Recent discoveries concerning regulation of Cholesterol Biosynthesis and Sterol Regulated Element Binding Proteins (SREBPs).

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Abstract

Cholesterol is an important component of cellular membranes and plays an important role in several physiological processes. Atypical accumulation of cholesterol in the blood elevates the risk of cardiovascular disease, so its homeostasis has to be tightly regulated.

Cholesterol is synthesized (de novo) via the mevalonate pathway, and the clearance of plasma cholesterol is highly dependent on the expression of LDL-receptors in the liver. Cellular cholesterol levels are tightly controlled by a family of membrane-bound transcription factors: Sterol-Regulatory Element-Binding Proteins (SREBPs). SREBP isoforms SREBP-2 and SREBP-1c bind to the DNA of promotor/enhancer regions of genes involved in cholesterol- and lipid synthesis, respectively. SREBPs transcription factor activity is regulated by SREBP processing, which produces nuclear SREBP (nSREBPs). nSREBPs regulate transcription of SREBPs themselves, by binding to the promotor/enhancer region in the *Srebf2* and *Srebf1* genes. Transcription of *Srebf1* is also dependent on activation of the Liver-X-Receptor (LXR).

Cholesterol levels are also regulated by controlling the flux over the mevalonate pathway, via degradation of 3-hydroxy-3-methylglutaryl-CoA Reductase (HMGCR).

There are still a lot of questions surrounding the regulation of SREBPs and cholesterol. More research has to be done to fully understand the mechanisms involved. Said mechanisms might eventually be manipulated for pharmaceutical purposes, which could aid in the treatment against cardiovascular diseases.

This paper focused on methods of cholesterol regulation in the liver that were recently discovered.

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Introduction

Cholesterol is an important biological molecule, it's a precursor for the synthesis of steroid hormones, bile acids and vitamin D. Cholesterol has a key role in membrane structure and function, its synthesis is tightly regulated to prevent over-accumulation in the body, which – as stated above – elevates the risk of cardiovascular disease.

Cardiovascular- and heart disease are the leading causes of death in the Western World. Atherosclerosis is the accumulation of cholesteryl esters in the arteries, which results in a higher concentration of cholesterol in the blood. This could form a plaque, which elevates the risk of cardiovascular disease [1].

Cholesterol is a nonpolar lipid molecule and is transported by lipoproteins in the blood. Lipoproteins are they classified based on their density, content of cholesterol, triglycerides and proteins. The five classes are chylomicrons; very-low-density lipoproteins (VLDL); intermediate-density lipoproteins (IDL); low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [2].

Cellular cholesterol homeostasis is tightly controlled by *de novo* synthesis via the mevalonate pathway or receptor-mediated uptake of low-density lipoproteins cholesterol (LDL-c) [2]. Both will be discussed in more detail below. Biosynthesis of cholesterol via the mevalonate pathway is regulated by the family of transcription factors Sterol Regulated Element Binding Proteins (SREBPs) [3].

This review will provide an overview of the latest developments involved in the regulation of SREBPs, cholesterol- and lipid homeostasis in the liver.

Chapter 1: Cholesterol metabolism

1.1 Biosynthesis of cholesterol

Almost 50% of the cholesterol in our body is biosynthesized *de novo*. Cholesterol biosynthesis takes place in the cytoplasm, the ER and peroxisomes. The reactions of the cholesterol biosynthesis is collectively called the mevalonate pathway (depicted in figure 1). Cholesterol synthesis can be divided in to five major steps, with minor steps in between:

1. Acetyl-CoA is transported from the mitochondria to the cytosol. Acetyl-CoA is converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) in the cytosol via HMGCS1 (HMG-CoA synthase 1).

2. HMG-CoA is then converted to mevalonate by HMG-CoA reductase, which is bound to the ER. The reaction requires NADPH as co-factor for HMGCR, this is the rate-limiting step in cholesterol synthesis. HMGCR is tightly regulated, which will be discussed further on. Mevalonate is then activated by two successive phosphorylation's, which produces mevalonate-5-diphosphate (M5D).

3. M5D is subjected to ATP-dependent decarboxylation, which leads to isopentenyl pyrophosphate (IPP). IPP is an isoprene based molecule and is in equilibrium with dimethylallyl-PP (DMAPP), which is IPP's isomer.

4. IPP and DMAPP merge to produce geranyl pyrophosphate (GPP). GPP merges with another IPP molecule, which forms farnesyl pyrophosphate (FPP). NADPH is required by the enzyme farnesyl-diphosphate farnesyltransferase (FDFT1), which merges two FPP molecules to produce squalene.

5. Squalene is subjected to a two-step cyclization, which produces lanosterol. The first step is catalysed by squalene epoxidase, which uses NADPH as cofactor to produce an epoxide. The epoxide intermediate is converted to lanosterol via lanosterol synthase. 19 successive reactions convert lanosterol to cholesterol [4].

