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REVIEW

Lipid Signaling in the Atherogenesis Context

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Abstract—Normally macrophages localized in the arterial vessel wall perform the "reverse transfer" of cholesterol, which includes endocytosis of low density lipoproteins (LDL), cholesterol transfer to newly formed high density lipoprotein particles, and their following elimination by the liver. The homeostatic function of macrophages for cholesterol involves a system of lipid sensors. Oxysterol sensors LXRs, oxysterol and cholesterol sensors INSIG and SCAP acting through controlled transcription factors SREBP, as well as sensors for oxidized fatty acids and their derivatives, PPAR, are the best studied. Activation of LXR and PPAR is also accompanied by inhibition of macrophage functions related to inflammation. Accumulation of oxidized and otherwise modified LDL in the subendothelial space induced by endothelium injury, infection, or other pathogenic factors instead of stimulation of the homeostatic functions of macrophages leads to their weakening with a concurrent increase in the inflammatory potential of these cells. These shifts seem to drive the transformation of macrophages into foam cells, which form the core of sclerotic plaques. The intervention of another lipid sensor, TLR4, can trigger such a radical change in the functional activity of macrophages. The interaction of modified LDL with this signaling receptor results in inhibition of the homeostatic oxysterol signaling, induction of additional LDL transporters, and activation of the phagocytic function of macrophages. The re-establishment of cholesterol homeostasis under these circumstances can be achieved by administration of LXR and PPARγ agonists. Therefore, it is urgent to design ligands with reduced side effects.

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Key words: atherogenesis, macrophages, cholesterol, lipid sensors, oxysterols, modified low density lipoproteins

Atherogenesis is a complex of events in the wall of large arteries finally resulting in formation of a lipid plaque, which in the case of an unfavorable scenario of further development can promote formation of a thrombus with ischemia of the corresponding tissues. According to hypotheses of "response to damage" [1] and "response to retention" [2], primary lipid streaks appear after a damage of the endothelium (provoking factors are smoking, atherogenic diet, heredity, high arterial pressure, infection). This results in activation of the endothelium and increase in the expression of adhesion molecules that promotes the infiltration of atherogenic lipoproteins and entrance of monocytes and T-cells into the subendothelial space. Under the influence of the macrophage colony-stimulating factor (M-CSF) secreted by the endothelium and smooth muscle cells, monocytes are differentiated into macrophages with the prevalent phenotype of M1. Accumulation of lipids activates macrophages and converts them into foam cells. Smooth muscle cells migrate into the intima and acquire the proliferative phenotype with expressed growth factors and adhesion molecules that promotes the retention of the migrating macrophages and T-cells in the intima [3].

Macrophages localized in the vascular wall perform a double function. First, these cells are important contributors to the immune response, both innate and adaptive, to endothelium damage. If the damage is caused by a bacterial infection, the interaction of bacterial cell lipopolysaccharide with the signaling Toll-like receptor

Abbreviations: HDL, high density lipoproteins; HMGR, 3-hydroxy-3-methylglutaryl-CoA-reductase; HNF, hepatocyte nuclear factor; INSIG, product of the insulin-induced gene; LDL, low density lipoproteins; LDLR, LDL receptor; LPL, lipoprotein lipase; LRP, LDLR related protein; LXR, liver X-receptor; M-CSF, macrophage colony-stimulating factor; ORP, OSBP related protein; PPAR, peroxisome proliferator-activated receptor; ROR, retinoid acid receptor-related orphan receptor; SCAP, SREBP cleavage activating protein; SRE, sterol-responsive element; SREBP, sterol-responsive element-binding protein; TLR, Toll-like receptor; VLDL, very low density lipoproteins; VLDLR, very low density lipoprotein receptor.

(TLR4) activates the phagocytic, antigen-presenting, and cytokine-producing functions of macrophages. Second, macrophages are the most important elements of normal and pathological lipid metabolism. A small number of macrophages in the intact vascular wall provides for reverse transport of cholesterol from normal low density lipoproteins (nLDL) into the liver to be utilized and excreted. If modified LDL (mLDL) is accumulated in the subendothelial space, macrophages become unable to perform this function, begin to intensively accumulate lipids, and transform into foam cells. The interaction with mLDL increases the inflammatory potential of macrophages and, by contrast, inflammatory stimuli inducing production of reactive oxygen species by macrophages (a form of defense against infection) induce an increased production of mLDL in the vascular wall, thus generating a vicious circle of inflammation–lipid accumulation. Thus, lipid metabolism in macrophages of the vascular wall plays the main role in the development of atherosclerosis, and knowledge of the lipid signaling pathways is important for creating efficient tools for combating this common pathology.

LIPID SIGNALING UNDER NORMAL CONDITIONS

The term "sensor" denotes a protein or a protein complex directly interacting with a lipid and/or lipoprotein and transmitting a signal about this interaction to effector systems. Lipoprotein receptors, scavenger receptors of the plasma membrane, intracellular membrane proteins, and transcription factors of the nuclear receptor family can act as sensors. The majority of data presented concern lipid signaling in macrophages, but many elements of this signaling function in other cells of the vascular wall and also in other tissues involved in lipid metabolism. In the present review the term "lipid signaling" is applied only to signaling initiated by lipids not specialized for signaling function. The signaling that involves specially synthesized signaling lipids, such as eicosanoids, endocannabinoids, and sphingolipids, is beyond the scope of this review.

In an intact vascular wall a small population of macrophages (mainly with the M2 phenotype) eliminates nLDL that enter into the subendothelial space by capturing these particles and their components (triglycerides, phospholipids, free and esterified cholesterol), metabolizing them and transporting free cholesterol on newly produced particles of high density lipoproteins (HDL) that are then transferred into the liver by the blood flow. The nLDL are also captured otherwise, either by receptor-mediated (saturated uptake) or by receptor-unmediated (pinocytosis, unsaturated uptake) pathways [4]. The receptor-mediated capture involves the LDL receptor (LDLR), the very low density lipoprotein receptor (VLDLR), and partially some scavenger receptors via endocytosis of lipoprotein–receptor complexes. This capture is promoted by a preliminary hydrolysis of LDL triglycerides by lipoprotein lipase (LPL) expressed by macrophages and localized on their surface. LPL can also form a triple complex with lipoprotein and its receptor that is favorable for endocytosis. In addition to transporting lipoproteins into the cell, LDLR and VLDLR also seem to perform a signaling function due to their ability to interact with the adaptor protein Dab1. The role of this signaling for lipid metabolism, in particular in macrophages, remains unclear.

A multifunctional LDLR-related protein 1 (LRP-1) is also responsible for endocytosis of lipids containing apolipoprotein E (apoE) and acts as a signaling receptor for these ligands [5, 6]. The larger than in LDLR and VLDLR intracellular domain of the LRP group proteins is responsible for the interaction of LRP with a significantly broader spectrum of adaptor proteins and transmission of signals through additional pathways [4]. Experiments with *LRP-1* gene deletion in macrophages and smooth muscle cells have revealed its atheroprotective activity, which requires the signaling function of LRP-1 [7]. LRP-1 of macrophages was also shown to facilitate oxidation of LDL under the influence of 12/15-lipoxygenase [8].

Scavenger receptors CD36 (fatty acid translocase), SR-A (scavenger receptor A), LOX-1 (a lectin-like receptor of oxidized LDL), and some others provide for endocytosis of mLDL, especially oxidized ones. The signaling function can also be performed by lipid components of mLDL and mLDL themselves as ligands of scavengers. Because production of mLDL mainly occurs under pathologic conditions, data on mLDL signaling mediated through scavenger receptors will be considered in the section concerning the lipid signaling in disease.

Upon entrance into the cell within endosomes, LDL undergo further degradation accompanied by release of fatty acids, phospholipids, free cholesterol, and its esters. Polyunsaturated fatty acids, e.g. arachidonic acid, and products of partial oxidation of fatty acids, such as hydroxyeicosatetraenic acids, possess signaling features as ligands of transcription factors PPARs (peroxisome proliferator-activated receptors) of the nuclear receptor class. PPARs are involved in the control of the transcription activity of genes associated with lipid metabolism including biosynthesis and degradation of fatty acids. Polyunsaturated fatty acids (ω3 and ω6) and their hydroxy derivatives (15-hydroxyeicosatetraenic acid (15-HETE) and 13-hydroxyoctadecadienic acid (13-HODE)) can also be agonists of another nuclear receptor, testicular receptor 4 (TR4), which, in particular, mediates the stimulatory effect of these compounds on expression of the scavenger receptor CD36 with the corresponding acceleration of production of foam cells and atherogenesis [9]. γ-Linoleic acid and other unsaturated fatty acids

(from 16:1 to 20:4) also can stimulate expression of the anti-atherogenic apolipoprotein E (ApoE) through TR4 [10].

Functioning as a sensor of fatty acids of the hepatocyte nuclear factor 4α (HNF4α), which is another transcription factor of the nuclear receptor class, is still under question. This factor is involved in the control of transcription of a broad variety of genes including those associated with lipid metabolism [11]. Information about potential ligands, mechanisms of their interaction with HNF4α, and consequences of this interaction have been published [11-14].

Cholesterol esters, cholesterol itself, and also products of its partial oxidation to oxysterols, which act as ligands of some intracellular sensors, seem also to possess signaling features. The first group of oxysterol sensors includes two isoforms (α and β) of liver X-receptor (LXR), which is another transcription factor of the nuclear receptor class. The LXR-controlled genes are associated first of all with cholesterol metabolism and also with fatty acid metabolism.

Members of the second group of oxysterol sensors localized in the endoplasmic reticulum are pairs of mutually interacting proteins – SCAP (the SREBP (sterol-responsive element-binding protein) cleavage activating protein) and INSIG (product of the insulin-induced gene). SCAP and INSIG mediate negative effects of cholesterol and oxysterols, respectively, on activities of transcription factors SREBP-1 and SREBP-2, inhibiting their activation during the limited proteolysis of their precursors in the endoplasmic reticulum and Golgi complex. This inhibition includes stimulation by SCAP or by INSIG ligands of the interaction between these proteins, which is responsible for retention of the SREBP precursors in the endoplasmic reticulum [15]. SREBP-1 and SREBP-2 are involved in the regulation of genes mainly associated with the metabolism of fatty acids and cholesterol, respectively. 24(S),25-Epoxycholesterol acts as a common ligand of LXR and SCAP/INSIG. This oxysterol is unique by its generation concurrently with cholesterol as a result of *de novo* biosynthesis from the common precursor 2,3(S)-monooxidosqualene. Via suppression of SREBP precursor processing in the endoplasmic reticulum, 24(S),25-epoxycholesterol inhibits expression of LDLR and thus decreases the entrance of exogenous cholesterol into the cell and also inhibits expression of the cholesterol biosynthesis limiting factor 3-hydroxy-3-methylglutaryl CoA-reductase (HMGR) that decreases *de novo* cholesterol biosynthesis. Via activation of LXR, 24(S),25-epoxycholesterol also stimulates expression of the cholesterol transporter ABCA1 providing for accelerated export of cholesterol from the cell [16].

INSIG is also involved in a rapid negative regulation of HMGR. Sterols stimulate formation of INSIG/HMGR complexes that undergo ubiquitinylation and proteasomal degradation [17, 18].

Cholesterol was detected in the ligand-binding pocket of the purified recombinant retinoic acid receptor-related orphan receptor α (RORα) [19], and RORα could efficiently bind cholesterol sulfate [20]; therefore, it was suggested that this transcription factor of the nuclear receptor family could also serve as a sterol sensor. RORα and LXR inhibit the *in vitro* effects of each other on expression of some genes associated with lipid metabolism [21]; however, the *in vivo* deficiency of both types of receptors has the same consequence – a predisposition for development of atherosclerosis [22]. There are no data on the functional significance of sterol binding to RORα.

Other proteins can also act as oxysterol sensors. In particular, oxysterols and synthetic LXR ligands are shown to somehow influence hedgehog signaling [23, 24]. Although the significance of hedgehog for functioning of mature macrophages under normal conditions is unknown, hedgehog signaling in macrophages has been shown to prevent the development of atherosclerosis [25].

