
Regulation of HDL Genes: Transcriptional, Posttranscriptional, and Posttranslational

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A. von Eckardstein, D. Kardassis (eds.), *High Density Lipoproteins*, Handbook of Experimental Pharmacology 224, DOI 10.1007/978-3-319-09665-0_3

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Abstract

HDL regulation is exerted at multiple levels including regulation at the level of transcription initiation by transcription factors and signal transduction cascades; regulation at the posttranscriptional level by microRNAs and other noncoding RNAs which bind to the coding or noncoding regions of HDL genes regulating mRNA stability and translation; as well as regulation at the posttranslational level by protein modifications, intracellular trafficking, and degradation. The above mechanisms have drastic effects on several HDL-mediated processes including HDL biogenesis, remodeling, cholesterol efflux and uptake, as well as atheroprotective functions on the cells of the arterial wall. The emphasis is on mechanisms that operate in physiologically relevant tissues such as the liver (which accounts for 80 % of the total HDL-C levels in the plasma), the macrophages, the adrenals, and the endothelium. Transcription factors that have a significant impact on HDL regulation such as hormone nuclear receptors and hepatocyte nuclear factors are extensively discussed both in terms of gene promoter recognition and regulation but also in terms of their impact on plasma HDL levels as was revealed by knockout studies. Understanding the different modes of regulation of this complex lipoprotein may provide useful insights for the development of novel HDL-raising therapies that could be used to fight against atherosclerosis which is the underlying cause of coronary heart disease.

Keywords

High-density lipoprotein • Regulation • Transcriptional • Posttranscriptional • Posttranslational • miRNAs • Protein stability • Hormone nuclear receptors • Hepatocyte nuclear factors • apoA-I • ABCA1 • ABCG1 • ABCG5 • ABCG8 • apoE • SR-BI • CETP

List of Abbreviations

HMG-CoA	3-Hydroxy-3-methylglutaryl-coenzyme A
12/15LO	12/15-Lipoxygenase
AP1	Activator protein 1
AF1	Activation function 1
Ang	Angiotensin
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
apoE	Apolipoprotein E
apoM	Apolipoprotein M
ARP-1	Apolipoprotein A-I-regulated protein 1
DHHC8	Asp-His-His-Cys 8
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
BIG1	Brefeldin A-inhibited guanine nucleotide-exchange protein 1
JNK	c-Jun N-terminal kinase
C/EBP	CCAAT/enhancer-binding protein
CAV-1	Caveolin 1
CXCL	Chemokine (C-X-C motif) ligand
COUP-TFI	Chicken ovalbumin upstream promoter transcription factor I
CEH	Cholesterol ester hydrolase
CETP	Cholesterol ester transfer protein
3C	Chromosome conformational capture
CLA	Conjugated linoleic acid
CAR	Constitutive androstane receptor
CREB	Cyclic AMP response element-binding protein
DNase	Deoxyribonuclease
DR4	Direct repeat with 4 nucleotides in the spacer region
DBD	DNA-binding domain
EGR-1	Early growth response protein 1
LIPG	Endothelial lipase
EE	Early endosome
ER	Endoplasmic reticulum
ESCRT	Endosomal sorting complex required for transport
ER α and β	Estrogen receptors α and β
ERE	Estrogen response element
ESRRG	Estrogen receptor-related gamma
FXR	Farnesoid X receptor
FXRE	Farnesoid X receptor-responsive element
FF	Fenofibrate
FOXA2	Forkhead box A2
FOXO1	Forkhead box O1
GF	Gemfibrozil

GR	Glucocorticoid receptor
GSK3 β	Glycogen synthase kinase 3 β
HNF-4	Hepatocyte nuclear factor-4
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
Hcy	Homocysteine
HRE	Hormone response element
IRAK-1	IL-1 receptor-associated kinase 1
IDOL	Inducible degrader of the LDLR
IFN	Interferon
IL	Interleukin
KO	Knockout
KLF4	Kruppel-like factor 4
LCAT	Lecithin-cholesterol acyltransferase
LXR	Liver X receptor
LXRE	Liver X receptor response element
LRH-1	Liver receptor homologue-1
LBD	Ligand-binding domain
LPS	Lipopolysaccharide
LDLR	Low-density lipoprotein receptor
LAL	Lysosomal acid lipase
MCSF	Macrophage colony-stimulating factor
miRNAs	microRNAs
MEKK	Mitogen-activated protein kinase/ERK kinase kinase
MCP-1	Monocyte chemoattractant protein-1
ME	Multienhancer
MVB	Multivesicular bodies
DMHCA	N,N-Dimethyl-3 β -hydroxycholeamide
NHERF	Na ⁺ /H ⁺ exchanger regulator factor
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NFY	Nuclear factor Y
ORP1S	Oxysterol-binding protein-related protein 1S
PDZK1	PDZ domain-containing adaptor protein
PPAR	Peroxisome proliferator-activated receptors
PPRE	Peroxisome proliferator-activated receptor-responsive element
PMA	Phorbol 12-myristate 13-acetate
PI3K	phosphatidylinositol 3- kinase
PLD	Phospholipase D
PGC-1	PPARgamma coactivator 1
PLTP	Phospholipid transfer protein
pri-miRNA	Primary long miRNA
PREB	Prolactin regulatory element-binding
PEST	Proline, glutamic acid, serine, threonine
PKC	Protein kinase C
PCA	Protocatechuic acid

PCSK9	Proprotein convertase subtilisin/kexin type 9
RAR	Retinoic acid receptor
ROR α	Retinoic acid receptor-related orphan receptor α
RXR α	Retinoid X receptor α
RISC	RNA-induced silencing complex
SR-BI	Scavenger receptor class B type I
SPTLC1	Serine palmitoyltransferase 1
STAT	Signal transducer and activator of transcription
SHP	Small heterodimer partner
siRNAs	Small interfering RNAs
SP1	Specificity protein 1
S1P	Sphingosine 1 phosphate
SRC-1	Steroid receptor coactivator-1
SF-1	Steroidogenic factor-1
SREBP	Sterol regulatory element-binding protein
SREs	Sterol-responsive elements
TR β	Thyroid hormone receptor β
TAD	Transactivation domain
TGF β 1	Transforming growth factor β 1
TNF α	Tumor necrosis factor α
Tp12	Tumor progression locus 2
UTR	Untranslated region
URE	Upstream regulatory element
USF	Upstream stimulatory factors
VDR	Vitamin D receptor
WT	Wild type
YY1	Yin Yang 1
ZIC	Zinc finger of the cerebellum
ZNF202	Zinc finger protein 202

1 Regulation of Genes Involved in HDL Metabolism at the Transcriptional Level

A large body of work generated over the past four decades has revealed that eukaryotic gene transcription is a remarkably intricate biochemical process that is tightly regulated at many levels by the ordered assembly of multiprotein transcription initiation complexes to specific regulatory regions in the promoters of genes (Roeder 1998, 2005; Lemon and Tjian 2000). Despite the progress made, still limited knowledge regarding the details exists. It is believed that specificity in gene regulation is determined by the unique order of *cis*-acting regulatory regions which are recognized by sequence-specific DNA-binding transcription factors. Recent advances in gene regulation technologies including the powerful chromatin

immunoprecipitation assay have enabled the monitoring in real time of the ordered assembly and the disassembly of transcription factor complexes on the promoters and the enhancers of genes in response to extracellular or intracellular cues (Christova 2013; Rodriguez-Ubreva and Ballestar 2014). High-throughput sequencing technologies have revolutionized the fields of genomics, epigenomics, and transcriptomics and have provided novel insights into the transcription signatures of human diseases (Churko et al. 2013). Furthermore, using new powerful methodologies such as chromosome conformation capture (3C) and its derivatives, we are at a position to monitor dynamic intra- and interchromosomal interactions that allow the optimal expression of genes at a given time and space (Gavrilov et al. 2009; Wei et al. 2013).

Transcription factors may be constitutively active in a cell or work in an inducible mode in response to various ligands and signal transduction pathways. The cross talk between different signaling pathways which orchestrate the cellular responses can be facilitated by the physical and functional interactions between transcription factors, and these interactions can be monitored by various methods both *in vivo* and *in vitro*. All known transcription factors are modular in nature and contain a DNA-binding domain and a transcriptional activation domain (Mitchell and Tjian 1989; Lemon and Tjian 2000). In addition, several factors contain a dimerization domain that permits them to form homodimers and/or heterodimers. A variety of nuclear receptors for steroids, thyroids, retinoids, etc. contain a ligand binding site. Via their transcription activation domains, transcription factors appear to facilitate the recruitment of the proteins of the coactivator complex and the basal transcription complex to the transcription initiation site of each gene and thus initiate transcription (Roeder 2005). Importantly, the activity of transcription factors can be modulated by drugs against diseases such as cancer and cardiovascular disease as exemplified by the drugs that activate or repress the hormone nuclear receptors (Gronemeyer et al. 2004).

It is beyond the scope of this chapter to provide a thorough review of the different mechanisms of transcriptional regulation of eukaryotic genes or to describe extensively the different classes of transcription factors, their structures, and their mode of regulation. We will only focus on those classes of transcription factors that have been shown to play key roles in the regulation of the genes involved in lipid and lipoprotein metabolism and more specifically on those involved in the metabolism of high-density lipoproteins (HDL) such as the hormone nuclear receptors.

1.1 General Introduction to Hormone Nuclear Receptors

Hormone nuclear receptors belong to a superfamily of transcription factors that are activated by steroid hormones (estrogens, androgens, glucocorticoids, etc.), retinoids, thyroids, and products of intermediate metabolism such as bile acids, fatty acids, and cholesterol derivatives, among others (Gronemeyer et al. 2004). Some members of this family do not need ligand binding to regulate transcription

and are classified as “orphans” (Blumberg and Evans 1998). Nuclear receptors are structurally highly conserved. In terms of primary structure, the highest degree of homology among family members is in the DNA-binding domain that contains two zinc fingers (Helsen et al. 2012). Nuclear receptors also contain two transactivation domains (TADs), one N-terminal ligand-independent TAD called activation function 1 (AF1) and a ligand-dependent TAD called AF2 located close to the ligand-binding domain (LBD) (Rochel et al. 2011). Nuclear receptors bind to hormone response elements (HREs) on the promoters of target genes either as homodimers or as heterodimers with the retinoid X receptor (RXR). The HREs consist of direct repeats (DRs), inverted repeats (IRs), or palindromic repeats (PRs) of the consensus sequence 5' AG(G/T)TCA 3'. The repeats are separated by 1, 2, 3, 4, or 5 nucleotides and are designated DR1, DR2, etc. (for the direct repeats); IR1, IR2, etc. (for the inverted repeats); and PR1, PR2, etc. (for the palindromic repeats) as described previously (Kardassis et al. 2007; Helsen et al. 2012). The HRE type and inter-repeat spacing determine to a large degree the specificity in nuclear receptor binding, but this rule is not strict at all. For instance, both LXR/RXR and T3R/RXR heterodimers prefer to bind to DR4 HREs, whereas RAR/RXR heterodimers bind to DR5. The direct repeats with one base spacing (DR1 type) appear to be very promiscuous as they bind RXR, COUP-TFI, ARP-1, and HNF-4 homodimers and PPAR/RXR, RAR/RXR, COUP-TFI/RXR, and ARP-1/RXR heterodimers (Nakshatri and Bhat-Nakshatri 1998). The elucidation of the three-dimensional structure of the ligand-binding domain of several nuclear receptors by X-ray crystallography in the absence and in the presence of ligands has allowed a good understanding of the modulation of nuclear receptor action by ligands and the development of very potent agonists and antagonists, some of which have been used therapeutically (Bourguet et al. 2000). Chromatin immunoprecipitation studies usually reveal that nuclear receptors are constitutively nuclear and bound to chromatin but they are transcriptionally silent in the absence of ligand. The binding of the ligand to the LBD causes a major conformational change to this domain which culminates in the recruitment of nuclear receptor coactivators such as PGC-1 and CBP/p300 and the displacement of corepressors (Chen and Li 1998; Liu and Lin 2011). Nuclear receptors can cross talk with other transcription factors in a positive or a negative manner as exemplified by the negative regulation of Jun or NF- κ B transcription factors by the glucocorticoid receptors during inflammation, a mechanism termed *trans*-repression (Adcock and Caramori 2001).

1.2 Transcriptional Regulation of the apoA-I Gene in the Liver

The hypothesis that apolipoprotein (apo) A-I overexpression positively influences plasma concentrations of HDL cholesterol (HDL-C) has been validated experimentally in transgenic mice expressing human apoA-I under homologous or heterologous regulatory sequences. These mice have significantly elevated plasma levels of HDL-C and human apoA-I (Rubin et al. 1991; Kan et al. 2000). These “humanized” apoA-I transgenic mice are valuable tools for the study of apoA-I gene regulation

in vivo. Furthermore, it was demonstrated that the overexpression of apoA-I in apoE KO or LDLR KO mice via transgene- or adenovirus-mediated gene transfer reduced atherosclerosis development confirming the anti-atherogenic role of apoA-I upregulation (Paszty et al. 1994; Belalcazar et al. 2003; Valenta et al. 2006).

In humans, the apoA-I gene is expressed abundantly in the liver and intestine and to a lesser extent in other tissues (Zannis et al. 1985). Early studies had established that the human apoA-I promoter containing 250 bp upstream from the transcription start site of the gene is sufficient to drive liver-specific gene expression both in cell cultures and in mice (Walsh et al. 1989; Tzameli and Zannis 1996; Hu et al. 2010a). This promoter region is rich in nuclear factor binding sites and responds to various intracellular as well as extracellular ligands (Zannis et al. 2001a; Haas and Mooradian 2010). As shown in Fig. 1, prominent role in the regulation of the apoA-I promoter play two hormone response elements (HREs) located at positions $-210/-190$ and $-132/-120$ that bind members of the hormone nuclear receptor superfamily in a competitive manner (Tzameli and Zannis 1996).

One of the nuclear receptors that plays a prominent role in apoA-I gene regulation in the liver and the intestine is the hepatocyte nuclear factor-4 (HNF-4).

HNF-4 was discovered as a rat liver nuclear protein that binds to the promoters of liver-specific genes such as transthyretin and apolipoprotein C-III (Sladek 1994). In the adult organism, HNF-4 is expressed in the liver, kidney, intestine, and pancreas (Sladek 1994). The total disruption of the HNF-4 gene in mice leads to an embryonic lethal phenotype due to the impairment of endodermal differentiation and gastrulation (Chen et al. 1994). This early developmental arrest was rescued by the complementation of the HNF-4 $\alpha^{-/-}$ embryos with a tetraploid embryo-derived

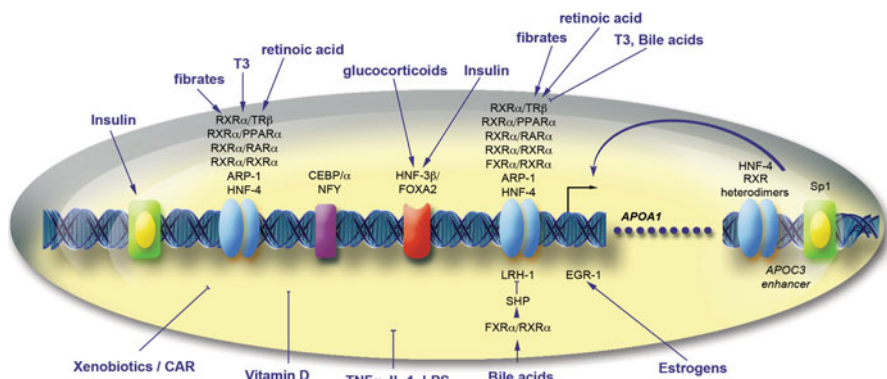


Fig. 1 Regulatory elements and transcription factors that control the expression of the apoA-I gene. *Arrows and block lines* denote activation and repression, respectively. The mechanisms are described in detail in the text. Abbreviations: retinoic acid receptor (RAR); retinoid X receptor (RXR); thyroid receptor β (TR β); farnesoid X receptor (FXR); hepatocyte nuclear factor (HNF); apoA-I regulatory protein 1 (ARP-1); forkhead box 2 (FOXA2); nuclear factor Y (NFY); CCAAT/enhancer-binding protein (CEBP); liver receptor homologue-1 (LRH-1); small heterodimer partner (SHP); specificity protein 1 (Sp1); tumor necrosis factor α (TNF α), interleukin-1 (IL-1); lipopolysaccharide (LPS)

wild-type visceral endoderm (Li et al. 2000). The analysis of the rescued mice showed that the expression of the apoA-I gene as well as of other apolipoprotein genes, shown previously to be regulated by HNF-4 including apoA-II, apoB, apoC-III, and apoC-II, was abolished confirming the cell culture data (Li et al. 2000). Experiments in mice in which the HNF-4 gene was disrupted in the adult liver using Alb-Cre revealed that HNF-4 is essential not only for the establishment but also for the maintenance of hepatic differentiation status (Hayhurst et al. 2001). Lipid and lipoprotein analysis of plasma of these mice revealed a dramatic reduction in total cholesterol, HDL cholesterol, and triglycerides as well as a dramatic increase in the concentration of bile acids (Hayhurst et al. 2001). Furthermore, FPLC analysis showed that HDL cholesterol from the HNF-4 Liv KO mice eluted later than that from controls indicative of the presence of smaller HDL populations. Interestingly, the expression of the two essential genes for HDL biogenesis, namely apoA-I and ABCA1, was not affected in the livers of the HNF-4 Liv KO, suggesting that the reduction in the plasma HDL levels was the result of altered HDL remodeling rather than reduced biosynthesis. In agreement with this, the expression level of the HDL receptor SR-BI gene was dramatically increased (Hayhurst et al. 2001).

