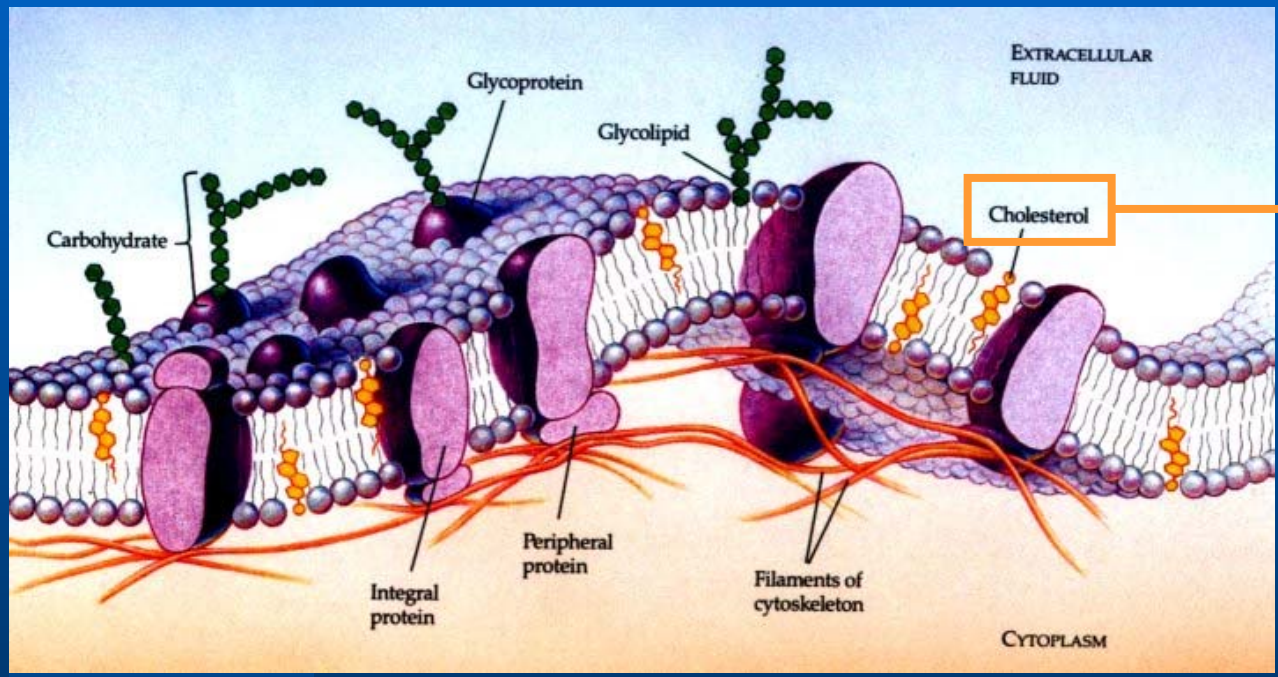
Cholesterol transporters as new therapeutic targets