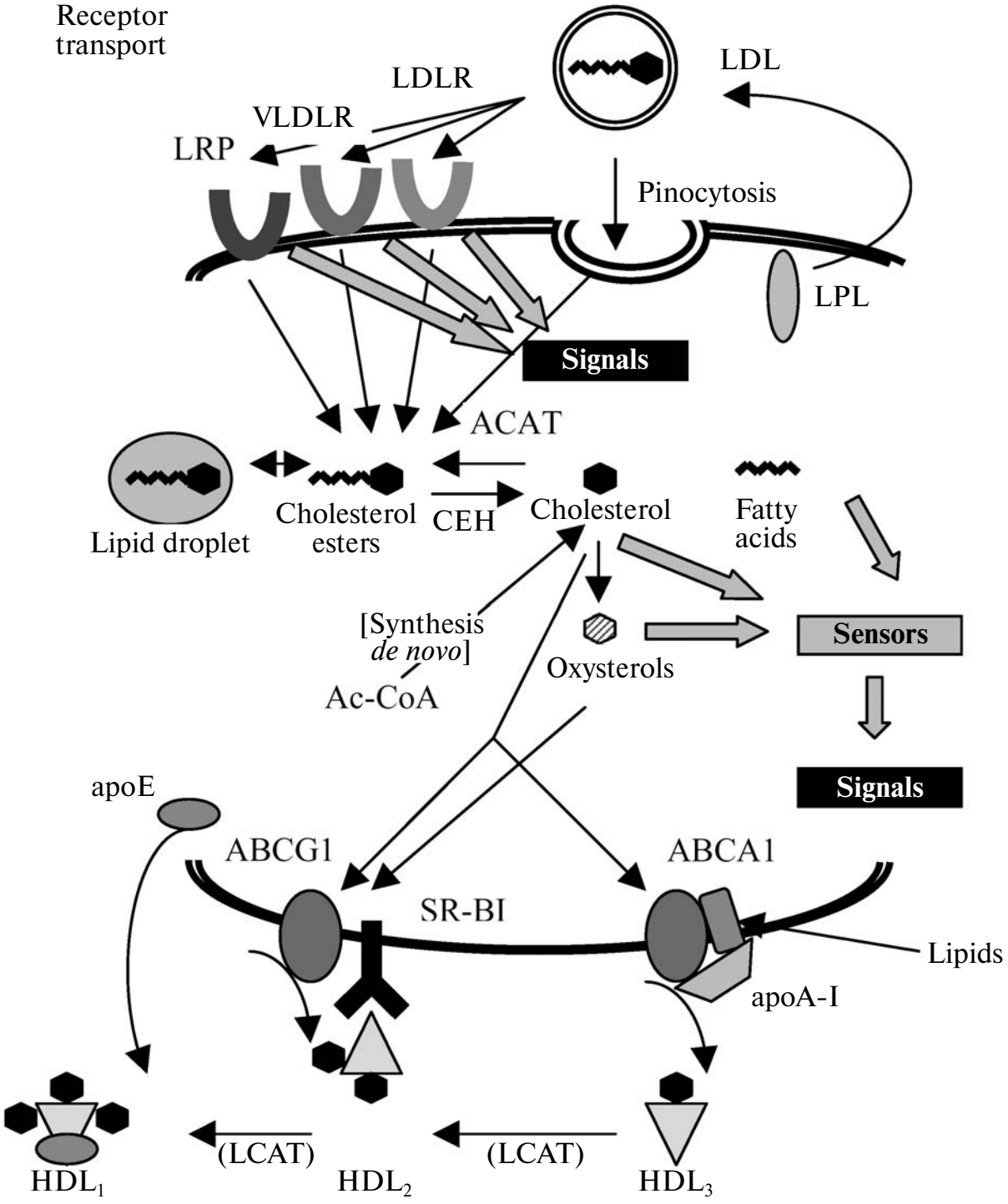
The amyloid precursor protein (APP) probably acts as a cholesterol sensor. It is suggested that due to interaction with cholesterol within the membrane, this protein undergoes proteolytic processing with generation of peptide products that inhibit expression of the LRP1 gene and HMGR activity [26].

The oxysterol-binding protein (OSBP) seems to react to the cholesterol/oxysterol balance in the cell. Cholesterol binding to OSBP through the associated protein phosphatase and extracellularly regulated kinase (ERK) is shown to influence the activity of SREBP and of the genes controlled by it [27, 28].

It seems that OSBP related proteins (ORP) also can act as cholesterol sensors. In particular, ORP8 expressed in macrophages and specifically binding 25-hydroxycholesterol can inhibit expression of the ATP-binding cassette transporter ABCA1 of cholesterol through an unclear mechanism [29]. However, the modulating function of cholesterol has not been demonstrated in this case. ORP2 can interact with 25- and 22(R)-hydroxycholesterols, 7-ketocholesterol, and cholesterol. Based on experiments with ORP2 knockdown, this protein is supposed to be involved in the coupling of cholesterol with the metabolism of neutral lipids [30].

The scavenger receptor BI (SR-BI) is also suggested to function as a cholesterol sensor. The SR-BI expressed on the macrophage plasma membrane can interact with a cholesterol acceptor (HDL) and serves as an element of the cholesterol reverse transport system. In particular, the SR-BI signal is closed on activating endothelial nitroxide synthase [31].

Interaction of lipid ligands with the majority of the abovementioned sensors is accompanied by homeostatic reactions supporting the lipid content in the cell at a stationary level. The free cholesterol content in the cell is especially controlled because excess of cholesterol itself



Known elements of the cholesterol metabolism system in macrophages. ABCA1 and ABCG1, cassette ATP-binding cholesterol transporters; SR-BI, scavenger receptor BI; apoA-I, apolipoprotein A-I; apoE, apoliporotein E; LCAT, lecithin:cholesterol acyltransferase I

Scheme 1

and of its oxidized derivatives is cytotoxic and, in particular, induces apoptosis [32, 33]. Major components of systems of cholesterol entrance into the cell, its storage, mobilization, and reverse transport are presented in Scheme 1.

Accumulation of cholesterol and its oxidized derivatives in the cell is accompanied by inhibition of cholesterol entrance from outside due to decrease in the expression of LDLR; suppression of *de novo* cholesterol biosynthesis, in particular, due to accelerated degradation of

HMGR and decrease in its expression on the transcription level; the prevalence of generation and storage in lipid droplets of cholesterol esters compared to their hydrolysis due to reciprocal regulation of acyl-coenzyme A:cholesterol acyltransferase (ACAT) and cholesterol ester hydrolase (CEH) activities; an accelerated elimination of cholesterol and oxysterols from the cell due to increased activities of the cassette ATP-binding transporters A1 and G1 (ABCA1, ABCG1) and expression of accessory proteins, of the scavenger receptor SR-BI (responsible for bringing together the cholesterol transporter ABCA1 with its acceptor HDL_3), and of apolipoprotein E (apoE) increasing the capacity of the acceptor. Paradoxically, the capture of cholesterol esters from HDL_3 by cells increases concurrently with acceleration of cholesterol reverse transport [34].

LXR: at the center of homeostatic lipid signaling. LXRα and LXRβ are transcription factors of the nuclear receptor class that act as permissive heterodimers with retinoid X receptors (RXR, isoforms α, β, γ, of the nuclear receptor class). LXRα is mainly expressed in cells involved in lipid metabolism (liver, adrenal cortex, intestine, adipose tissue, macrophages) and also in lungs and kidneys; LXRβ is distributed more widely. In a typical case LXR/RXR heterodimers interact with hormone-responsive elements (LXRE) of the regulated genes represented by direct hexanucleotide repeats (consensus AGGTCA) with the four-member spacer (DR4). No consensus LXRE were found in some oxysterol-repressed genes. It seems that transrepression can occur without a direct interaction of LXR with DNA.

The functional significance of *in vivo* oxysterol signaling through LXRα is shown in mice with *LXRα* gene knockout: these animals fed with cholesterol-enriched diet manifested a rapid accumulation of cholesterol in the liver mainly because of inability of oxysterols to induce the expression of cytochrome CYP7A, which is a limiting enzyme of bile acids biosynthesis from cholesterol [35]. In the regulatory region of the *CYP7A* gene of mice and rats, several LXRE are found that are absent in the human gene, and this explains the species-related differences in tolerance to cholesterol excess [36]. The functioning of LXR in oxysterol regulation of cholesterol reverse transport from peripheral tissues into the liver is similarly important for cholesterol metabolism in the whole organism. LXR ligands stimulate expression of cholesterol reverse transport components (Scheme 1) and thus increase HDL content. Moreover, LXR ligands also inhibit absorption of alimentary cholesterol in the intestine [37]. Note that contributions of LXR isoforms to regulation of cholesterol metabolism can vary depending on the genetic background and with alimentary cholesterol load. Under conditions of normal cholesterol entrance no changes in cholesterol metabolism were recorded in *LXRα*(−/−) mice, which indicated a compensation of LXRα insufficiency due to functioning of LXRβ. However, in the case of concurrent insufficiencies of LXRα and apoE (disorders in peripheral production of HDL and in their capture by the liver) and under conditions of cholesterol load, LXRβ became unable to fit the need in the regulation, and cholesterol accumulation in the peripheral tissues significantly increases accompanied by development of atherosclerosis. In such a situation injection of synthetic ligands of LXR attenuates these symptoms [38].

In addition to organization of autoregulation of cholesterol metabolism, LXR provides for relations between cholesterol content and other types of metabolism [39]. LXR ligands induce accumulation of triglycerides in the liver and triglyceridemia due to stimulation of biosynthesis of fatty acids [40]. This effect includes both the direct effect of LXR on the fatty acid synthase gene promoter and induction of expression of SREBP-1c, which also activates the fatty acid synthase gene [41]. LXR ligands also increase glucose tolerance, acting on hepatocytes (suppression of gluconeogenesis, stimulation of glucose utilization), adipocytes (stimulation of GLUT4), and on β-cells of the pancreas (stimulation of glucose-induced secretion of insulin) [39]. In the absence of LXR the intensity of energy metabolism increases [42].

There are some pathways for regulating the transcription activity of LXR: a synergic activation by ligands of both partners of the LXR/RXR heterodimer, i.e. by oxysterols and 9-*cis*-retinoic acid; regulation of the LXR content in the cell on the *LXRα* gene level; modulation of the interaction of LXR with coregulators through sumoylation; and a selective modulation of the activity via phosphorylation.

According to X-ray crystallographic analysis, the ligand-binding pockets of LXRα and LXRβ are virtually identical. Experiments with mutagenesis and docking have shown that the role of the major determinant on the interaction of the ligand with LXR is played by the oxysterol hydroxyl in positions 22(R), 24(S), or 27, which acts as a proton acceptor from amino acid residues H421 and W443 LXRα. This interaction is supposed to stabilize an active conformation of the 12th α-helix of the receptor ligand-binding domain corresponding to activating function 2 (AF2). The oxysterol hydroxyl in position 3 seems to form the hydrogen bond through R305 or directly with E267 of the receptor. This interaction increases the efficiency of ligands but does not play the main role for manifestations of the biological activity. The above-mentioned arginyl R305 is important also for the interaction of the receptor with antagonistic fatty acids. Other amino acids lining the pocket are involved in hydrophobic interactions with the oxysterol ligand [43].

Oxysterols can enter the organism as constituents of food products. Moreover, oxysterols are generated endogenously as a result of enzymatic or nonenzymatic oxidation of cholesterol or its precursors. Major blood oxysterols (mainly as LDL components) include 7-keto-

and 7β-hydroxycholesterol [44]. However, these oxysterols have low affinities for LXR [45]. The content of oxysterols determining the transcription activity of LXR depends on the import into the cell of exogenous cholesterol; on endogenous cholesterol biosynthesis *de novo*; on the entrance of oxysterols as constituents of modified (oxidized) lipoproteins; on the level of reactive oxygen species in the cell; on activities of P450 cytochromes hydroxylating cholesterol at positions 22, 24, and 27; on activities of enzymes modifying the oxysterol structures (e.g. CYP7A1 promoting oxysterol conversion into bile acids in the liver, sulfotransferases or UDP glucuronosyl transferases catalyzing formation of oxysterol conjugates with sulfuric and glucuronic acids, and steroid sulfatases and glucuronidases hydrolyzing these conjugates); and also on activities of transporters of oxysterols and their derivatives.

The regulatory significance of formation of the oxysterol conjugates for the LXR-mediated signaling can be illustrated by sulfation. Sulfation of 24-hydroxycholesterol is catalyzed by three isozymes of human sulfotransferase (SULT2A1, SULT1E1, and SULT2B1b) with production of 3- and 24-monosulfates and 3,24-disulfate of 24-hydroxycholesterol [46]. The ester bond of sulfate in position 3 can be hydrolyzed by steroid sulfatase, whereas a similar bond in position 24 is resistant to the enzyme. Both 3- and 24-sulfation of 24-hydroxycholesterol result in the loss of its agonistic activity and arising of an antagonistic activity with respect to LXR; efficiencies of 3- and 24-sulfates of 24-hydrocholesterol as antagonists (IC_{50} = 0.15 and 0.31 μM) are about an order of magnitude higher than the agonistic activity of the initial oxysterol (EC_{50} = 1.99 μM). Obviously, inhibition of oxysterol signaling in lymphocytes under the influence of proliferative stimuli via induction of SULT2B1b [47] includes not only elimination of LXR agonists but also accumulation of antagonists. Induction of SULT2B1b expression by LXR ligands in human keratinocytes [48] indicates that this sulfotransferase can act as a component of the system of autoregulation of cholesterol metabolism. 25-Hydroxy-cholesterol-3-sulfate also can act as an LXR antagonist. In the THP-1 line of human macrophages, this compound suppresses effects of its biosynthetic precursor 25-hydroxycholesterol on lipid metabolism through the LXR-SREBP axis [49].