Studies in transgenic mice expressing the human apoA-I gene under its own regulatory sequences and clinical studies in humans have shown that fibrates have a positive effect on apoA-I gene transcription as well as on plasma HDL levels. The increase in human apoA-I gene transcription by fibrates is mediated by peroxisome proliferator-activated receptor α (PPAR α) which binds to a PPRE on the proximal apoA-I promoter as a heterodimer with RXR α (Tzamelis and Zannis 1996; Staels and Auwerx 1998). This was confirmed by *in vivo* experiments performed in mice that express human apoA-I under the control of its own promoter but lack the expression of PPAR α . When these mice were given fenofibrate (FF) or gemfibrozil (GF) for 17 days, an increase in plasma HDL-C levels was observed by FF and to a lesser extent by GF only in the mice that express endogenous PPAR α (Duez et al. 2005). The fibrate-treated mice had larger HDL particles possibly due to the upregulation of phospholipid transfer protein and downregulation of SR-BI (Duez et al. 2005). Interestingly, the apoA-I gene cannot be upregulated by fibrates in rodents due to a three base pair difference in the PPRE rendering the rodent apoA-I PPRE nonfunctional (Vu-Dac et al. 1994). In line with the above findings, liver-specific inactivation of the PPAR α heterodimer partner retinoid X receptor α (RXR α) gene in mice was associated with increased expression of the apoA-I gene (Wan et al. 2000).

In a clinical study involving 234 patients with combined hyperlipidemia, both FF and GF reduced triglycerides and increased HDL-C to a similar extent, but only FF treatment increased apoA-I plasma levels, and this was in agreement with the previous clinical trials (Schaefer et al. 1996; Durrington et al. 1998; Sakai et al. 2001; Duez et al. 2005).

A humanized apoA-I transgenic mouse model expressing human apoA-I under its own regulatory sequences in a mouse apoA-I null background was recently used to identify by global gene expression profiling candidate genes that affect lipid and lipoprotein metabolism in response to fenofibrate treatment (Sanoudou et al. 2009).

Bioinformatical analysis and stringent selection criteria (twofold change, 0 % false discovery rate) identified 267 significantly changed genes. In contrast to the study by Duez et al. discussed above (Duez et al. 2005), fenofibrates (FF) did not significantly alter the levels of hepatic human apoA-I mRNA and plasma apoA-I protein. This could be due to differences either in the mouse models used (for instance, the humanized apoA-I mouse of Sanoudou et al. has 2.1 kb apoA-I promoter fragment, whereas the model of Duez et al. has a 5.5 kb apoA-I promoter) or the doses of fibrates (0.2 % w/w in the paper of Duez et al. and 0.03 % in the paper by Sanoudou et al.). Despite the lack of apoA-I responsiveness, the FF treatment increased cholesterol levels 1.95-fold mainly due to the increase in HDL-C. The observed changes in HDL were associated with the upregulation of genes involved in phospholipid biosynthesis and lipid hydrolysis, as well as in the phospholipid transfer protein. The gene encoding the estrogen receptor-related gamma (ESRRG) transcription factor was upregulated 2.36-fold by FF and had a significant positive correlation with genes of lipid and lipoprotein metabolism and mitochondrial functions, indicating an important role of this orphan receptor in mediating the FF-induced activation of a specific subset of its target genes (Sanoudou et al. 2009).

In addition to HNF-4 and PPAR α , the two HREs of the proximal human apoA-I promoter bind apoA-I regulatory protein 1 (ARP-1) and liver receptor homologue-1 (LRH-1) which repress and activate the apoA-I promoter, respectively, as illustrated in Fig. 1 (Ladiaz and Karathanasis 1991; Delerive et al. 2004). LRH-1 is a member of the *fushi tarazu* subfamily of nuclear receptors that is highly expressed in the liver, intestine, pancreas, and ovary (Fayard et al. 2004). In the liver, LRH-1 plays a key role in cholesterol homeostasis, through the control of the expression of genes that are implicated in bile acid biosynthesis and enterohepatic circulation such as CYP7A1, CYP8B1, and ABCG5/8 (del Castillo-Olivares and Gil 2000; Freeman et al. 2004; Kir et al. 2012; Back et al. 2013), reverse cholesterol transport (SR-BI, apoA-I) (Schoonjans et al. 2002; Delerive et al. 2004), and HDL remodeling (CETP) (Luo et al. 2001). However, mice with targeted inactivation of the LRH-1 gene in the liver are characterized by physiological levels of HDL cholesterol, LDL cholesterol, and triglycerides but have a profound effect on bile acid composition in the liver which leads to reduced intestinal reuptake of bile acids and to the enhanced removal of lipids from the body (Mataki et al. 2007). Recent data suggest that LRH-1 functions in a compensatory safeguard mechanism for adequate induction of bile salt synthesis under conditions of high bile salt loss (Out et al. 2011).

The two HREs of the apoA-I promoter also mediate the response of apoA-I to thyroids, retinoids, and bile acids via heterodimers of RXR α with thyroid hormone receptor β (TR β), retinoic acid receptor α (RAR α), and farnesoid X receptor α (FXR α), respectively (Rottman et al. 1991; Hargrove et al. 1999). Although retinoids activate apoA-I gene expression, thyroids have dual effects on apoA-I promoter activity, whereas bile acids inhibit apoA-I gene expression (Taylor et al. 1996; Tzameli and Zannis 1996; Srivastava et al. 2000; Claudel et al. 2002). As shown in Fig. 1, in response to bile acids, FXR downregulates apoA-I gene transcription by two complementary mechanisms: (a) a direct binding

to the apoA-I HRE and (b) an indirect mechanism via the induction of small heterodimer partner (Bavner et al. 2005) which, in turn, represses the activity of LRH-1 (Delerive et al. 2004).

The nuclear receptor constitutive androstane receptor (CAR) regulates the detoxification of xenobiotics and endogenous molecules. In mice, the specific CAR agonist 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) decreased HDL cholesterol and plasma apoA-I levels in a CAR-specific manner (Masson et al. 2008). In transient transfections, CAR decreased the activity of the human apoA-I promoter in the presence of TCPOBOP, but the mechanism by which this repression is facilitated remains unknown (Masson et al. 2008).

Ligands of the vitamin D receptor (VDR) were also shown to affect negatively apoA-I gene expression in hepatic cells (Wehmeier et al. 2005). In VDR KO mice, serum HDL-C levels were 22 % higher and the mRNA levels of apoA-I were 49.2 % higher compared with WT mice. The mechanisms by which VDR ligands affect HDL levels remain unclear (Wang et al. 2009).

1.2.1 The Role of the Distal Enhancer in apoA-I Gene Transcription

In addition to its own promoter, optimal expression of the human apoA-I gene in hepatic and intestinal cells requires the presence of a 200 bp transcriptional enhancer element located downstream of the apoA-I gene, 500 bp upstream of the first exon of the adjacent apoC-III gene. This regulatory region, which coordinates the expression of all three genes of the apoA-I/C-III/A-IV cluster, contains two hormone response elements that bind HNF-4 and different combinations of ligand-dependent nuclear receptors as well as two binding sites for the ubiquitous transcription factor specificity protein 1 (Sp1) (Kardassis et al. 1997; Lavrentiadou et al. 1999). The mutagenesis of the HREs and of the Sp1 sites reduced the activity of the apoA-I promoter/C-III enhancer cassette in cell cultures and abolished the binding of the corresponding factors (Kardassis et al. 1997; Lavrentiadou et al. 1999).

The contribution of the HREs and the Sp1 binding sites to the tissue-specific expression of the apoA-I gene *in vivo* was addressed using transgenic mice bearing the WT apoA-I/apoC-III gene cluster under the control of their regulatory regions or the same cluster bearing mutations in different regulatory elements (Georgopoulos et al. 2000; Kan et al. 2000, 2004). It was shown that mutations in one of the two HREs of the enhancer (element I4) abolished the intestinal expression and reduced the hepatic expression of the adjacent apoA-I gene to 20 % of the control. Mutations in the two HREs of the proximal apoA-I promoter reduced the hepatic and intestinal expression of the apoA-I gene to approximately 15 % of the control, whereas combined mutations in all three HREs totally eliminated the intestinal and hepatic expression of the apoA-I gene (Kan et al. 2000). Studies in cell cultures established that HNF-4 and Sp1 factors are both required for the synergy between the apoA-I promoter and the enhancer by physically interacting with each other and forming transcriptional complexes in order to facilitate the recruitment of the basal transcriptional machinery (Kardassis et al. 2002). The aforementioned mouse model that expresses human apoA-I under its own promoter

and enhancer (Kan et al. 2000) is very useful for the *in vivo* characterization of the mechanisms that regulate the expression of the apoA-I gene under physiological or pathological conditions as well as for the identification and validation of novel compounds that are designed to upregulate human apoA-I gene transcription and serve as HDL-raising drugs. This is especially important in light of the differences between the mouse and the human apoA-I promoters. For instance, the mouse gene cannot be upregulated by fibrates due to a three base pair difference in the PPRE compared to the human promoter which responds to 0.2 % fibrates but not to 0.03 % fibrates as mentioned above.

1.2.2 Other Factors Regulating apoA-I Gene Transcription

Further upstream from the two apoA-I HREs, an insulin response core element (IRCE) was identified and shown to bind Sp1 (Murao et al. 1998). Insulin-activated signaling pathways including the Ras/raf and the phosphatidylinositol 3-kinase (PI3K) have been shown to posttranslationally modify Sp1, and this leads to increased apoA-I promoter activity (Mooradian et al. 2004).

Early growth response protein 1 (EGR-1) is another transcription factor that regulates apoA-I expression via the proximal HREs (Kilbourne et al. 1995; Cui et al. 2002). Mice with experimental nephrotic syndrome are characterized by a fivefold increase in the levels of EGR-1, and these changes were associated with high plasma apoA-I and HDL-C levels as well as apoA-I gene transcription in the liver (Zaiou et al. 1998). In line with these findings, mice deficient in EGR-1 have reduced plasma HDL-C and apoA-I as well as hepatic apoA-I mRNA levels (Zaiou et al. 1998). EGR-1 was shown to mediate the response of the apoA-I promoter to estrogens (Hargrove et al. 1999).

The proximal apoA-I promoter also contains one element that binds the basic leucine zipper (bZip) factor CCAAT/enhancer-binding protein (C/EBP) and nuclear factor Y (NFY) and another element that binds hepatocyte nuclear factor-3 β /FOXA2 (Papazafiri et al. 1991; Novak and Bydlowski 1997). Nuclear factor HNF-3 β was shown to mediate the response of the apoA-I promoter to glucocorticoids (Hargrove et al. 1999).

Several natural compounds with antioxidant, pro-estrogenic, or other activities were shown to affect apoA-I and HDL-C levels, and these studies are summarized in Haas and Mooradian (2010).

Pro-inflammatory cytokines including tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β) were previously shown to inhibit apoA-I gene expression both in cell cultures and in animals (Ettinger et al. 1994; Song et al. 1998). Furthermore, plasma levels of HDL-C and apoA-I were shown to be highly increased in mice deficient in the p50 subunit of the pro-inflammatory transcription factor NF- κ B (Morishima et al. 2003). In agreement with this observation, the activation of NF- κ B by lipopolysaccharide (LPS) caused a reduction in apoA-I mRNA and protein levels in HepG2 cells, whereas the inhibition of NF- κ B via adenovirus-mediated overexpression of I κ B α abolished the reduction (Morishima et al. 2003). This I κ B α -induced apoA-I increase was blocked by preincubation with MK886, a selective inhibitor of peroxisome proliferator-activated receptor α , and

mutations in the PPAR α binding site in the apoA-I promoter abrogated these changes (Morishima et al. 2003). In a recent study, it was shown that apoA-I promoter activity in HepG2 cells is inhibited by TNF α in a c-Jun-dependent manner but no API-responsive element within the apoA-I promoter was reported to mediate this effect (Parseghian et al. 2013). The inhibition of the expression of apoA-I and other HDL genes in hepatocytes during inflammation could also be mediated by HNF-4 which was previously shown to be negatively regulated by the TNF α /NF- κ B signaling pathway by physically interacting with NF- κ B (Nikolaidou-Neokosmidou et al. 2006).

1.3 Transcriptional Regulation of the ABCA1 Gene

The gene encoding the ATP-binding cassette transporter A1 (ABCA1) is expressed in the liver, small intestine, macrophages, kidney, and various other tissues (Langmann et al. 1999, 2003; Kielar et al. 2001; Wellington et al. 2002). ABCA1 is an important regulator of HDL biogenesis in the liver and facilitates the removal of excess cholesterol from macrophages.

ABCA1 is particularly abundant in macrophages (Langmann et al. 1999). ABCA1 expression in macrophages has little influence on HDL-C plasma levels (Haghighpassand et al. 2001) but is an important factor in the prevention of cholesterol accumulation in the macrophages found in the atherosclerotic plaque and their transformation into foam cells (Aiello et al. 2002). ABCA1 mRNA and protein are very unstable, having a half-life of 1–2 h in murine macrophages (Wang and Oram 2002). Fine-tuning regulatory mechanisms (transcriptional regulation as well as posttranscriptional and posttranslational modifications) are involved to ensure the constant and inducible ABCA1 expression in macrophages. In this section, we will focus on the transcriptional regulation of the ABCA1 gene with emphasis on macrophages. Posttranscriptional and posttranslational regulation of this gene will be discussed in later sections.

The human ABCA1 gene mapped to chromosome 9q31.1 is composed of 50 exons, which encode 2261-amino-acid residues (Santamarina-Fojo et al. 2000). The ABCA1 gene promoter contains a TATA box localized 24 bp upstream of the transcription initiation site, essential for promoter activity in macrophages as well as in hepatocytes (Langmann et al. 2002). The engagement of alternative promoters and transcription initiation sites localized upstream of the first exon or inside the first intron of the gene enables the inducible and tissue-specific expression regulation of ABCA1 gene (Huuskonen et al. 2003; Singaraja et al. 2005). In addition, other transcriptional response elements of the promoter influence the constitutive and tissue-specific expression of ABCA1 (Fig. 2).

