging to the basic-helix–loop–helix–leucine zipper (bHLH-Zip) family [20]. They were identified in Brown and Goldstein's laboratory to bind to SRE, which is not only present in the promoter of LDL receptor gene but also in promoters of other genes that code for enzymes participating in cholesterol or fatty acids biosynthesis, such as the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase gene and the acetyl coenzyme A synthetase gene [19–22]. There are three members of the SREBPs in mammalian cells, SREBP-1a, SREBP-1c, and SREBP-2. They have different selectivity for different target genes, with SREBP-2 as a major activator of the LDL receptor gene [23]. The SREBPs are synthesized as inactive precursors embedded in the ER membrane with a molecular mass of about 125 kDa. They consist of approximately 1,150 amino acid residues and can be divided into three functional domains forming a hairpin structure, within which the amino terminal domain contains the bHLH-Zip transcription activator (Fig. 2) [24].

To enter the nucleus and activate the transcription of target genes, the SREBP precursors must travel to the Golgi apparatus where the amino terminal domain is released [25]. The transfer of SREBPs needs the escort of another ER membrane protein termed SREBP cleavage-activating protein (SCAP). SCAP consists of 1,276 amino acids and can be divided into two functional domains (Fig. 2) [26]. The amino terminal forms eight membrane-spanning helices with short loops between them; five of the helices (2–6) serve as sterol sensors and are designated as sterol sensing domain (SSD) [26]. The carboxy terminal