Protein phosphorylation is another mechanism for regulating LXR activity. The site S198 located in the hinge region of LXRα is preferentially phosphorylated in macrophages. The phosphorylation is stimulated by LXR ligands and suppressed by 9-*cis*-retinoic acid, which is a ligand of the heterodimerization partner. Casein kinase 2 seems to act as an effector kinase, and the content of its subunit β in the cell nucleus is reciprocally regulated by LXR and RXR ligands. Biological consequence of LXRα phosphorylation is a differentiated decrease in the efficiency of the stimulating effect of LXR ligands on transcription of some of the regulated genes (LPL, the chemokine CCL24, AIM (apoptosis inhibitor expressed in macrophages)) but without influencing the activation of transcription of other regulated genes (ABCA1, ABCG1, SREBP-1c, PLTP (phospholipid transfer protein)). The phosphorylation of LXRα is supposed to prevent detachment of the NCoR corepressor from the LXR/RXR dimer and attachment of the coactivator. This mechanism can be a form of self-limiting of the oxysterol effects on the cell [50].

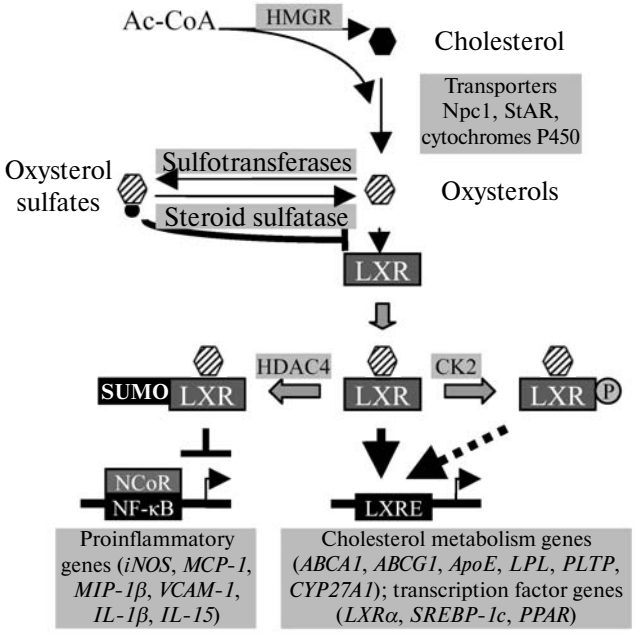
The transrepressor action of LXR on proinflammatory genes also including NCoR is activated by LXR sumoylation. In primary macrophages and their line RAW264.7, LXR ligands stimulate the interaction of LXRα and LXRβ with the promoter region of the inducible nitroxide synthase (iNOS) gene and inhibit the release of NCoR and increase in LPS-induced promoter activity. These effects of LXR ligands depend on activities of the sumoylation enzymes Ubc9 (E2-conjugating enzyme) and HDAC4 (E3-ligase). Similar results were obtained for the MCP-1 gene. The ligand-dependent sumoylation of LXRβ occurs at lysine residues 410 or 448 in the ligand-binding domain, and substitution of these residues by arginyl inhibits the transrepressor effect of LXR ligands on iNOS and MCP-1 but does not influence the transactivating effect of LXR on the gene *ABCA1* promoter. Similar results were obtained for LXRα. Note that 22(R)-hydroxycholesterol, 24(S),25-epoxycholesterol, and 24-hydroxycholesterol caused transrepression of iNOS and activation of ABCA1, whereas 25- and 27-hydroxycholesterols displayed only an activating effect on ABCA1. Thus, oxysterol agonists of LXR are different in the transrepressor activity and, consequently, the inhibitory effect of oxysterols on expression of proinflammatory proteins can vary depending on the pathways of oxysterol generation. The transrepressor effect of LXR ligands can depend on the nature of the stimulus activating the gene [51]. Other pathways of transrepression mediated by the ligand-dependent sumoylation of LXR also seem to exist. Thus, in astrocytes the LXR sumoylation prevents binding of the activated STAT1 to promoters of proinflammatory genes induced by interferon γ. The inhibition includes interaction of LXRβ and LXRα with complexes of SUMO ligases, PIAS1 and HDAC4, respectively, and phosphorylated STAT1 [52].

LXR activity is also regulated through modulation of *LXR* gene activities. LXRα expression is induced both *in vivo* and *in vitro* in different cells including macrophages by their own agonists, whereas the LXRβ expression is resistant to LXR agonists. The effect of LXR on the human gene *LXRα* is mediated by LXRE in the 5′-regulatory region (–4139/–4115 nucleotide pairs) [53, 54]. LXRβ expression is also resistant to some other factors influencing the LXRα content [55].

For elucidation of the regulatory role of lipids in atherogenesis, a presence of a positive PPAR-responsive element (PPRE) in the 5′-regulatory region of the *LXRα*

gene is important [56]. Ligands of PPARα and PPARγ accelerate the reverse transport of cholesterol from macrophages partially due to induction of LXRα expression, and PPARγ insufficiency in macrophages promotes atherogenesis [57, 58].

The main pathways of LXR-mediated regulatory effect of oxysterols on gene expression are presented in Scheme 2.



LXR in oxysterol signaling. HMGR, 3-hydroxy-3-methylglutaryl-CoA-reductase; Npc1, Niemann–Pick protein C1; StAR, steroidogenic acute regulatory protein; HDAC, histone deacetylase; CK, casein kinase; NcoR, nuclear receptor corepressor; NF-κB, nuclear factor κB

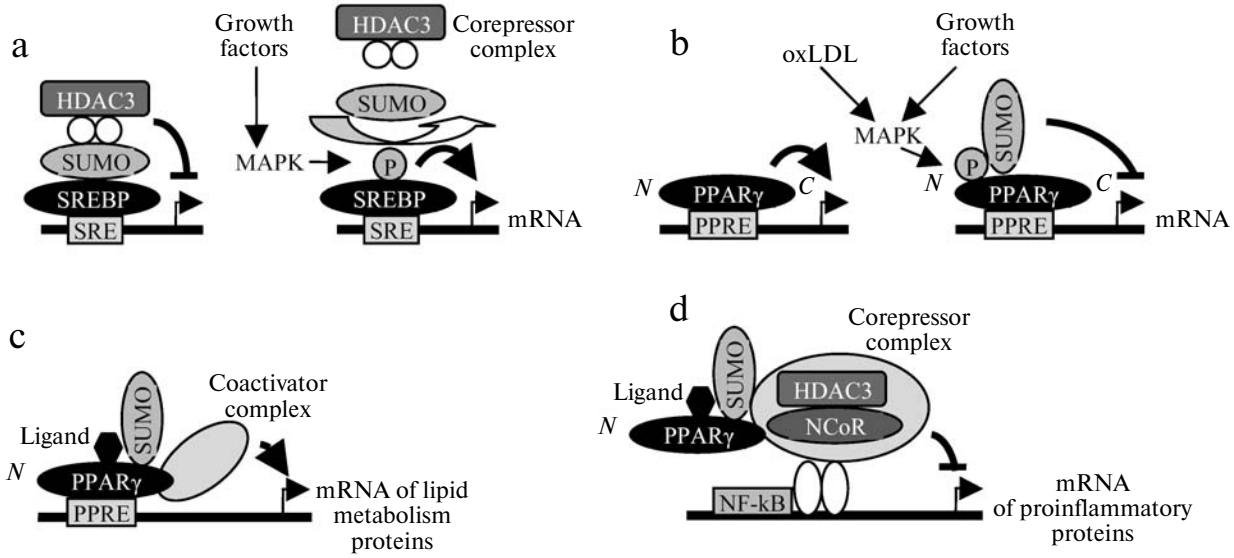
Scheme 2

SREBP: threshold switches. SREBP-1c and SREBP-2 are transcription factors, and their activities are suppressed on the post-translational level by cholesterol and oxysterols, which inhibit the proteolytic processing of the precursors in the endoplasmic reticulum and Golgi complex. The inhibitory system functions according to the "all or none" principle with the threshold value depending on expression of sterol sensors SCAP and INSIG [59]. In the absence of such inhibition, i.e. when the free cholesterol content in the endoplasmic reticulum is below the threshold value, regulation of the SREBP activities is mainly determined by intensities of transcription of their genes.

Lipids including fatty acids and oxysterols are important regulators of expression of the *SREBP* genes. A tandem of LXR-response elements that mediate the stimulating effect of oxysterols on the *SREBP-1c* expression is found in the 5′-regulatory region of the mouse *SREBP-1c* gene [60, 61]. These regulatory elements can also mediate the inhibitory effect of PPAR ligands on the gene expression [62]. Ligands of PPARα/βδ/γ can influence the level of the active nuclear form of SREBP, directly and indirectly inducing expression of the *INSIG-1/2* genes through PPRE in the 5′-regulatory region of the *INSIG* gene [63, 64]. The importance of this pathway of regulation of SREBP-2 activity by fatty acids is indicated by the absence of a hypocholesterolemic effect of eicosapentaenic acid in mice with *PPARα* gene knockout [65]. Within the *INSIG-1* gene a sterol-responsive element (SRE) is also found that provides for functioning of the negative feedback in the system of SREBP activation. On using a reporter construct with the INSIG-1 promoter, the nuclear form of SREBP-1c (nSREBP-1c) stimulated transcription more efficiently than nSREBP-2 [66]. SRE found in the proximal region of the *SREBP-1* gene promoter provides for positive feedback between SREBP activity and biosynthesis of its precursor [67]. It seems that this regulatory element is necessary for compensating the depletion in the precursor content during its processing to nSREBP. A similar system of autoregulation has also been found for expression of the *SREBP-2* gene. This system is activated on a decrease in the endogenous biosynthesis of cholesterol (and oxysterols) [68].

The transcription activity of SREBP can be also regulated by other mechanisms. Thus, growth factors stimulate the activating phosphorylation of nuclear forms SREBP-1a [69] and SREBP-2 [70] through protein kinases ERK1/2. Inhibition of the attachment of SUMO to adjacent lysine residues can be a mechanism of positive influence of phosphorylation on SREBP activity. Sumoylation of SREBP recruits the histone deacetylase 3 (HDAC3)-containing corepressor complex, which inhibits transcription of the controlled genes [71] (Scheme 3a). It seems that the physiological purpose of this regulatory system is provision of proliferating cells with lipids.

As mentioned above, SREBP isoforms are different in preferential transcriptional regulation of steroidogenic and lipogenic enzymes and proteins. This is due to the presence of several types of regulatory elements available for interaction with SREBP. The classical sterol-responsive element (SRE) ATCACCCCAC or its variant (SRE3) CTCACACGAG are specific for genes involved in the control of cholesterol biosynthesis (3-hydroxymethyl glutaryl-CoA synthase, 3-hydroxy-3-methylglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase), and also that of SREBP-1c, SREBP-2, and LDLR. These regulatory elements are usually located close to the binding sites of the transcription factors Sp1 and NF-Y. SRE-like elements and palindromic E-blocks ATCACGTGAT or E-block-like sequences capable of interacting with SREBP are detected in the regulatory regions of the liposynthesis controlling genes (acetyl-CoA carboxylase, ATP-citrate lyase, fatty acid synthase, stearoyl-CoA desaturase-1 and -2, glycero-3-phosphate acyltransferase, acyl-CoA-binding protein, S14, malic



Variants of regulation of SREBP and PPAR activities by phosphorylation and sumoylation. a, b) Opposite influences of phosphorylation of SREBP-1/2 and the PPARγ N-terminus on their sumoylation and transcription activities. c, d) Ligand-induced sumoylation of the PPARγ C-terminus has no influence on the transactivating function of PPARγ but induces its transrepressor effects on two groups of genes. oxLDL, oxidized LDL; MAPK, mitogen-activated protein kinase

Scheme 3

enzyme, glucose-6-phosphate dehydrogenase). These elements can also confer carbohydrate or insulin effects on transcription. Experiments with reporter constructs containing the corresponding promoters revealed the selectivity of SREBP isoforms relative to different regulatory elements [72]. This finding explains the results of experiments with knockout of SREBP isoforms [73] and expression of their transgenes [74, 75].

SREBP activity can be regulated on the posttranslational level by oxysterols through the anchor proteins INSIG-1 and -2. The interaction of oxysterols with these proteins has not been analyzed in detail. Cholesterol derivatives with oxidized side chain, such as 25-, 22(R)-, 24(S)-, 27-hydroxycholesterols, and 24(S),25-epoxycholesterol are efficient INSIG ligands. Cholesterol derivatives with the oxidized ring B, 7α- and 7β-hydroxycholesterols, and 7-ketocholesterol have low affinities for INSIG [15]. Overall, the ligand specificity of INSIG is similar to the specificities of LXRα and LXRβ [45, 76].