1.3.1 Upregulatory Mechanisms of ABCA1 Gene Expression

The major transcription factors that upregulate ABCA1 gene expression in macrophages are the nuclear receptors liver X receptors α and β (LXR α and LXR β), both expressed by this cell type. LXRs heterodimerize with the retinoic

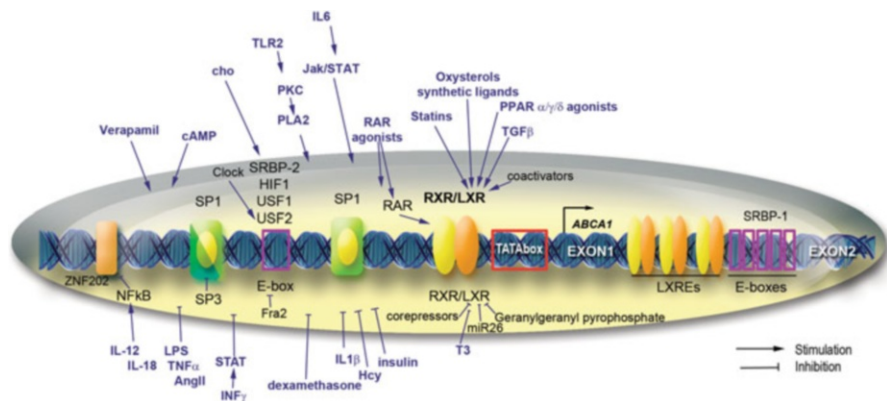


Fig. 2 Regulatory regions, transcription factors, and signaling molecules (cytokines, growth factors, metabolites, drugs) that modulate the expression of the ABCA1 gene in macrophages and other tissues. *Arrows and block lines* denote activation and repression, respectively. The mechanisms are described in detail in the text. Abbreviations: retinoic acid receptor (RAR); retinoid X receptor (RXR); liver X receptor (LXR); sterol regulatory element-binding protein (SREBP); specificity protein 1 (Sp1); tumor necrosis factor α (TNF α), interleukin (IL); lipopolysaccharide (LPS); interferon γ (IFN γ); angiotensin II (AngII); nuclear factor kappa beta (NF- κ B); upstream stimulatory factor (USF); hypoxia-inducible factor (HIF); transforming growth factor β (TGF β); peroxisome proliferator-activated receptor (PPAR); Janus kinase (Jak); signal transducer and activator of transcription (STAT)

X receptor (RXR α) to bind to the direct repeats separated by four nucleotides (direct repeat 4; DR4 elements) found at position $-62/-47$ of the ABCA1 promoter (Costet et al. 2000). Additional but not well-characterized LXREs are present at the intron 1 promoter of the ABCA1 gene (Singaraja et al. 2001). Since the LXR α promoter is subject to autoregulation, the LXR-mediated gene expression is auto-amplified (Laffitte et al. 2001a). The natural ligands of LXR are several hydroxylated derivatives of cholesterol (oxysterols) and include 27-hydroxycholesterol (Schwartz et al. 2000; Fu et al. 2001), 22(R)-hydroxycholesterol (Costet et al. 2000), 20(S)-hydroxycholesterol (Schwartz et al. 2000), and desmosterol (Yang et al. 2006). Among these ligands, 27-hydroxycholesterol which is endogenously produced by the action of CYP27A1 enzyme represents the sensor of cholesterol loading in macrophages. In addition to these natural ligands, synthetic LXR ligands such as TO901317 have also been developed. These synthetic LXR agonists upregulate ABCA1 in cultured macrophages more efficiently than cholesterol loading via modified LDL particles (Sparrow et al. 2002).

LXR tissue-selective gene transcription is dependent on co-regulatory proteins. For example, data showed that the activation of the ABCA1 promoter by LXR α /RXR α heterodimers and their ligands require Sp1 (Thymiakou et al. 2007). The overexpression of Sp1 increased ABCA1 mRNA level in HeLa cells and enhanced cellular cholesterol and phospholipid efflux in RAW 246.7 macrophages. Gel shift experiments revealed *in vitro* binding of Sp1 to $-85/-91$ and $-151/-156$ GC

boxes. Moreover, it was shown that Sp3 competed with Sp1 for binding to the latter GC box, acting as a repressor (Langmann et al. 2002). Physical interactions between Sp1 and LXR α require the N-terminal region of LXR α , which includes the DNA-binding domain and two different domains of Sp1: the transactivation domain B and the DNA-binding domain (Thymiakou et al. 2007). LXR agonists, such as the natural steroidal ligand 22(R)-hydroxycholesterol and the weak non-steroidal ligand GSK418224, differentially recruit coactivators and corepressors compared with full LXR agonists, such as the nonsteroidal ligand T0901317 (Albers et al. 2006; Peng et al. 2008, 2011; Phelan et al. 2008). The synthetic oxysterol N, N-dimethyl-3 β -hydroxycholeamide (DMHCA) caused a differential induction of the ABCA1 and the sterol regulatory element-binding protein (SREBP)-1c genes in hepatic and macrophage cell lines, as well as in mice (Quinet et al. 2004). In cholesterol-loaded or unloaded peritoneal macrophages, DMHCA increased ABCA1 mRNA, whereas SREBP-1c mRNA levels were downregulated (Quinet et al. 2004). Cineole, a small aromatic compound found in teas and herbs, considerably stimulated the transactivation potential of LXR α and LXR β and induced ABCA1 expression in macrophages but significantly reduced the expression of LXR α - and LXR α -responsive genes in hepatocytes (Jun et al. 2013). Another LXR agonist, ATI-111, had a strong effect on ABCA1 expression in macrophages as well as in the intestine and small effect on ABCA1 expression in the liver. ATI-111 significantly stimulated SREBP-1c mRNA in some tissues but inhibited the conversion of SREBP-1c precursor form into its active form (Peng et al. 2011).

These findings revealed that LXR agonists have a promising potential for the upregulation of the ABCA1 transporter and the promotion of the cellular lipid efflux capacity of macrophages. Due to the concomitant LXR-mediated upregulation of two genes involved in the fatty acid biosynthesis, fatty acid synthase (Joseph et al. 2002a), and SREBP-1c (Yoshikawa et al. 2001), the development of LXR agonists for therapeutic uses has been limited by their adverse effects that include hepatic steatosis and hypertriglyceridemia. In order to dissociate the positive effects of LXR agonists on cholesterol homeostasis from the adverse effects on fatty acid metabolism, the next step will be the discovery of new LXR β -selective agonists and the synthesis of novel tissue-specific LXR ligands with weaker transcriptional effects on SREBP-1c. SREBP-1a, a different member of the SREBP family of proteins, binds to several sites present inside intron 1; however, the role of these elements in ABCA1 gene regulation is still unknown (Thymiakou et al., unpublished observations) (Fig. 2).

Besides LXR, other nuclear receptors and transcription factors are involved in ABCA1 gene regulation in macrophages. Retinoic acid receptor (RAR) activators such as all-*trans*-retinoic acid and 4-[(E)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (arotinoid acid) were found to increase ABCA1 mRNA and protein levels in macrophages (Costet et al. 2003). Co-transfection experiments showed that the same DR4 promoter element of the ABCA1 promoter binds RXR heterodimers in the following order: RAR γ /RXR α bound stronger and activated the human ABCA1 promoter, and RAR α /RXR α bound weaker, while no RAR β /RXR α binding was detected. However, in

macrophages from $RAR\gamma^{-/-}$ mice, rotinoid acid still induced ABCA1 gene expression and caused marked upregulation of $RAR\alpha$, suggesting that high levels of $RAR\alpha$ can compensate for the absence of $RAR\gamma$ (Costet et al. 2003).

Peroxisome proliferator-activated receptors α ($PPAR\alpha$) and γ ($PPAR\gamma$) are both expressed in human macrophages where they exert anti-inflammatory effects. The hydroxylated derivative of linoleic acid, 13-hydroxy linoleic acid, a natural $PPAR$ agonist, and pioglitazone ($PPAR\gamma$ agonist) increased $PPAR$ transcriptional activity and induced ABCA1 gene expression in macrophages (Kammerer et al. 2011; Ozasa et al. 2011). However, data suggest that these effects are indirect and most probably are mediated by $LXR\alpha$ (Chinetti et al. 2001). $PPAR\delta$ activators appeared to induce ABCA1 gene expression and cholesterol efflux moderately and to increase HDL levels in an obese-monkey model (Oliver et al. 2001).

Recent data showed that Clock, a key transcription factor that controls circadian rhythm, is involved in ABCA1 regulation in macrophages (Pan et al. 2013). The dominant-negative Clock mutant protein (Clock $\Delta 19/\Delta 19$) enhanced plasma cholesterol and atherosclerosis. Mutant Clock $\Delta 19/\Delta 19$ /apoE $^{-/-}$ mice had macrophage dysfunction, expressed low levels of ABCA1, and had higher levels of scavenger receptors. Molecular studies revealed that Clock regulated ABCA1 expression in macrophages by modulating the activity of upstream stimulatory factor 2 (Pan et al. 2013).

Experimental data showed that signaling molecules upregulate ABCA1 expression in macrophages, but the fine-tuning mechanism of modulation and the regulatory regions involved remain to be elucidated. Transforming growth factor $\beta 1$ (TGF $\beta 1$) increased ABCA1 mRNA levels in cholesterol-loaded macrophages (Argmann et al. 2001; Panousis et al. 2001). It was demonstrated that in THP-1 macrophage-derived foam cells, the $LXR\alpha$ pathway is involved in TGF β -mediated upregulation of ABCA1 expression (Hu et al. 2010b). Interleukin-6 (IL-6) significantly increased ABCA1 at both the mRNA and protein levels. This effect was abolished by selective inhibition of the JAK2/STAT3 signaling pathway (Frisdal et al. 2011). ABCA1 mRNA levels were significantly increased by estradiol treatment of macrophages for a short period of time, suggesting a direct activation of the ABCA1 promoter via the estrogen receptor β (Schmitz and Langmann 2005). Membrane-permeable analogues of cyclic adenosine monophosphate (cAMP) induced the ABCA1 mRNA in macrophages and other cells by an unknown mechanism (Lin and Bornfeldt 2002). Inhibitors that are able to block the action of phosphodiesterase 4 on cAMP have been found to increase ABCA1 mRNA and cellular cholesterol efflux (Lin and Bornfeldt 2002). Verapamil, a calcium channel blocker, enhanced ABCA1 transcription by an LXR -independent process (Suzuki et al. 2004). Toll-like receptor 2 agonist Pam(3)CSK(4) upregulated ABCA1 gene expression in RAW 264.7 macrophages via the activation of the PKC η /phospholipase D2 signaling pathway (Park et al. 2013). S-Allylcysteine, the most abundant organosulfur compound in aged garlic extract, also elevated ABCA1 content in human THP-1 macrophages (Malekpour-Dehkordi et al. 2013). The data showed that stimulation with CXCL5 that has a protective role in atherosclerosis (Rousselle

et al. 2013) induced ABCA1 expression in alternatively activated (M2) macrophages but not in classically activated (M1) macrophages.

1.3.2 Negative Regulation of ABCA1 Gene Transcription

The thyroid hormone T3 strongly suppressed ABCA1 gene transcription (Huuskonen et al. 2004). It was demonstrated that T3 significantly inhibited the ability of oxysterols to activate LXR. Moreover, the TR/RXR heterodimers competed with LXR/RXR for the DR4 element in the ABCA1 promoter (Huuskonen et al. 2004). A reciprocal negative cross talk exists between LXRs and STAT1 based on the competition for CREB-binding protein (Pascual-Garcia et al. 2013). This may explain the IFN γ -mediated downregulation of ABCA1 in cholesterol-loaded macrophages (Panousis and Zuckerman 2000; Argmann et al. 2001; Ma et al. 2013). Moreover, TNF α downregulates ABCA1 as well as LXR α expression in macrophages via a PKC θ -dependent pathway (Ma et al. 2013).

Unsaturated fatty acids decrease the expression of ABCA1 in RAW 264.7 macrophages by a mechanism that involves LXR/RXR binding to the promoter (Uehara et al. 2007) and by modulation of the histone acetylation state (Ku et al. 2012). In MCSF-activated human monocytes, linoleic acid decreases ABCA1 gene expression (Mauerer et al. 2009). Geranylgeranyl pyrophosphate, a product of the mevalonate pathway that is used for protein isoprenylation, suppresses LXR-induced ABCA1 synthesis in two ways: as an antagonist of the LXR interaction with the steroid receptor coactivator-1 (SRC-1) and as an activator of Rho GTP-binding proteins (Gan et al. 2001).

Among the transcription factors that downregulate ABCA1 expression in macrophages in an LXR-independent manner is the SCAN domain-containing zinc finger transcription factor ZNF202 which binds to a G μ T motif in the region -234/-215 of the ABCA1 promoter (Porsch-Ozcurumez et al. 2001). High intracellular levels of ZNF202 prevented LXR/RXR-mediated induction of the ABCA1 promoter in response to oxysterols (Porsch-Ozcurumez et al. 2001).

The conserved E-box at position -140 binds transcription factors that modulate the ABCA1 gene expression. Experimental data indicated that upstream stimulatory factors (USF) 1 and 2, hepatocyte nuclear factor-1 α (HNF-1 α), and fos-related antigen (Fra)2 bind to the intact E-box of the human ABCA1 promoter and differentially modulate the gene expression: USF1 and USF2 enhanced and Fra2 repressed ABCA1 promoter activity (Langmann et al. 2002; Yang et al. 2002). The same E-box element also binds the sterol regulatory element-binding protein 2 (SREBP-2), a key regulator of cholesterol metabolism, which suppresses ABCA1 gene transcription in response to cholesterol depletion (73).

ABCA1 gene expression was severely decreased in the liver and peritoneal macrophages of diabetic mice (Uehara et al. 2002). This observation was explained using *in vitro* models in which it was revealed that acetoacetate downregulates ABCA1 mRNA and protein in HepG2 hepatocytes and RAW 264.7 macrophages (Uehara et al. 2002) and thus high glucose concentration decreases ABCA1 gene expression in MCSF-activated monocytes (Mauerer et al. 2009). Data showed that

the treatment of THP-1 macrophages with 100 nM dexamethasone, a potent synthetic ligand of the glucocorticoid receptor, decreases the expression of the ABCA1 gene (Sporstol et al. 2007).

LPS downregulates ABCA1 in macrophages; this inhibition was reverted by the treatment with betulinic acid acting via the downregulation of miR-33 and suppression of NF- κ B pathway (Zhao et al. 2013). IL-18 and IL-12 synergistically decrease ABCA1 levels in THP-1 macrophage-derived foam cells through the IL-18 receptor/NF- κ B/ZNF202 signaling pathway (Yu et al. 2012). ABCA1 expression was strongly suppressed by angiotensin (Ang) II at both mRNA and protein levels in a dose-dependent manner in THP-1-derived macrophages, whereas ABCG1 expression was not affected. The effect of Ang II on ABCA1 expression could be mediated by the angiotensin II type 1 (AT1) receptor (Chen et al. 2012a). It was demonstrated that clinically relevant concentrations of homocysteine (Hcy) decreased the mRNA and protein expression levels of ABCA1 in macrophages. It was revealed that mRNA expression and the activity of DNA methyltransferase were increased by Hcy, which may explain the higher DNA methylation level of ABCA1 gene in macrophages incubated with Hcy (Liang et al. 2013).

1.4 Transcriptional Regulation of the ABCG1 Gene

ABCG1 mediates cholesterol removal from macrophages to HDL particles, but not to lipid-free apoA-I (Kennedy et al. 2005; Fitzgerald et al. 2010). Although recent data showed that the combined macrophage deficiency of ABCA1/G1 is pro-atherogenic, probably by promoting plaque inflammation (Westerterp et al. 2013), the data concerning the role of ABCG1 expression in macrophages is controversial. Two independent groups reported that LDLR^{-/-} mice lacking macrophage ABCG1 show decreased atherosclerotic lesions (Baldan et al. 2006; Ranalletta et al. 2006), while others reported that the absence of macrophage ABCG1 causes a modest increase in atherosclerotic lesions (Out et al. 2006). These contradictory results may be explained by recent data showing that the absence of ABCG1 leads to increased lesions in early stages of atherosclerosis but causes retarded lesion progression in more advanced stages of atherosclerosis in LDLR^{-/-} mice, suggesting that the influence of ABCG1 deficiency on lesion development depends on the stage of atherogenesis (Meurs et al. 2012).

The human ABCG1 gene has been mapped to chromosome 21q22.3 and encodes for a 678-amino-acid protein of 75.5 kDa molecular mass (Chen et al. 1996). The ABCG1 gene spans more than 70 kb and includes 15 exons, each containing between 30 and 1,081 bp, while the intron size is between 137 bp and more than 45 kb. All exon-intron boundaries display the canonical GT/AG sequences. In contrast to the ABCA1 gene, the ABCG1 gene does not contain a canonical TATA box in the promoter (Langmann et al. 2000).

