Regulation and Feedback of Cholesterol Metabolism
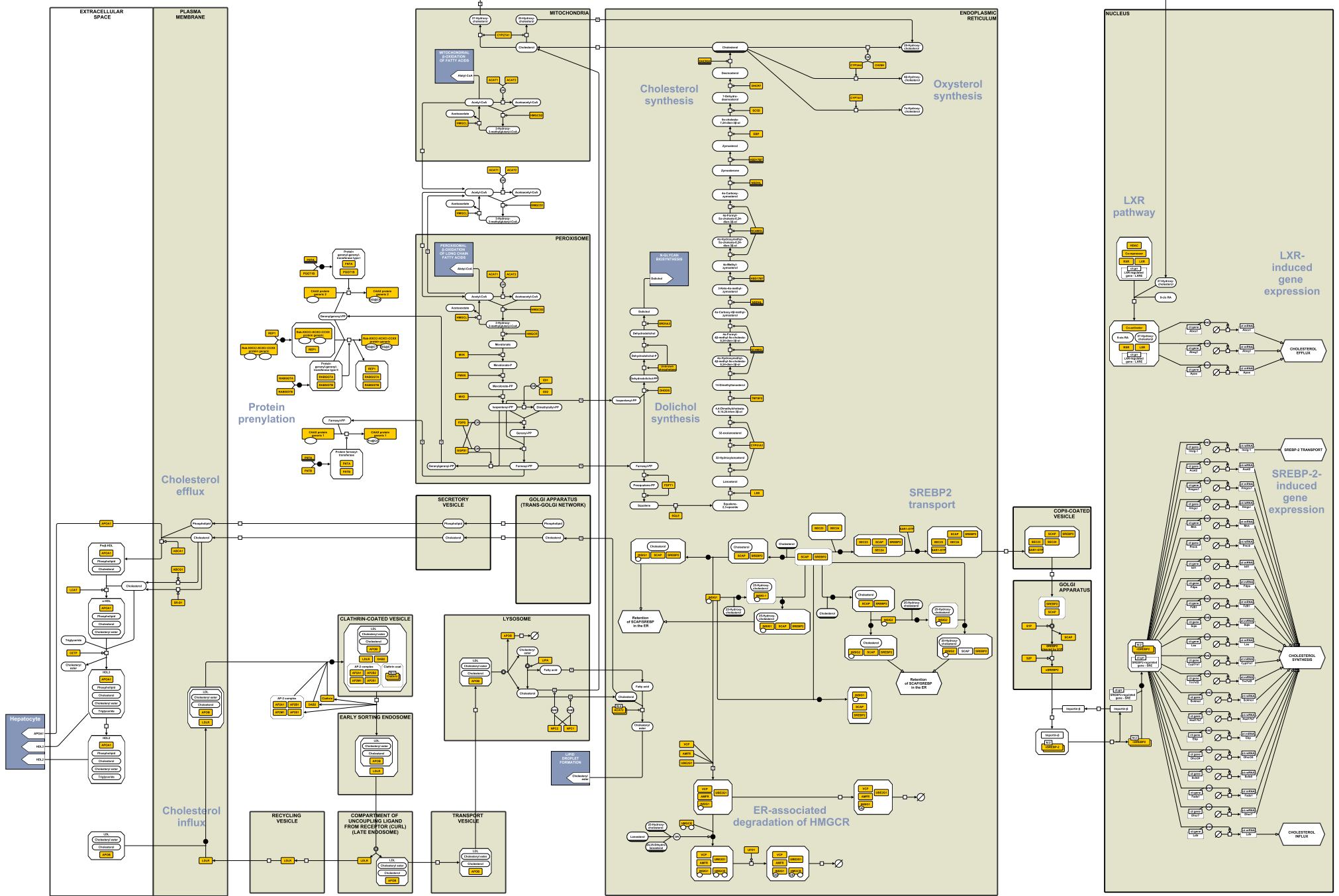
A Mazein[1,2], S Watterson[1,2], H Gibbs[1], W Hsieh[1], M Ba Abdullah[1], M Blanc[1], WJ Griffiths[4], TC Freeman[1,3] and P Ghazal[1,2]

[1]Division of Pathway Medicine, Chancellor's Building, Little France Crescent, Edinburgh, EH16 4SB, Scotland, UK;[2]Centre for Systems Biology at Edinburgh, CH Waddington Building, King's Buildings Campus, Mayfield Road, Edinburgh, EH9 3JD, Scotland, UK
[3]The Roslin Institute, R (D) SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK;[4]Institute of Mass Spectrometry, School of Medicine, Grove Building, Swansea University, Singleton Park, Swansea SA2 8PP, UK