PPAR: mysteries remain. Nuclear PPAR receptors (α, β/δ, γ) of fatty acids as heterodimers with retinoid X receptors (RXR) interact with PPAR-response elements (PPRE) of the controlled genes, which are direct repeats with a spacer of one nucleotide pair (DR-1). The PPRE consensus sequence AGGTCAaAGGTCA provides for efficient binding of all three PPAR isotypes. Combinations of nucleotide substitutions lead to a definite differentiation of PPRE, but the majority of natural PPRE can be regulated by more than one PPAR isotype [77, 78]. Analysis of the influence of selective PPAR ligands on gene expression in fibroblast cell lines expressing different isotypes of PPAR transgenes has shown that expression of different groups of PPAR-dependent genes is stimulated by all three PPAR isotypes, two isotypes, or by each isotype specifically. Different transcription efficiencies of PPAR isotypes with respect to different genes are not associated with the tissue-specific level of different coregulators but could be at least partially explained by preferences of PPAR isoforms for various PPRE and coregulators [79]. The transcription activity of a PPAR isotype can also depend on the cell type, and this suggests differences of the PPAR isotypes in the interactions with coregulators and additional transcription factors. Microarray analysis of interactions between ligand-binding domains of the three PPAR isotypes and peptides corresponding to the receptor-recognizable sequences of the broad spectrum of coactivators (motif LxxLL) or corepressors (motif LxxxIxxxL) revealed that PPAR isotypes were different in the preferential interaction with different coregulators, whereas PPAR isotype agonists were different in efficiencies of influence on the PPAR interactions with some coregulators. In particular, PPARβ/δ was characterized by a very weak interaction with coregulators in the absence of ligand, whereas in the case of PPARγ these interactions were rather strong also in the absence of agonist and in some cases (e.g. PGC1α) the agonist did not additionally stimulate the PPARγ binding to a coactivator [80]. Thus, specificity of effects of PPAR isoforms on expression of different genes is determined by different recognition of PPRE variants and coregulators.

Similarly to cases of LXR and SREBP, activities of PPARs can be regulated differently, including activation by ligands, posttranslational modifications (e.g. phosphorylation and sumoylation), and expression on the transcription level.

It is agreed that endogenous ligands of PPAR include fatty acids and their derivatives [81], but which compounds are really physiological regulators of PPAR is still unclear. X-Ray crystallographic analysis of the PPAR ligand-binding domain has shown that the ligand-binding pocket of PPAR is different from that of other nuclear receptors by a significantly larger volume and Y-shape with two entrances. This provides for the possibility of the interaction of PPAR with ligands of very different structures. One of the pocket sections is adjacent to the 12th α-helix of the domain triggering induction of the transcription activity of PPAR due to formation of a network of hydrogen bonds with involvement of the conservative tyrosine residue in the 12th helix of PPAR and carboxyl group of fatty acids. The aliphatic "tail" of a ligand can be located in one of the two remaining sections of the pocket and form hydrophobic bonds [82]. The size of the fatty acid aliphatic chain plays a certain role: too short a chain ($C < 14$) does not provide a sufficient number of hydrophobic interactions, and too long a chain ($C > 20$) can sterically prevent the accommodation of the ligand inside the pocket [81]. The affinity of ligands for PPARγ correlates with the degree of their oxidation: keto derivatives, nitro derivatives > hydroxy derivatives > polyunsaturated fatty acids > saturated fatty acids and their derivatives. Double bonds localized in the middle of the fatty acid molecule are responsible for its 90° bend with the hydrocarbon tail directed into the hydrophobic section of the pocket. In the central region of PPARγ there are some potential donors of hydrogen bonds with the ligand that provide increased affinity of PPAR for hydroxylated and nitrated fatty acids [83]. The keto-group coupled with the double bond in the middle of the fatty acid molecule is able to covalently interact with a cysteine residue of PPAR located in the central region of the ligand-binding pocket. Substitution of this cysteine by another amino acid significantly decreases the affinity of keto-derivatives for PPAR [84, 85] and prevents induction by the ligand of an active conformation of the receptor [86, 87]. Efficient ligands of PPARγ are 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, 15-ketoprostaglandins E_2, E_1, $F_{2\alpha}$, $F_{1\alpha}$, 5- and 15-oxoeicosatetraenoic acids, 15-oxoeicosadecaenic acid, 9- and 13-oxooctadecadienic acids.

The large size of the PPAR ligand-binding pocket provides for a concurrent accommodation of two ligand molecules. In fact, X-ray crystallographic analysis of PPARγ reveals the presence of two fatty acid molecules within the ligand–receptor complex [85]. This finding explains an atypical competition between the selective synthetic full agonist of PPARγ rosiglitazone and the partial agonist 1-O-oleyl-glycero-3-phosphate, which is a biosynthetic precursor of a platelet-activating factor. This analog of lysophosphatide acid, despite the high affinity for PPARγ, even in 2000-fold excess is unable to displace all the PPAR-bound rosiglitazone [88].

Unlike PPARγ, PPARα can also interact with saturated fatty acids. Endocannabinoids are supposed to be endogenous ligands of PPARα [89]. However, a very high effective concentration (EC_{50} = 10-30 μM) of endocannabinoids from various chemical groups (noladin ether, anandamide, and virodhamine) towards PPARα makes doubtful the physiological significance of the revealed interaction.

In the case of PPARβ/δ there is also a question about specific endogenous ligands. Products of fatty acid peroxidation such as 13(S)-hydroxyoctadecadienic acid and aldehyde products of their cleavage such as 4-hydroxynonenal can bind to PPARβ/δ and activate it. However, in this case high values of EC_{50} (for 4-hydroxynonenal EC_{50} = 16 μM) [90] also lead us to doubt whether such compounds can really be involved in the regulation of PPARβ/δ activity. Unsaturated nitrated fatty acids can be considered as possible natural regulators of PPARβ/δ (and also of PPARα). Their total content in human blood approaches 1 μM, and this concentration has a pronounced influence on the activity of PPARs. PPARγ is the most sensitive among PPARs to nitro-derivatives of fatty acids [91]; therefore, just this PPAR isoform can first of all mediate the influence of changes in contents of nitrated fatty acids on cell functions.

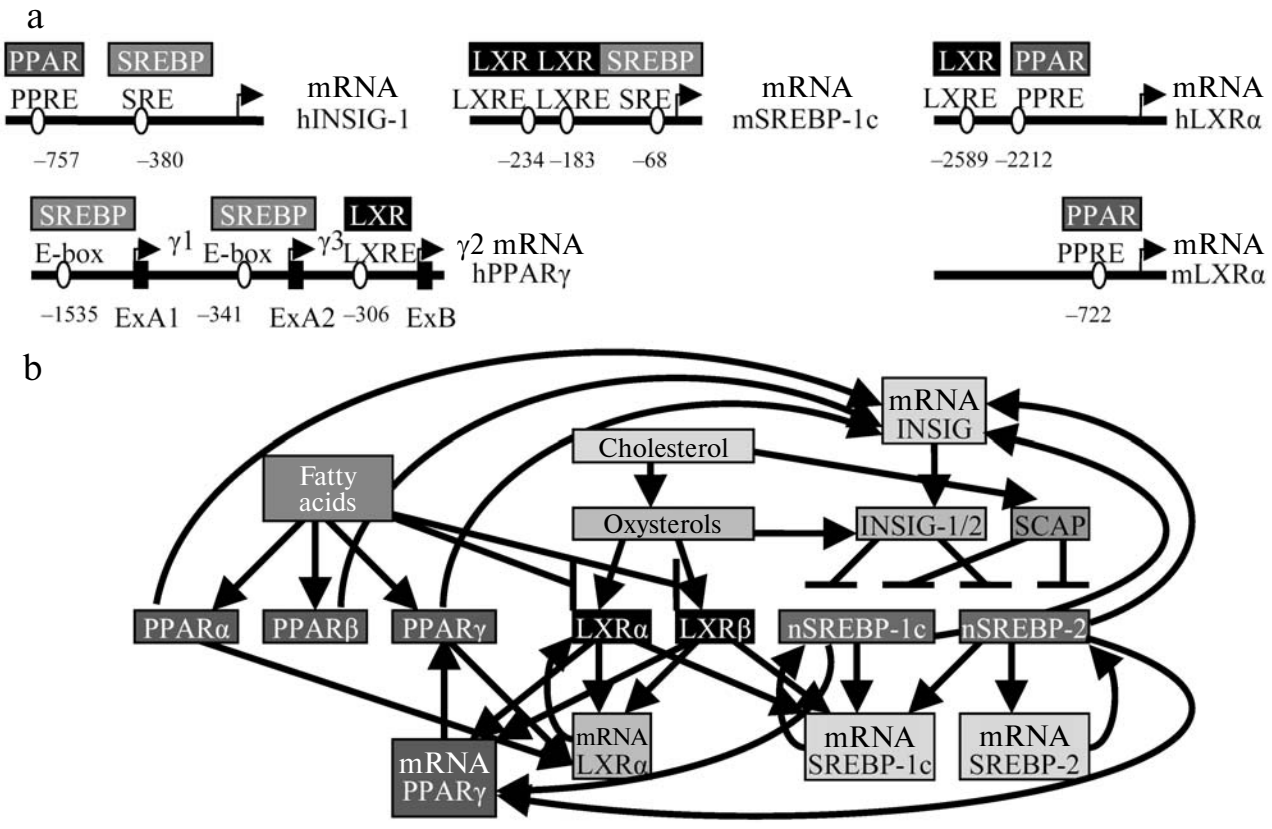
Notwithstanding a significant similarity of the three PPAR isoforms, small differences in the structure of their ligand-binding pockets open a possibility for creating selective ligands for clinical application. Thus, these differences include the narrower section for the head part of the fatty acids in PPARβ/δ than in PPARα and PPARγ and also the presence of a number of non-conservative amino acid residues in the lining of pockets in different PPARs [92]. These differences can also influence the ability of the same ligand to manifest differently directed effects on the interactions of PPAR isotypes with coregulators [93].

In the same cell (e.g. in a macrophage) at least two PPAR isoforms can be expressed that regulate different gene constellations with activities leading to different final biological responses; therefore, it is reasonable to expect that selective endogenous agonists of PPAR isoforms can exist that allow the cell to adequately react to signals. In the case of PPARγ such agonists can be keto- and nitro-derivatives of fatty acids. In the case of PPARα in mouse liver, a phospholipid 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine can be such a regulator [94]. This compound has a high affinity for PPARα, interacts less with PPARβ/δ, and does not bind to PPARγ.

PPARs contain a number of consensus sequences for some serine/threonine protein kinases (PKA, PKC, MAPK, AMPK, etc.). Phosphorylation of serine residues

in some of these sequences has been confirmed experimentally. Functional consequences of phosphorylation depend on the PPAR isoform and localization of the modified residues in the receptor molecule. Phosphorylation can influence the interaction of PPAR with ligands, coregulators, the heterodimerization partner RXR, PPRE DNA, and also the receptor stability; also, phosphorylation can influence either only ligand-induced activity or both induced and basal activities of PPAR [95]. In the case of macrophages the induction of MAPK activity under the influence of oxidized LDL results in the inhibitory phosphorylation of PPARγ at the N-terminus, which counteracts the direct activation of PPARγ by oxidized derivatives of fatty acids of oxidized LDL [96]. The possible modulation of PPAR activity by phosphorylation promotes intervention of various hormones, growth factors, cytokines, and nutrition regimens in the regulatory systems controlled by fatty acids through PPARs.

PPAR ligands stimulate sumoylation of PPAR, which is supposed to provide for the anchorage of PPAR complexes with corepressors in the region of binding sites of crucial activating transcription factors (e.g. AP-1, NF-κB) on promoter inflammation-associated genes and thus to prevent their activation. A direct binding of PPAR to DNA is not required for transrepression. Based on X-ray crystallographic analysis of PPARγ, the ligand is supposed to induce changes in the orientation of Lys365 in the ligand-binding domain and to make it available for sumoylation. The attachment of SUMO by E3-SUMO ligase PIAS1 increases the affinity of PPARγ for the corepressor NCoR (in the absence of sumoylation the ligand inhibits PPARγ binding to NCoR). The attachment of PPARγ to the repressor complex prevents the detachment of the complex from the promoter. Note that the ligand-dependent sumoylation of PPARγ does not prevent the stimulation of positively regulated PPAR genes [97, 98]. In addition, the PPARγ ligand induces sumoylation not only of its receptor but also of the heterodimerization partner RXR, whereas the RXR ligand in turn stimulates sumoylation of both its receptor and PPARγ [99]. The ligand-independent sumoylation of the N-terminal region (Lys107) of PPARγ inhibits the transcription activity of the receptor. Sumoylation at Lys107 is favored by the prior phosphorylation of the adjacent Ser112 [100]. Variants of PPAR phosphorylation and sumoyla-



Localization of regulatory elements in promoters of genes of some sensors of fatty acids and sterols (a) responsible for the complicated network of mutual influences of these sensors (b). Note that the positive autoregulation of LXRα expression is present in humans (h) and absent in mice (m) (in (a), to the right). Ex, exon; nSREBP, nuclear form of SREBP

Scheme 4

tion influencing transcription activity are shown in Scheme 3.

The expression of PPARγ is stimulated by low content of cholesterol/oxysterols through activation of SREBP-1/2, which act via E-blocks in two promoters (1 and 3) of the *PPARγ* gene [101]. This influence of oxysterols is supplemented by their stimulation of PPARγ expression through LXRα/β and LXRE of the PPARγ promoter 2 [102]. The activity of PPARγ promoters is tissue specific: in macrophages promoters 1 and 3 are active, in adipocytes promoters 1 and 2 are prevalent [103], and this, in particular, contributes to the tissue specificity of regulation of PPARγ expression.