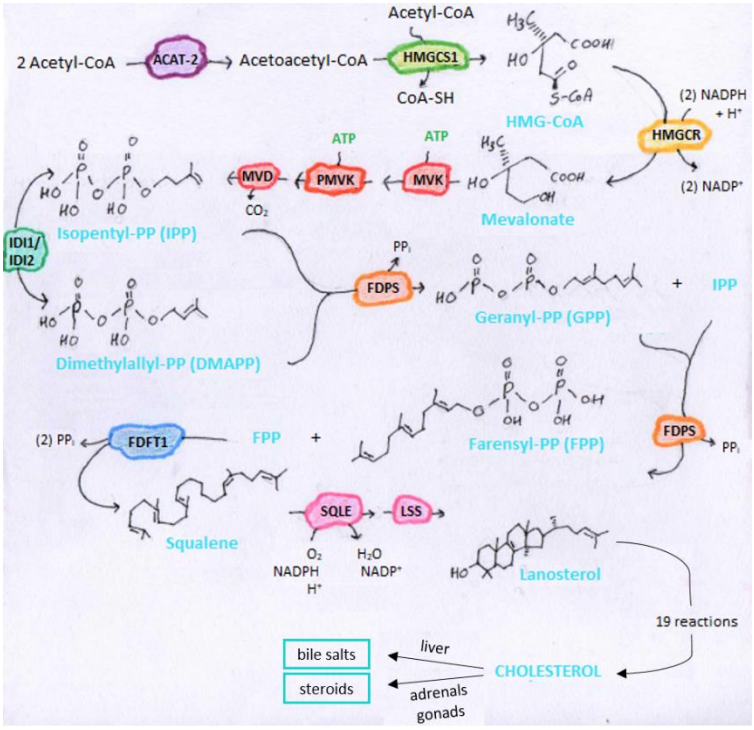

***Figure 1*. Cholesterol biosynthesis via the mevalonate pathway.** The pathway follows successive reactions which converts acetyl-CoA to cholesterol. Cholesterol can then be converted to bile salts in the liver and excreted as bile, or to steroids in adrenals and glands [5].

1.2 Regulation of cholesterol synthesis

The range of plasma cholesterol is 150-200 mg/dL. Adults have a cholesterol intake of approximately 0.3g/day, so the range of cholesterol in blood is mainly maintained by controlling the level of *de novo* synthesis (which is normally around 1g/day). The level of *de novo* synthesis is controlled by regulation of HMGCR activity; plasma cholesterol levels and processing of Sterol Regulated Element Binding Proteins (SREBPs) [4].

Regulation of HMGCR activity and levels (primary)

HMGCR is a poly-topic ER membrane protein and is the enzyme used during the rate-limiting step in the mevalonate pathway (as seen in figure 1). HMGCR is subjected to acute sterol-stimulated degradation in reaction to oxysterols and cholesterol pathway intermediates. Its degradation is controlled by feed-back inhibition, control of gene expression, rate of enzyme degradation and phosphorylation-dephosphorylation [4].

The stability of HMGCR is regulated by the flux through the mevalonate pathway. A high flux results in an increase in sterols (including cholesterols) in the cell, which triggers an increase in HMGCR degradation. Regulation dependent on sterol levels in the cell is facilitated by HMGCRs sterol sensing domain (SSD). Cholesterol mediates feed-back inhibition of existing HMGCR and induces enzyme degradation via ER-associated protein degradation (ERAD). Ubiquitination of HMGCR takes place on its conserved lysine residues. HMGCR is then extracted from the ER and degraded by the proteasome, which stops the metabolic flux through the Mevalonate pathway [5].

HMGCR is also controlled via phosphorylation-dephosphorylation. HMGCR is most active in its dephosphorylated state. Phosphorylation of HMGCR is controlled by AMP-activated protein kinase (AMPK). Dephosphorylation of HMGCR is controlled by HMGCR phosphatase (PP2A). This mechanism is influenced by the level of cAMP. Various stimuli, such as insulin, decrease the level of cAMP, resulting in increased dephosphorylation of HMGCR. Which gives rise to a higher level of HMGCR activity and cholesterol synthesis [4].

Regulation of plasma cholesterol levels via LDL receptor-mediated endocytosis and HDL-mediated reverse transport

Cholesterol is transported through the plasma in the form of cholesteryl esters in lipoproteins.
Chylomicrons transport dietary cholesterol from the small intestine to the liver and triglycerides to adipose and muscle tissue. Triglycerides are converted to fatty acids and are stored or used for oxidation. In the liver, cholesterol is either converted to bile acids, excreted in bile or incorporated in lipoproteins (VLDL). Once VLDL arrives in the capillaries,

lipoprotein lipase hydrolyses the core triglycerides, which leads to a smaller density lipoprotein (IDL). After release of the remaining triglycerides and substituting them for cholesteryl esters, IDL is converted to LDL – which is rich in cholesterol. LDL serves as the agonist for LDL-receptors (LDLR). Excess cholesterol in plasma is transported to the liver by HDL, which prevents accumulation of cholesterol in the body [6].

If the cholesterol synthesized via the mevalonate pathway is insufficient, the expression of LDL receptors (LDLR) is induced via SREBP-2. Newly synthesized LDLR is transported to the plasma membrane. At the plasma membrane, LDL-c binds to LDLR and both are internalized (depicted in figure 2A). The vesicles containing the LDLR-LDL complex are transported to the lysosome, where hydrolysis of LDLs cholesterol esters takes place. The cholesterol is released and used in the synthesis of membranes, steroid hormones and bile acids. Excess cholesterol is excreted in the bile after conversion to bile acids in the liver. The LDL receptors are returned to the plasma membrane and repeat the previously described cycle.
In addition to LDLR, proprotein convertase subtilisin/kexin type 9 (PCSK9) expression is also induced under low intracellular cholesterol conditions. PCSK9 – which degrades LDLR after enough cholesterol has been taken up [7].

LDL controls the cholesterol metabolism in the cell. An increase in LDL cholesterol drives cholesterol storage in the cell, inhibits HMGCR synthesis, and thus inhibits cholesterol synthesis. This increase also inhibits the SREBP-2-mediated LDLR expression, which inhibits cholesterol uptake [6].

Reverse cholesterol transport (depicted in figure 2B) describes the peripheral cholesterol that returns to the liver through HDL. Cholesterol in plasma membranes can be extracted by HDLs and esterified by cholesterol esterifying enzyme lecithin cholesterol acyltransferase (LCAT). Cholesteryl esters in HDL are transported to the liver and can be taken up or transferred to triglyceride-rich lipoprotein remnants in the liver by cholesteryl ester transfer protein (CETP) [8].