Unité de Pharmacologie
cellulaire et moléculaire

F. Van Bambeke

Physiological roles of cholesterol

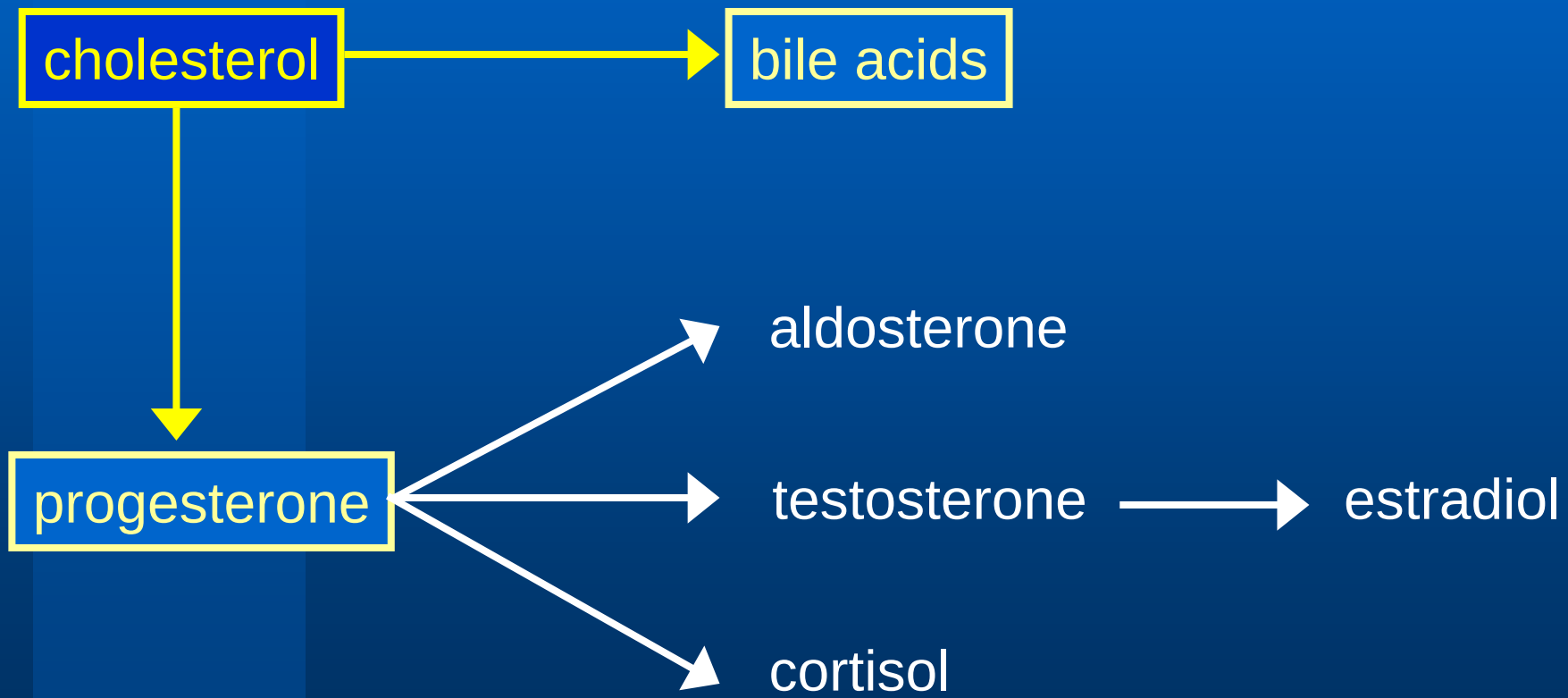
Physical role: structure and functions of membranes



- fluidity
- domains (rafts)
- protein function (pumps)

Physiological roles of cholesterol

Biochemical role:
precursor of steroid hormones and bile acids



Pathological roles of cholesterol

- increase in storage
- alteration of cellular fate
- HDL/LDL dysbalance
- increase in absorption
- alteration of excretion

- obesity, dyslipidemia
- Niemann-Pick disease
- atheromatosis, Tangier dis.
- sitosterolemia
- stones

current therapeutic options…	and their limitations
• lipid adsorbants	adsorption of liposoluble vitamins
• statins	risk of side effects
• fibrates	useful if high triglycerides