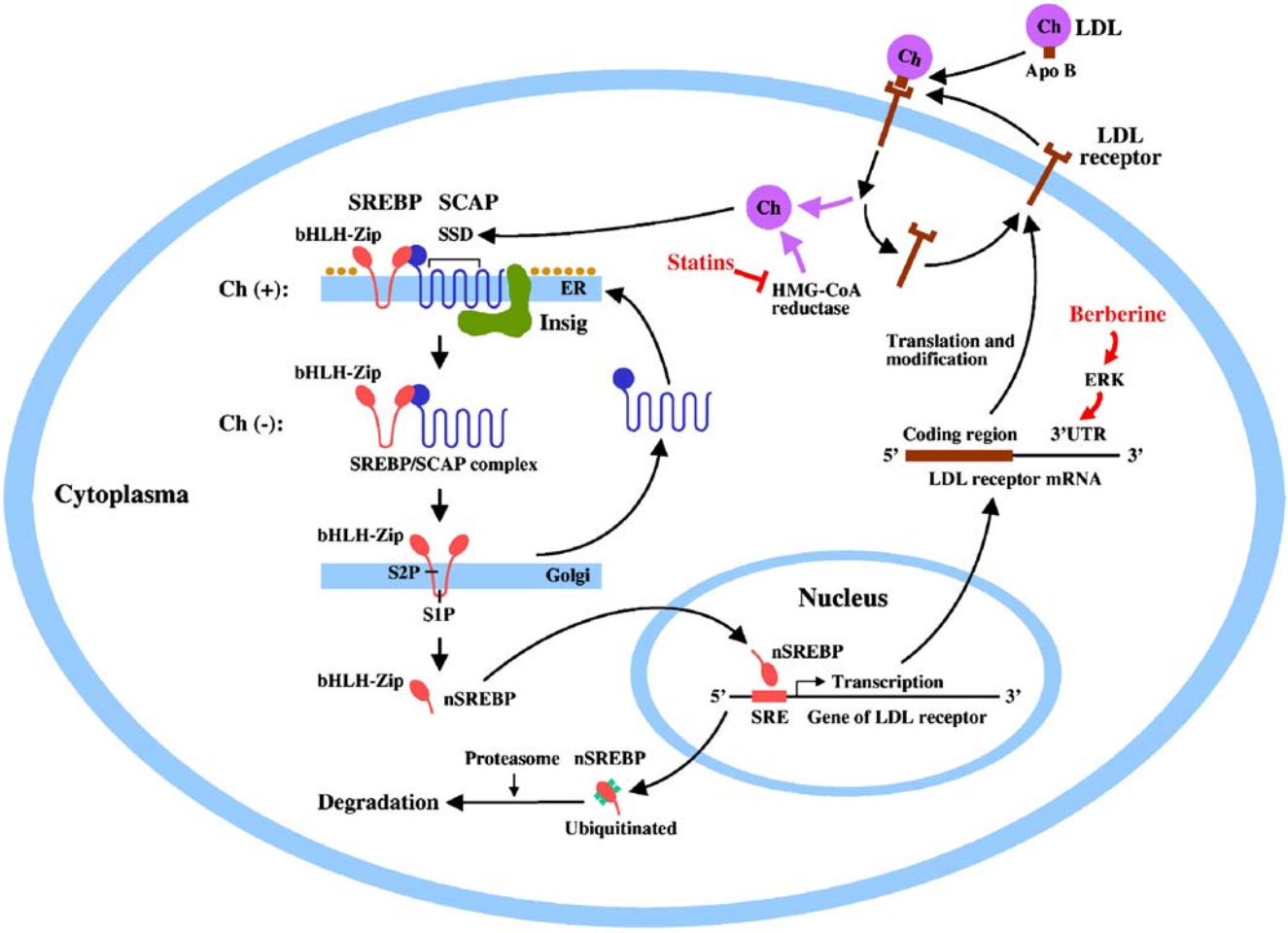

Fig. 2 Intracellular regulation of LDL receptor gene expression. This figure illustrates how LDL receptor gene expression is regulated in the cells. As indicated in the text and shown in this figure, the SREBP pathway plays an important role for the transcriptional regulation, while the 3′ UTR of LDL receptor mRNA is a key factor for the posttranscriptional regulation. Targets and/or pathways for the clinical agents with cholesterol-lowering effect are demonstrated in the figure. *bHLH-Zip*, basic-helix–loop–helix–leucine zipper; *Ch*, cholesterol; *ER*, endoplasmic reticulum; *ERK*, extracellular signal-regulated kinase; *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *Insig*, insulin-induced gene; *LDL*, low density lipoprotein; *nSREBP*, nuclear SREBP; *S1P*, site-1 protease; *S2P*, site-2 protease; *SCAP*, SREBP cleavage-activating protein; *SRE*, sterol regulatory element; *SREBP*, sterol regulatory element-binding protein; *SSD*, sterol sensing domain; *UTR*, untranslated region

domain of SCAP mediates protein–protein interaction with the SREBP precursor and forms SREBP/SCAP complex [26, 27].

When cholesterol or its derivatives are abundant in cells, the SREBP pathway is suppressed, and the transcription of the LDL receptor gene or other genes required for lipids synthesis are turned off. Cholesterol can bind directly to the SSD of SCAP, causing a conformational change of SCAP, which permits it to bind to a pair of ER membrane proteins named insulin-induced genes (Insig) 1 and 2 then forms SREBP/SCAP/Insig ternary complex (Fig. 2) [28–32]. The binding of Insig proteins traps SREBP/SCAP in ER membrane so that the SREBPs are not able to get to the Golgi apparatus for cleavage, and the expression levels of their target genes decrease accordingly. As a result, the uptake and synthesis of cholesterol are inhibited, and the cells reach a cholesterol homeostasis [28–32].

On the contrary, when the cells are absent of sterols, SCAP does not interact with the Insig proteins. In this case, the SREBP/SCAP complex is free to leave the ER [33]. After getting into the Golgi apparatus, the transcriptional active domain of the SREBP precursor will be released by two sequential proteolytic cleavages catalyzed by two proteases residing in the Golgi membrane, while SCAP will return to the ER for recycling (Fig. 2) [34, 35]. The two proteases are site-1 protease (S1P) and site-2 protease (S2P), representing a serine protease and a zinc metalloprotease, respectively [36, 37]. The cleavage of SREBP precursor results in the release of a fragment containing the bHLH-Zip domain; its molecular weight is about 68 kDa and termed as nuclear SREBP (nSREBP), or the mature form of SREBP. The nSREBP enters into the nucleus and activates the transcription of target genes [34–37]. As a result, the cells uptake more cholesterol-containing lipoproteins and increase the cholesterol production to reach a new level of cholesterol homeostasis. The nSREBP is not stable and is polyubiquitinated and rapidly degraded by the proteasome with an estimated half-life of 3 h [38].