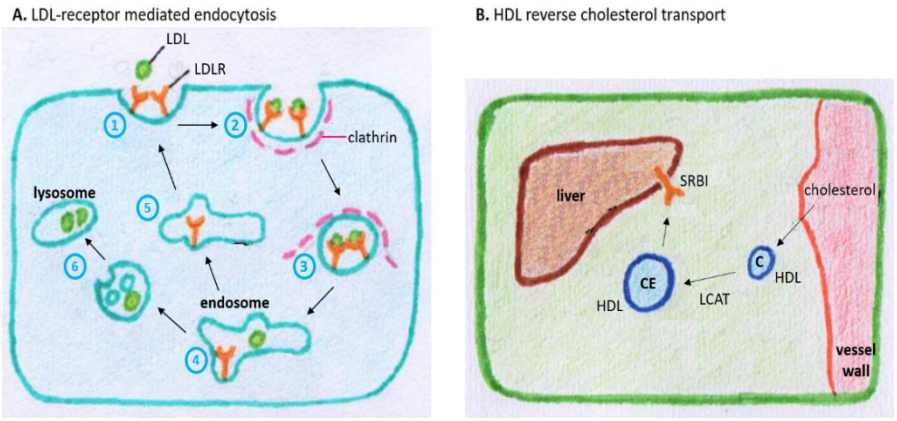

Figure 2. **A: LDL-receptor mediated endocytosis.** 1; LDL binds to the LDLR. 2 + 3; A Clathrin-coated pit forms and endocytosis of the pit takes place. 4; The vesicle merges with an endosome. 5; LDLR recycling to the surface. 6; cholesterol is passed on from the endosome to the lysosome and is used for further cellular processes [26]. **B: HDL reverse cholesterol transport**. Cholesterol is picked up from the vessel wall by HDL. LCAT esterifies the cholesterol. The cholesterol esters are taken up by liver via the SRBI receptor [27].

Chapter 2: Sterol Regulated Element Binding Proteins (SREBPs)

Biosynthesis via the mevalonate pathway and uptake of cholesterol are regulated by the transcription factors Sterol Regulated Element Binding Proteins (SREBPs). The cholesterol regulatory machine in the ER is very sensitive for changes in the cholesterol level of lipoprotein-derived cholesterol. A change of 1-2% in cholesterol concentrations in the ER leads to SREBP processing [3]. This chapter will focus on the isoforms of SREBPs, their processing and regulation.

2.1 SREBPs and SREBP processing

SREBPs are a family of transcription factors that regulate the expression of >30 target genes required for biosynthesis and uptake of cholesterol, triglyceride (TG), phospholipids, fatty acids (FA) and the NADPH cofactor necessary for cholesterol synthesis. SREBPs are part of the basic helix-loop-helix-leucine zipper (bHLH-Zip) family of transcription factors. They differ from other bHLH-Zip proteins, as they synthesize as inactive precursors bound to the ER.
The SREBP precursors consist of about 1150 amino acids (aa) and are composed of three domains: the NH_2-terminal domain of 480 aa with a bHLH-Zip region for the binding of DNA; two hydrophobic transmembrane-spanning segments

with an intermittent loop of 30 aa that projects to the lumen of the ER; a COOH-terminal domain of 590 aa that has important regulatory functions [9]. The structure of SREBPs is depicted in figure 3A.

Newly synthesized SREBP is transported to the ER membrane, where its COOH-terminal regulatory domain binds to the COOH-terminal domain of SREBP cleavage-activating protein (SCAP). SREBP-SCAP forms a complex with insulin induced genes (INSIG). INSIG anchors the complex to the ER-membrane. SCAP is a post-transcriptional regulator of SREBP and plays a key role in SREBP processing by escorting SREBP and measuring the level of sterols in the membrane of the ER. When the level of sterols in the ER membrane drops below a certain level, it is measured by SCAP via its membrane sterol-sensing domain (SSD) and SCAP is subjected to a conformational change. The SREBP-SCAP complex dissociates from INSIG. COPII vesicles bind to SCAP and SCAP transports SREBP to the Golgi Apparatus [10]. Progestin and adipoQ receptor 3 (PAQR3) anchors the SREBP-SCAP complex to the Golgi Apparatus, in a similar way that INSIG anchors the complex to the ER-membrane. Both of these interaction play a key role in cholesterol homeostasis [11]. Site-1- and site-2-proteases (S1P and S2P) reside in the Golgi Apparatus and provide the proteolytic processing of SREBP. S1P is a membrane bound serine protease and cleaves SREBP in the intermittent luminal loop, which bisects SREBP. The NH_2-terminal bHLH-Zip domain is released from the membrane via successive cleavage of S2P – which is a membrane bound zinc metalloproteinase. This gives rise to the mature transcription factor: the N-terminal of SREBP, which is translocated to the nucleus. Nuclear SREBP (nSREBP) binds to Sterol Response Elements (SREs) in the promotor/enhancer region of target genes and induces expression of said genes (such as *HMGCR*, *Srebf*, *LDLR*, etc). The processing mechanism of SREBP is depicted in figure 3B.
A high level of sterols in the ER-membrane is measures by SCAPs SSD and induces a conformational change in SCAP, which inhibits SREBP-SCAP transport to the Golgi. Thus, transcription of SREBs target genes is stopped via sterol-mediated suppression of SREBP cleavage [2, 5, 9, 10, 12].