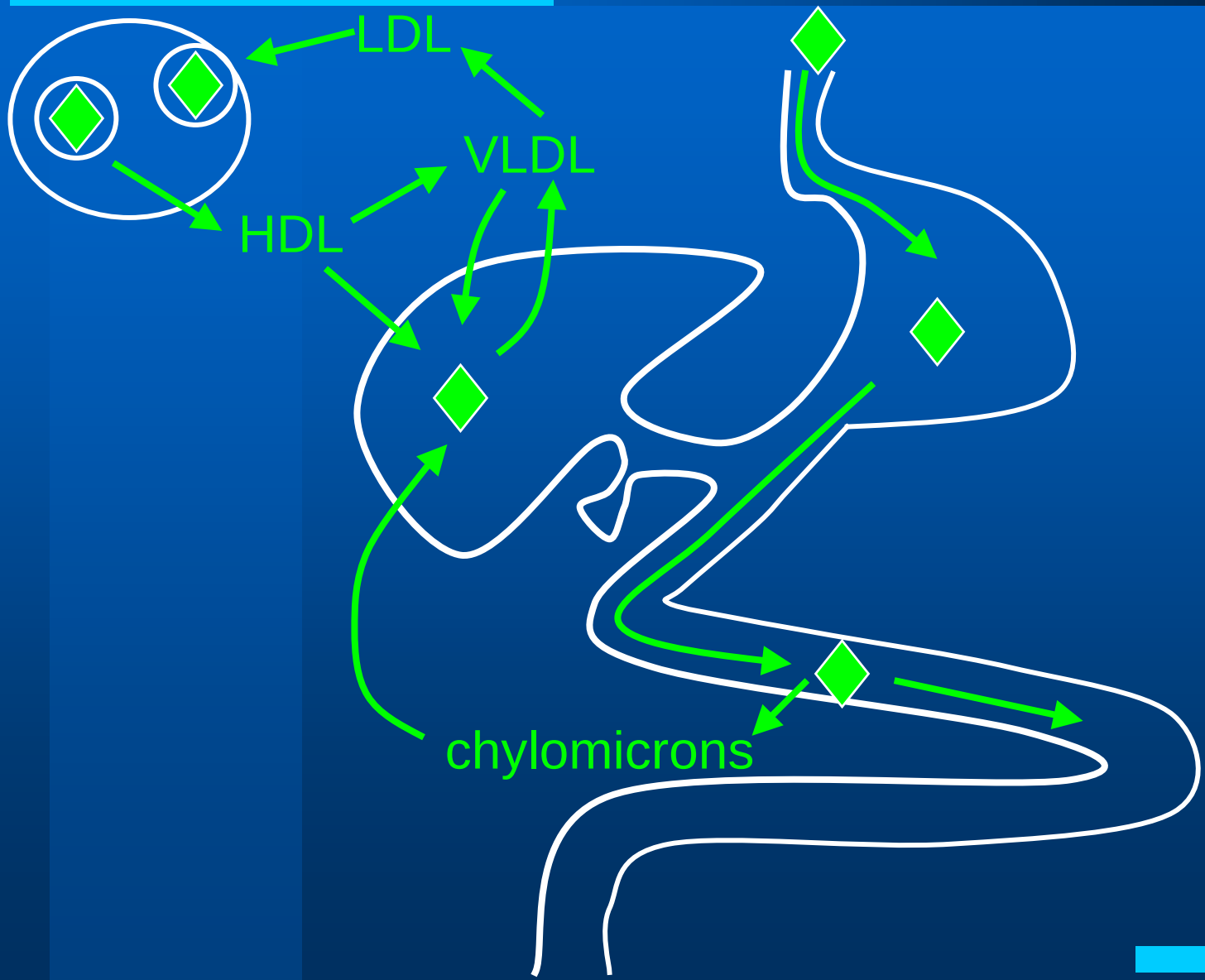
Pathological roles of cholesterol transporters

- NPC1L1
- NPC1-NPC2
- ABCA1
- ABCG5-ABCG8
- ABCG8

- obesity, dyslipidemia
- Niemann-Pick disease
- atheromatosis, Tangier dis.
- sitosterolemia
- stones