Pharmacological modulation of the LDL receptor gene expression through the SREBP pathway has been proven to be effective in treating hypercholesterolemia. Among the currently available lipid-altering drugs, cholesterol biosynthesis inhibitors, bile acid sequestrants, and cholesterol absorption inhibitors are effective in decreasing the plasma cholesterol and subsequently upregulate liver LDL receptor expression. These agents have been used in monotherapy and in combination [11], and they all reduce the plasma LDL-c level to a certain extent in the clinic, with statins being the most promising agents among them [11, 39]. Statins are currently the most prescribed lipid-lowering drugs around the world. Statins competitively inhibit HMG-CoA reductase and block cholesterol biosynthesis in the liver. Secondary to this action, the expression of LDL receptor is upregulated (Fig. 2). Statins are very effective in lowering LDL-c level and have been proven beneficial in preventing and ameliorating atherosclerosis and CHD in large-scale clinical trials [39].

Nonsterol mediators

In addition to cholesterol and derivatives, a number of nonsterol mediators such as hormones, cytokines, growth factors, and second messengers also regulate the transcription of the LDL receptor gene [17, 18, 40]. Some of them are of physiological or pharmacological significance. Their effects on the LDL receptor gene expression are summarized in Table 1.

Among hormones, estrogens such as 17 beta-estradiol dramatically increase liver LDL receptor expression [41, 42].

Table 1 Upregulation of LDL receptor gene expression by different agents

Agent(s)	Sites of action	Sterol dependence	*cis*-Acting element(s)	*trans*-Acting factors	Signaling pathway
Statins [34–37]	Transcription	Dependent	SRE	SREBPs (activated by proteolytic cleavage)	–
Estrogens [44–46]	Transcription	Independent	Repeat 3	Estrogen receptor-alpha and Sp1	–
Insulin/growth factors [53, 54, 56]	Transcription	Independent	SRE/SRE+repeats 1 and 3	SREBPs (activated by phosphorylation)/ SREBPs+Sp1	ERK
TNF-alpha/IL-1 [58, 64]	Transcription	Dependent	Unidentified	Unidentified	ERK
OM [62, 63, 65]	Transcription	Independent	SIRE	Egr1 and c/EBP beta	ERK
PMA [16]	Transcription/posttranscription	Independent	Unidentified/3′ sequence of LDL receptor 3′ UTR	Unidentified	PKC
Berberine [80]	Posttranscription	Independent	5′ sequence of LDL receptor 3′ UTR	Unidentified	ERK

SRE sterol regulatory element, *SREBP* sterol regulatory element-binding protein, *ERK* extracellular signal-regulated kinase, *TNF* tumor necrosis factor, *IL* interleukin, *OM* oncostatin M, *SIRE* sterol-independent regulatory element, *Egr1* early growth response gene 1, *c/EBP* CCAAT/enhancer binding protein, *PMA* phorbol-12-myristate-13-acetate, *LDL* low-density lipoprotein, *UTR* untranslated region, *PKC* protein kinase C

Therefore, estrogens lower plasma LDL-c and have atheroprotective effects both in animal models and in clinical studies [42, 43]. The estrogen-responsive element in the LDL receptor promoter has been located to repeat 3, which contains a consensus Sp1 binding site [44]. Estrogen receptor-alpha, a member of the family of nuclear hormone receptors, is necessary for the LDL receptor expression induced by estrogens. It interacts with Sp1 and forms a protein complex to activate the transcription of the LDL receptor gene [44–46]. Another hormone that acts through nuclear receptor and increases the LDL receptor transcription is triiodothyronine [47]. Through sequential deletion analysis, a potential thyroid hormone responsive element was found in the promoter region of the LDL receptor gene [48]. An obvious clinical feature of hypothyroidism in animals and man is hypercholesterolemia, mainly because of a reduced expression of the LDL receptor in the liver. Therefore, thyroid hormone therapy can improve the lipids' profile in the circulation by increasing the hepatic LDL receptor expression [49, 50]. Insulin is also able to upregulate the expression of the LDL receptor gene both in cultured hepatoma cells and in mononuclear cells of type 2 diabetic patients [51, 52]. The induction of the LDL receptor gene transcription by insulin needs an intact SRE and the participation of SREBPs [53]. Moreover, activation of the extracellular signal-regulated kinase (ERK) in cells is essential for insulin's induction because inhibition of the ERK pathway completely abolishes the effect of insulin on the LDL receptor gene promoter [54, 55]. Evidences have shown that SREBP-1a and SREBP-2 are direct substrates of ERK [56, 57]. Insulin-activated ERK can phosphorylate specific serine residues in the amino terminal domain of SREBP-1a and SREBP-2, followed by an enhanced transcriptional activity of the mature form of these SREBPs without affecting their nuclear abundance. Then, the transcription of the LDL receptor gene is activated accordingly. Mutations of the serine residues at the phosphorylation sites in SREBPs abolish the effect of insulin [54–57]. These evidences suggest that besides sterol-mediated proteolytic cleavage, SBEBPs are also regulated by the phosphorylation process.