The above-described system of regulation of PPAR expression is a component of a complicated network of influences of lipids on the contents of their sensors (Scheme 4). The physiological reasonability of this network seems to be supporting balance between different types of lipid metabolism.

Considering lipid signaling in vascular wall macrophages, one has to take into account the heterogeneity of macrophages. The same monocytes of peripheral blood can be differentiated *in vitro* under the influence of the granulocyte-macrophage colony-stimulating factor (GM-CSF) and of the macrophage colony-stimulating factor (M-CSF) into two subpopulations of macrophages (GM- and M-macrophages). Similar effects on macrophage differentiation are displayed, respectively, by Th2- and Th1-cytokines. Macrophages of these two subpopulations are significantly different in morphology and expression levels of different proteins and, consequently, in functions. The M-macrophage-like $CD68^+/CD14^+$ cells and the GM-macrophage-like $CD68^+/CD14^-$ cells are found in both undamaged regions of vessels and in atherosclerotic lesion but in different ratios: in the first case $CD68^+/CD14^-$ cells and in the other case $CD68^+/CD14^+$ cells are prevalent. Due to some features M-macrophages seem to be more aggressive in the atherogenesis context: they secrete more proinflammatory cytokines and growth factors, proteinases, which destroy matrix, and procoagulants and due to expression of CD14 are more active in triggering innate immunity reactions in response to bacterial infection. These cells accumulate cholesterol from LDL more intensively than GM-macrophages and are less active in reverse cholesterol transport because of weaker expression of lipid sensors PPARγ and LXRα and their targets (CYP27A1, ApoE, and ABCG1) [104]. Note that PPARγ acts as a mediator in formation of the macrophage alternative phenotype and plays an important role in stimulation of β-oxidation of fatty acids in macrophages [105] that seems to prevent their transformation into foam cells. Unlike the expression of PPARγ, PPARα and PPARβ/δ expression is not associated with the phenotype of macrophages, and their activation does not influence the direction of monocyte differentiation [106].

ATHEROGENIC LIPID SIGNALING

The above-presented data indicate that in macrophages a system of autoregulation of lipid metabolism is functioning, mainly directed to limiting the free cholesterol content. This system comprises a group of closely interrelated sensors of lipids mainly consisting of oxidized cholesterol derivatives and fatty acids. A significantly higher efficiency of oxylipids as agonists of LXR, PPAR, and INSIG as compared to the initial cholesterol and fatty acids should provide for more effective protection against lipid accumulation in macrophages and their transformation into foam cells in the presence of modified (oxidized) LDL as compared to unmodified LDL. But in fact, just the modified LDL are atherogenic [107]. Obviously, the basic autoregulatory system is superimposed by other factors, including effect of oxylipids through other sensors counteracting this system.

The role of pro-atherogenic sensors of oxylipids can be performed by TLR4, which in addition to lipopolysaccharides of bacterial pathogens recognizes minimally modified LDL and hydroperoxides of cholesterol esters inside them. The binding of these ligands to TLR4 of macrophages via activation of cascades of protein kinases and small G-proteins leads to reorganization of the actin cytoskeleton and induction of macropinocytosis of the LDL-containing fluid fragments [108]. Production of proinflammatory cytokines is concurrently stimulated [109]. Macrophages themselves could be a source of hydroperoxides of cholesterol esters inside oxidized LDL. Cholesterol esters from intact LDL are supposed to be transferred into the cell by means of LRP1, to be oxidized under the influence of 12/15-lipoxygenase, and to be retransferred onto LDL with involvement of LRP1 [110]. Accumulation of oxidized LDL in the subendothelial space reflecting a local inflammation serves as a signal for the further development of the inflammation. Oxysterols entering into the cell via their LXR sensor increase expression of the *TLR4* gene, the promoter of which contains LXRE [111]. By contrast, the PPARγ and PPARα ligands inhibit the expression of TLR4 (and TLR2) [112, 113]. Other components of oxidized LDL – oxidized phospholipids – also inhibit the TLR4 signalization, but oxidized phospholipids act otherwise because they are competitive antagonists of lipopolysaccharides in binding to receptor complex components – CD14 and lipopolysaccharide-binding protein (LBP) [114]. Obviously, these negative influences of oxidized phospholipids and (presumably) fatty acids on TLR4 expression and signaling cannot fully compensate for the stimulatory effect of oxysterols on TLR4-mediated macropinocytosis of LDL.

Mutual regulation of LXR and TLR signaling is now considered to be a leading mechanism of atherogenesis [115]. TLR are members of a family of signaling receptors that react to determinants common for a number of pathogens and play the most important role in develop-

ment of the innate immune response and preparing for induction of acquired immunity. Thus, the activation of TLR4 by a lipopolysaccharide is accompanied by induction of IL-1β and IL-6 expression in macrophages, production of chemoattractants and oxidizers, and stimulation of phagocytosis [116]. The stimulation of phagocytosis provides for not only the elimination of a pathogen but also an increase in the capture of cholesterol-containing lipoprotein particles. The TLR-mediated activation of phagocytosis is a complicated and poorly studied process that includes induction of expression of retinoic acid-induced gene 1 (RIG-1) and activation of the RIG-1-dependent small GTPases, polymerization of actin, and recruiting of actin nucleation regulator Arp2/3 [117]. Experiments with homo- and heterozygous mice and their cells with *TLR4*, *TLR2*, and *SR-A* gene deletions indicate that stimulation of phagocytosis by TLR activators includes the cooperation of TLR with the scavenger receptor SR-A [118]. The TLR4 and TLR2 ligands increase SR-A expression in the line of macrophages [109] that leads to amplification of induced phagocytosis. Although an increase in the cholesterol content in macrophages as an element of autoregulation includes inhibition of the SR-A expression [119], this negative effect seems to be overcome by effects of pro-atherogenic factors. In particular, interferon γ can be such a factor, and the site for its activation has been found in the *SR-A* gene promoter [120].

Inhibition of the LXR signaling directed to cholesterol homeostasis under conditions of bacterial or viral activation of TLR4 and TLR3 is also mediated by the transcription factors IRF3 [121] and the adipocyte enhancer binding protein 1 (AEBP1) [122]. The activation of TLR2 and TLR4 stimulates the accumulation of cholesterol in macrophages and their transformation into foam cells in the presence of LDL. The LXR agonist inhibits these effects [123], and this suggests an insufficiency of signaling of endogenous ligands of LXR under conditions of TLR activation. In mouse kidneys the lipopolysaccharide (apparently via TLR4) concurrently decreases the expression of LXRα (but not of LXRβ) and its heterodimerization partners RXRα, RXRβ, and LXR targets: transporters ABCA1 and ABCG1, ApoE, and of the transcription factor SREBP-1c, as well as of CYP27. CYP27 also provides for the formation of 27-hydroxycholesterol, which is an agonist of LXR [55]. Thus, TLR activation can suppress LXR functions through a number of pathways including inhibition of expression of LXR itself, its heterodimerization partners, and the enzyme of LXR agonist biosynthesis. Cytokines TNFα and IL-1β secreted by immune cells in response to lipopolysaccharide also inhibit the expression of LXRα and RXRα in cell culture of human proximal tubules (HK-2). Using a reporter construct, the ability of cytokines was shown in this system to decrease the transactivation potential of LXR. This is probably associated with inhibition of expression of coactivators such as PGC-1α and SRC-2 [55].

Although an increase in lipid accumulation in macrophages is a step toward formation of foam cells, this accumulation is concurrently a factor restraining atherogenesis via suppression of inflammation. The stimulatory effect of *Chlamydia pneumoniae* infection on atherogenesis and the level of proinflammatory cytokines in mice with the apoE deficiency were shown to depend on TLR2 and TLR4 and to be restrained by LXRα [124]. Induction by bacterial lipopolysaccharide, TNFα, and IL-1β of the inflammation genes *iNOS*, *COX2*, and *MMP9*, and of proinflammatory cytokines in macrophages is inhibited by LXR ligands and depends on the presence of LXRα and LXRβ. Inhibition of the inflammation genes under the influence of sumoylated or phosphorylated LXR seems to be realized through transrepression without a direct interaction of LXR with DNA and includes blockade of dissociation of the transcription factor NF-κB and AP-1 complexes with the corepressor NCoR. By a similar mechanism LXR ligands can inhibit the proinflammatory cytokine-induced expression of the acute phase proteins: the C-reactive protein (CRP) and the serum amyloid P component (SAP) in liver cells [54].

Some other consequences of oxysterol signaling in atherogenesis can also be considered as positive, e.g. inhibition of apoptosis of macrophages [125, 126], suppression of production of autoantibodies [127], and stimulation of arginase II expression [128].

Mutual influences of LXR and acquired immunity components can play an essential role in the context of atherogenesis. Activation and stimulation of T- and B-lymphocytes induced by mitogens and antigens result in suppression of reverse cholesterol transport dependent on LXRβ and its endogenous ligands (LXRα is not expressed in these cells) [47]. And on the contrary, LXR ligands can inhibit lymphocyte proliferation that results in lymphoid hyperplasia and an enhanced immune response to antigens in mice with *Lxrβ* gene knockout. The mechanism of LXR signaling suppression under the influence of proliferative stimuli includes the induction of sulfotransferase SULT2B1 inactivating oxysterols with production of their antagonists. The inhibitory effect of oxysterols on cell proliferation seems to include stimulation of cholesterol reverse transport with involvement of the ABCG1 transporter because the inhibition is not observed in cells with the *ABCG1* gene knockout. A physiological basis for the above-described system of mutual regulation of lymphocyte proliferation and their cholesterol metabolism is the need for cholesterol for formation of proliferating cell membranes. The more selective involvement of LXR in functioning of B-cells has also been shown. Activation of LXR suppressed the secretion by human B-cells only of isotype E immunoglobulins but not of isotypes G, M, and A [129]. Thus, oxysterols restrain the cellular components of acquired immunity.

The entrance of atherogenic oxidized LDL into cells, in particular, is provided for by a lectin-like recep-

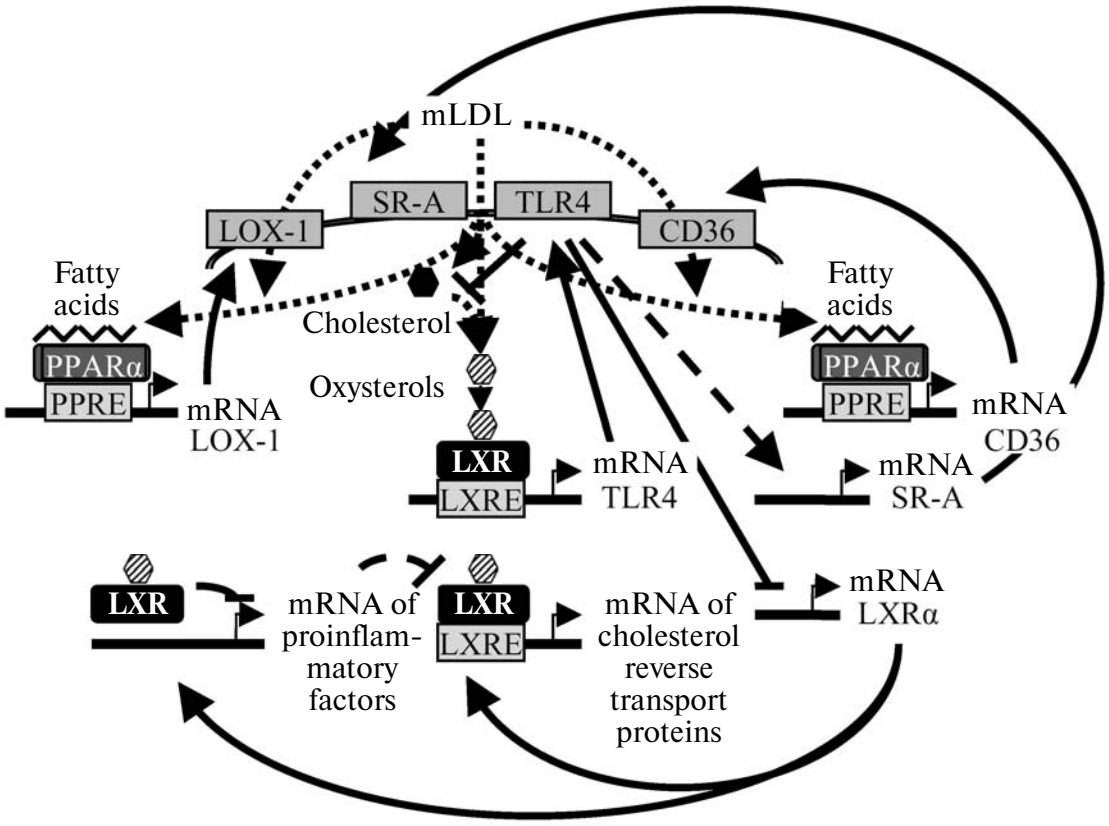
tor of oxidized LDL (LOX-1). The role of LOX-1 in atherogenesis is indicated by data on a significantly decreased atherosclerotic lesion in mice with the *LOX-1* gene deletion [130]. In macrophages, endothelial cells, and smooth muscular cells of blood vessels, oxidized LDL can induce LOX-1 expression [131]. This effect is also reproduced by fatty acids and lysophosphatidylcholine, which can be components of oxidized LDL [132, 133]. In this case PPARα can act as a sensor of these components of LDL (or of their derivatives): just PPARα agonists but not agonists of PPARβ/δ and PPARγ induce LOX-1 expression in macrophages and endothelial cells [134, 135]. In contrast, PPARγ ligands can suppress TNFα-induced expression of LOX-1 [136]. PPARα stimulates the *LOX-1* gene directly, and this stimulation includes the interaction of PPARα with the GC block, which is different from the classical PPRE in the proximal part of the 5′-regulatory region of the gene [137]. Stimulation of LOX-1 expression by fatty acids can be associated with a predisposition for atherogenesis of persons with diabetes mellitus and obesity due to increased content of free fatty acids in blood. The expression of LOX-1 *in vitro* and *in vivo* can be suppressed by inhibitors (statins) of HMG-CoA reductase, which is a key enzyme of cholesterol biosynthesis [138]; therefore, oxysterols of oxidized LDL are supposed to also stimulate LOX-1, but there are no direct proofs of the action of oxysterol and LXR or SREBP on LOX-1. The interaction of oxidized LDL with LOX-1 of endothelial cells is accompanied by changes in expression of various genes associated with inflammation, cell adhesion, and intracellular signal transmission [139]. The signaling function of LOX-1 is not limited to mediation of the action of oxidized LDL. In particular, LOX-1 is required for the stimulatory effect of monocyte adhesion on endothelial cells [140]. The mechanism of LOX-1 signal transmission has not been studied. Thus, oxidized LDL can stimulate lipid accumulation in the blood vessel cells due to induction of LOX-1 expression.