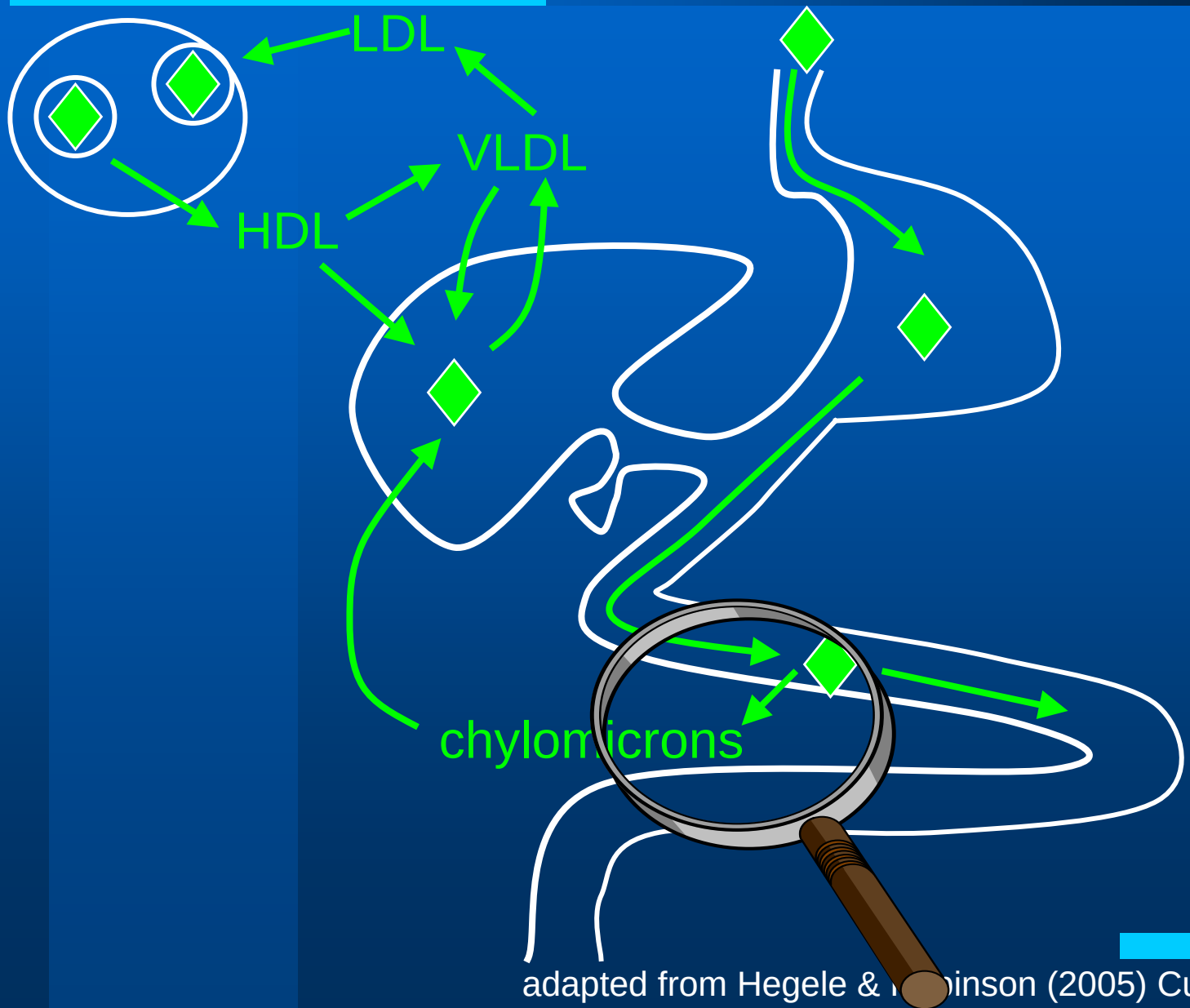
Cholesterol transporters as new drug targets ?