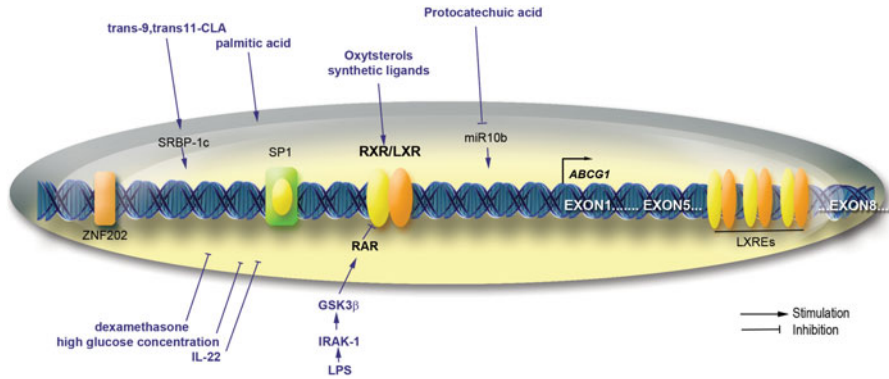


Fig. 3 Regulatory regions, transcription factors, and signaling molecules (cytokines, growth factors, metabolites, drugs) that modulate the expression of the ABCG1 gene. *Arrows and block lines* denote activation and repression, respectively. The mechanisms are described in detail in the text. Abbreviations: conjugated linoleic acid (CLA); glycogen synthase kinase 3 β (GSK3 β); sterol regulatory element-binding protein (SREBP); specificity protein 1 (Sp1)

The regulation of ABCG1 expression has similarities with that of ABCA1 (Fig. 3). Thus, LXR plays an important role in ABCG1 promoter activation. Experimental data indicated that various LXR ligands upregulate ABCG1 expression. ATI-111, a novel steroidal LXR agonist, induces ABCG1 mRNA expression in peritoneal macrophages more potently than T0901317 and inhibits its expression in the liver, suggesting tissue selectivity (Peng et al. 2011). Different natural compounds, such as cineole and fucosterol that are LXR activators, are able to increase ABCG1 levels in macrophages (Hoang et al. 2012; Jun et al. 2013). Recent data showed that the knockdown of LXR α impaired cholesterol efflux in human primary macrophages, while LXR β silencing had no detectable impact on the expression of LXR target genes such as ABCA1 and ABCG1 and did not affect cholesterol efflux (Ishibashi et al. 2013). The indirect effects of LXR in ABCG1 regulation were recently shown. Adiponectin treatment significantly increased ABCG1 mRNA and protein levels in macrophages from diabetic patients, whereas the pharmacological or genetic inhibition of LXR abrogated this enhancement; these data demonstrated that the mechanism of adiponectin-mediated upregulation of ABCG1 includes LXR α (Wang et al. 2013b). Similar with ABCA1, TGF β upregulates the expression of ABCG1, while unsaturated fatty acids suppress ABCG1 expression via the LXR pathway (Uehara et al. 2007; Hu et al. 2010b).

The analysis of potential regulatory elements in the promoter region carried out using the MatInspector program identified multiple Sp1 sites at positions -184, -382, and -566, an AP2 binding site at position -222, a NF- κ B site at position -338, an E-box motif at position -233, a sterol regulatory element at position -660, and the NFY binding site at position -198 in ABCG1 proximal promoter (Langmann et al. 2000). A functional genetic variant of the ABCG1 promoter associated with an increased risk of myocardial infarction and ischemic heart disease in the general population was revealed (Schou et al. 2012). This study

showed that the ABCG1 expression was decreased by approximately 40 % in g.-376C>T heterozygotes versus noncarriers. This gene polymorphism is included in a Sp1 binding site located at position -382/-373 in the ABCG1 promoter. Thus, the presence of the -376 T allele reduced the binding and transactivation of the promoter by Sp1, leading to a decreased ABCG1 expression (Schou et al. 2012).

ZNF202 was identified as a transcriptional repressor of ABCG1 gene which binds at position -560 in the ABCG1 promoter (Porsch-Ozcurumez et al. 2001).

Recently, microbiotic and dietary factors were shown to regulate the ABCG1 expression. Protocatechuic acid (PCA), a gut microbiota metabolite of cyanidin-3 formed by 0- β -glucoside, exerts an anti-atherogenic effect partially through the inhibition of miR-10b-mediated downregulation of ABCG1 expression (Wang et al. 2012a). Extra-virgin olive oil intake has been shown to improve the capacity of HDL to mediate cholesterol efflux and increased ABCG1 and ABCA1 expression in human macrophages (Helal et al. 2013). Conjugated linoleic acids (CLAs) are minor components of the diet with many reported biological activities. It was revealed that in MCSF-differentiated monocytes, *trans*-9,*trans*-11-CLA, but not *cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA, activated ABCG1 via SREBP-1c (Ecker et al. 2007). In addition, it was demonstrated that palmitic acid upregulates the ABCG1 gene, while high glucose concentration decreased ABCG1 gene expression in MCSF-activated human monocytes (Mauerer et al. 2009).

Among the downregulators of ABCG1, low doses of LPS strongly reduce the expression of ABCG1 in bone marrow-derived macrophages through IL-1 receptor-associated kinase 1 (IRAK-1)/glycogen synthase kinase 3 β (GSK3 β)/retinoic acid receptor α (RAR α) signaling pathway (Maitra and Li 2013). HMG-CoA reductase inhibitors, simvastatin and atorvastatin, decreased ABCG1-mediated cholesterol efflux in human macrophages, despite the fact that the protein expression remained unaltered (Wang et al. 2013c). In THP-1 monocytes, 100 nM dexamethasone, a synthetic glucocorticoid, inhibited the mRNA expression of ABCG1, although the glucocorticoid receptor expression was very low in this cell line (Sporstol et al. 2007). IL-22, a member of the IL-10 cytokine family secreted primarily by Th17 and Th22 subsets of T lymphocytes, was induced by S100/calgranulin and impaired cholesterol efflux in macrophages by downregulation of ABCG1 (Chellan et al. 2013).

1.5 Transcriptional Regulation of the Apolipoprotein E Gene

Apolipoprotein E (apoE), a glycoprotein of 35 kDa, plays an important role in plasma cholesterol level regulation and in cholesterol efflux, as documented by studies in patients and animal models with apoE deficiency or mutated apoE genes (Nakashima et al. 1994; Linton et al. 1995; von Eckardstein 1996; Van Eck et al. 1997; Grainger et al. 2004; Ali et al. 2005; Davignon 2005; Raffai et al. 2005). ApoE is mainly synthesized by the liver but also by various cells and peripheral tissues (Zannis et al. 2001b). ApoE is a marker for the developmental state of macrophages; the culture of mouse bone marrow cells in vitro showed that

macrophages, but not their monocytic precursors, synthesized apoE (Werb and Chin 1983c). At the site of atherosclerotic lesion, apoE is provided by infiltrated macrophages. Transgenic mice expressing apoE only in macrophages are protected against atherosclerosis, even though the plasma levels of apoE are exceedingly low and the animals are hypercholesterolemic (Bellosta et al. 1995). In contrast, transgenic mice with normal levels of apoE in plasma, but not in macrophages, are more susceptible to atherosclerosis (Fazio et al. 1997). ApoE secreted by macrophages within the atherosclerotic plaque facilitates the cholesterol efflux to exogenous acceptors (such as HDL), thus assisting the reverse cholesterol transport to the liver. The uptake of acetylated LDL or cholesterol ester-rich β -VLDL into peritoneal macrophages stimulates apoE synthesis and secretion (Basu et al. 1981).

The human apoE gene is located on chromosome 19 at the 5' end of a cluster containing also apoC-I, apoC-IV, and apoC-II genes (Myklebost and Rogne 1988; Smit et al. 1988; Allan et al. 1995a, b). The regulation of apolipoprotein E gene transcription is a highly complex process and requires the interaction of transcription factors with the proximal promoters but also with the distal regulatory regions (Fig. 4).

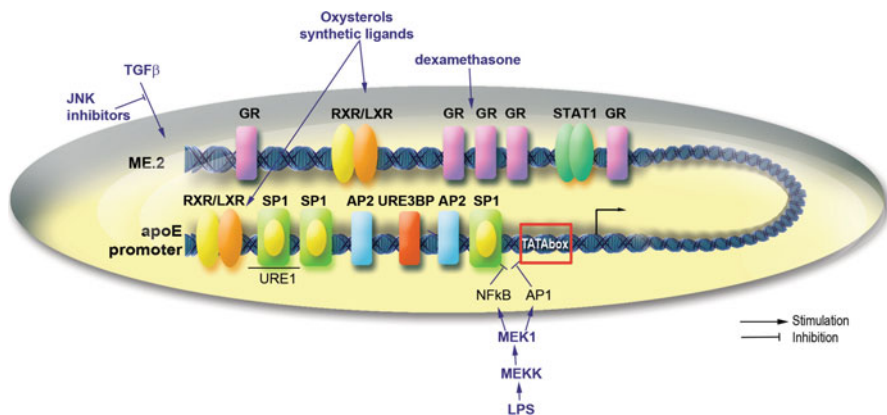


Fig. 4 Regulatory regions, transcription factors, and signaling molecules (cytokines, growth factors, metabolites, drugs) that modulate the expression of the apoE gene. *Arrows and block lines* denote activation and repression, respectively. The mechanisms are described in detail in the text. Abbreviations: glucocorticoid receptor (GR); signal transducer and activator of transcription (STAT); Jun N-terminal kinase (JNK); retinoid X receptor (RXR); liver X receptor (LXR); specificity protein 1 (Sp1); activator protein 2 (AP2); nuclear factor kappa beta (NF- κ B); upstream regulatory region 3 binding protein (URE3BP); lipopolysaccharide (LPS); multienhancer 2 (ME.2); transforming growth factor β (TGF β)

1.5.1 Proximal Regulatory Binding Sites Involved in the apoE Gene Expression

The proximal apoE promoter is well conserved in humans and mice, having the same localization of TATA box and GC box (Rajavashisth et al. 1985; Horiuchi et al. 1989). Multiple positive and negative elements that modulate apoE gene expression have been detected on the apoE promoter, using different *in vitro* systems (Larkin et al. 2000; Zannis et al. 2001b). Smith et al. analyzed the apoE promoter in both expressing (HepG2) and non-expressing (HeLa) cells (Smith et al. 1988). Within the proximal 5'-flanking sequence and the first intron, eight regions were identified which had a positive effect and three regions with a negative effect on apoE expression, in both HepG2 and HeLa cells (Smith et al. 1988). The proximal apoE promoter contains a GC box transcriptional control element at -59/-45, a nonspecific enhancer element at -366/-246, an upstream regulatory element (URE1) at -193/-124, and a downstream regulatory element at +44/+262 (Paik et al. 1988). Within URE1, a sequence spanning -161/-141, defined as a positive element for transcription, has the ability to act alone as an enhancer element (Chang et al. 1990). This element interacts with Sp1 transcription factor that constitutively binds the GC box motif, suggesting that Sp1 may play an important role in the basal level of apoE expression, as well as in the activity of this enhancer element. Another regulatory element, termed URE3, was identified at position -101/-89 and found to bind a 300 kDa protein from placental nuclear extracts termed URE3 BP (Jo et al. 1995). DNase I footprinting revealed the existence of two binding sites for recombinant AP2 in the regions from -48/-74 and from -107/-135 of the apoE promoter (Olaisen et al. 1982; Smith et al. 1988; Garcia et al. 1996; Salero et al. 2001, 2003). Gel mobility-shift assays showed the direct binding of LXR α /RXR α and LXR β /RXR α to a low-affinity LXRE present in the region -494/-465 of the proximal promoter (Laffitte et al. 2001b). Other studies revealed that USF binds to an atypical E-box located in the -101/-91 region of the apoE promoter (Salero et al. 2003). The same group found that Zic1 and Zic2 transcription factors can bind to three binding sites located at -65/-54, -136/-125, and -185/-174 in the apoE promoter and stimulate apoE gene expression (Salero et al. 2001).

Bacterial endotoxin and other inflammatory agents decrease apoE production (Werb and Chin 1983a, b; Gafencu et al. 2007). The apoE downregulation in macrophages impaired the local beneficial effect of apoE during the plaque development. As a result, despite the fact that macrophages are present in the lesion, their ability to regress atherosclerosis is seriously compromised. We have previously reported the mechanisms of apoE downregulation in macrophages exposed to inflammatory conditions, similar to those found at the atherosclerotic site (Gafencu et al. 2007). Tumor progression locus 2 (Tpl2) and mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1) were identified as the kinases that are primarily responsible for the downregulation of apoE promoter activity by LPS. Tpl2 and MEKK1 signaling pathways converge to NF- κ B and AP1, acting on the apoE core promoter -55/+73 (Gafencu et al. 2007).

1.5.2 Distal Regulatory Binding Sites That Modulate apoE Gene Expression in Macrophages

Despite this complex transcription factor machinery that may be targeted to the apoE promoter, the promoter itself lacks the ability to direct gene transcription *in vivo* in any cells, in the absence of the distal enhancers (Shih et al. 2000). In many tissues, cell-specific distal enhancers regulate the expression of genes in the apoE/apoC-I/apoC-I'/apoC-IV/apoC-II gene cluster (Shih et al. 2000). The expression of apoE in macrophages is controlled by two homologous enhancers (95 % identical in sequence), designated as multienhancer 1 (ME.1) and multienhancer 2 (ME.2), containing 620 and 619 nucleotides, respectively (Shih et al. 2000). These enhancers are located at 3.3 and 15.9 kb downstream of the apoE gene, respectively. We demonstrated by chromosome conformational capture (3C) and transient transfections that both ME.1 and ME.2 can interact with the apoE promoter only in phorbol 12-myristate 13-acetate (PMA)-differentiated macrophages, but not in undifferentiated monocytes (Trusca et al. 2011). The results showed that the interactions take place in antisense orientation of the promoter and ME.1/2. Our data obtained using a series of deletion mutants of the promoter or of the ME.2 identified the fragment $-100/+73$ as the minimal region of the apoE promoter that is activated by the ME.2. We showed that the entire sequence of ME.2 is necessary for an optimal interaction with the apoE promoter, but the 5' region of ME.2 is more important than 3' region for enhancing apoE promoter activity (Trusca et al. 2011). The interaction of the apoE promoter with ME.1/2 facilitates the transcriptional enhancement of the apoE gene by various transcription factors.

LXR α and LXR β and their oxysterol ligands are key regulators of apoE expression in macrophages (Laffitte et al. 2001b; Joseph et al. 2002b; Mak et al. 2002b). The ability of oxysterols and synthetic ligands to regulate apoE expression in peritoneal macrophages as well as in adipose tissue is reduced in LXR $\alpha^{-/-}$ or LXR $\beta^{-/-}$ mice and abolished in double knockouts. However, basal expression of apoE is not compromised in LXR null mice, suggesting that LXRs mediate lipid-inducible expression rather than tissue-specific expression of this gene (Laffitte et al. 2001b). Data revealed that LXR/RXR binds to a low-affinity LXRE present in the apoE promoter as well as a high-affinity site conserved in both human ME.1 and ME.2 (Laffitte et al. 2001b). Experimental data revealed that the ligand activation of the LXR/RXR heterodimer enhanced the activity of the reporter constructs under the control of human ME.1 or ME.2 fused to the apoE proximal promoter (Laffitte et al. 2001b). Oxysterol-binding protein-related protein 1S (ORP1S) translocates from the cytoplasm to the nucleus in response to sterol binding and then binds to LXRs, promoting the binding of LXRs to LXREs. Thus, ORP1S mediates the LXR-dependent transcription via the ME.1 and ME.2 of the apoE gene (Lee et al. 2012). An interesting finding was that the induction of apoE gene expression by LXR agonists is attenuated by inhibitors of JNK and PI3K pathways (Huwait et al. 2011). A similar inhibition was noticed in the case of TGF β -induced expression of apoE, which was prevented by pharmacological inhibitors of JNK, p38 kinase, and casein kinase 2 (Singh and Ramji 2006).

The synthetic glucocorticoid receptor (GR) ligand, dexamethasone, increased apoE mRNA levels in mature macrophages up to sixfold over basal levels (Zuckerman et al. 1993). In silico analysis of the ME.1 and ME.2 revealed some transcription factor binding motifs for the GR (Shih et al. 2000). The presence of these GR binding sites in the multienhancers may explain the apoE upregulation by GR ligands, but the biological activity of these GR binding sites remains to be revealed.

TRANSFAC analysis of the apoE promoter, ME.1, and ME.2 showed that STAT1 transcription factor has a binding site only on the ME.2. Our data showed that this binding site is biologically active and STAT1 specifically upregulates apoE gene expression via ME.2, in macrophages, but not in hepatocytes. The STAT1 binding site was located in the 174/182 region of ME.2 (Trusca et al. 2011). Interestingly, a simultaneous increase in the expression of apoE and STAT1 was recorded after monocyte differentiation with PMA treatment (for 4 h). Our model proposed that after DNA bending, which probably takes place during monocyte differentiation, STAT1 bound on ME.2 interacts with the transcription initiation complex, leading to the activation of apoE expression. In addition, STAT1 can interact and cooperate with other transcription factors bound on the ME.2 or on the apoE promoter, for the modulation of apoE gene expression. Recently, we have revealed that STAT1 can interact with RXR and modulate gene expression of the apoC-II gene (Trusca et al. 2012). Since RXR α binds to the ME.2, we can speculate that apoE expression in macrophages may be modulated by the STAT1-RXR α interactions, similarly with apoC-II.