The scavenger receptor CD36 plays an important role in the capture of oxidized lipoproteins by macrophages. The capture is similar to macropinocytosis but the size of vesicles generated in the cell and the spectrum of the participating proteins are different [141]. The CD36-dependent capture of oxidized LDL is accompanied by inhibition of cell migration that can be important for early stages of atherogenesis [142]. The latter work described the influence of oxidized LDL on activities of some proteins associated with the cell adhesion and polymerization of actin of the cytoskeleton (protein kinase FAK, phosphotyrosine phosphatase SHP-2), but mechanism of the signal transmission (due to the signaling function of CD36 or to components of oxidized LDL accumulated in the cell) remains unclear. It is also known that CD36 has signaling function either as it is or as a coreceptor of other signaling receptors such as TLR and integrins [143]. Capture by macrophages of oxidized LDL with involvement of CD36 can also be accompanied by activation of other signaling pathways. In particular, the endocannabinoids anandamide and 2-arachidonoyl glycerol are accumulated and expression of their receptors CB1 and CB2 is increased, and via stimulation of PPARγ this results in the increase in the CD36 level, in suppression of the cholesterol transporter ABCA1 activity, and finally in increased accumulation of cholesterol in the cells [144]. Oxidized phospholipids can also act as CD36 ligands [145]. Expression of CD36 is induced by hydroperoxides of cholesterol esters, which are components of oxidized LDL through activation of PPARα and PPRE of the CD36 gene promoter, as well as by some proinflammatory cytokines secreted by neointima cells [146, 147].

Thus, modified (oxidized) LDL and their components weaken homeostatic lipid signaling acting through several pathways. The main pathways of atherogenic lipid signaling are presented in Scheme 5.

It seems that lipoprotein signaling from the cell surface is not confined to the signaling by the abovementioned receptors. The proinflammatory cytokine – vascular endothelium growth factor (VEGF) – is induced in macrophages under the influence of oxidized LDL also in the absence of their scavenger receptors CD36, SR-A, and LOX-1 and does not require the entrance of LDL into the cell [148].

So-called stress of the endoplasmic reticulum is now believed to play an important role in pathogenesis of atherosclerosis. Normally this phenomenon is an adaptation of the endoplasmic reticulum for increased need for folding of newly synthesized proteins and as a component of the response includes an increase in expression of genes responsible for biosynthesis of lipids, which are constituents of the enlarging endoplasmic reticulum. This induction is supposed to be associated with the removal of INSIG-1 from the endoplasmic reticulum and the corresponding release of nuclear forms of SREBP. Macrophages in atherosclerotic lesions demonstrate signs of endoplasmic reticulum stress (possibly partially due to the influence of accumulated lipids on physicochemical properties of the endoplasmic reticulum). This suggests that stress contributes to formation of foam cells [149]. The stress of endoplasmic reticulum contributes to progress of atherosclerotic lesions also by stimulating apoptosis of macrophages, the subsequent necrosis of the plaque core cells, and its instability, i.e. processes directly leading to tissue ischemia. The stress is supposed, via an increase in the level of intracellular calcium and activation of calmodulin-dependent protein kinase (CaMKII), to induce some proapoptotic factors such as expression of the death receptor Fas, release of cytochrome *c* from mitochondria, and generation of reactive oxygen species. To transform these changes into apoptosis, the activation of TLR, SR-A, and CD36 is required [150].



Main pathways of atherogenic lipid signaling. An increase in entrance of modified lipids into the cell is amplified by induction of mLDL transporters and inhibits oxysterol homeostatic and anti-inflammatory signaling

Scheme 5

PROSPECTS OF THERAPEUTIC INTERVENTION

Ligands of lipid sensors, e.g. of LXR and PPARγ, display a pronounced atheroprotective effect, first of all due to their negative influence on the two crucial processes of atherogenesis: accumulation of lipids and inflammation in vascular wall cells. Unfortunately, all known agonists of lipid sensors cause pronounced side effects associated with their action on other organs and systems. Thus, LXR ligands induce accumulation of triglycerides in the liver, triglyceridemia, and an increase in cholesterol of LDL [40, 151]. Therefore, new attempts are being made to create selective analogs that would reproduce only the desirable effect of full agonists. Such analogs can be created based first of all on the structure–functional organization of nuclear receptors and their conformational plasticity. Functions of binding of ligands, hormone-response elements of DNA, coregulators, and dimerization partners within molecules of nuclear receptors are closely interrelated. Coupling of these combinations with tissue specificity of coregulator expression could lead to separation of gene constellations regulated by the given receptor into subgroups selectively sensitive to different agonists. Ligand-dependent phosphorylation and sumoylation of receptors present additional possibilities for creating selective analogs. Both modifications are determined by the ligand-induced change in the receptor conformation with exposition of the corresponding amino acid sequences on the surface of the protein molecule. Ligands of LXR and PPAR possessing an increased ability for stimulating sumoylation of their receptors could probably be used for treatment and/or prevention not only of atherosclerosis, but also of other disorders associated with inflammation.

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REFERENCES

1. Ross, R., and Glomset, J. (1974) *Adv. Exp. Med. Biol.*, **43**, 265-279.
2. Schwenke, D. C., and Carew, T. E. (1989) *Arteriosclerosis*, **9**, 895-907.
3. Lawson, C., and Wolf, S. (2009) *Pharmacol. Rep.*, **61**, 22-32.
4. Buono, C., Anzinger, J. J., Amar, M., and Kruth, H. S. (2009) *J. Clin. Invest.*, **119**, 1373-1381.

5. Zhu, Y., and Hui, D. Y. (2003) *J. Biol. Chem.*, **278**, 36257-36263.
6. Lillis, A. P., van Duyn, L. B., Murphy-Ullrich, J. E., and Strickland, D. K. (2008) *Physiol. Rev.*, **88**, 887-918.
7. Gordts, P. L., Reekmans, S., Lauwers, A., van Dongen, A., Verbeek, L., and Roebroek, A. J. (2009) *Arterioscler. Thromb. Vasc. Biol.*, **29**, 1258-1264.
8. Xu, W., Takahashi, Y., Sakashita, T., Iwasaki, T., Hattori, H., and Yoshimoto, T. (2001) *J. Biol. Chem.*, **276**, 36454-36459.
9. Xie, S., Lee, Y. F., Kim, E., Chen, L. M., Ni, J., Fang, L. Y., Liu, S., Lin, S. J., Abe, J., Berk, B., Ho, F. M., and Chang, C. (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 13353-13358.
10. Tsai, N. P., Huq, M., Gupta, P., Yamamoto, K., Kagechika, H., and Wei, L. N. (2009) *Biochim. Biophys. Acta*, **1789**, 734-740.
11. Gonzalez, F. J. (2008) *Drug Metab. Pharmacokinet.*, **23**, 2-7.
12. Rajas, F., Gautier, A., Bady, I., Montano, S., and Mithieux, G. (2002) *J. Biol. Chem.*, **277**, 15736-15744.
13. Dhe-Paganon, S., Duda, K., Iwamoto, M., Chi, Y. I., and Shoelson, S. E. (2002) *J. Biol. Chem.*, **277**, 37973-37976.
14. Yuan, X., Ta, T. C., Lin, M., Evans, J. R., Dong, Y., Bolotin, E., Sherman, M. A., Forman, B. M., and Sladek, F. M. (2009) *PLoS One*, **4**, e5609.
15. Radhakrishnan, A., Ikeda, Y., Kwon, H. J., Brown, M. S., and Goldstein, J. L. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 6511-6518.
16. Wong, J., Quinn, C. M., Gelissen, I. C., and Brown, A. J. (2008) *J. Biol. Chem.*, **283**, 700-707.
17. Lange, Y., Ory, D. S., Ye, J., Lanier, M. H., Hsu, F. F., and Steck, T. L. (2008) *J. Biol. Chem.*, **283**, 1445-1455.
18. Leichner, G. S., Avner, R., Harats, D., and Roitelman, J. (2009) *Mol. Biol. Cell.*, **20**, 3330-3341.
19. Bitsch, F., Aichholz, R., Kallen, J., Geisse, S., Fournier, B., and Schlaeppi, J. M. (2003) *Anal. Biochem.*, **323**, 139-149.
20. Kallen, J., Schlaeppi, J. M., Bitsch, F., Delhon, I., and Fournier, B. (2004) *J. Biol. Chem.*, **279**, 14033-14038.
21. Wada, T., Kang, H. S., Angers, M., Gong, H., Bhatia, S., Khadem, S., Ren, S., Ellis, E., Strom, S. C., Jetten, A. M., and Xie, W. (2008) *Mol. Pharmacol.*, **73**, 891-899.
22. Boukhtouche, F., Mariani, J., and Tedgui, A. (2004) *Arterioscler. Thromb. Vasc. Biol.*, **24**, 637-643.
23. Dwyer, J. R., Sever, N., Carlson, M., Nelson, S. F., Beachy, P. A., and Parhami, F. (2007) *J. Biol. Chem.*, **282**, 8959-8968.
24. Kim, W. K., Meliton, V., Park, K. W., Hong, C., Tontonoz, P., Niewiadomski, P., Waschek, J. A., Tetradis, S., and Parhami, F. (2009) *Mol. Endocrinol.*, **23**, 1532-1543.
25. Beckers, L., Heeneman, S., Wang, L., Burkly, L. C., Rousch, M. M., Davidson, N. O., Gijbels, M. J., de Winther, M. P., Daemen, M. J., and Lutgens, E. (2007) *J. Pathol.*, **212**, 420-428.
26. Beel, A. J., Mobley, C. K., Kim, H. J., Tian, F., Hadziselimovic, A., Jap, B., Prestegard, J. H., and Sanders, C. R. (2008) *Biochemistry*, **47**, 9428-9446.
27. Wang, P. Y., Weng, J., and Anderson, R. G. (2005) *Science*, **307**, 1472-1476.
28. Yan, D., Lehto, M., Rasilainen, L., Metso, J., Ehnholm, C., Yla-Herttuala, S., Jauhiainen, M., and Olkkonen, V. M. (2007) *Arterioscler. Thromb. Vasc. Biol.*, **27**, 1108-1114.
29. Yan, D., Mayranpaa, M. I., Wong, J., Perttila, J., Lehto, M., Jauhiainen, M., Kovanen, P. T., Ehnholm, C., Brown, A. J., and Olkkonen, V. M. (2008) *J. Biol. Chem.*, **283**, 332-340.
30. Hynynen, R., Suchanek, M., Spandl, J., Back, N., Thiele, C., and Olkkonen, V. M. (2009) *J. Lipid Res.*, **50**, 1305-1315.
31. Assanasen, C., Mineo, C., Seetharam, D., Yuhanna, I. S., Marcel, Y. L., Connelly, M. A., Williams, D. L., de la Llera-Moya, M., Shaul, P. W., and Silver, D. L. (2005) *J. Clin. Invest.*, **115**, 969-977.
32. Cui, D., Thorp, E., Li, Y., Wang, N., Yvan-Charvet, L., Tall, A. R., and Tabas, I. (2007) *J. Leukoc. Biol.*, **82**, 1040-1050.
33. Terasaka, N., Wang, N., Yvan-Charvet, L., and Tall, A. R. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 15093-15098.
34. Bultel, S., Helin, L., Clavey, V., Chinetti-Gbaguidi, G., Rigamonti, E., Colin, M., Fruchart, J. C., Staels, B., and Lestavel, S. (2008) *Arterioscler. Thromb. Vasc. Biol.*, **28**, 2288-2295.
35. Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E., and Mangelsdorf, D. J. (1998) *Cell*, **93**, 693-704.
36. Chiang, J. Y., Kimmel, R., and Stroup, D. (2001) *Gene*, **262**, 257-265.
37. Repa, J. J., Turley, S. D., Lobaccaro, J. A., Medina, J., Li, L., Lustig, K., Shan, B., Heyman, R. A., Dietschy, J. M., and Mangelsdorf, D. J. (2000) *Science*, **289**, 1524-1529.
38. Bradley, M. N., Hong, C., Chen, M., Joseph, S. B., Wilpitz, D. C., Wang, X., Lusis, A. J., Collins, A., Hseuh, W. A., Collins, J. L., Tangirala, R. K., and Tontonoz, P. (2007) *J. Clin. Invest.*, **117**, 2337-2346.
39. Zelcer, N., and Tontonoz, P. (2006) *J. Clin. Invest.*, **116**, 607-614.
40. Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D., and Shan, B. (2000) *Genes Dev.*, **14**, 2831-2838.
41. Joseph, S. B., Laffitte, B. A., Patel, P. H., Watson, M. A., Matsukuma, K. E., Walczak, R., Collins, J. L., Osborne, T. F., and Tontonoz, P. (2002) *J. Biol. Chem.*, **277**, 11019-11025.
42. Kalaany, N. Y., Gauthier, K. C., Zavacki, A. M., Mammen, P. P., Kitazume, T., Peterson, J. A., Horton, J. D., Garry, D. J., Bianco, A. C., and Mangelsdorf, D. J. (2005) *Cell. Metab.*, **1**, 231-244.
43. Svensson, S., Ostberg, T., Jacobsson, M., Norstrom, C., Stefansson, K., Hallen, D., Johansson, I. C., Zachrisson, K., Ogg, D., and Jendeberg, L. (2003) *EMBO J.*, **22**, 4625-4633.
44. Vejux, A., Malvitte, L., and Lizard, G. (2008) *Braz. J. Med. Biol. Res.*, **41**, 545-556.
45. Janowski, B. A., Grogan, M. J., Jones, S. A., Wisely, G. B., Kliewer, S. A., Corey, E. J., and Mangelsdorf, D. J. (1999) *Proc. Natl. Acad. Sci. USA*, **96**, 266-271.
46. Cook, I. T., Duniac-Dmuchowski, Z., Kocarek, T. A., Runge-Morris, M., and Falany, C. N. (2009) *Drug Metab. Dispos.*, **37**, 2069-2078.
47. Bensinger, S. J., Bradley, M. N., Joseph, S. B., Zelcer, N., Janssen, E. M., Hausner, M. A., Shih, R., Parks, J. S.,