Sterol fate in the body



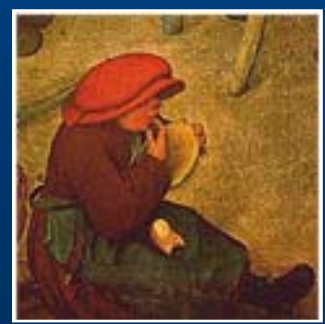
adapted from Hegele & Robinson (2005) Cur. Drug Tragets 5:31-7

Sterol fate in the body



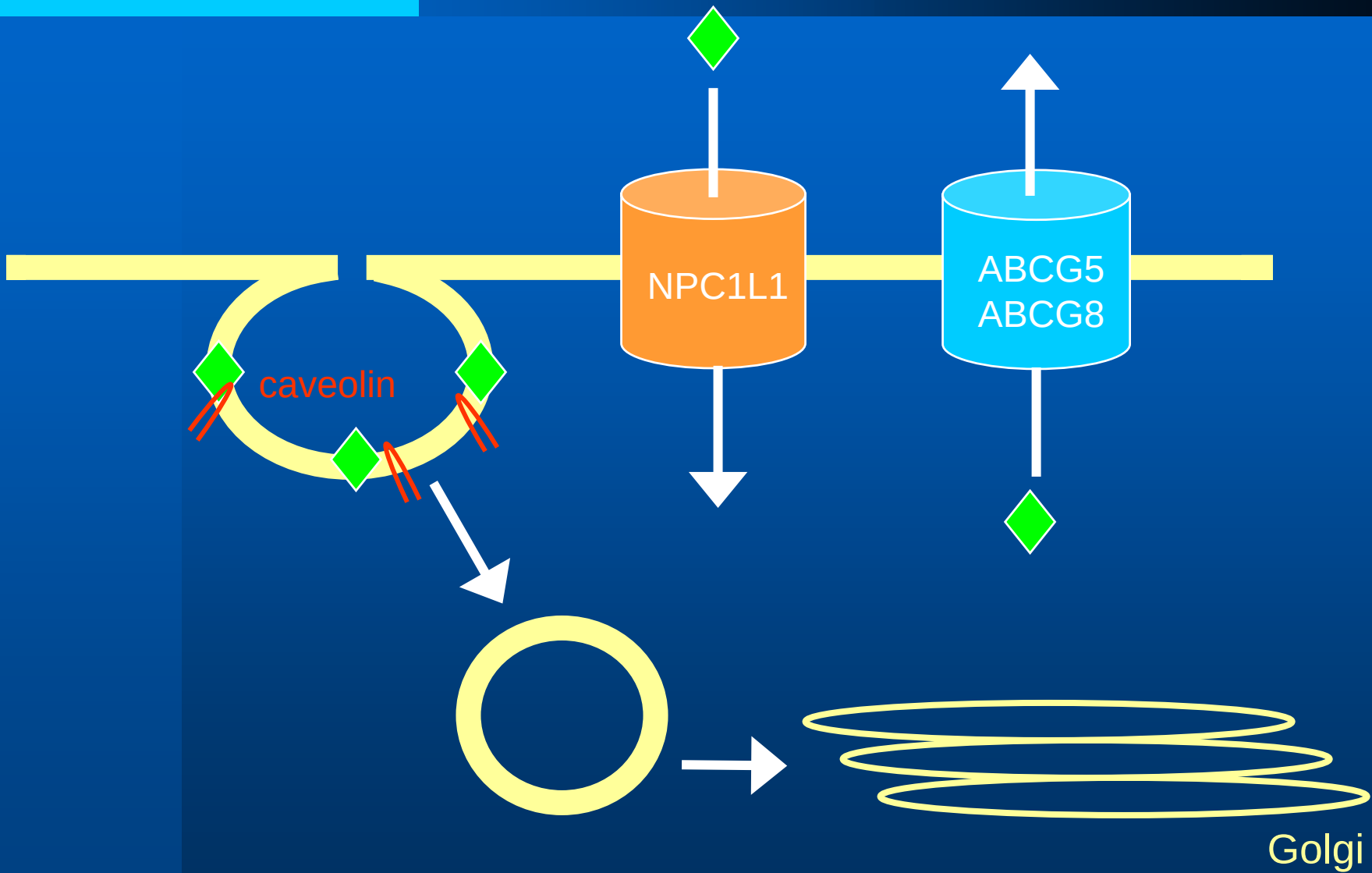
inhibition of unfavorable transport

cholesterol absorption



adapted from Hegele & Robinson (2005) Cur. Drug Tragets 5:31-7

Transport of sterols in intestinal cells

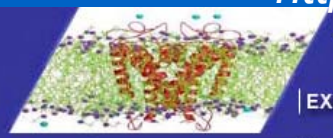


Razani et al (2002) Pharmacol. Rev. 54: 431-67; Sudhop *et al*. (2005) Pharmacol. Ther. 105:333-41