1.6 Transcriptional Regulation of the Human apoM Gene in the Liver

Apolipoprotein M (apoM) belongs to the lipocalin protein superfamily and differs from typical water-soluble apolipoproteins by its tertiary structure (Dahlback and Nielsen 2009; Nielsen et al. 2009). ApoM is secreted primarily by the liver and associates with HDL particles through its retained N-terminal signal peptide (Axler et al. 2008; Christoffersen et al. 2008). The silencing of the endogenous apoM gene in mice showed a loss of pre- β -HDL particles and formation of large HDL particles (Wolfrum et al. 2005). In addition to its role in HDL remodeling, it was shown that apoM is the sole carrier of the bioactive lipid sphingosine 1 phosphate (S1P) in HDL, thus mediating many of the atheroprotective properties of HDL in the endothelium (Christoffersen et al. 2011; Arkensteijn et al. 2013; Christoffersen and Nielsen 2013).

The expression of apoM in the liver is primarily controlled by hepatocyte nuclear factor-1 α (HNF-1 α) (Richter et al. 2003). HNF-1 α ^{-/-} mice are characterized by the complete absence of apoM from plasma. The plasma concentrations of other apolipoproteins in HNF-1 α ^{-/-} mice were either similar (apoA-II, apoB, apoC) or increased (apoA-I, apoE) compared to wild-type mice. This was not due to the absence of apoM since restoration of apoM gene expression

in the liver via adenovirus-mediated gene transfer could not rescue the abnormal apolipoprotein profile (Wolfrum et al. 2005). The analysis of the plasma lipoprotein profile of HNF-1 α ^{-/-} mice showed that similar to the apoM gene-silenced mice, plasma cholesterol was primarily associated with the HDL fraction. In addition, an abnormal large apoE-enriched HDL fraction that was identified as HDLc or HDL1 was observed, suggesting that this abnormal lipid profile in HNF-1 α ^{-/-} mice may be caused by the lack of apoM (Shih et al. 2001). In humans, HNF-1 α regulates apoM gene expression through direct binding to a conserved DNA element located in the proximal apoM promoter region between nucleotides -55 and -41 (Richter et al. 2003).

ApoM gene expression in the liver is negatively regulated during inflammation or infection via pro-inflammatory cytokines such as TNF α or IL-1 β (Feingold et al. 2008). The HNF-1 α binding element in the proximal human apoM promoter is a dual-specificity regulatory element that mediates the activation or repression of apoM promoter activity by HNF-1 and by activator protein 1 (AP1) proteins (c-Jun and JunB), respectively, in hepatic cells (Mosialou et al. 2011). Competition experiments showed that the binding of Jun proteins and HNF-1 α to the apoM promoter is mutually exclusive and chromatin immunoprecipitation assays established that AP1 activation leads to the recruitment of c-Jun and JunB proteins to the proximal apoM promoter with the simultaneous displacement of HNF-1 (Mosialou et al. 2011). A similar mechanism of transcriptional repression via dual-specificity AP1/HNF-1-responsive elements has been demonstrated in the case of the promoter of the human apolipoprotein A-II gene (Mosialou et al. 2011). AP1 factors were shown to inhibit the promoters of the apolipoprotein C-III (Hadzopoulou-Cladaras et al. 1998) and ABCA1 (Mosialou and Kardassis unpublished) genes in hepatic cells suggesting a broader role of AP1 factors in lipoprotein metabolism in the liver during inflammation.

Besides HNF-1 α , apoM gene transcription in the liver has been shown to be controlled positively by liver receptor homologue-1 (LRH-1) and forkhead box A2 (FOXA2) transcription factors which bind to distinct sites on the proximal apoM promoter (Venteclef et al. 2008; Wolfrum et al. 2008). Bile acids suppress apoM expression in vivo by inhibiting LRH-1 transcriptional activity via the recruitment of small heterodimer partner (SHP) to the apoM promoter (Venteclef et al. 2008).

Insulin, insulin-like growth factor I (IGF-I), and IGF-I potential peptide (IGF-IPP) were all shown to inhibit apoM gene expression in a dose- and time-dependent manner in primary human and murine hepatocytes via a signal transduction pathway that involves the serial activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (PKB) and the inactivation of Foxa2 (Xu et al. 2006). In HepG2 cells, glucose and insulin inhibited apoM gene expression in an additive manner, while in hyperglycemic rats, serum apoM concentrations and hepatic apoM mRNA levels were significantly reduced (Zhang et al. 2007).

The human apoM gene is under the control of various orphan- and ligand-dependent nuclear receptors (Mosialou et al. 2010). The overexpression via adenovirus and silencing via siRNA established that HNF-4 is an important regulator of apoM gene transcription in hepatic cells (Mosialou et al. 2010). In addition to

HNF-4, homodimers of retinoid X receptor and heterodimers of retinoid X receptor with receptors for retinoic acid, thyroid hormone, fibrates (peroxisome proliferator-activated receptor), and oxysterols (liver X receptor) were shown to bind with different affinities to the proximal HRE in vitro and in vivo (Mosialou et al. 2010). These findings provide novel insights into the role of apoM in the regulation of HDL by steroid hormones and into the development of novel HDL-based therapies for diseases such as diabetes, obesity, metabolic syndrome, and coronary artery disease that affect a large proportion of the population in Western countries.

1.7 Transcriptional Regulation of the CETP Gene

The gene encoding the cholesterol ester transfer protein (CETP) plays an important role in human HDL metabolism because it facilitates the transfer of cholesteryl esters from mature spherical HDL particles to VLDL/IDL lipoproteins in exchange of triglycerides and its activity determines the plasma levels of HDL cholesterol (von Eckardstein et al. 2005; Tall et al. 2008).

The CETP gene is expressed mainly in the liver, adipose tissue, and spleen and at lower levels in the small intestine, adrenal, kidneys, and heart (Jiang et al. 1991; Radeau et al. 1995). Atherogenic diets were shown to increase CETP mRNA levels in rabbits and in human CETP transgenic mice (Quinet et al. 1990; Jiang et al. 1992). Both LXRs and SREBPs were shown to bind to regulatory elements on the promoter of the CETP gene and regulate its transcription in response to intracellular cholesterol levels (Gauthier et al. 1999; Luo and Tall 2000). It was shown recently that synthetic LXR agonists enhanced plasma CETP activity and decreased HDL-C levels in cynomolgus monkeys and human CETP transgenic mice (Honzumi et al. 2010). The induction of CETP gene expression by the LXR agonist was significantly reduced by knocking down the expression of LXR α but not LXR β both ex vivo and in mice (Honzumi et al. 2010). In another study, it was shown that the LXR agonist T0901317 markedly increased CETP mRNA levels and CETP production in human differentiated macrophages but not in human peripheral blood monocytes (Lakomy et al. 2009). In inflammatory mouse and human macrophages, LXR-mediated CETP gene upregulation was inhibited and this inhibition was independent of lipid loading. It was concluded that LXR-mediated induction of human CETP expression is switched on during monocyte-to-macrophage differentiation and is abrogated in inflammatory macrophages (Lakomy et al. 2009).

Other factors that regulate CETP promoter activity include Yin Yang 1 (YY1) that binds to the same element as SREBPs (Gauthier et al. 1999), the LRH-1 that potentiates the sterol-mediated induction of the CETP gene by LXRs (Luo et al. 2001), the orphan nuclear receptor ARP-1 (Gaudet and Ginsburg 1995) that inhibits CETP promoter activity, the retinoic acid receptor (RAR) which regulates CETP gene expression in response to all-*trans*-retinoic acid (Jeoung et al. 1999), and the CCAAT/enhancer-binding protein (C/EBP) which is an activator of CETP

gene expression (Agellon et al. 1992). Binding sites for the ubiquitous transcription factors SP1 and SP3 have been identified on the CETP promoter at positions -690 , -623 , and -37 and seem to be essential for the basal CETP promoter activity (Le Goff et al. 2003).

CETP gene expression was shown recently to be under regulation by bile acids and their nuclear receptor farnesoid X receptor (FXR) (Gautier et al. 2013). It was shown that plasma CETP activity and mass was higher in patients with cholestasis than controls and this was associated with lower HDL-C levels (Gautier et al. 2013). In agreement with this observation, bile acid feeding of APOE3*Leiden mice expressing the human CETP transgene controlled by its endogenous promoter decreased HDL-C and increased plasma CETP activity and mass. An FXR response element (FXRE) was identified in the first intron of the human CETP gene which could be responsible for the upregulation of CETP gene expression in response to bile acids (Gautier et al. 2013). In another study, it was shown that FXR α binds to DR4 LXRE that is present in the proximal CETP promoter and represses LXR-mediated transactivation of the CETP promoter by a competition mechanism (Park et al. 2008).

1.8 Transcriptional Regulation of the PLTP Gene

Phospholipid transfer protein (PLTP) belongs to the lipopolysaccharide (LPS) binding/lipid transfer gene family that includes the LPS-binding protein (LBP), the neutrophil bactericidal/permeability-increasing protein (BPI), and the cholesteryl ester transfer protein (CETP). PLTP is essential in the transfer of very low-density lipoprotein phospholipids into HDL (Jiang et al. 2012).

PLTP is expressed ubiquitously, but the highest expression levels in human tissues were observed in the ovary, thymus, placenta, and lung (Day et al. 1994). Taking into account the organ size involved, the liver and small intestine appear to be important sites for the overall PLTP expression. A high-fat, high-cholesterol diet causes a significant increase in PLTP activity and in mRNA levels. Plasma PLTP activity and PLTP mRNA levels in the liver and adipose tissues were significantly decreased following LPS administration (Jiang and Bruce 1995).

An FXR-responsive element (FXRE) has been found in the proximal PLTP promoter that binds FXR α /RXR α heterodimers and mediates the response of the PLTP promoter to bile acids (Urizar et al. 2000). Fibrates were shown to increase PLTP gene expression by activating PPARs which bind to three PPAR-responsive elements on the PLTP promoter (Tu and Albers 1999; Bouly et al. 2001). Two of these PPAR-responsive elements also seem to be responsible for the induction of PLTP expression by high glucose (Tu and Albers 2001).

The human PLTP promoter contains at least two LXR-responsive elements, one in the proximal and one in the distal region, that were shown to mediate PLTP gene regulation by oxysterols *ex vivo* and *in vivo* (Cao et al. 2002; Mak et al. 2002a; Laffitte et al. 2003). It was recently demonstrated that LXR agonists activate triglyceride synthesis and PLTP gene transcription by activating SREBP-1c

(Okazaki et al. 2010). In concert with the increase in triglyceride synthesis, the increased PLTP caused triglyceride incorporation into abnormally large VLDL particles which were removed from plasma by LDL receptors, whereas in the absence of LDL receptors, the large VLDLs accumulated and caused massive hypertriglyceridemia (Okazaki et al. 2010).

Recently, microarray analysis following alteration of p53 status in several human- and mouse-derived cells identified a group of 341 genes whose expression was induced by p53 in the liver-derived cell line HepG2 (Goldstein et al. 2012). Twenty of these genes encode proteins involved in many aspects of lipid homeostasis including PLTP (Goldstein et al. 2012).

1.9 Transcriptional Regulation of the Bile Acid Transporters ABCG5/ABCG8

ATP-binding cassette half-transporters G5 and G8 (ABCG5 and ABCG8) play important roles in the control of sterol excretion from the liver (Fitzgerald et al. 2010; Tarling and Edwards 2012; Li et al. 2013; Yu et al. 2014). Mutations in either of these transporters leads to β -sitosterolemia, an autosomal recessive disease characterized by premature coronary atherosclerosis and elevated levels of phytosterols in plasma (Fitzgerald et al. 2010; Tarling and Edwards 2012; Li et al. 2013; Yu et al. 2014). Mice lacking ABCG5 and ABCG8 proteins have decreased ability to secrete sterols into the bile (Yu et al. 2002a). The overexpression of ABCG5 and ABCG8 in the liver increases biliary cholesterol secretion and decreases dietary cholesterol absorption (Yu et al. 2002b). The human ABCG5 and ABCG8 genes are oriented in a head-to-head configuration, they are transcribed in opposite directions, and their transcription is coordinated by a short 374 bp bidirectional promoter in the intergenic region (Remaley et al. 2002).

The bidirectional promoter of ABCG5/ABCG8 genes contains a binding site for LRH-1 at positions 134–142 which is required for the activity of both the ABCG5 and ABCG8 promoters (Freeman et al. 2004). Mutating this LRH-1 binding site reduced promoter activity of the human ABCG5/ABCG8 intergenic region in HepG2 and Caco2 cells. Bile acids such as deoxycholic acid repressed ABCG5 and ABCG8 promoters via the FXR-SHP-LXR pathway that was described above (Sumi et al. 2007).

Dietary cholesterol feeding was shown to increase duodenal, jejunal, and hepatic expression levels of ABCG5 and ABCG8 mRNA in wild-type mice (Berge et al. 2000). The increase in ABCG5 or ABCG8 gene expression by diet was compromised in mice lacking either LXR α or both LXR α and LXR β (Repa et al. 2002). Both the RXR-specific agonist LG268 and the LXR-specific agonist T0901317 caused upregulation of ABCG5 and ABCG8 mRNA expression in the liver and intestine of wild-type mice but not in LXR α/β ^{-/-} mice (Repa et al. 2002). To identify functional LXREs that control the expression of the ABCG5/ABCG8 genes in response to oxysterols, a recent study searched for evolutionarily conserved regions (ECRs) between the human and the mouse genes and identified

23 ECRs which were studied by luciferase assays for LXR responsiveness (Back et al. 2013). Two ECRs were found to be responsive to the LXR and binding of LXR α to these regions was verified (Back et al. 2013).

The bidirectional promoter of the ABCG5/G8 genes was shown to bind HNF-4 and GATA transcription factors and to be regulated by these factors in a cooperative manner and independent of the orientation of the bidirectional promoter (Sumi et al. 2007).

It was shown that the expression of both ABCG5 and ABCG8 genes is upregulated in the livers of mice with genetic ablation of the insulin receptor gene (LIRKO mice) both at the mRNA and the protein levels (Biddinger et al. 2008). In agreement with these findings, insulin suppressed the expression of ABCG5 and ABCG8 genes at subnanomolar concentrations and in a dose-responsive manner in rat hepatoma cells (Biddinger et al. 2008). The observation that the short intergenic region responded to insulin in both the ABCG5 and ABCG8 orientations suggested the presence of an element in the intragenic region of the ABCG5 and ABCG8 genes that responds to insulin. Using *ex vivo* and *in vivo* approaches, it was shown that insulin resistance leads to the activation of the forkhead box 1 (FOXO1) transcription factor which binds to the bidirectional promoter and activates the transcription of both genes severalfold (Biddinger et al. 2008).

1.10 Transcriptional Regulation of the HDL Receptor SR-BI

The gene encoding the HDL receptor scavenger receptor class B type I (SR-BI) is expressed at high levels in the liver and steroidogenic tissues. Several transcription factors have been shown to bind to the human or rodent SR-BI promoter and to regulate SR-BI gene transcription in a positive or negative manner.

The steroidogenic factor-1 (SF-1) has been shown to regulate both the human and rat SR-BI promoters and to serve as mediator of the cAMP-dependent regulation of the SR-BI gene in response to steroidogenic hormones (Lopez et al. 1999).

Liver X receptors α and β and PPAR α and γ were shown to bind to distal LXRE and PPARE, respectively, on the human and rat SR-BI promoters and regulate the expression of the human SR-BI gene in response to oxysterols and fibrates (Lopez and McLean 1999; Malerod et al. 2002, 2003), whereas HNF-4 enhances the PPAR γ -mediated SR-BI gene transcription (Malerod et al. 2003; Zhang et al. 2011). As discussed above, conditional inactivation of the HNF-4 gene in the liver of adult mice was associated with a significant increase in hepatic SR-BI mRNA levels and a decrease in plasma HDL-C levels, suggesting that HNF-4 influences negatively the expression of the HDL receptor (Hayhurst et al. 2001).

LRH-1 binds to a proximal response element on the human SR-BI promoter in an overlapping manner with SF-1 and activates the SR-BI promoter (Schoonjans et al. 2002). Retrovirus-mediated overexpression of LRH-1 in hepatic cells induced SR-BI gene expression, and this was associated with histone H3 acetylation on the SR-BI promoter. In agreement with these findings, the SR-BI mRNA levels were

decreased in the livers of LRH-1(+/-) animals providing evidence that LRH-1 regulates SR-BI gene expression in vivo (Schoonjans et al. 2002).

Estrogens regulate the activity of the rat SR-BI promoter via estrogen receptors α and β (ER α and β) which bind to three different estrogen response elements (ERE) on the SR-BI promoter (Lopez et al. 2002; Lopez and McLean 2006). In endothelial cells, 17 β -estradiol (E2) increased the mRNA levels of the human SR-BI gene and the activity of the hSR-BI promoter, and this upregulation was protein kinase C (PKC) dependent since it was blocked by the PKC inhibitor bisindolylmaleimide I and a dominant-negative mutant of PKC (Fukata et al. 2013).