Edwards, P. A., Jamieson, B. D., and Tontonoz, P. (2008) *Cell*, **134**, 97-111.
48. Jiang, Y. J., Kim, P., Elias, P. M., and Feingold, K. R. (2005) *J. Lipid Res.*, **46**, 57-66.
49. Ma, Y., Xu, L., Rodriguez-Agudo, D., Li, X., Heuman, D. M., Hylemon, P. B., Pandak, W. M., and Ren, S. (2008) *Am. J. Physiol. Endocrinol. Metab.*, **295**, E1369-E1379.
50. Torra, I. P., Ismaili, N., Feig, J. E., Xu, C. F., Cavasotto, C., Pancratov, R., Rogatsky, I., Neubert, T. A., Fisher, E. A., and Garabedian, M. J. (2008) *Mol. Cell. Biol.*, **28**, 2626-2636.
51. Ghisletti, S., Huang, W., Ogawa, S., Pascual, G., Lin, M. E., Willson, T. M., Rosenfeld, M. G., and Glass, C. K. (2007) *Mol. Cell*, **25**, 57-70.
52. Lee, J. H., Park, S. M., Kim, O. S., Lee, C. S., Woo, J. H., Park, S. J., Joe, E. H., and Jou, I. (2009) *Mol. Cell*, **35**, 806-817.
53. Li, Y., Bolten, C., Bhat, B. G., Woodring-Dietz, J., Li, S., Prayaga, S. K., Xia, C., and Lala, D. S. (2002) *Mol. Endocrinol.*, **16**, 506-514.
54. Blaschke, F., Takata, Y., Caglayan, E., Collins, A., Tontonoz, P., Hsueh, W. A., and Tangirala, R. K. (2006) *Circ. Res.*, **99**, e88-e99.
55. Wang, Y., Moser, A. H., Shigenaga, J. K., Grunfeld, C., and Feingold, K. R. (2005) *J. Lipid Res.*, **46**, 2377-2387.
56. Laffitte, B. A., Joseph, S. B., Walczak, R., Pei, L., Wilpitz, D. C., Collins, J. L., and Tontonoz, P. (2001) *Mol. Cell. Biol.*, **21**, 7558-7568.
57. Chawla, A., Boisvert, W. A., Lee, C. H., Laffitte, B. A., Barak, Y., Joseph, S. B., Liao, D., Nagy, L., Edwards, P. A., Curtiss, L. K., Evans, R. M., and Tontonoz, P. (2001) *Mol. Cell*, **7**, 161-171.
58. Li, A. C., Binder, C. J., Gutierrez, A., Brown, K. K., Plotkin, C. R., Pattison, J. W., Valledor, A. F., Davis, R. A., Willson, T. M., Witztum, J. L., Palinski, W., and Glass, C. K. (2004) *J. Clin. Invest.*, **114**, 1564-1576.
59. Radhakrishnan, A., Goldstein, J. L., McDonald, J. G., and Brown, M. S. (2008) *Cell. Metab.*, **8**, 512-521.
60. Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. (2000) *Genes Dev.*, **14**, 2819-2830.
61. Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A. H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Kimura, S., Ishibashi, S., and Yamada, N. (2001) *Mol. Cell. Biol.*, **21**, 2991-3000.
62. Yoshikawa, T., Ide, T., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Matsuzaka, T., Yatoh, S., Kitamine, T., Okazaki, H., Tamura, Y., Sekiya, M., Takahashi, A., Hasty, A. H., Sato, R., Sone, H., Osuga, J., Ishibashi, S., and Yamada, N. (2003) *Mol. Endocrinol.*, **17**, 1240-1254.
63. Konig, B., Koch, A., Spielmann, J., Hilgenfeld, C., Stangl, G. I., and Eder, K. (2007) *Biochem. Pharmacol.*, **73**, 574-585.
64. Qin, X., Xie, X., Fan, Y., Tian, J., Guan, Y., Wang, X., Zhu, Y., and Wang, N. (2008) *Hepatology*, **48**, 432-441.
65. Sugiyama, E., Ishikawa, Y., Li, Y., Kagai, T., Nobayashi, M., Tanaka, N., Kamijo, Y., Yokoyama, S., Hara, A., and Aoyama, T. (2008) *Life Sci.*, **83**, 19-28.
66. Kast-Woelbern, H. R., Dana, S. L., Cesario, R. M., Sun, L., de Grandpre, L. Y., Brooks, M. E., Osburn, D. L., Reifel-Miller, A., Klausing, K., and Leibowitz, M. D. (2004) *J. Biol. Chem.*, **279**, 23908-23915.
67. Amemiya-Kudo, M., Shimano, H., Yoshikawa, T., Yahagi, N., Hasty, A. H., Okazaki, H., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Sato, R., Kimura, S., Ishibashi, S., and Yamada, N. (2000) *J. Biol. Chem.*, **275**, 31078-31085.
68. Sato, R., Inoue, J., Kawabe, Y., Kodama, T., Takano, T., and Maeda, M. (1996) *J. Biol. Chem.*, **271**, 26461-26464.
69. Roth, G., Kotzka, J., Kremer, L., Lehr, S., Lohaus, C., Meyer, H. E., Krone, W., and Muller-Wieland, D. (2000) *J. Biol. Chem.*, **275**, 33302-33307.
70. Kotzka, J., Lehr, S., Roth, G., Avci, H., Knebel, B., and Muller-Wieland, D. (2004) *J. Biol. Chem.*, **279**, 22404-22411.
71. Arito, M., Horiba, T., Hachimura, S., Inoue, J., and Sato, R. (2008) *J. Biol. Chem.*, **283**, 15224-15231.
72. Amemiya-Kudo, M., Shimano, H., Hasty, A. H., Yahagi, N., Yoshikawa, T., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Sato, R., Kimura, S., Ishibashi, S., and Yamada, N. (2002) *J. Lipid Res.*, **43**, 1220-1235.
73. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., and Brown, M. S. (2002) *J. Biol. Chem.*, **277**, 9520-9528.
74. Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., and Shimano, H. (1998) *J. Clin. Invest.*, **101**, 2331-2339.
75. Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., Brown, M. S., and Goldstein, J. L. (2003) *Proc. Natl. Acad. Sci. USA*, **100**, 12027-12032.
76. Reschly, E. J., Ai, N., Welsh, W. J., Ekins, S., Hagey, L. R., and Krasowski, M. D. (2008) *J. Steroid Biochem. Mol. Biol.*, **110**, 83-94.
77. Heinaniemi, M., Uski, J. O., Degenhardt, T., and Carlberg, C. (2007) *Genome Biol.*, **8**, R147.
78. Viswakarma, N., Yu, S., Naik, S., Kashireddy, P., Matsumoto, K., Sarkar, J., Surapureddi, S., Jia, Y., Rao, M. S., and Reddy, J. K. (2007) *J. Biol. Chem.*, **282**, 18613-18624.
79. Hummasti, S., and Tontonoz, P. (2006) *Mol. Endocrinol.*, **20**, 1261-1275.
80. Koppen, A., Houtman, R., Pijnenburg, D., Jeninga, E. H., Ruijtenbeek, R., and Kalkhoven, E. (2009) *Mol. Cell. Proteom.*, **8**, 2212-2226.
81. Kliewer, S. A., Xu, H. E., Lambert, M. H., and Willson, T. M. (2001) *Recent Prog. Horm. Res.*, **56**, 239-263.
82. Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sternbach, D. D., Lehmann, J. M., Wisely, G. B., Willson, T. M., Kliewer, S. A., and Milburn, M. V. (1999) *Mol. Cell*, **3**, 397-403.
83. Li, Y., Zhang, J., Schopfer, F. J., Martynowski, D., Garcia-Barrio, M. T., Kovach, A., Suino-Powell, K., Baker, P. R., Freeman, B. A., Chen, Y. E., and Xu, H. E. (2008) *Nat. Struct. Mol. Biol.*, **15**, 865-867.
84. Shiraki, T., Kamiya, N., Shiki, S., Kodama, T. S., Kakizuka, A., and Jingami, H. (2005) *J. Biol. Chem.*, **280**, 14145-14153.
85. Itoh, T., Fairall, L., Amin, K., Inaba, Y., Szanto, A., Balint, B. L., Nagy, L., Yamamoto, K., and Schwabe, J. W. (2008) *Nat. Struct. Mol. Biol.*, **15**, 924-931.
86. Waku, T., Shiraki, T., Oyama, T., and Morikawa, K. (2009) *FEBS Lett.*, **583**, 320-324.