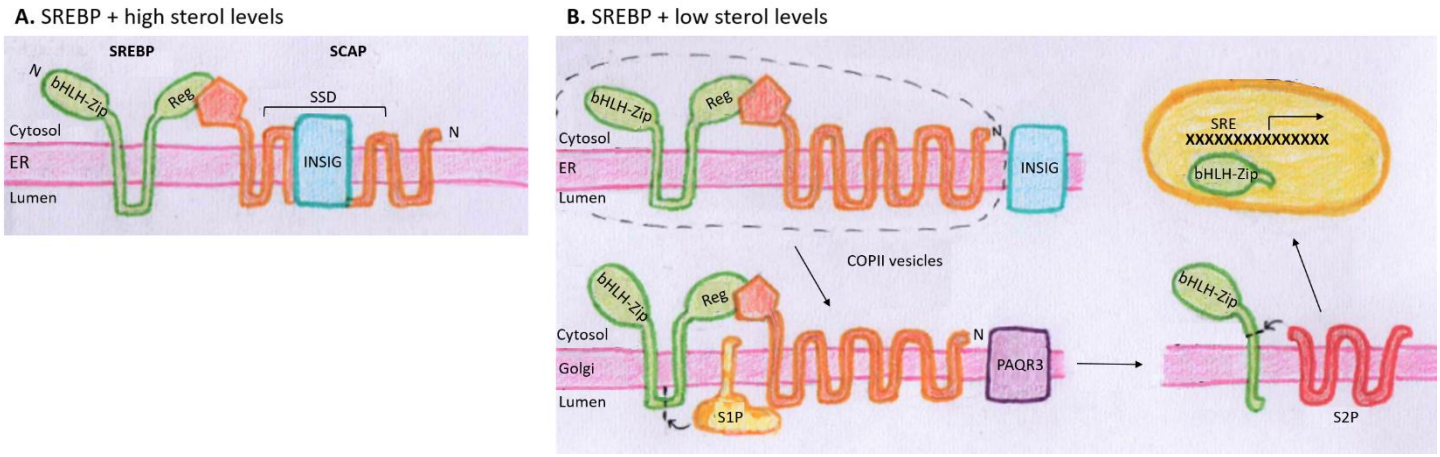

Figure 3. **A: SREBP + high cholesterol levels.** SREBP with its N-terminal bHLH-Zip domain and COOH-terminal regulatory domain is ER membrane-bound. SREBP is bound to SCAP and INSIG. INSIG anchors the SREBP-SCAP complex to the ER membrane. High cholesterol levels inactivate SCAP via its Sterol Sensing Domain (SSD). **B: SREBP + low cholesterol levels.** A drop in cholesterol levels is noticed by SCAPs SSD domain and induces a conformational change which breaks the attachment of SCAP to INSIG. The SREBP-SCAP complex is transported to Golgi via COPII vesicles and is anchored to Golgi by PAQR3. SREBP is subsequently cleaved by S 1P and S2P and frees the bHLH-Zip domain of SREBP. The N-terminal bHLH-Zip domain translocates to the nucleus, binds to the SRE domain in the promotor/enhancer of target genes and activates their transcription [28].

2.2 SREBP isoforms and target genes

There are three isoforms of SREBPs: SREBP-1a, SREBP-1c and SREBP-2. SREBP-1a and -1c are transcribed from the *Srebf1* gene on chromosome 17p11.2. Two different independent promotors with alternative transcription start sites. This produces SREBP-1a and SREBP-1c, which differ from each other at exon1: exon-1a and exon-1c. SREBP-2 is transcribed from the *Srebf2* gene on chromosome 22q13. The isoforms and their genes are depicted in figure 4. SREBP-1a and -2 are the predominant isoforms of SREBP in most cell lines, whilst SREBP-1c and -2 are dominantly active in the liver [13,14].

SREBP-1a has a strong transcriptional effect on all SREBP-responsive genes, which synthesize FA, TG and cholesterol. The high level of transcription activation is due to exon-1a, which codes for a longer transactivation domain than exon-1c. Overexpression of SREBP-1a causes accumulation of cholesterol and triglycerides [9, 15].

SREBP-1c was found to be the dominant SREBP-1 isoform the liver. It preferentially increases the transcription of genes used in lipogenesis (FA and TG synthesis), but not of genes involved in cholesterol synthesis. SREBP-1c dependent lipogenesis pathway is stimulated by feeding (nutrient and energy status) [9, 15, 16]. Insulin activates mTORC1 via the insulin-receptor Akt, which in turn triggers SREBP-1c processing. Successively, SREBP-1c starts lipogenesis in the liver. Overexpression of SREBP-1c leads to increased *de novo* lipogenesis, which gives rise to the development of fatty livers.

SREBP-2 is the primary transcription factor of cholesterol biosynthesis in the liver. SREBP-2 preferentially prompts the activation of genes involved in cholesterol biosynthesis; HMG-CoA reductase, farnesyl diphosphate and squalene synthase, LDLR and PCSK9. The level of cholesterol in the ER membrane is a key signal in activating SREBP-2 [12].

In conclusion: SREBP-1c favours the activation of genes involved in FA biosynthesis whilst SREBP-2 favours genes involved in cholesterol biosynthesis. Both SREBP isoforms produce genes necessary for the formation of NADPH, which is used in multiple stages of cholesterol- and lipid biosynthesis [9].

A rise in hepatic sterol levels inhibits the activation of SREBP-2 target genes, but not of SREBP-1c target genes. This implies that there are key differences in the activation of SREBP-2 and SREBP-1c by cellular lipids, which will be discussed later on [15].

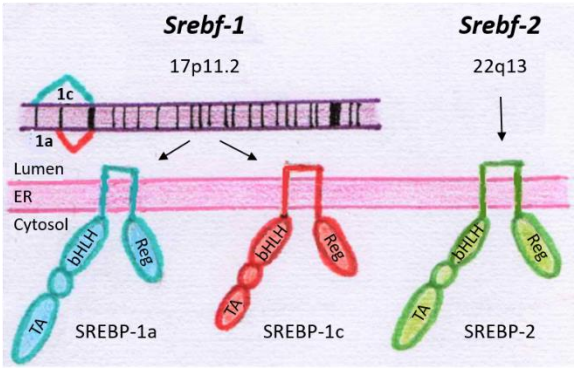

Figure 4. *Srebf* genes that encode for their respective SREBP isoforms. *Srebp-1* – located on chromosome 17p11.2 – encodes for SREBP-1a and SREBP-1c, which differ from each other in the starting sites of transcription. The different transcriptional start site leads to a difference in exon 1 of SREBP-1a and SREBP-1c, which results in a longer transactivation domain in the case of SREBP-1a. *Srebf-2* – located on chromosome 22q13 – encodes for SREBP-2 [29].