A number of cytokines such as tumor necrosis factor (TNF) alpha, interleukin (IL) 1, IL-6, and oncostatin M (OM) are able to activate the transcription of the LDL receptor gene in hepatocytes [58–60] (Table 1). The plasma levels of these proinflammatory cytokines are often elevated in inflammation, infection, or trauma, during which hypocholesterolemia status is frequently observed [61]. Systemic infusion of cytokines also lowers plasma cholesterol level in animals, and this effect is partially explained by their induction of the liver LDL receptor expression [61]. Cytokines activate the transcription of the LDL receptor gene through different mechanisms. TNF-alpha and IL-1 are capable of regulating the LDL receptor gene transcription only when cells are cultured in sterol-free media, and their induction is repressed after sterols or LDL is added [58]. In contrast, OM or IL-6 upregulates the LDL receptor gene expression in a sterol-independent manner, similar to that by insulin and some growth factors [59, 60]. OM has been shown to increase the LDL receptor gene transcription by recruiting transcription factors early growth response gene 1 (Egr1) and CCAAT/enhancer binding protein beta (c/EBP beta) to bind to a DNA motif termed sterol-independent regulatory element (SIRE), which overlaps the TATA-like sequences in the promoter region of the LDL receptor gene (Fig. 1) [62, 63], whereas IL-6 needs SRE and the repeat 3 Sp1 binding site for mediating its transcriptional activation effect [59]. The *cis*-acting DNA elements and transcription factors involved in the induction of LDL receptor expression by TNF-alpha and IL-1 remain to be identified. Although cytokines regulate the LDL receptor gene expression through diverse mechanisms, they all act through the ERK pathway [64, 65].

Growth factors, including the platelet-derived growth factor (PDGF), EGF, and the fibroblast growth factor (FGF) also upregulate LDL receptor gene expression [66–68]. It was observed previously that the induction of the LDL receptor gene transcription by growth factors was related to the effects of their stimulation on cell growth, as upregulation of LDL receptor can provide proliferating cells with more cholesterol for biomembrane synthesis [69]. However, recent data suggests that LDL receptor gene expression and cell growth can be regulated independently [70]. The stimulation effect of growth factors on the LDL receptor gene promoter requires SRE and the Sp1 binding sites as *cis*-acting elements and is related to the ERK-mediated phosphorylation and activation of SREBPs, as growth factors potently activate this signaling pathway just like insulin [54, 56, 66]. Second messenger analog phorbol esters regulate the LDL receptor gene expression as well. For example, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) increases LDL receptor gene transcription in a protein kinase C (PKC) dependent manner in hepatocytes. TPA-activated PKC induces hyperphosphorylation of histone H3 at the LDL receptor gene promoter region, thereby increasing its transcription [71]. On the other hand, phorbol-12-myristate-13-acetate (PMA) increases LDL receptor gene expression both at the transcriptional level and at the posttranscriptional level by stabilizing its mRNA [16].

While a variety of abovementioned extracellular stimuli upregulate the transcription of the LDL receptor gene, the activation of the ERK signaling cascade is crucial for these activities because blocking of this pathway closes down their effects [64, 65, 72]. ERK belongs to the subfamilies of the mitogen-activated protein kinases (MAPK), the activation of which by successive phosphorylation is secondary to the extracellular stimuli binding to their receptors on cell surface. Those receptors either have intrinsic tyrosine kinase activity themselves (like growth factor receptors and insulin receptor) or are coupled to another protein–tyrosine kinase (like receptors for cytokines) [73]. Upon activation, ERK phosphorylates and activates numerous cytoplasmic or nuclear protein factors and mediates multiple biological responses, including those control cell growth and differentiation [73]. But how the ERK pathway links to the promoter of the LDL receptor gene and increases its transcription through different mechanisms is not fully elucidated. It is speculated that transcription factors or

coactivators participating in LDL receptor gene expression may be modulated by ERK or its downstream signals; therefore, increasing the LDL receptor transcription through a mechanism similar to that of insulin and some of the growth factors [72, 74].