NPC1L1, a RND sterol transporter

http://www-biology.ucsd.edu/~msaier/transport/

TCDB UCSD



Transport Classification Database

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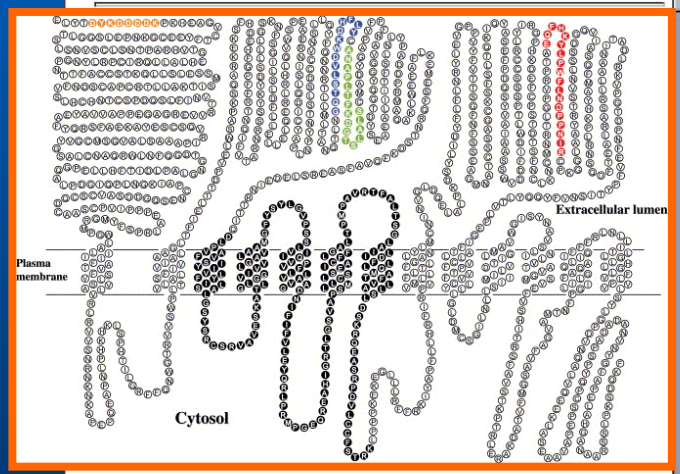
2.A.6. The Resistance-Nodulation-Cell Division (RND) Superfamily

View Proteins Read Description



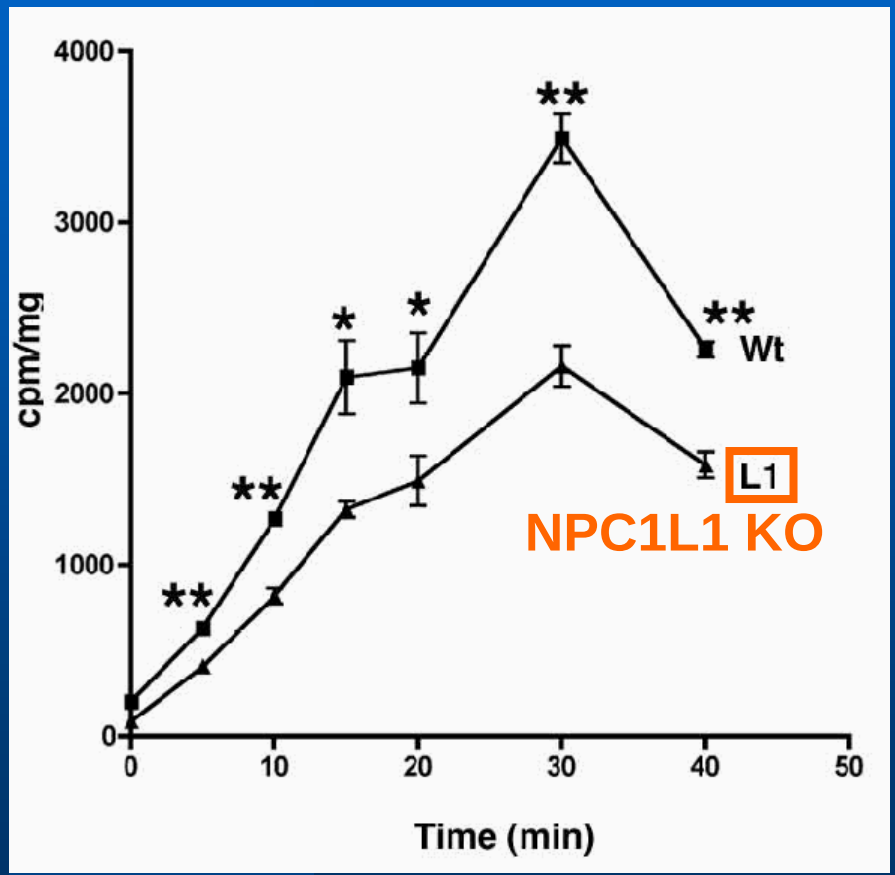
2.A.6.6. The Eukaryotic (Putative) Sterol Transporter (EST) Family