2.3 Regulation of SREBPs

Transcriptional regulation of SREBPs

Transcriptional regulation of SREBP-1c and SREBP-2 is partly controlled by feed-forward regulation mediated by SREs in the enhancer or promotor region of the *Srebf* genes. nSREBPs activate the transcription of its own genes.

Transcriptional regulation of SREBP-1c is also controlled by three other factors: liver-X-activating receptors (LXRs); insulin and glucagon [4].

There are two isoforms of the LXRs: LXRα and LXRβ. They are nuclear receptors that dimerise with retinoid X receptors (RXR). The LXRs are activated by a range of different sterols, including oxysterol intermediate produced during cholesterol biosynthesis. If there is an abundance of sterols, the agonist binds to LXR and LXR-RXR hetero-dimerization occurs. The heterodimer binds to the promotor region of *Srebp-1c*, which contains a LXR-binding site and SREBP-1c transcription starts. The LXR-mediated activation of SREBP-1c leads to the production of oleate, which is the preferential fatty acid used in the synthesis of cholesteryl esters (which is necessary for transport and storage of cholesterol) [9].

Insulin and glucagon ratio affect the transcription of SREBP-1c. The liver is responsible for the transition of carbohydrates to fatty acids, which are then stored in triglycerides or burned in muscle. An intake of food produces an increase in carbohydrates and insulin. Insulin induces the expression of E4-binding protein 4 (E4BP4) via the AKT-mTORC1-SREBP-1c pathway mentioned in 2.2. E4BP4 deficient hepatocytes do not exhibit signs of *de novo* lipogenesis. E4BP4 protects SREBP-1c against ubiquitination-dependent degradation. Thus insulin mediates *de novo* lipogenesis after an increase in carbohydrates is established by feeding. Insulin mediates this via E4BP4-stabilization of nSREBP-1c, which

leads to the activation of target genes involved in fatty acid synthesis [17].

Glucagon has the opposite effect. An increase in glucagon induces an increase in cAMP, which activates protein kinase A (PKA). PKA activates downstream signalling and reduces the production of mRNA of *SREBP-1c* and its target genes. This facilitates the conversion of fatty acids to carbohydrates [9].

The composition of phospholipids in the ER-membrane influences SREBP-1c processing

As described in 2.2, SREBP-1c is a key regulator in *de novo* lipogenesis in the liver. After SREBP-1c processing, the mature transcription factor nSREBP-1c binds directly to the genes involved in fatty acid synthesis and activates their transcription. The SREBP-1c pathway is influenced by the shifts in cellular levels of phospholipids and fatty acids, but the mechanisms these factors use to regulate SREBP-1c and lipogenesis is -as of yet- unknown [15].

Prior studies revealed that processing of a SREBP-1c orthologue in *Drosophila* S2 cells could be inhibited by saturated phosphatidylethanolamine (PE). PE is the primary phospholipid in *Drosophila* membranes and it controls the release of SREBP from *Drosophila* cell membranes, thus giving it control over fatty acid synthesis and phospholipids. They found that SREBP processing in mammals and flies are regulated by different lipids, namely sterols and PE. This lead the researchers to conclude that a function of SREBP is to supervise the framework of cell membranes and to alter lipid synthesis suitably [18].

The major phospholipid in membranes of mammals is phosphatidylcholine (PC) in contrast to PE in *Drosophila*. A different study found that blocking PC synthesis in *C. elegans*, mouse liver and human cells caused a rise in SREBP-1-dependent transcription and lipogenesis, which lead to lipid accumulation. This data proposed a feedback mechanism wherein processing of SREBP-1c is regulated by the levels of PC. It was found that lowering the phospholipid levels in cells activated SREBP-1c processing by interfering with the transport of SREBP from ER to Golgi and promoting mislocalization of S1P and S2P to the ER [3, 15].

The effect of LXR on SREBP-1c activity were thought to only be related to its effect on *Srebf1* transcription. A recent study found that LXR also regulates processing of SREBP-1c by inducing transcription of the phospholipid remodelling enzyme LPCAT3. This led to the discovery that some phospholipids in the ER membrane – particularly linoleoyl and arachidonyl PC – stimulate SREBP-1c processing and maturation. LPCAT3 is required to incorporate the phospholipids in to membranes, incorporation gives the lipids regulatory abilities over lipogenesis homeostasis. LPCAT3-mediated phospholipid remodelling influences the maturation of SREBP-1c and SREBP-2 by changing the amount of specific PC species in the ER membrane. The exact mechanism with which ER membrane phospholipid composition regulates SREBP-1c processing is unclear. It is thought to depend on membrane fatty acyl chain composition and its effect on SCAP and thus on transport of SREBP-1c. Flexible polyunsaturated fatty acyl chains in the vicinity of the SREBP-1c-SCAP-INSIG complex increase membrane dynamics and promote release of the complex from the ER, which leads to SREBP-1c processing [15].

Rong X; Wang B, et al (2017) link hyperinsulinemia and a higher level of membrane polyunsaturated PC to SREBP-1c hyperactivation, which in turn leads to excess lipogenesis. Obesity showed an increase in *Lpcat3* mRNA and an increase in polyunsaturated PC in the livers ER membrane. A change in membrane composition has been observed in patients with metabolic liver diseases. This leads to speculation that an increase in polyunsaturated PC in obesity could be due to a combination of excessive uptake of essential fatty acids that increase LPCAT3 expression in an LXR-dependent manner. Blocking the incorporation of polyunsaturated PCs in membranes decreases lipogenesis and hepatic triglyceride levels. Thus, LPCAT3 inhibition might be a possible therapy for fatty liver disease, as it reduces SREBP-1c processing and subsequently reduces lipogenesis [15].