The mRNA levels of the mouse SR-BI gene were decreased in mice lacking the FXR nuclear receptor (FXR^{-/-} mice) (Lambert et al. 2003). When WT mice were placed on a diet containing 0.4 % of the FXR agonist cholic acid, the hepatic SR-BI mRNA and protein levels increased in the wild-type but not in the FXR^{-/-} mice, indicating that bile acids positively regulate SR-BI gene expression via FXRs (Lambert et al. 2003). In agreement with these findings, treatment of human hepatoma HepG2 cells with FXR ligands resulted in the upregulation of SR-BI both at the mRNA and protein levels via FXR binding to a novel FXRE, a direct repeat 8 at position -703/-684 of the promoter (Chao et al. 2010). A natural ligand of FXR administered to mice increased hepatic SR-BI expression (Chao et al. 2010). However, in another study, it was reported that bile acids inhibit SR-BI gene expression in the liver of mice and reduce the SR-BI promoter activity (Malerod et al. 2005). It was proposed that this inhibition was due to the FXR-mediated activation of SHP, which repressed the activity of LRH-1 that binds to the proximal SR-BI promoter (Malerod et al. 2005).

The zinc finger transcription factor Kruppel-like factor 4 (KLF4) was shown to bind to a putative KLF4 element on the SR-BI promoter at position -342/-329 and upregulate its activity in peripheral blood mononuclear cells and PMA-differentiated THP-1 cells treated with HDL (Yang et al. 2010).

Using high-throughput screening of 6,000 microbial secondary metabolite crude extracts for the identification of compounds that upregulate the SR-BI promoter in HepG2 cells, several putative SR-BI upregulators were identified: hoxy1-3',5,7-hydroxyl isoflavone; (9R,13S)-7-deoxy-13-dihydrodaunomycinone; pratensein; the isoflavones formononetin, genistein, and daidzein; and the histone deacetylase inhibitor trichostatin A (Yang et al. 2007, 2009; Bao et al. 2009). Some of these compounds were shown to increase the uptake of DiI-labeled HDL and the efflux of cholesterol to HDL by cells.

In steroidogenic tissues, SR-BI supplies the cells with exogenous cholesterol for storage or for the synthesis of steroid hormones. In these tissues, SR-BI expression was shown to be upregulated by adrenocorticotrophic hormone (ACTH) (Sun et al. 1999). The suppression of ACTH by the synthetic corticosteroid dexamethasone (which inhibits the hypothalamic-pituitary axis and decreases ACTH secretion) decreased SR-BI levels. However, the mechanism by which ACTH and glucocorticoid regulate the expression of the SR-BI gene in steroidogenic tissues is unclear. It was shown that the transcription of the human SR-BI gene is subject to feedback inhibition by glucocorticoids in adrenal and ovarian cells. SR-BI mRNA

levels were increased in adrenals from corticosterone-insufficient $\text{Crh}^{-/-}$ mice, whereas corticosterone replacement by oral administration inhibited SR-BI gene expression in these mice (Mavridou et al. 2010). The glucocorticoid-mediated inhibition of SR-BI gene transcription required de novo protein synthesis and the glucocorticoid receptor (GR). No direct binding of GR to the SR-BI promoter could be demonstrated in vitro and in vivo, suggesting an indirect mechanism of repression of SR-BI gene transcription by GR in adrenal cells (Mavridou et al. 2010).

In the rat ovary, the uptake of cholesterol by the theca-interstitial cells is mediated by SR-BI. It was shown that insulin and the trophic hormone LH/cGH stimulate the expression of SR-BI in theca-interstitial cells and increase intracellular cholesterol, which is subsequently mobilized for androgen biosynthesis (Li et al. 2001; Towns et al. 2005).

In the adrenocortical cell line Y-1, adenovirus-mediated overexpression of prolactin regulatory element-binding (PREB) protein, a transcription factor that regulates prolactin expression in the anterior pituitary and is induced by cAMP, increased the levels of SR-BI protein and the activity of the SR-BI promoter by binding to a PREB-responsive *cis*-element of the SR-BI promoter. Using small interfering RNA against PREB in Y-1 cells, the effects of cAMP on SR-BI expression were attenuated. It was concluded that in the adrenal gland under conditions of cAMP increase, PREB regulates the transcription of the SR-BI gene (Murao et al. 2008).

Intracellular sterol levels regulate SR-BI gene expression via SREBP-1a which binds to two sterol-responsive elements (SREs) present on the rat SR-BI promoter (Cao et al. 1997; Lopez and McLean 1999). The ubiquitous transcription factors SP1 and SP3 bind to several GC-rich boxes present on the proximal SR-BI promoter and have been shown to be important for the basal activity as well as the SREBP-1a-mediated transactivation of the SR-BI promoter (Shea-Eaton et al. 2001).

The expression of the SR-BI gene is also subject to negative regulation by transcription factors including the orphan nuclear receptor dorsal-sensitive sex adrenal hypoplasia congenital critical region on the X chromosome gene 1 (DAX-1), which was shown to repress the rat SR-BI promoter by interfering with the SF-1- and SREBP-1a-mediated transactivation of the this promoter (Lopez et al. 2001). In addition, YY1 transcription factor binds directly to two sites on the SR-BI promoter and downregulates its activity by interfering with the binding of SREBP-1a to the SR-BI promoter (Shea-Eaton et al. 2001).

2 Posttranscriptional Regulation of HDL Genes by Noncoding RNAs and microRNAs

According to the Encyclopedia of DNA Elements (ENCODE project), 76 % of the human genome is transcribed (Bernstein et al. 2012; Djebali et al. 2012). While novel promoter and enhancer regions have been described (Bernstein et al. 2012; Sanyal et al. 2012), some of the newly described genes encode for noncoding RNAs

including microRNAs (miRNAs), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), circular RNAs (circRNAs), long noncoding RNAs (lncRNAs), *trans*-acting siRNAs (tasiRNAs), and several other noncoding RNAs. Their abundance and their recognized roles in transcriptional and epigenetic gene regulation and their involvement in several diseases suggest the existence of an extensive regulatory network on the basis of RNA signaling (Mattick and Makunin 2005). An important class of these endogenous noncoding RNAs is miRNAs. miRNAs are small noncoding RNAs (~22 nt) that have emerged as important posttranscriptional regulators of different protein-coding genes (Bartel 2009) including those related to HDL metabolism (Davalos and Fernandez-Hernando 2013).

2.1 miRNAs: Biogenesis and Function

Although originally described as regulators of developmental timing in *Caenorhabditis elegans* (Lee et al. 1993; Wightman et al. 1993), miRNAs did not receive special attention until their widespread identification in different species (Lagos-Quintana et al. 2002; Reinhart et al. 2002) and their role in human diseases was uncovered (Calin et al. 2002). miRNAs are important posttranscriptional regulators of gene expression through sequence-specific complementary binding to the 3' untranslated region (UTR) of the target mRNA (Bartel 2009), even though certain miRNAs can interact with other target mRNA regions including the 5' UTR, coding region, or intron–exon junction and even increase rather than decrease target mRNA expression (Vasudevan et al. 2007; Orom et al. 2008; Tay et al. 2008; Schnall-Levin et al. 2010). The interaction of a miRNA with its target mRNA results in the inhibition of translation and/or degradation of mRNAs (Guo et al. 2010; Krol et al. 2010).

miRNA biogenesis and function have been intensively studied in recent years (Bartel 2009; Krol et al. 2010). Briefly, in most mammals, these small RNAs are transcribed by RNA polymerase II into a primary long miRNA (pri-miRNA). The pri-miRNA is then processed in the nucleus into an ~70 nucleotide precursor hairpin (pre-miRNA) by a multiprotein complex containing different cofactors and two core components, a ribonuclease III (Drosha) and a double-stranded RNA-binding domain protein (DGCR8/Pasha). Some intronic miRNAs that bypass Drosha-mediated processing are produced by splicing and debranching (miRtrons). Pre-miRNAs are transported to the cytoplasm by Exportin 5 in a Ran-GTP-dependent manner (Lund et al. 2004) where they are further cleaved by an RNase III enzyme called Dicer, generating the mature ~22 nt long miRNA/miRNA* duplex. Dicer-independent miRNA biogenesis has also been described (Cifuentes et al. 2010). The duplex miRNA is separated and one of the strands associates with an argonaute protein (Ago) within the RNA-induced silencing complex (RISC), guiding the complex to the complementary sites within the 3' UTR of the target mRNA to induce mRNA repression and/or degradation. Although the miRNA/miRNA* duplex is initially produced in equal amounts, the miRNA*

strand (passenger strand) is usually degraded. However, it also contains target recognition sites and is thus functional (Yang et al. 2011). Argonaute proteins are major players in miRNA-mediated gene regulation (Meister 2013). Although several aspects of miRNA biogenesis and function are well characterized, the different factors that regulate their decay or turnover are not well known (Krol et al. 2010).

Sequencing studies have identified ~2,000 miRNAs encoded in our human genome, and prediction algorithms suggest that 60 % of human protein-coding genes have conserved targets for pairing with miRNAs within their 3' UTR (Friedman et al. 2009). Factors that influence miRNA target selection include the “seed” sequence which consists of nucleotides 2–8 at the 5' end of the mature miRNA (Bartel 2009) and tissue distribution or developmental stage, as certain miRNAs are highly expressed or even restricted to certain cell types and can only target their mRNA target if they are co-expressed in the same tissue at the same time (Lagos-Quintana et al. 2002; Small and Olson 2011). In contrast to most plant miRNAs, which bind with near perfect complementarity to their targets, most mammalian miRNAs bind with mismatches and bulges and the mode of binding determines the type of posttranscriptional repression (Carthew and Sontheimer 2009). Recent experimental data suggest that around 60 % of seed interactions are noncanonical, containing bulged or mismatched nucleotides which are accompanied by specific, non-seed base pairing (Helwak et al. 2013). Some miRNA-mRNA interactions also involve the 3' end of the miRNA (Helwak et al. 2013). Moreover, pseudogenes (Poliseno et al. 2010), long noncoding RNAs (lncRNAs) (Cesana et al. 2011), and circular RNAs (Hansen et al. 2013) that contain miRNA binding sites also influence miRNA activity, acting as competing endogenous RNAs (ceRNAs) and thus sequestering miRNAs and preventing them from binding to their mRNA targets (Salmena et al. 2011).

While the primary role of miRNAs seems to be the “fine-tuning” of gene expression, their capacity to simultaneously bind and repress multiple target genes and similarly the possibility of a single mRNA to be targeted by multiple miRNAs provide a mechanism to synchronize the coordinated regulation of a large number of transcripts that govern an entire biological process, thus resulting in strong phenotypic output (Mendell and Olson 2012). The high redundancy among related and non-related miRNAs in regulating gene expression reduces the importance of a particular miRNA under conditions of normal cellular homeostasis. However, compelling evidence suggests that under conditions of stress the function of miRNAs become especially pronounced (Mendell and Olson 2012) and their pharmacological modulation represents a novel approach to treat disease by modulating entire biological pathways as described in Davalos and Chroni (2014).

Compelling evidences have shown that miRNAs are present in the systemic circulation and other human biological fluids associate with extracellular microvesicles, exosomes (Valadi et al. 2007; Hunter et al. 2008), Ago2 complex (Arroyo et al. 2011), or HDL (Vickers et al. 2011). Even exogenous miRNAs have been described in our circulating plasma (Wang et al. 2012b; Zhang et al. 2012). Although several circulating miRNAs have been implicated as disease biomarkers

in several diseases (Allegra et al. 2012; Kinet et al. 2013) or secreted for intercellular communication (Chen et al. 2012b), the biological significance, the factors that modulate their extracellular secretion, and the mechanisms by which they reach the target tissue still remain elusive. HDL has been shown to transport and deliver endogenous miRNAs to recipient cells with functional targeting capabilities (Vickers et al. 2011). Although the HDL miRNA signature from normal subjects was found to be different from hypercholesterolemic subjects (Vickers et al. 2011), the extent by which HDL-bound miRNAs contribute to total circulating miRNAs and thus regulate target cell gene function is not clear (Wagner et al. 2013).

In summary, miRNAs play a major role in almost every aspect of cellular function by posttranscriptional regulation of gene expression. Whether other non-coding RNAs (that modulate miRNA activity) or circulating or extracellular miRNAs might modulate HDL metabolism is not known. However, it seems obvious that by modulating miRNA activity, they might also have a role in HDL metabolism.

2.2 Posttranscriptional Modulation of HDL Metabolism by miRNAs

For normal function, mammalian cells must maintain cellular cholesterol levels within tight limits. Thus, complex mechanisms have been evolutionarily developed to control both cellular input from endogenous cholesterol biosynthesis or the uptake from circulating lipoproteins and cellular output by controlling cholesterol efflux. miRNAs have emerged as important regulators of HDL and cholesterol metabolism, and their functions are summarized in Fig. 5 and described in detail below. Although miRNAs have increased the complexity of HDL metabolism regulation, our understanding of every single step in their regulation will provide better opportunities to develop novel targets for their therapeutic modulation.

2.2.1 Targeting ABCA1 and ABCG1

ABCA1 is one of the most important proteins directly involved in the elimination of excess cholesterol from cells (cholesterol efflux). As described in this chapter, ABCA1 is regulated at the transcriptional, posttranscriptional, and posttranslational level. Its regulation is in accordance with the cellular need to handle cholesterol levels within tight limits, as excess of free cholesterol is deleterious to cells. ABCA1 mRNA contains a particularly long 3' UTR (>3.3 kb) as compared to other common genes involved in cholesterol and HDL metabolism including LCAT (20 bp), apoA-I (55 bp), ApoA-II (112 bp), CETP (178 bp), apoB (301 bp), PDZK1 (583 bp), ABCG1 (852 bp), SR-BI (959 bp), PCSK9 (1,269 bp), IDOL (1,496 bp), CAV-1 (1,898 bp), LIPG (2,386 bp), and LDLR (2,513 bp) (Davalos and Fernandez-Hernando 2013). This unusually long 3' UTR of ABCA1 clearly raises the probability of posttranscriptional regulation by miRNAs (and probably other noncoding RNAs). Different prediction algorithms indicate that ABCA1 can potentially be targeted by ~100 miRs. Some of them have been experimentally validated