EXTRACELLULAR SPACE

PLASMA MEMBRANE

MITOCHONDRIA

ENDOPLASMIC RETICULUM

NUCLEUS

MITOCHONDRIAL β-OXIDATION OF FATTY ACIDS

Cholesterol synthesis

Oxysterol synthesis

LXR pathway

LXR-induced gene expression

PEROXISOMAL β-OXIDATION OF LONG CHAIN FATTY ACIDS

N-GLYCAN BIOSYNTHESIS

Protein prenylation

Dolichol synthesis

SREBP-2-induced gene expression

SREBP2 transport

Cholesterol efflux

SECRETORY VESICLE

GOLGI APPARATUS (TRANS-GOLGI NETWORK)

COPII-COATED VESICLE

GOLGI APPARATUS

CLATHRIN-COATED VESICLE

LYSOSOME

Hepatocyte

EARLY SORTING ENDOSOME

LIPID DROPLET FORMATION

ER-associated degradation of HMGCR

Cholesterol influx

RECYCLING VESICLE

COMPARTMENT OF UNCOUPLING LIGAND FROM RECEPTOR (CURL) (LATE ENDOSOME)

TRANSPORT VESICLE

CHOLESTEROL EFFLUX

CHOLESTEROL SYNTHESIS

CHOLESTEROL INFLUX

SREBP-2 TRANSPORT

Regulation and feedback of cholesterol metabolism

A Mazein[1,2], S Watterson[1,2], H Gibbs[1], W Hsieh[1], M Ba Abdullah[1], M Blanc[1], WJ Griffiths[4], TC Freeman[1,3] and P Ghazal[1,2]

[1]Division of Pathway Medicine, University of Edinburgh, Chancellor's Building, Little France Crescent, Edinburgh, EH16 4SB, UK, [2]Centre for Systems Biology at Edinburgh, University of Edinburgh, CH Waddington Building, King's Buildings Campus, Mayfield Road, Edinburgh, EH9 3JD, UK, [3]The Roslin Institute, R (D) SVS, University of Edinburgh, Easter Bush, Midlothian, EH25 9RG, UK [4]Institute of Mass Spectrometry, School of Medicine, Grove Building, Swansea University, Singleton Park, Swansea SA2 8PP, UK.

Cholesterol biosynthesis serves as a central metabolic hub for numerous biological processes in health and disease. An integrative knowledge representation of how the cholesterol pathway is structured and how it interacts with other pathway systems is lacking. Here we provide using Systems Biology Graphical Notation the research synthesis of a process diagram integrating the regulatory and feedback systems for cholesterol synthesis.
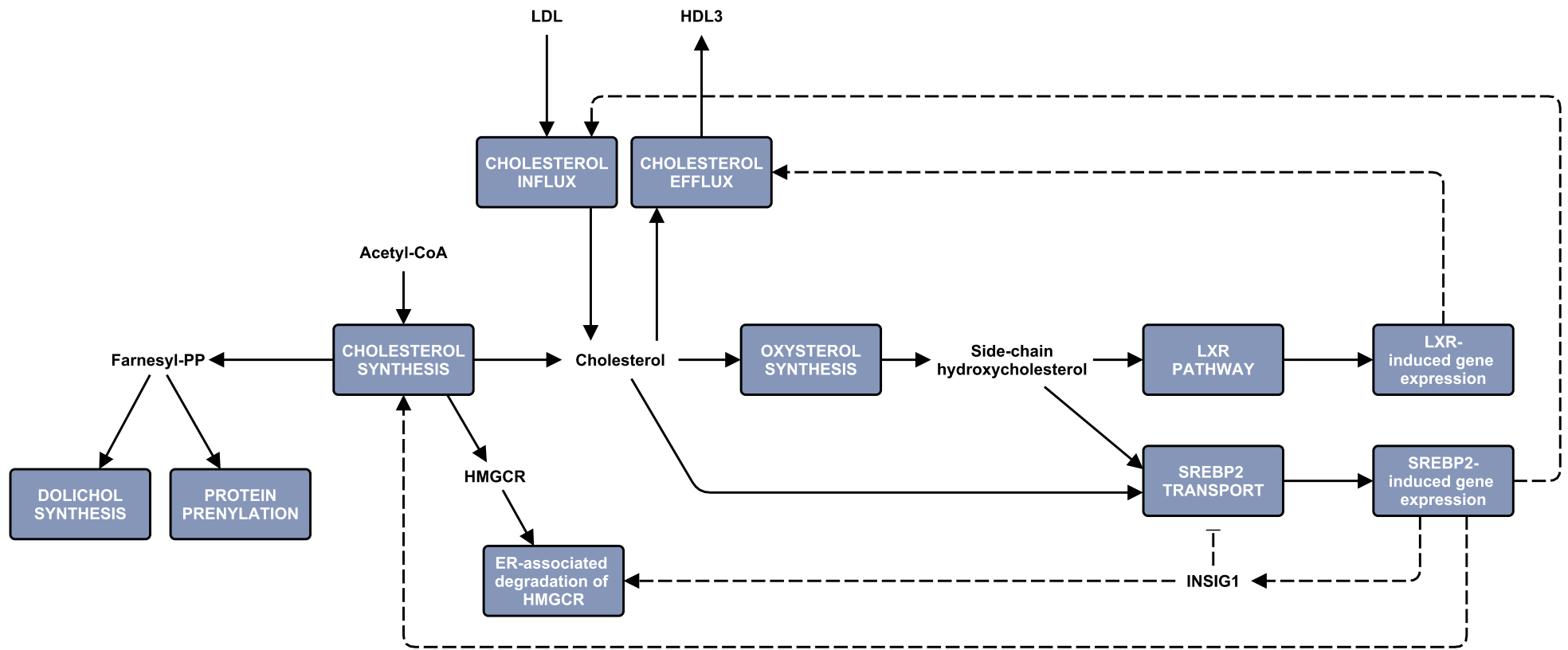
Cholesterol is an intensively studied, multi-functional lipid that is key to many aspects of immunological, neuronal, viral and hepatocyte biology. It is an essential component of cellular membranes and is a precursor to steroids, bile acids and vitamin D whilst its own precursors contribute to prenylation and dolichylation. As a consequence of its broad role, tightly regulated mechanisms have evolved to ensure intracellular homeostasis of the cholesterol biosynthesis pathway.