In contrast to the ERK pathway, p38 decreases the transcription of the LDL receptor gene [64, 75]. There is a cross-talk mechanism between the p38 and ERK pathways, in which p38 exerts its inhibitory effect on the LDL receptor gene promoter by suppressing ERK activity. Blocking solely the p38 pathway activates ERK and induces LDL receptor gene transcription in hepatocytes [75]. Inhibiting p38 in IL-1 treated liver cells causes the superinduction of ERK activity and LDL receptor gene transcription [64, 72]. These data suggest that the transcription of the LDL receptor gene can be regulated through an interlinked signal network in response to various extracellular stimuli.

Posttranscriptional regulation of the LDL receptor gene

In addition to transcriptional regulation by sterols and various nonsterol mediators, the LDL receptor expression can be regulated at posttranscriptional level as well, although the mechanism and significance requires further investigation and appreciation. Current data suggest that the mRNA stability is the major mechanism for the posttranscriptional regulation of the LDL receptor gene expression [16]. The LDL receptor mRNA has a constitutively short half-life of about 45 min in HepG2 cells [76]. The stability of a specific mRNA is largely determined by the structure of its 3′ UTR. There are three AREs being identified in the 5′ proximal region of the LDL receptor 3′ UTR [16]. AREs have also been found in 3′ UTRs of many other short-lived mRNA species, like the mRNAs for cytokines. These elements confer their destabilizing effects on mRNAs, including the LDL receptor mRNA [16, 77]. Evidence has shown that after fusing the 5′-most ARE of the LDL receptor 3′ UTR to the coding region of the beta-globin cDNA, the degradation rate of the fusion transcript increases threefold as compared to the wild-type beta-globin mRNA. Fusion of LDL receptor 3′ UTR fragment containing all three AREs with the beta-globin gene increases the degradation rate of the fusion transcript by more than tenfold [16]. The destabilizing effect of AREs has also been demonstrated in animals in vivo. Transgenic mice expressing human LDL receptor gene, with two AREs in the 3′ UTR being truncated, can increase its mRNA stability up to threefold as compared to the native mice LDL receptor mRNA, resulting in a total of 2.5-fold increase in the LDL receptor expression in the mice liver [78].

The stability of the LDL receptor mRNA can be regulated by several mediators, including phorbol ester PMA, chenodeoxycholic acid (CDCA), berberine, and a fibrate drug, gemfibrozil [16, 76, 79–81]. PMA prolongs the half-life of the LDL receptor mRNA by more than twofold in HepG2 cells. The *cis*-acting element of PMA in the 3′ UTR of LDL receptor mRNA has been located to the 3′ distal region which contains three *Alu*-like repeats, and the stabilization effect of PMA also associates with the actin cytoskeleton in HepG2 cells [16, 76]. Berberine is a natural compound isolated from herbs such as *Coptis chinensis*. It was recently shown to be capable of upregulating LDL receptor expression through a posttranscriptional and sterol-independent mechanism in hepatocytes (Fig. 2). The 5′ proximal region of the LDL receptor 3′ UTR containing three AREs is indispensable for berberine to stabilize the receptor mRNA. Berberine has also shown a promising LDL-c-lowering effect and safety both in an animal model and in hypercholesterolemic patients [80]. The *trans*-acting protein factors used by these mediators to stabilize LDL receptor mRNA have not been identified yet and need extensive investigation. Interestingly, the stabilization of LDL receptor mRNA by berberine and CDCA also need the activation of the ERK signaling pathway in cells. Blocking this pathway abolishes their stabilization effects [79, 80]. But how the ERK pathway, activated by these mediators, links to the LDL receptor 3′ UTR to stabilize the receptor's mRNA remains to be clarified.

Taken together, significant progression has been made to elucidate the mechanisms underlying the regulation of the LDL receptor gene expression since its discovery. Concomitantly, lipid-lowering agents working through upregulation of liver LDL receptor expression have been used in clinical practice [11, 80] and/or are in development in laboratories [82]. However, several key questions remain unresolved, including the ones regarding cross talks among the signal molecules or pathways, the other cytoplasmic factors that might participate in regulating LDL receptor mRNA stability, and whether or not other posttranscriptional mechanisms exist. Answers to these questions may lead to the discovery of new treatments for dyslipidemia.

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