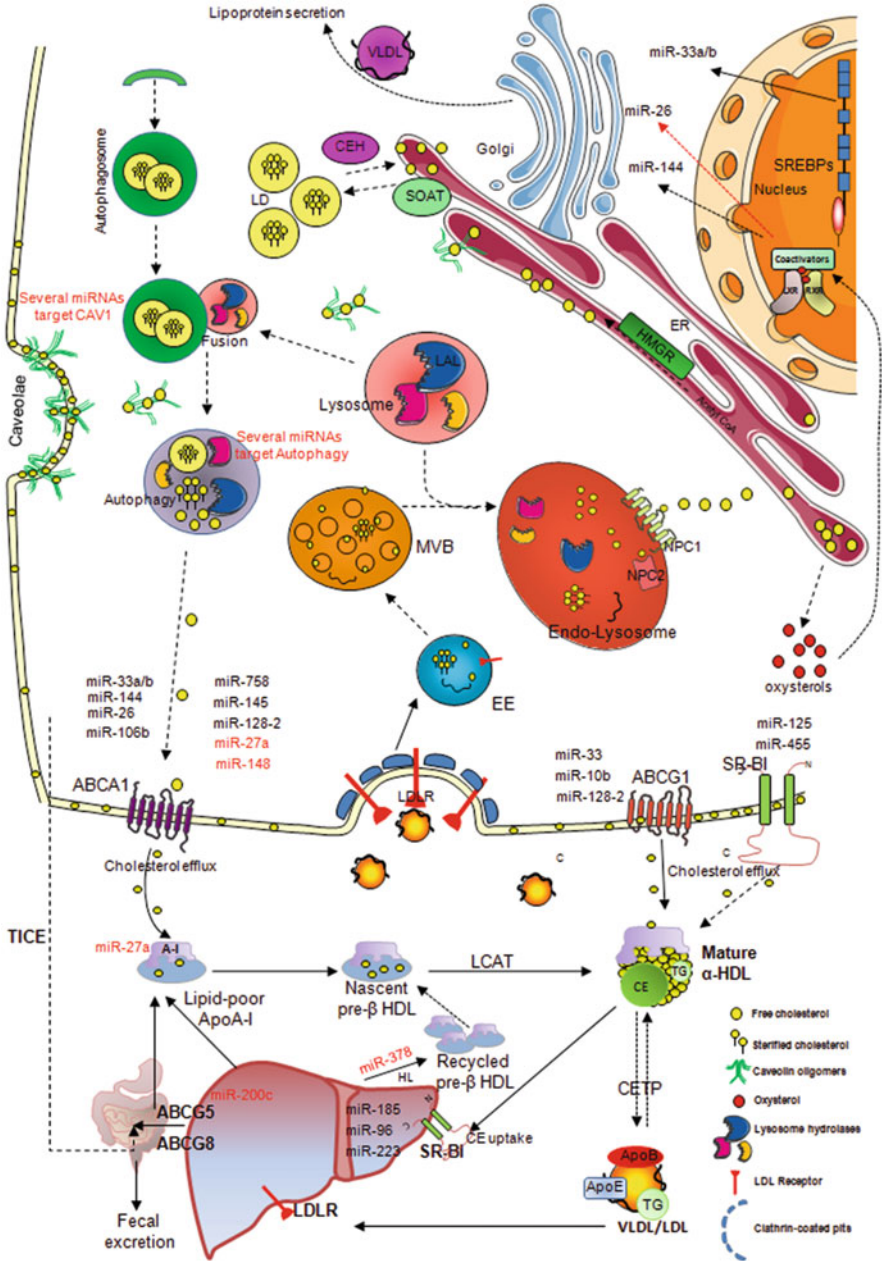


Fig. 5 Overview of posttranscriptional regulation by miRNAs of proteins involved in cholesterol efflux, RCT, and HDL metabolism. HDL metabolism and the role of individual proteins such as plasma enzymes, membrane transporters, and receptors are described in the text and more extensively in other chapters. Autophagy, the cell catabolism process through the **lysosomal machinery**, has been proposed to participate in cholesterol efflux. The induction of SREBPs induces the expression of miR-33 family. The induction of LXR induces the expression of miR-144 and represses miR-26. The induction of FXR also induces miR-144. miRNAs in black

for their importance in cholesterol efflux, reverse cholesterol transport, and cardiovascular disease, whereas other miRNAs still remain to be elucidated. Validated miRNAs that directly target ABCA1 are miR-33 family, miR-758, miR-106b, miR-26, miR-144, miR-10b, miR-128-2, and miR-145 (Fig. 5).

miR-33a and miR-33b play a crucial role in controlling cholesterol efflux and HDL function in concert with their host genes, the SREBP transcription factors (Najafi-Shoushtari et al. 2010; Horton et al. 2002; Horie et al. 2010; Marquart et al. 2010; Rayner et al. 2010). While both mature miRNAs only differ in two nucleotides, they are predicted to have largely overlapping sets of target genes in rodents (Rayner et al. 2011b; Horie et al. 2012) and nonhuman primates (Rayner et al. 2011a; Rottiers et al. 2013). The inhibition of the two miR-33 isoforms, either genetically or therapeutically, resulted in increased cholesterol efflux, increased HDL levels, increased reverse cholesterol transport, reduced atherosclerosis, and reduced VLDL triglyceride levels. Although miR-33a and miR-33b target different genes involved in lipid homeostasis, fatty acid β -oxidation, insulin signaling, and biliary transporters (Gerin et al. 2010; Davalos et al. 2011; Rayner et al. 2011a; Allen et al. 2012; Horie et al. 2013), their effects are mediated mainly by ABCA1. The human 3' UTR of ABCA1 has three functional binding sites for miR-33. While most of the studies have been performed in tissues/cells directly related to lipoprotein metabolism and CVD (i.e., hepatocytes, macrophages), it is possible that from these ~100 miRs that potentially target ABCA1 3' UTR, some of them might be relevant to other cells and tissues as well.

Regarding other tissues, miR-106b (Kim et al. 2012) and miR-758 (Ramirez et al. 2011) were found to regulate neuronal cholesterol excess. While miR-758 expression is particularly high in the brain and its expression is mediated by high cholesterol levels, the expression of miR-106 in brain tissues is low. However, their role in neuronal cholesterol efflux suggests that these miRNAs may contribute to the regulation of cholesterol levels in the brain. miR-758 and miR-106 directly target the 3' UTR of ABCA1 by binding to two sites and one site, respectively. Moreover, miR-758 has other targets involved in neurological functions (Ramirez et al. 2011). miR-106b also targets the amyloid precursor protein (APP) and increases the amyloid β (A β) peptide secretion and clearance (Kim et al. 2012).

HDL metabolism is regulated by the LXRs which control the transcription of several genes related to lipid metabolism in response to hydroxylated products of cholesterol as described above (Calkin and Tontonoz 2012). Recent data suggest that LXRs also regulate HDL metabolism posttranscriptionally (Sun et al. 2012; de Aguiar Vallim et al. 2013; Ramirez et al. 2013; Vickers and Rader 2013). The induction of LXR resulted in the repression of miR-26 and induction of miR-26-a-1,

Fig. 5 (continued) labels are validated miRNAs for HDL metabolism. miRNAs in red labels are suggested miRNAs, but not fully validated, in HDL metabolism. All biological processes may not necessarily happen in the same cell type. Abbreviations: endoplasmic reticulum (ER); early endosome (EE); cholesterol ester hydrolase (CEH); multivesicular bodies (MVB); lysosomal acid lipase (LAL) (Figure courtesy of Dr. Alberto Canfrán-Duque)

and miR-144 that directly target the 3' UTR of ABCA1 and thus regulate cholesterol efflux, RCT, and HDL levels. While having one binding site for miR-26-a-1 (Sun et al. 2012), the human 3' UTR of ABCA1 has several (up to seven) predicted binding sites for miR-144 (Ramirez et al. 2013). miR-26-a-1 also targets the ADP-ribosylation factor-like 7 (Arl7), which participates in cellular cholesterol efflux (Engel et al. 2004). miR-144 expression is also induced by the nuclear receptor FXR (Vickers and Rader 2013). By controlling bile acid levels, FXR activation will not only repress the expression of ABCA1 posttranscriptionally but will also induce the expression of SR-BI, thereby resulting in an increased uptake of plasma HDL cholesterol and increased cholesterol biliary excretion via ABCG5/ABCG8 (de Aguiar Vallim et al. 2013).

Evolutionarily, the dual effect of LXR activation on miR-144 induction and miR-26-a-1 repression seems obvious, as cells must maintain cellular cholesterol levels within tight limits and these miRNAs might work as buffers against deleterious variation in gene expression programs.

Other miRNAs including miR-10b, miR-128-2, and miR-145 also regulate cellular cholesterol efflux by directly targeting the 3' UTR of ABCA1. Protocatechuic acid, an intestinal microbiota metabolite of cyanidin-3-O-glucoside, was found to repress miR-10b and thus regulate cholesterol efflux (Wang et al. 2012a). While in hepatocytes the inhibition of miR145 increases cholesterol efflux, in pancreatic islet beta cells it improves glucose-stimulated insulin secretion (Kang et al. 2013). The proapoptotic miRNA miR-128-2 was also found to regulate cholesterol efflux by targeting (one binding site) the 3' UTR of ABCA1 (Adlakha et al. 2013). Recent data suggest that this miRNA family governs neuronal excitability and motor behavior in mice (Tan et al. 2013) and that miR-128-2 might control neuronal cholesterol levels. RXR α is also a direct target of miR-128-2 (Adlakha et al. 2013). miR-27a and miR-148 were found to repress an ABCA1 3' UTR luciferase reporter construct; however, their physiological role in regulating cholesterol efflux has not been described (Kang et al. 2013).

ABCG1, the other member of the ATP-binding cassette transporter family, which also participates in cellular cholesterol efflux to HDL, was found to be directly regulated by some of these miRNAs. miR-33, miR-10b, and miR-128-2 regulate cholesterol efflux by posttranscriptionally regulating ABCG1 through binding to its 3' UTR (Rayner et al. 2010; Wang et al. 2012a; Adlakha et al. 2013).

2.2.2 Targeting SR-BI

Liver regulation of SR-BI is primarily achieved posttranslationally through a PDZ domain-containing adaptor protein (PDZK1) (Kocher et al. 2003; Kocher and Krieger 2009) as described in detail in a later section of this chapter. Increasing evidence suggests that other posttranscriptional mechanisms, such as targeting by miRNAs, also regulate SR-BI protein levels. Prediction miRNA analysis (www.targets.org) suggests that ~25 miRNA families might target SR-BI, most of which are poorly conserved among mammals and vertebrates. miR-125a and miR-455 were found to repress the lipoprotein-supported steroidogenesis by directly targeting the 3' UTR of SR-BI (Hu et al. 2012). In hepatic cells, where

SR-BI is expressed at higher levels, the expression of miR-125a was also high and its overexpression resulted in a reduced SR-BI-mediated selective cholesterol ester uptake. In contrast, miR-455 did not show any effect on hepatic SR-BI expression (Hu et al. 2012). miR-185, miR-96, and miR-223 were also found to directly target the 3' UTR of SR-BI and to repress HDL cholesterol uptake in hepatic cells (Wang et al. 2013a).

2.2.3 Targeting Other miRNAs Related to HDL Biogenesis and Function

miR-27a was suggested to regulate apoA-I plasma levels, but the direct interaction to its 3' UTR was not evaluated (Shirasaki et al. 2013). Several other miRNAs were proposed to regulate genes involved in cholesterol efflux, RCT, or HDL metabolism, including ABCG5 (Liu et al. 2012) or endothelial lipase (Kulyte et al. 2013), but either their direct interaction with the 3' UTR of these genes or their physiological effects on HDL metabolism were not directly assessed.

Autophagy is the catabolic process by which unnecessary or dysfunctional cellular components are degraded in the lysosome. During starvation, this breakdown of cellular components contributes to the maintenance of cellular energy levels. The hydrolysis of cytoplasmic cholesteryl ester of lipid droplets is normally mediated by the action of neutral cholesteryl ester hydrolases. It has been recently recognized that lipid droplets can also be delivered to lysosomes via autophagy, where lysosomal acid lipase can hydrolyze lipid droplet cholesteryl esters to generate free cholesterol for cholesterol efflux (Ouimet et al. 2011).

Several miRNAs have been described that regulate different targets in autophagy (Frankel and Lund 2012). miRNAs that target key pathways in lipid-loaded macrophage autophagy genes and/or cholesterol ester hydrolases might be promising targets to promote cholesterol efflux (Davalos and Fernandez-Hernando 2013). Caveolin, the major protein coat of caveolae, has been proposed to contribute to cellular cholesterol efflux (Truong et al. 2010; Kuo et al. 2011). There is accumulating evidence that several miRNAs including miR-103, miR-107, miR-133a, miR-192, miR-802, and others target caveolin (Nohata et al. 2011; Trajkovski et al. 2011) but their contribution to cholesterol efflux, RCT, and HDL metabolism and their real physiological contribution to HDL function remain to be elucidated.

3 Posttranslational Mechanisms of HDL Regulation

3.1 ABCA1

In addition to the transcriptional and posttranscriptional mechanisms described above, the expression of ABCA1 is also regulated at the posttranslational level. Following its synthesis, ABCA1 is inserted into the ER where it undergoes proper folding, N-glycosylation, dimerization, and disulfide bond formation and transport to the plasma membrane via the ER-Golgi system (Fig. 6) (Kang et al. 2010).

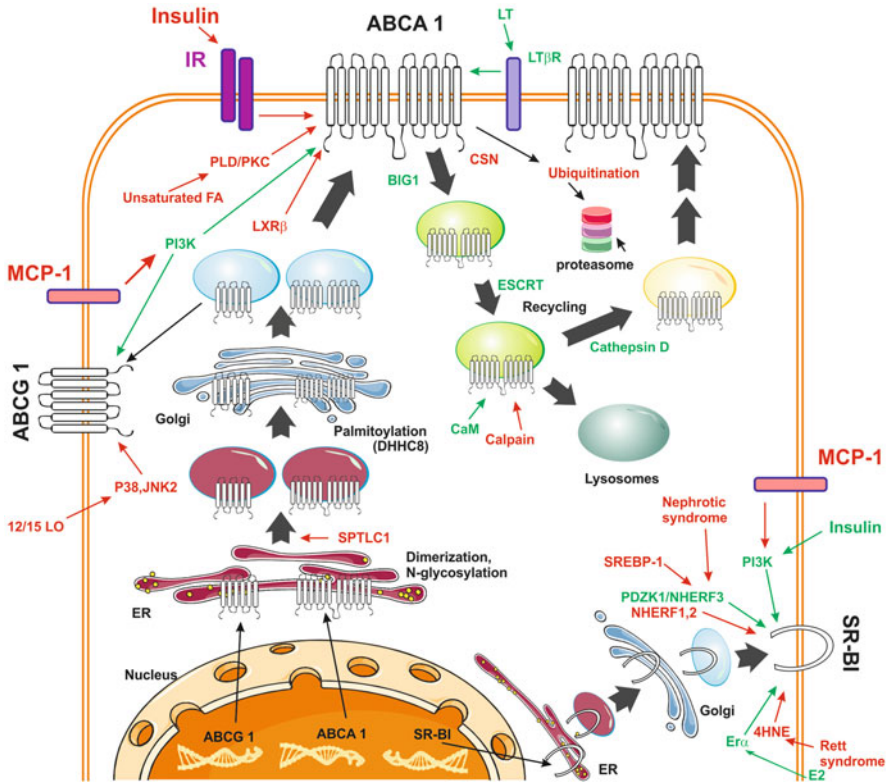


Fig. 6 Overview of the mechanisms that regulate the expression of ABCA1, ABCG1, and SR-BI genes at the posttranslational level. The mechanisms are described in the text. Abbreviations: endoplasmic reticulum (ER); Asp-His-His-Cys 8 (DHHC8); serine palmitoyltransferase 1 (SPTLC1); guanine nucleotide-exchange protein 1 (BIG1); endosomal sorting complex required for transport (ESCRT); Pro-GLu-Ser-Thr (PEST); calmodulin (Cam); lymphotoxin (LT); LT- β receptor (LT β R); phospholipase D (PLD); protein kinase C δ (PKC δ); insulin receptor (IR); phosphatidylinositol 3-kinase (PI3K); 12/15-lipoxygenase (12/15LO); PDZ-containing kidney protein 1 (PDZK1); Na⁺/H⁺ exchanger regulator factor-3 (NHERF3); 4-hydroxy-2-nonenal (4HNE); monocyte chemoattractant protein-1 (MCP-1)

Transport from the ER to the Golgi is prevented when ABCA1 interacts with the enzyme serine palmitoyltransferase 1 (SPTLC1) which participates in the biosynthetic pathway of sphingomyelin, a major phospholipid component of membranes. The ABCA1/SCPTL1 interaction may regulate the levels of intracellular pools of sphingolipids when the cellular demands for these lipids are high. Pharmacological inhibition of SPTLC1 with myriocin, an atypical amino acid and antibiotic derived from thermophilic fungi (Miyake et al. 1995), increases both the ABCA1 levels at the plasma membrane and the ABCA1-mediated cholesterol efflux (Tamehiro et al. 2008). Mutations in ABCA1 that prevent normal glycosylation cause failure

of ABCA1 to exit the ER (Singaraja et al. 2006). In the Golgi compartment, ABCA1 undergoes palmitoylation at cysteine residues 3, 23, 1110, and 1111 by the palmitoyltransferase Asp-His-His-Cys 8 (DHH8) (Singaraja et al. 2009). Inhibiting this enzyme by drugs or preventing ABCA1 palmitoylation by site-specific mutagenesis reduced ABCA1 levels at the plasma membrane and decreased efflux to apoA-I (Singaraja et al. 2009). The gene encoding cathepsin D, a lysosomal protease, was identified by comparative transcriptomic analysis to be associated with low HDL-C levels in humans (Haidar et al. 2006). Blocking the activity or expression of cathepsin D reduced ABCA1 expression and protein abundance as well as apoA-I-mediated lipid efflux by more than 70 % and caused retention of ABCA1 in lysosomal compartments (Haidar et al. 2006). Very recently, BIG1 (brefeldin A-inhibited guanine nucleotide-exchange protein 1) was found to modulate ABCA1 trafficking and functions in the liver cells (Lin et al. 2013). BIG1 depletion reduced surface ABCA1 on HepG2 cells and inhibited by 60 % cholesterol efflux, whereas BIG1 overexpression had the opposite effects. RNA interference with BIG1 dramatically decreased the internalization and recycling of ABCA1. This novel function of BIG1 was dependent on the guanine nucleotide-exchange activity and achieved through the activation of ADP-ribosylation factor 1 (Lin et al. 2013).