Despite the importance of the sterol biosynthesis pathway to cellular function and its value in pharmaceutical therapies, an integrative picture of how the pathway is structured and how it interacts with other pathway systems has not been well described in the literature.

Here, we present a pathway diagram that provides an integrative consensus view of the current level of understanding of the sterol biosynthesis pathway, its relationship with other pathways of note and its associated feedback mechanisms. This model of the sterol biosynthesis pathway has been assembled using a variety of publicly available resources including the research findings of the LipidMaps consortium (http://www.lipidmaps.org) and results obtained from thorough searches of the published literature that have been manually curated and validated by domain experts. It is most relevant to macrophage biology.

The model that we present here is described using the Systems Biology Graphical Notation[1] (SBGN), a community driven consensus graphical schema for capturing the molecular details of pathway systems, although many other schema exist that can be used to provide alternative descriptions, such as mEPN[2]. The Systems Biology Graphical Notation Process Description language Level 1 scheme[3] was used in a slightly modified form. We used a different shape for simple chemicals in order for compound names to fit within. The pathway names such as "cholesterol synthesis" or "SREBP transport" are also not a part of SBGN PD language and are used on the scheme for the readers' benefit.

Together with the SBGN model, we include a simplified schematic that shows the relationship between the pathway subsystems that are related to cholesterol biosynthesis.

LDL

HDL3

**CHOLESTEROL
INFLUX**

**CHOLESTEROL
EFFLUX**

Acetyl-CoA

Farnesyl-PP ◄── **CHOLESTEROL
SYNTHESIS** ──► Cholesterol ──► **OXYSTEROL
SYNTHESIS** ──► Side-chain
hydroxycholesterol ──► **LXR
PATHWAY** ──► **LXR-
induced gene
expression**

**DOLICHOL
SYNTHESIS**

**PROTEIN
PRENYLATION**

HMGCR

**ER-associated
degradation of
HMGCR**

**SREBP2
TRANSPORT** ──► **SREBP2-
induced gene
expression**

INSIG1

The model can be broadly described as a system that combines: cholesterol influx, cholesterol efflux, protein prenylation, dolychol synthesis, cholesterol synthesis, ER associated degradation of HMGCR, SREBP2 transport, oxysterol synthesis, the LXR pathway, SREBP2-induced gene expression and LXR-induced gene expression.

We also include a list of the published literature from which the model has been assembled and a list of UniProt Ids for the proteins captured in the model.

This is a multi-scale model that captures the details of absorption and efflux of cholesterol between the cell and the blood plasma. However, it is not completely exhaustive. The LDL and VDL transports systems are not captured. It describes the cholesterol processing that occurs in each organelle of the cell and the transcriptional control that mediates feedback to ensure cholesterol homeostasis.