Regulation of nSREBP-2s transcriptional activity

mTORC1 functions as a nutrient-, energy-, stress-sensor and activates SREBP-1a, SREBP-1c and SREBP-2 via the insulin-AKT-mTORC-SREBP-1c pathway as described in 2.2. This leads to activation of lipogenesis and cholesterol synthesis. mTORC1 is hyperactive in the case of obesity, this causes a continual activation of SREBP-1c and an increase in *de novo* lipogenesis. An overproduction of lipids could lead to hepatic steatosis and hypertriglyceridemia.

mTOCR1 modulates SREBP-2 maturation by influences the cholesterol levels in the ER via two membrane trafficking events: autophagy and endosome recycling. Autophagy is a self-degradation mechanism of the cell that is used in cases of nutrient stress. Intra-cellular structures are broken down inside lysosomes, which are transported to the ER. The lysosome delivers the degradation products to the ER, which restores balances sources of energy [19].
mTORC1 is mostly active in dividing cells. Active mTORC1 supresses autophagy and promotes endosome recycling in the plasma membrane. Membrane organelles and cell debris do not reach the lysosomes. The lysosomes contain little cholesterol that can be transported to the ER. This leads to a drop in ER membrane cholesterol, which activates SREBP-2 processing and maturation and subsequently cholesterol synthesis.

mTORC1 blocks nuclear entry of lipin-1 (depicted in figure 5). If lipin-1 enters the nucleus – in the absence of mTORC1 – it promotes the degradation of nSREBP proteins and silencing of nSREBPs target genes. This degradation is thought to be carried out using an autophagy-related process. Lipin-1 could prove to be an extra mode of regulation over SREBPs transcriptional activity [16].

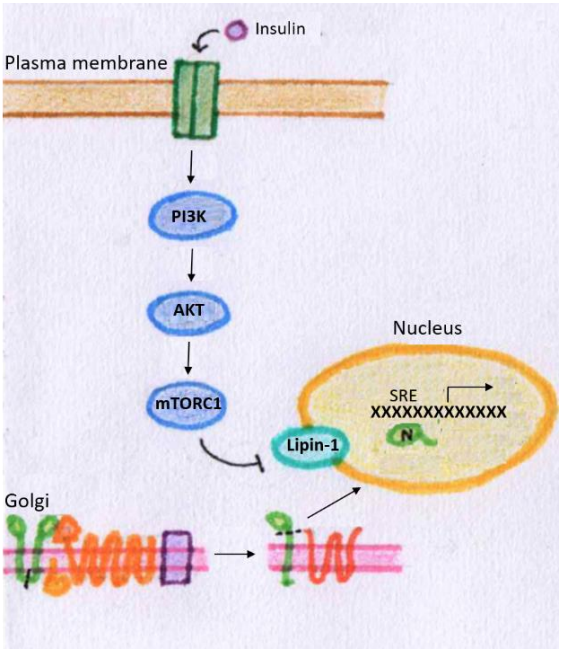

Figure 5. Insulin-Akt-mTORC1-SREBP-1c pathway. Insulin binds to the receptor on the plasma membrane. This activates the PI3K-Akt-mTORC pathway. mTORC1 block the entry of Lipin-1 to the nucleus, this inhibits nSREBP degradation. At the same time, SREBP processing takes place thanks to the increase in insulin. The N-terminal bHLH-Zip domain of SREBP translocates to the nucleus and regulates its own expression [30].

Chapter 3: Regulation of cholesterol levels

SREBP-2 is not the only manner of regulating cholesterol levels in the cell. This chapter will focus on other methods of regulating cholesterol homeostasis.

3.1 HMGCR degradation and control over flux of the mevalonate pathway

Flux of metabolites through the mevalonate pathway is strongly controlled by metabolic regulation via feedback mechanisms ensuring that the precise amount of cholesterol is synthesized. Rate-limiting enzyme and major control point of the pathway is centred around the enzyme HMG-CoA Reductase (HMGCR). HMGCR is controlled by SREBP-dependent transcription in response to sterols and post-translational via INSIG-dependent protein degradation [5].

The INSIG proteins react to metabolic signals and promote ER-associated protein degradation (ERAD). HMGCR is ubiquitinated on conserved lysine residues by ER-resident ubiquitin ligases (E3s) gp78 and TRC8. Ubiquitinated HMGCR is extracted out of the ER in a VCP/p97-dependent manner and degraded by proteasomes (depicted in figure 6). This stops the metabolic flux of the pathway [5, 20]. A different study found that the E3 MARCH6 also regulates the level of HMGCR. It was known that MARCH6 regulated the levels of the enzyme squalene monooxygenase (SM), which converts squalene to cholesterol in another rate-limiting step in the cholesterol biosynthesis. MARCH6 controlled the levels of SM by negative feedback: degradation was dependent on the sterol levels in the cell. However, the study found that

knockdown of MARCH6 also led to a decrease in HMGCR degradation. This suggests that MARCH6 directly controls the levels of SM and HMGCR, making it a major regulator of flux through the mevalonate pathway [21].

A recent study identified ubiquitin regulator X domain-containing protein 8 (UBXD8) as key determinant of sterol-stimulated HMGCR degradation. UBXD8 is a membrane-bound recruitment factor of VCP/97 – which facilitates extraction of ubiquitinated HMGCR during ERAD. UBXD8 has a VCP-interacting UBX domain. Knockout of UBXD8 showed an accumulation of HMGCR in ER membrane and a lack of HMGCR in the cytosol. This resulted in disruption of the cholesterol metabolism.
It was also proposed that UBXD8 has an unsaturated fatty acid sensing domain. It guides the production of triglycerides and release of fatty acids from triglycerides. Mice with knockdown of UBXD8 developed hepatosteatosis through lipid accumulation. Altogether, these results indicate that UBXD8 has an important regulator role in cellular lipid homeostasis, due to its control over fatty acid- and cholesterol synthesis [5].