The transport of ABCA1 to the plasma membrane is also facilitated by certain members of the family of small GTPase including Rab8, Rab4A, and Rab4B (Linder et al. 2009) (Jean and Kiger 2012).

Calpain, a cysteine protease, cleaves ABCA1 at the PEST (proline, glutamic acid, serine, threonine) sequence (amino acids 1283–1306) that is located in the cytosolic loop of the molecule, and this cleavage takes place at the early endosomes (Fig. 6) (Yokoyama et al. 2012; Miyazaki et al. 2013). The deletion of the PEST sequence increases ABCA1 levels on the plasma membrane and apoA-I binding (Chen et al. 2005). The interaction with apoA-I stabilizes ABCA1 against this degradation (Arakawa and Yokoyama 2002; Wang et al. 2003; Arakawa et al. 2004). Importantly, the inhibition of calpain was shown to increase HDL biogenesis in cultured cells, indicating that the inhibition of proteolytic degradation of ABCA1 could be a promising strategy for increasing HDL (Lu et al. 2008). The phosphorylation of the PEST sequence of ABCA1 at Thr-1286 and Thr-1305 regulates calpain-mediated proteolysis of ABCA1 since the ABCA1-T1286A/T1305A mutant could not be degraded by calpain (Martinez et al. 2003). The interaction of ABCA1 with apoA-I results in the dephosphorylation of the ABCA1 PEST sequence and inhibition of calpain degradation, leading to an increase of ABCA1 cell-surface expression (Martinez et al. 2003). Calmodulin was also shown to interact with ABCA1 in the presence of Ca^{2+} and to protect from calpain-mediated degradation (Iwamoto et al. 2010).

In addition to the calpain-mediated proteolytic degradation of ABCA1 described above, ABCA1 is subject to ubiquitin-mediated proteolysis. The presence of ubiquitinated ABCA1 in the plasma membrane of several cell lines was recently demonstrated (Mizuno et al. 2011). In HuH-7 cells, the degradation of cell-surface-resident ABCA1 was inhibited by the overexpression of a dominant-negative form

of ubiquitin. Moreover, the disruption of the endosomal sorting complex required for transport (ESCRT) pathway by the knockdown of hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) delayed the degradation of ABCA1 (Mizuno et al. 2011). These findings suggested that ubiquitination mediates the lysosomal degradation of plasma membrane ABCA1 and thereby regulates the expression and cholesterol efflux functions of ABCA1 independently of the calpain-mediated pathway.

Unsaturated fatty acids such as oleate and linoleate were shown to destabilize ABCA1 protein and to inhibit ABCA1-mediated cholesterol efflux in macrophages by a mechanism that depends on the activity of the enzyme acyl-CoA synthetase 1 which is responsible for their activation to their CoA derivatives (Kanter et al. 2012). At the same time, unsaturated fatty acids increase the Ser phosphorylation of ABCA1 via a phospholipase D (PLD)/protein kinase C δ pathway which contributes to ABCA1 destabilization (Fig. 6) (Wang and Oram 2007).

Two additional proteins that were identified by two-hybrid screen to physically interact with ABCA1 and to regulate ABCA1 intracellular localization and turnover by interacting with its PDZ (PSD-95, Dlg, ZO-1) domain are the a1 and b1 syntrophins (Munehira et al. 2004). Given the short half-life of ABCA1 ($t_{1/2} = 1\text{--}2$ h) (Wang et al. 2003), interfering with the accessory proteins or the enzymes that posttranslationally modify ABCA1 may prove a valuable strategy to regulate ABCA1-mediated HDL biogenesis in the liver and the intestine or to enhance cholesterol efflux in peripheral cells.

Insulin enhances ABCA1 protein degradation in HepG2 cells via PI3K. In addition, it inhibits ABCA1 activity by phosphorylation at Tyr1206 (Nonomura et al. 2011). The kinase that is responsible for this Tyr phosphorylation of ABCA1 is not known, but it was hypothesized that it is the insulin receptor itself.

TNF α and lymphotoxin α (LT) are key inflammatory mediators which also contribute to the atherogenic process (Schreyer et al. 1996, 2002; Pamiir et al. 2012). TNF induces ABCA1 mRNA and protein levels as well as cholesterol efflux from cultured macrophages to apoA-I (Gerbod-Giannone et al. 2006). The induction of ABCA1 by TNF α depended primarily on NF- κ B (Gerbod-Giannone et al. 2006). It was also shown that the expression of the two TNF receptors is required to mediate full ABCA1 induction by TNF α . In addition, LT increased ABCA1 protein levels by inhibiting protein degradation through the LT- β receptor (LT β R) (Edgel et al. 2010).

ABCA1-mediated cholesterol efflux and ABCA1 protein levels were shown to be decreased by interferon γ (IFN γ) in murine macrophages and macrophage-derived foam cells (Panousis and Zuckerman 2000). This ABCA1 downregulation was an early event in IFN γ -mediated activation of macrophages (Alfaro Leon et al. 2005) and was dependent on signal transducer and activator of transcription 1 (STAT1) since similar effects were not observed in macrophages from STAT1 KO mice (Wang et al. 2002).

Additional protein kinases were shown to affect the activity and stability of ABCA1 at the posttranslational level including protein kinase A (PKA), protein kinase C (PKC), Janus kinase 2 (JAK2), and casein kinase (CK2) (Tang et al. 2004).

ABCA1 activity was shown to be regulated by the nuclear receptor LXR β by a mechanism that is distinct from the transcriptional induction of the ABCA1 gene caused by cholesterol accumulation. It was shown that at low cholesterol levels, LXR β binds to ABCA1 and the ABCA1-LXR β complex is stably localized at the plasma membrane but is unable to facilitate apoA-I-mediated cholesterol efflux. Exogenously added LXR ligands, which mimic cholesterol accumulation, cause LXR β to dissociate from ABCA1, thus freeing ABCA1 for apoA-I binding and subsequent cholesterol efflux (Hozoji et al. 2008; Hozoji-Inada et al. 2011).

3.2 ABCG1

Similar to ABCA1, the levels of ABCG1 transporter were shown to be subject to regulation at the posttranslational level (Fig. 6) (Tarling and Edwards 2012). Recent studies showed that the presence of additional 12 amino acids between the Walker B motif and the first transmembrane domain of ABCG1 can affect ABCG1 protein stability (Engel et al. 2006). It was shown that stable overexpression of 12/15-lipoxygenase (12/15LO) in macrophages was associated with a 30 % reduction in HDL-mediated cholesterol efflux and reduced ABCG1 protein expression (Nagelin et al. 2008). Treatment of macrophages with the 12/15LO eicosanoid product 12SHETE increased serine phosphorylation of ABCG1 and affected the stability of the protein (Nagelin et al. 2008). Proteasomal inhibitors blocked the downregulation of ABCG1 and resulted in the accumulation of phosphorylated ABCG1 (Nagelin et al. 2009). Macrophages that lack 12/15LO had enhanced ABCG1 expression, reduced ABCG1 phosphorylation, and increased cholesterol efflux. Conversely, macrophages that overexpress 12/15LO have reduced ABCG1 expression, increased transporter phosphorylation, and reduced cholesterol efflux (Nagelin et al. 2009). It was also shown that 12/15LO regulates ABCG1 expression and function through p38- and JNK2-dependent mechanisms (Nagelin et al. 2009).

The activation of adenosine monophosphate-activated protein kinase (AMPK) in human aortic endothelial cells resulted in increased levels of ABCG1 protein via a posttranscriptional mechanism that involved increased stability of ABCG1 mRNA (Li et al. 2010a, b). The aminoimidazole carboxamide ribonucleotide (AICAR)-dependent induction of ABCG1 was associated with increased efflux of cellular cholesterol and 7-ketocholesterol to HDL and protected nitric oxide synthase activity and vascular reactivity (Li et al. 2010a, b).

The protein levels of ABCG1 were shown to be controlled by palmitoylation in both human embryonic kidney 293 cells and in mouse macrophage, J774 (Gu et al. 2013). Five cysteine residues located at positions 26, 150, 311, 390, and 402 in the NH₂-terminal cytoplasmic region of ABCG1 were shown to be palmitoylated. The removal of palmitoylation at Cys311 by mutating the residue to Ala (C311A) or Ser significantly decreased ABCG1-mediated cholesterol efflux. On the other hand, the removal of palmitoylation at sites 26, 150, 390, and 402 had no significant effect (Gu et al. 2013).

3.3 SR-BI

The stability of the HDL receptor SR-BI in the hepatocyte plasma membrane is very important for its functions and is subject to posttranscriptional regulation (Fig. 6). SR-BI stability is primarily controlled by its adapter protein, PDZ-containing kidney protein 1 (PDZK1 or NHERF3 for Na⁺/H⁺ exchanger regulator factor-3), since PDZK1-knockout mice exhibit a >95 % reduction in hepatic SR-BI protein (but not mRNA) and are characterized by hypercholesterolemia and the presence of large cholesterol-rich HDL particles in the plasma (Kocher et al. 2003). In adrenal cells where the PDZK1 levels are very low, additional members of the NHERF family such as NHERF1 and NHERF2 (but not NHERF4) were shown to specifically interact with SR-BI and reduce its protein levels (Hu et al. 2013). The data showed that the downregulation of SR-BI by these NHERF1/2 factors significantly inhibits both HDL-CE uptake and steroid hormone production by adrenal cells. It was concluded that the PDZK1 homologues act as physiological translational/posttranslational regulators of the functional expression of SR-BI (Hu et al. 2013).

Feeding mice with an atherogenic diet was accompanied by a threefold post-translational downregulation of hepatic SR-BI at the protein level (Niemeier et al. 2009). A similar SR-BI downregulation was observed in transgenic mice overexpressing SREBP-1a and SREBP-1c on chow diet, and it was associated with a decrease in the expression levels of PDZK1 (Niemeier et al. 2009).

The levels of SR-BI protein were shown to be affected in two syndromes: the nephrotic syndrome and the Rett syndrome. It was shown that animals with nephrotic syndrome which is characterized by dyslipidemia, impaired high-density lipoprotein (HDL)-mediated reverse cholesterol transport, and atherosclerosis (Vaziri et al. 2003) exhibited severe hypercholesterolemia, hypertriglyceridemia, reduced HDL/total cholesterol ratio, significant upregulation of the endocytic HDL receptor ATP synthase mRNA and protein, and significant reduction of SR-BI protein despite its normal mRNA abundance. The reduction in SR-BI protein levels in animals with NS was accompanied by parallel reductions in PDZK1 mRNA and protein levels (Vaziri et al. 2011). Rett syndrome, a genetic form of infantile autism, is the second most common cause of mental retardation in women (Amir et al. 1999). When compared to healthy subjects, patients with Rett syndrome present with significant increases in total cholesterol (12 %), LDL cholesterol (15 %), and HDL-C (18 %) (Sticozzi et al. 2013). Skin fibroblasts isolated from patients with Rett syndrome exhibited low levels of SR-BI as a consequence of its association with 4-hydroxy-2-nonenal (4HNE), a product of lipid peroxidation that is increased in patients with Rett syndrome, and increased ubiquitination (Sticozzi et al. 2013). The role of the proteasome in SR-BI stability was confirmed using the proteasomal inhibitor (MG132). When Rett syndrome fibroblasts were pretreated with MG132, the loss of SR-BI was reversed demonstrating that SR-BI is degraded via the proteasome machinery (Sticozzi et al. 2013).

In hepatic cells and fibroblasts, SR-BI protein levels and SR-BI-mediated cholesterol transport (export and uptake) were shown to be affected by the Ras/MEK/ERK signaling cascade. This effect was mediated via PPAR α -inducible

degradation pathways (Wood et al. 2011). Metabolic labeling experiments in primary hepatocytes from mice demonstrated that fenofibrate enhances the degradation of SR-BI in a post-endoplasmic reticulum compartment (Lan and Silver 2005). Moreover, fenofibrate-induced degradation of SR-BI was independent of the proteasome, the calpain protease, or the lysosome (Lan and Silver 2005).

In primary macrophages and cell lines derived from female but not from male mice, tamoxifen and 4-hydroxytamoxifen increased SR-BI protein expression via the estrogen receptor α (Dong et al. 2011). Because SR-BI mRNA expression and promoter activity were not influenced by tamoxifen and 4-hydroxytamoxifen, it was demonstrated that the regulation of SR-BI by these substances takes place at the level of protein stability (Dong et al. 2011).

The phosphatidylinositol 3-kinase (PI3K) pathway was shown to affect positively the SR-BI-mediated HDL selective cholesterol ester uptake into human HepG2 cells as this process was compromised in the presence of the PI3K inhibitors wortmannin and LY294002 (Shetty et al. 2006). These inhibitors also blocked the positive effect of insulin on the SR-BI-dependent selective uptake. HDL cell-surface binding, receptor biotinylation studies, and confocal fluorescence microscopy of HepG2 cells expressing green fluorescent protein-tagged SR-BI demonstrated changes in SR-BI subcellular localization and cell-surface expression as a result of PI3K activation (Shetty et al. 2006). These data indicate that PI3K activation stimulates hepatic SR-BI function posttranslationally by regulating the subcellular localization of SR-BI. It was recently shown that monocyte chemoattractant protein-1 (MCP-1), a protein expressed by endothelial cells under inflammatory conditions, decreases the cell-surface protein expression of ABCA1, ABCG1, and SR-BI in a dose-dependent and time-dependent manner (Huang et al. 2013). It was shown that PI3K activation corrected the MCP-1-induced decreases in the cell-surface protein expression of the three transporters. MCP-1 decreased the lipid uptake by HepG2 cells and the ABCA1-mediated cholesterol efflux to apoA-I, and this was reversed by the activation of PI3K. These data suggested that MCP-1 impairs RCT activity in HepG2 cells by a PI3K-/AKT-mediated posttranslational regulation of ABCA1, ABCG1, and SR-BI cell-surface expression (Huang et al. 2013).

Finally, it was shown that hormones such as triiodothyronine (T3) and thyromimetics and some pharmacological agents such as probucol increase SR-BI levels posttranscriptionally (Leiva et al. 2011).

Conclusions

As discussed earlier in this chapter as well as in other chapters of this handbook, plasma HDL cholesterol levels are determined by the relative rates of HDL biogenesis and HDL catabolism which in turn are mediated by several lipid-carrying proteins, plasma enzymes, and membrane transporters. The deregulation of the activity or the expression of these proteins by mutations or during diseases is associated with non-physiological levels of HDL cholesterol, HDL dysfunction, and predisposition to coronary heart disease. Epidemiological studies as well as experiments in genetically modified animals have provided unequivocal proof that raising HDL cholesterol levels may be therapeutic. Drugs

that could specifically increase the rates of HDL biogenesis by inducing the levels of apoA-I, ABCA1, or LCAT are anticipated to be of great clinical benefit. Other HDL proteins such as ABCG1, ABCG5/G8, CETP, and LPL could also provide useful targets for HDL-based therapies that will interfere with HDL maturation or catabolism without compromising HDL functionality. The development of novel HDL-raising drugs that will capitalize on HDL biogenesis will certainly require a thorough understanding of the mechanisms and the molecules that are involved in HDL regulation at the transcriptional, posttranscriptional, or posttranslational level in hepatic cells as well as in other physiologically relevant cells such as the macrophages. For instance, understanding ABCA1 gene regulation in the macrophage has led to the elucidation of the important role of LXRs and their ligands in the reverse cholesterol transport and the development of novel LXR ligands which however have severe lipogenic effects in the liver and are thus of no clinical value today so the challenge here is to develop more specific drugs that will avoid lipogenesis. Novel single- or dual-specificity agonists of peroxisome proliferator-activated receptor isoforms are also anticipated. Understanding how the levels of HDL proteins and their functionality are affected by inflammatory factors will enable us to develop novel drugs to restore HDL levels and functionality and thus reduce the atherosclerotic burden in patients with chronic inflammatory diseases.

In summary, the current challenges in the HDL field are to develop novel therapies that would raise HDL cholesterol levels without compromising HDL functionality and to identify novel HDL-based biomarkers. The advances in the field of HDL regulation may provide crucial insights and tools to achieve this goal.

Acknowledgments This work was supported by the European Cooperation in Science and Technology (COST) Action BM0904; by grants from the Greek Secretariat for Research and Technology (SYNERGASIA-09 and THALIS MIS377286) to Dimitris Kardassis; by grants from the Spanish Instituto de Salud Carlos III (FIS, PI11/00315) to Alberto Dávalos; by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-ID-PCE-2011-3-0591, and the Romanian Academy to Anca Gafencu; and by an NIH grant HL-48739 to Vassilis I. Zannis. We thank Dr. Ovidiu Croitoru for the graphic design.

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