Cholesterol biosynthesis itself constitutes a sequence of metabolic transitions that occur across several organelles, starting with the processing of acetyl-CoA in the mitochondria. This pathway branches into the protein prenylation arm, which has been shown to be critical to optimal CMV infection[4], and the sterol arm that is responsible for cholesterol production. Feedback occurs through SREBP2 transport. The SCAP:SREBP2 complex, which ordinarily is chaperoned to the nucleus to activate the suite of enzymes associated with the cholesterol biosynthesis pathway, is instead retained in the endoplasmic reticulum, in the presence of relatively high concentrations of intracellular cholesterol or side-chain hydroxylated cholesterol, in particular 25-hydroxycholesterol. This acts to down-regulate transcription of the enzymes acting on the sterol biosynthesis pathway until ordinary levels of cholesterol and its derivatives have been reached.

It has been reported that SCAP:SREBP2 is significantly retained when cholesterol concentration exceeds 5% of total endoplasmic reticulum lipids[5]. However, it would appear that the sensitivity of the feedback mechanism to sterol levels is dependent on expression as overexpression of Insig-1 has been shown to lower this threshold to 3%[5].

INSIG1 is transcriptionally dependent on SREBP2, while INSIG2 is not[6]. When intracellular cholesterol rises, we would expect SREBP2 retention to lead to a fall in de novo INSIG1 synthesis. As the free and unbound INSIG1 protein declines, this would lead to INSIG2 becoming dominant in the process of SREBP2 retention. Differences between the affinity of INSIG1 and INSIG2 for complex formation will emerge at this point as a change to the feedback sensitivity. As cholesterol levels start to fall, retained SREBP2 will be increasingly transported to the nucleus, boosting INSIG1 synthesis and restoring the pool of INSIG1 protein available to respond to further fluctuations in cholesterol levels.

This regulation impacts not just upon cholesterol, but also upon the oxysterols downstream of cholesterol and upon the LXR genes, downstream of the oxysterols. In the diagram presented, the transcriptional output of the genes affected has not been directly connected to the corresponding enzymes in the cholesterol biosynthesis pathway for the benefit of clarity.

We hope that by elucidating and integrating the details of this pathway and its context, this will form the basis of a finer level of understanding of the pathway and its function and that this will add a greater insight to future studies of the sterol biosynthesis pathway.

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PROTEIN NAME	UNIPROT ID	SYNONYMS	COMMENTS
ABCA1	O95477		
ABCG1	P45844		
ACAT1	P35610	SOAT1	
ACAT2	O75908	SOAT2	
AMFR	Q9UKV5		
AP2A1	O95782		
AP2B1	P63010		
AP2M1	Q96CW1		
AP2S1	P53680		
APOA1	P02647		
APOB	P04114		
CAAX PROTEIN GENERIC 1	NA		GENERIC
CAAX PROTEIN GENERIC 2	NA		GENERIC
CETP	P11597		
CH25H	O95992		
CLATHRIN	NA		GENERIC
CYP27A1	Q02318		
CYP3A4	P08684		
CYP51A1	Q16850		
CYP7A1	P22680		
DAB2	P98082		
DHCR24	Q15392		
DHCR7	Q9UBM7		
EBP	Q15125		
FDFT1	P37268		
FDPS	P14324		
FNTA	P49354		
FNTB	P49356		
GGPS1	O95749		
HDAC	NA		GENERIC
HMGCL	P35914		
HMGCR	P04035		
HMGCS1	Q01581		
HMGCS2	P54868		
HSD17B7	P56937		
IDI1	Q13907		
IDI2	Q9BXS1		
INSIG1	O15503		
INSIG2	Q9Y5U4		
LCAT	P04180		

PROTEIN NAME	UNIPROT ID	SYNONYMS	COMMENTS
LDLR	P01130		
LIPA	P38571		
LSS	P48449		
LXR	NA		GENERIC
LXRA	Q13133	NR1H3	
LXRB	P55055	NR1H2	
MVD	P53602		
MVK	Q03426		
NPC1	O15118		
NPC2	P61916		
NSDHL	Q15738		
PGGT1B	P53609		
PMVK	Q15126		
RABGGTA	Q92696		
RABGGTB	P53611		
RAB-XXCC/-XCXC/-CCXX PROTEIN GENERIC	NA		GENERIC
REP1	P24386		
RXR	NA		GENERIC
RXRA	P19793		
RXRB	P28702		
RXRG	P48443		
S1P	Q14703	MBTPS1	
S2P	O43462	MBTPS2	
SAR1-GTP	Q9NR31		
SC4MOL	Q15800		
SC5D	O75845		
SCAP	Q12770		
SEC23	NA		GENERIC
SEC24	NA		GENERIC
SQLE	Q14534		
SR-B1	Q8WTV0		
SREBP2	Q12772		
TM7SF2	O76062		
UBE2G1	P62253		
UFD1	Q92890	UFD1L	