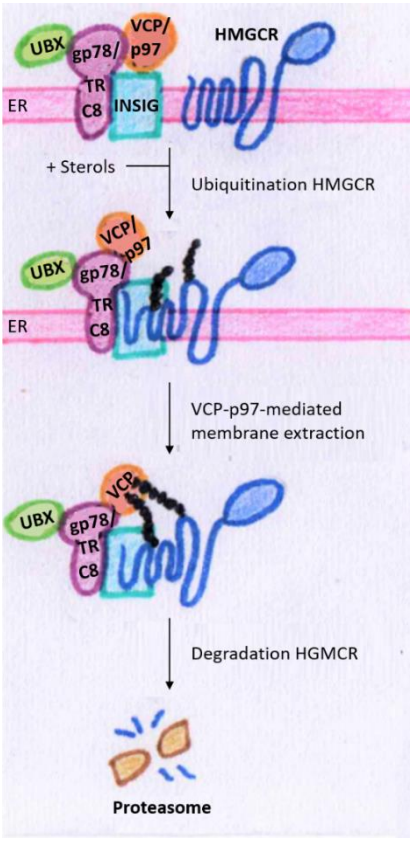

Figure 6. HMGCR degradation via ERAD. INSIG promotes ERAD based on metabolic signals. ERAD by HMGCR ubiquitination is carried out by gp78 and TRC8. The UBX domain of UBXD8 attracts VCP and p97. Which mediate HMGCRs membrane extraction after ubiquitination. HMGCR degradation is carried out by a proteasome [31].

3.2 ORP1L regulates cholesterol transport from late endosome/lysosome to ER, and supresses SREBP-2 processing

Cholesterol levels are regulated by the mevalonate pathway and the receptor-mediated endocytosis of LDL-c (chapter 1). After endocytosis of LDL-c, cholesterol esters are hydrolysed by acid lipase in the late endosome/lysosome (LEL) [7].

The released cholesterol is transported to the limiting outer membrane of LEL. The limiting outer membrane is a border between the inside of the LEL and the cytosolic compartment of the cell [22]. This transport of cholesterol is facilitated by Niemann-Pick C1 and -C2 (NPC1 and NPC2). NPC1 is a large polytopic membrane protein in the limiting membrane of LEL. NPC2 is a small soluble protein, which binds to the cholesterol in the lumen of LEL and transports it to the N-terminal lumen domain of NPC1. NPC1 transfers the cholesterol over the LELs glycocalyx –a lining of polysaccharides that protects the limiting membrane against degradative enzymes – to the limiting membrane [23]. From the limiting membrane, cholesterol is transported to the ER and other organelles. The mechanism by which cholesterol is transported from late endosomes/lysosomes (LELs) to the ER is not yet fully understood, but it is thought to be mediated by soluble transport proteins.

Two such groups of soluble transport proteins are oxysterol-binding protein (OSBP) and OSBP-related proteins (ORP1-11). They contain a conserved C-terminal OSBP-homologue domain (OHD), which binds to oxysterols, cholesterols and phospholipids. OSBPs and ORPs have two phenylalanine in acid tract (FFAT) domains that bind to vesicle-associated membrane protein-associated proteins (VAP) in the ER. They also contain pleckstrin homology (PH) domains that bind phosphatidylinositol polyphosphates (PIPs). These domains give OSBPs and ORPs the ability to anchor itself to the ER and other organelles and mediate lipid transport via their OHD. Multiple types of ORPs are known that facilitate cholesterol transfer from LEL to ER, this study focussed on the newly discovered ORP1L.
ORP1L (OSBPL1) is localized in the LELs and acts as a sensor for sterols. After transport of cholesterol of NPC1 to LELs limiting membrane, ORP1L binds to the cholesterol. This causes complex formation of ORP1L's N-terminal ankyrin (ANK) repeats with Rab7 and Rab7-interacting lysosomal protein (RILP) (depicted in figure 7) [24]. ORP1L mediates LEL-ER contact by binding to VAP on ER. This drives LDL-derived cholesterol transport from the limiting membrane of the LEL to the ER via ORP1L-VAP sites. The cholesterol concentration in the ER increases, which inhibits SREBP-2 processing. Loss of LEL-ER contact causes a drop in ER cholesterol homeostasis and activation of SREBP-2 processing occurs [7].

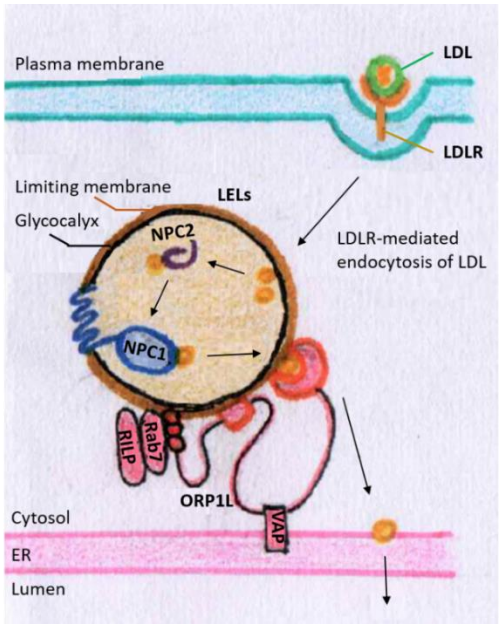

Figure 7. ORP1L mediates cholesterol transport from LELs to the ER. LDL-receptor mediated endocytosis facilitates transport of cholesterol to the LELs. NPC2 transports the cholesterol in the LELs to NPC1, which transports the cholesterol over the glycocalyx and to the limiting membrane of the LELs. Here ORP1L-VAP contact mediates cholesterol transport from the LELs to the ER [3].