87. Waku, T., Shiraki, T., Oyama, T., Fujimoto, Y., Maebara, K., Kamiya, N., Jingami, H., and Morikawa, K. (2009) *J. Mol. Biol.*, **385**, 188-199.
88. Tsukahara, T., Tsukahara, R., Yasuda, S., Makarova, N., Valentine, W. J., Allison, P., Yuan, H., Baker, D. L., Li, Z., Bittman, R., Parrill, A., and Tigyi, G. (2006) *J. Biol. Chem.*, **281**, 3398-3407.
89. Sun, Y., Alexander, S. P., Garle, M. J., Gibson, C. L., Hewitt, K., Murphy, S. P., Kendall, D. A., and Bennett, A. J. (2007) *Br. J. Pharmacol.*, **152**, 734-743.
90. Coleman, J. D., Prabhu, K. S., Thompson, J. T., Reddy, P. S., Peters, J. M., Peterson, B. R., Reddy, C. C., and Vanden Heuvel, J. P. (2007) *Free Radical Biol. Med.*, **42**, 1155-1164.
91. Baker, P. R., Lin, Y., Schopfer, F. J., Woodcock, S. R., Groeger, A. L., Batthyany, C., Sweeney, S., Long, M. H., Iles, K. E., Baker, L. M., Branchaud, B. P., Chen, Y. E., and Freeman, B. A. (2005) *J. Biol. Chem.*, **280**, 42464-42475.
92. Xu, H. E., Lambert, M. H., Montana, V. G., Plunket, K. D., Moore, L. B., Collins, J. L., Oplinger, J. A., Kliewer, S. A., Gampe, R. T., Jr., McKee, D. D., Moore, J. T., and Willson, T. M. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 13919-13924.
93. Stanley, T. B., Leesnitzer, L. M., Montana, V. G., Galardi, C. M., Lambert, M. H., Holt, J. A., Xu, H. E., Moore, L. B., Blanchard, S. G., and Stimmel, J. B. (2003) *Biochemistry*, **42**, 9278-9287.
94. Chakravarthy, M. V., Lodhi, I. J., Yin, L., Malapaka, R. R., Xu, H. E., Turk, J., and Semenkovich, C. F. (2009) *Cell*, **138**, 476-488.
95. Burns, K. A., and Vanden Heuvel, J. P. (2007) *Biochim. Biophys. Acta*, **1771**, 952-960.
96. Yin, R., Dong, Y. G., and Li, H. L. (2006) *Acta Pharmacol. Sin.*, **27**, 1146-1152.
97. Pascual, G., Fong, A. L., Ogawa, S., Gamliel, A., Li, A. C., Perissi, V., Rose, D. W., Willson, T. M., Rosenfeld, M. G., and Glass, C. K. (2005) *Nature*, **437**, 759-763.
98. Jennewein, C., Kuhn, A. M., Schmidt, M. V., Meilladec-Jullig, V., von Knethen, A., Gonzalez, F. J., and Brune, B. (2008) *J. Immunol.*, **181**, 5646-5652.
99. Burrage, P. S., Schmucker, A. C., Ren, Y., Sporn, M. B., and Brinckerhoff, C. E. (2008) *Arthritis Res. Ther.*, **10**, R139.
100. Shimizu, M., Yamashita, D., Yamaguchi, T., Hirose, F., and Osumi, T. (2006) *Mol. Cell. Biochem.*, **286**, 33-42.
101. Fajas, L., Schoonjans, K., Gelman, L., Kim, J. B., Najib, J., Martin, G., Fruchart, J. C., Briggs, M., Spiegelman, B. M., and Auwerx, J. (1999) *Mol. Cell. Biol.*, **19**, 5495-5503.
102. Seo, J. B., Moon, H. M., Kim, W. S., Lee, Y. S., Jeong, H. W., Yoo, E. J., Ham, J., Kang, H., Park, M. G., Steffensen, K. R., Stulnig, T. M., Gustafsson, J. A., Park, S. D., and Kim, J. B. (2004) *Mol. Cell. Biol.*, **24**, 3430-3444.
103. Ricote, M., Huang, J., Fajas, L., Li, A., Welch, J., Najib, J., Witztum, J. L., Auwerx, J., Palinski, W., and Glass, C. K. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 7614-7619.
104. Waldo, S. W., Li, Y., Buono, C., Zhao, B., Billings, E. M., Chang, J., and Kruth, H. S. (2008) *Am. J. Pathol.*, **172**, 1112-1126.
105. Odegaard, J. I., Ricardo-Gonzalez, R. R., Goforth, M. H., Morel, C. R., Subramanian, V., Mukundan, L., Red Eagle, A., Vats, D., Brombacher, F., Ferrante, A. W., and Chawla, A. (2007) *Nature*, **447**, 1116-1120.
106. Bouhlel, M. A., Brozek, J., Derudas, B., Zawadzki, C., Jude, B., Staels, B., and Chinetti-Gbaguidi, G. (2009) *Biochem. Biophys. Res. Commun.*, **386**, 459-462.
107. Steinberg, D., Parthasarathy, S., Carew, T. E., Khoo, J. C., and Witztum, J. L. (1989) *N. Engl. J. Med.*, **320**, 915-924.
108. Choi, S. H., Harkewicz, R., Lee, J. H., Boullier, A., Almazan, F., Li, A. C., Witztum, J. L., Bae, Y. S., and Miller, Y. I. (2009) *Circ. Res.*, **104**, 1355-1363.
109. Harkewicz, R., Hartvigsen, K., Almazan, F., Dennis, E. A., Witztum, J. L., and Miller, Y. I. (2008) *J. Biol. Chem.*, **283**, 10241-10251.
110. Takahashi, Y., Zhu, H., Xu, W., Murakami, T., Iwasaki, T., Hattori, H., and Yoshimoto, T. (2005) *Biochem. Biophys. Res. Commun.*, **338**, 128-135.
111. Fontaine, C., Rigamonti, E., Nohara, A., Gervois, P., Teissier, E., Fruchart, J. C., Staels, B., and Chinetti-Gbaguidi, G. (2007) *Circ. Res.*, **101**, 40-49.
112. Dasu, M. R., Park, S., Devaraj, S., and Jialal, I. (2009) *Endocrinology*, **150**, 3457-3464.
113. Xu, W. Y., Wang, L., Wang, H. M., Wang, Y. Q., Liang, Y. F., Zhao, T. T., and Wu, Y. Z. (2007) *Mol. Immunol.*, **44**, 2315-2323.
114. Von Schlieffen, E., Oskolkova, O. V., Schabbauer, G., Gruber, F., Bluml, S., Genest, M., Kadl, A., Marsik, C., Knapp, S., Chow, J., Leitinger, N., Binder, B. R., and Bochkov, V. N. (2009) *Arterioscler. Thromb. Vasc. Biol.*, **29**, 356-362.
115. Hong, C., and Tontonoz, P. (2008) *Curr. Opin. Genet. Dev.*, **18**, 461-467.
116. Wu, T. T., Chen, T. L., and Chen, R. M. (2009) *Toxicol. Lett.*, **191**, 195-202.
117. Kong, L., Sun, L., Zhang, H., Liu, Q., Liu, Y., Qin, L., Shi, G., Hu, J. H., Xu, A., Sun, Y. P., Li, D., Shi, Y. F., Zang, J. W., Zhu, J., Chen, Z., Wang, Z. G., and Ge, B. X. (2009) *Cell Host Microbe*, **6**, 150-161.
118. Amiel, E., Alonso, A., Uematsu, S., Akira, S., Poynter, M. E., and Berwin, B. (2009) *J. Leukoc. Biol.*, **85**, 595-605.
119. Llaverias, G., Lacasa, D., Vazquez-Carrera, M., Sanchez, R. M., Laguna, J. C., and Alegret, M. (2005) *Mol. Cell. Biochem.*, **273**, 185-191.
120. Grewal, T., Priceputu, E., Davignon, J., and Bernier, L. (2001) *Arterioscler. Thromb. Vasc. Biol.*, **21**, 825-831.
121. Castrillo, A., Joseph, S. B., Vaidya, S. A., Haberland, M., Fogelman, A. M., Cheng, G., and Tontonoz, P. (2003) *Mol. Cell*, **12**, 805-816.
122. Majdalawieh, A., and Ro, H. S. (2009) *Int. J. Biochem. Cell Biol.*, **41**, 1518-1525.
123. Cao, F., Castrillo, A., Tontonoz, P., Re, F., and Byrne, G. I. (2007) *Infect. Immun.*, **75**, 753-759.
124. Naiki, Y., Sorrentino, R., Wong, M. H., Michelsen, K. S., Shimada, K., Chen, S., Yilmaz, A., Slepenkin, A., Schroder, N. W., Crother, T. R., Bulut, Y., Doherty, T. M., Bradley, M., Shaposhnik, Z., Peterson, E. M., Tontonoz, P., Shah, P. K., and Arditi, M. (2008) *J. Immunol.*, **181**, 7176-7185.
125. Joseph, S. B., Bradley, M. N., Castrillo, A., Bruhn, K. W., Mak, P. A., Pei, L., Hogenesch, J., O'Connell, R. M., Cheng, G., Saez, E., Miller, J. F., and Tontonoz, P. (2004) *Cell*, **119**, 299-309.

126. Valledor, A. F., Hsu, L. C., Ogawa, S., Sawka-Verhelle, D., Karin, M., and Glass, C. K. (2004) *Proc. Natl. Acad. Sci. USA*, **101**, 17813-17818.
127. A-Gonzalez, N., Bensinger, S. J., Hong, C., Beceiro, S., Bradley, M. N., Zelcer, N., Deniz, J., Ramirez, C., Diaz, M., Gallardo, G., de Galarreta, C. R., Salazar, J., Lopez, F., Edwards, P., Parks, J., Andujar, M., Tontonoz, P., and Castrillo, A. (2009) *Immunity*, **31**, 245-258.
128. Marathe, C., Bradley, M. N., Hong, C., Lopez, F., Ruiz de Galarreta, C. M., Tontonoz, P., and Castrillo, A. (2006) *J. Biol. Chem.*, **281**, 32197-32206.
129. Heine, G., Dahten, A., Hilt, K., Ernst, D., Milovanovic, M., Hartmann, B., and Worm, M. (2009) *J. Immunol.*, **182**, 5276-5282.
130. Mehta, J. L., Sanada, N., Hu, C. P., Chen, J., Dandapat, A., Sugawara, F., Satoh, H., Inoue, K., Kawase, Y., Jishage, K., Suzuki, H., Takeya, M., Schnackenberg, L., Beger, R., Hermonat, P. L., Thomas, M., and Sawamura, T. (2007) *Circ. Res.*, **100**, 1634-1642.
131. Aoyama, T., Fujiwara, H., Masaki, T., and Sawamura, T. (1999) *J. Mol. Cell. Cardiol.*, **31**, 2101-2114.
132. Schaeffer, D. F., Riazy, M., Parhar, K. S., Chen, J. H., Duronio, V., Sawamura, T., and Steinbrecher, U. P. (2009) *J. Lipid Res.*, **50**, 1676-1684.
133. Ishiyama, J., Taguchi, R., Yamamoto, A., and Murakami, K. (2010) *Atherosclerosis*, **209**, 118-124.
134. Cabrero, A., Cubero, M., Llaverias, G., Jove, M., Planavila, A., Alegret, M., Sanchez, R., Laguna, J. C., and Carrera, M. V. (2003) *Metabolism*, **52**, 652-657.
135. Hayashida, K., Kume, N., Minami, M., Kataoka, H., Morimoto, M., and Kita, T. (2001) *Ann. N. Y. Acad. Sci.*, **947**, 370-372.
136. Chiba, Y., Ogita, T., Ando, K., and Fujita, T. (2001) *Biochem. Biophys. Res. Commun.*, **286**, 541-546.
137. Hayashida, K., Kume, N., Minami, M., Inui-Hayashida, A., Mukai, E., Toyohara, M., and Kita, T. (2004) *Biochem. Biophys. Res. Commun.*, **323**, 1116-1123.
138. Hofnagel, O., Luechtenborg, B., Eschert, H., Weissen-Plenz, G., Severs, N. J., and Robenek, H. (2006) *Arterioscler. Thromb. Vasc. Biol.*, **26**, 604-610.
139. Mattaliano, M. D., Huard, C., Cao, W., Hill, A. A., Zhong, W., Martinez, R. V., Harnish, D. C., Paulsen, J. E., and Shih, H. H. (2009) *Am. J. Physiol. Cell. Physiol.*, **296**, C1329-C1337.
140. Sakamoto, N., Ishibashi, T., Sugimoto, K., Sawamura, T., Sakamoto, T., Inoue, N., Saitoh, S., Kamioka, M., Uekita, H., Ohkawara, H., Suzuki, K., Teramoto, T., Maruyama, Y., and Takeishi, Y. (2009) *J. Cell. Physiol.*, **220**, 706-715.
141. Collins, R. F., Touret, N., Kuwata, H., Tandon, N. N., Grinstein, S., and Trimble, W. S. (2009) *J. Biol. Chem.*, **284**, 30288-30297.
142. Park, Y. M., Febbraio, M., and Silverstein, R. L. (2009) *J. Clin. Invest.*, **119**, 136-145.
143. Silverstein, R. L., and Febbraio, M. (2009) *Sci. Signal.*, **2**, re3.
144. Jiang, L. S., Pu, J., Han, Z. H., Hu, L. H., and He, B. (2009) *Cardiovasc. Res.*, **81**, 805-813.
145. Berliner, J. A., Leitinger, N., and Tsimikas, S. (2009) *J. Lipid Res.*, **50**, S207-S212.
146. Jedidi, I., Couturier, M., Therond, P., Gardes-Albert, M., Legrand, A., Barouki, R., Bonnefont-Rousselot, D., and Aggerbeck, M. (2006) *Biochem. Biophys. Res. Commun.*, **351**, 733-738.
147. Feng, J., Han, J., Pearce, S. F., Silverstein, R. L., Gotto, A. M., Jr., Hajjar, D. P., and Nicholson, A. C. (2000) *J. Lipid Res.*, **41**, 688-696.
148. Riazy, M., Chen, J. H., and Steinbrecher, U. P. (2009) *Atherosclerosis*, **204**, 47-54.
149. Marciniak, S. J., and Ron, D. (2006) *Physiol. Rev.*, **86**, 1133-1149.
150. Tabas, I., Seimon, T., Timmins, J., Li, G., and Lim, W. (2009) *Ann. N. Y. Acad. Sci.*, **1173**, E40-E45.
151. Groot, P. H., Pearce, N. J., Yates, J. W., Stocker, C., Sauermelch, C., Doe, C. P., Willette, R. N., Olzinski, A., Peters, T., d'Epagnier, D., Morasco, K. O., Krawiec, J. A., Webb, C. L., Aravindhan, K., Jucker, B., Burgert, M., Ma, C., Marino, J. P., Collins, J. L., Macphee, C. H., Thompson, S. K., and Jaye, M. (2005) *J. Lipid Res.*, **46**, 2182-2191.