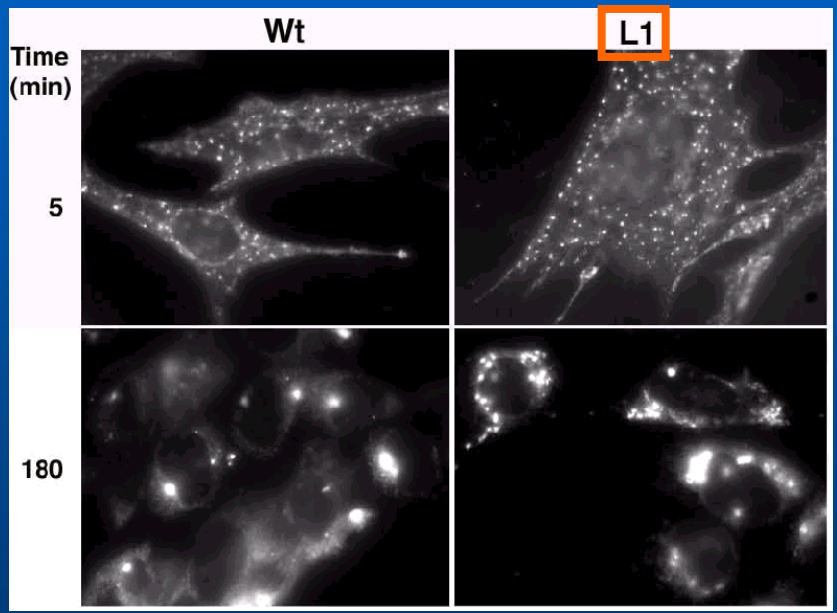
2.A.6.6.1	Niemann-Pick C1 AND C2 disease proteins (together to form a possible lipid/cholesterol exporter from lysosomes to other cellular sites) (Sleat et al., 2004).	Animals	NPC1 and NPC2 of *Homo sapiens* NPC1 (AAH63302) NPC2 (AAH02532)
2.A.6.6.2	Patched (Ptc) segmentation polarity protein	Animals	"Patched" of *Drosophila melanogaster*
2.A.6.6.3	Yeast membrane protein YPL006w	Protein, yeast	YPL006w of *Saccharomyces cerevisiae*
2.A.6.6.4	SREBP cleavage-activating protein, SCAP	Animals	SCAP of *Cricetulus griseus*
2.A.6.6.5	3-hydroxy-3-methylglutaryl (HMG)-CoA reductase	Animals	HMG-CoA reductase of *Homo sapiens*
2.A.6.6.6	Intestinal enterocyte brush border Niemann-Pick C1 like 1 (NPC1L1) protein; probably responsible for ezetimibe-sensitive absorption of luminal cholesterol (Altmann et al., 2004).	Animals	NPC1L1 of *Homo sapiens* (NP_037521)