Chapter 4: Crosstalk of SREBPs and LXRs

Both the LXR and SREBP pathways are important in lipid- and cholesterol homeostasis and mechanisms of crosstalk exist between these two pathways. One of the examples of crosstalk between SREBPs and LXRs is that expression of *Srebf-1c* and its target genes is dependent on SREBP-2 expression. SREBP-2 expression leads to the production of sterols and oxysterols are the target of LXRs, which activates *Srebf-1c* transcription [12]. Recent studies have found different mechanisms that use crosstalk between SREBPs and LXRs. They will be discussed in this chapters.

4.1 LXR modulates cholesterol biosynthesis by inducing expression of non-coding RNA *LeXis*

LXR regulates cellular cholesterol homeostasis on a transcriptional level. An excess of cholesterol facilitates LXR-mediated gene expression. LXRs target genes promote fatty acid synthesis, which leads to cholesterol esterification and promotes transcription of Idol – and E3 ligase that facilitates ubiquitination of LDLR, which inhibits cholesterol uptake. Inhibition of cholesterol synthesis is regulated by the expression of long non-coding RNA *Liver-expression LXR-induced sequence* (*LeXis*). Expression of *LeXis* is induced by Western Diet or by the synthetic LXR agonist GW3965. *LeXis* inhibits expression of genes involved in cholesterol biosynthesis by binding to RALY. RALY is a ribonucleoprotein and transcription cofactor of cholesterol biosynthesis genes. The binding of *LeXis* to RALY inhibits RALY to activate transcription of genes such as *Srebf-2* and subsequently its target genes (depicted in figure 8) [25].

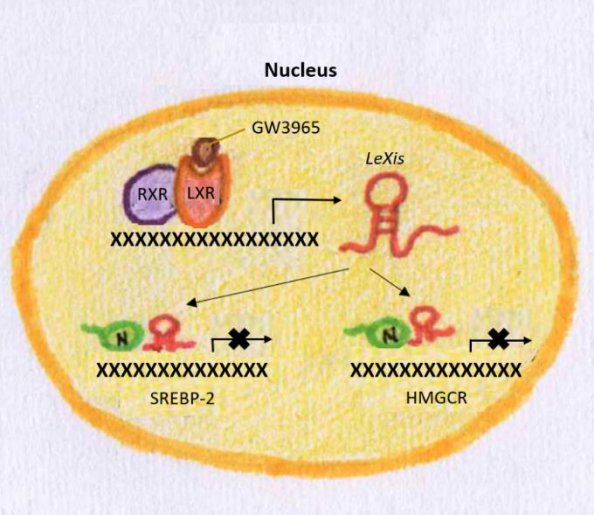

Figure 8. LXR induces expression of non-coding RNA *LeXis* which modulates cholesterol biosynthesis. GW3965 (a synthetic LXR agonist) activates dimerization of LXR and RXR. This activates the transcription of non-coding RNA *LeXis*. *LeXis* binds to the DNA of genes involved in cholesterol synthesis, such as SREBP-2 and HMGCR [25].

4.2 RNF145 inhibits SREBP-2 processing by post-translational modification of SCAP

A recent study identified RNF145 as a novel inhibitor of SREBP-2 processing in the liver.
RNF145 is an ER membrane ubiquitin E3 ligase. LXR is activated by a high level of cholesterol, leading to *Rnf145* expression. RNF145's structure is thought to contain 14 transmembrane domains and a RING domain at the C-terminus. RNF145 is structurally similar to the other E3 ligases, but their function in cholesterol homeostasis differs. RNF145 was the only E3 ligase that inhibited gene expression, whilst TRC8 and gp78 induce ERAD of certain enzymes (described in 3.1). RNF-145's RING domain induces ubiquitination of SCAP on two lysine residues near the COP-II binding site. This inhibits transport of the SREBP-SCAP complex from the ER membrane to the Golgi Apparatus, and thus blocks SREBP processing and inhibits gene expression of genes involved in cholesterol homeostasis [10].

Conclusion

Cholesterol is important for cellular processes, such as membrane formation. The cell will make sure the amount of cholesterol needed is produced or procured, via *de novo* synthesis or LDL-c uptake. If the cholesterol homeostasis is disrupted, it could have major consequences. SREBPs have a key role in this *de novo* synthesis of cholesterol, an overactive SREBP could result in an accumulation of cholesterol and lipids. The amount of cholesterol in plasma could greatly increase and significantly elevate the risk of heart- and vascular disease. The mechanisms described in this review could be manipulated to fit the pharmacological need of intercepting the cholesterol metabolism in different ways and decreasing the risk of various diseases associated with increased lipid- and cholesterol metabolisms.

For example: an increase in flexible polyunsaturated fatty acyl chains – courtesy of LPCAT3 – surrounding the SREBP-SCAP complex promote SREBP processing [15]. LPCAT3 inhibition might be a suitable therapy for fatty liver disease.
It might also be possible to block mTORC1 activity. This would enable translocation of lipin-1 to the nucleus, where it would degrade nSREBP-2 [16]. A different route could be increasing LEL-ER cholesterol transport, which would suppress SREBP-2 activation in a more natural way [3]. A less invasive route could be inhibiting SREBP processing via LXR-induced expression of *LeXis*, in the case of a disease associated with cholesterol accumulation [25].

Whilst recent developments have shone a light on new regulatory mechanisms of SREBP in the liver, there are still a lot of questions surrounding the regulation of SREBPs and cholesterol. More research would have to be done to determine the full physiological roles of the recently discovered pathways and to see if those pathways could be used as a possible therapy for disease in which the lipid- and cholesterol metabolisms play a major role.

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