Davies *et al.* (2000) Genomics 65:137-45; Yier *et al*. (2005) BBA in press

NPC1L1 mediates sterol absorption

NPC1L1 reduces cholesterol uptake and processing to the Golgi

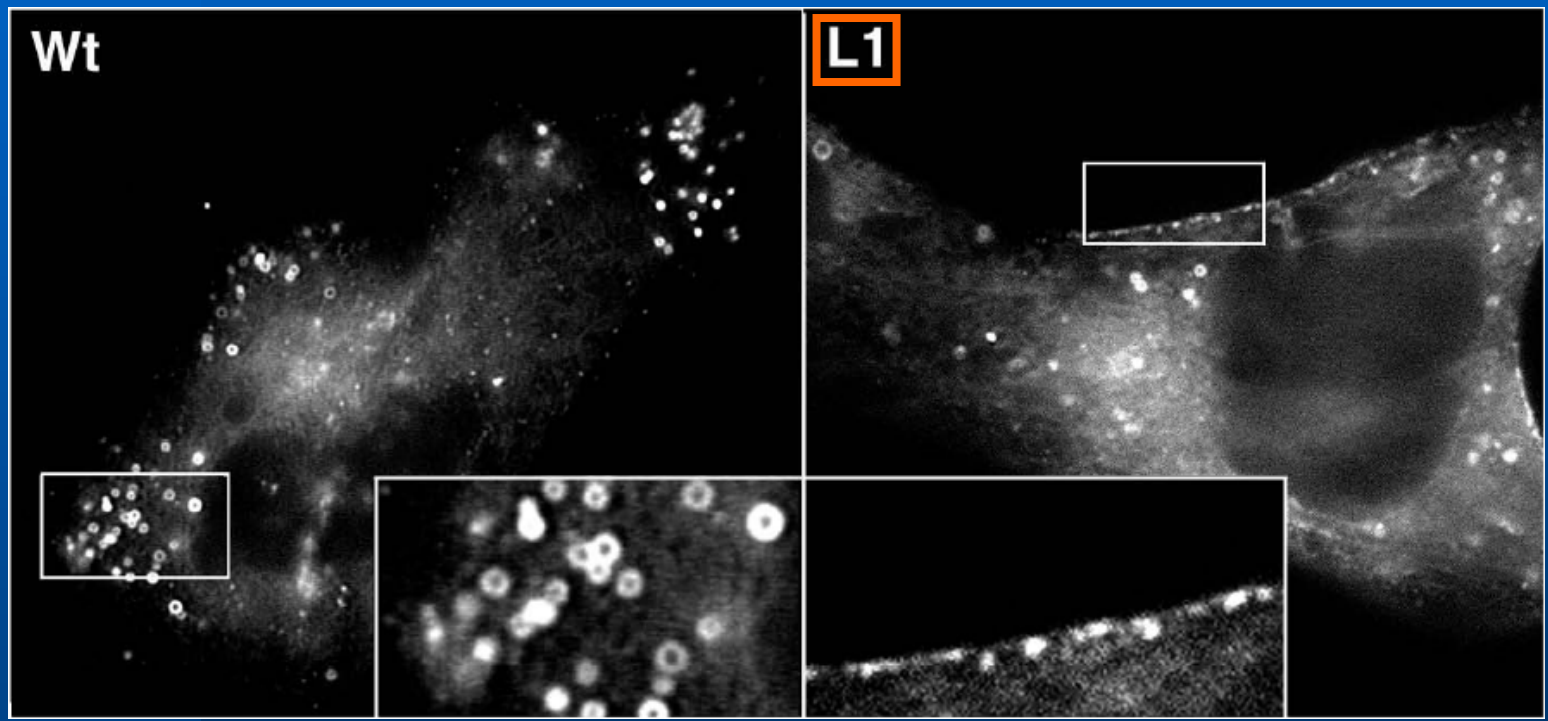




Davies *et al.* (2005) JBC 280:12710-20

NPC1L1 controls lipid endocytosis

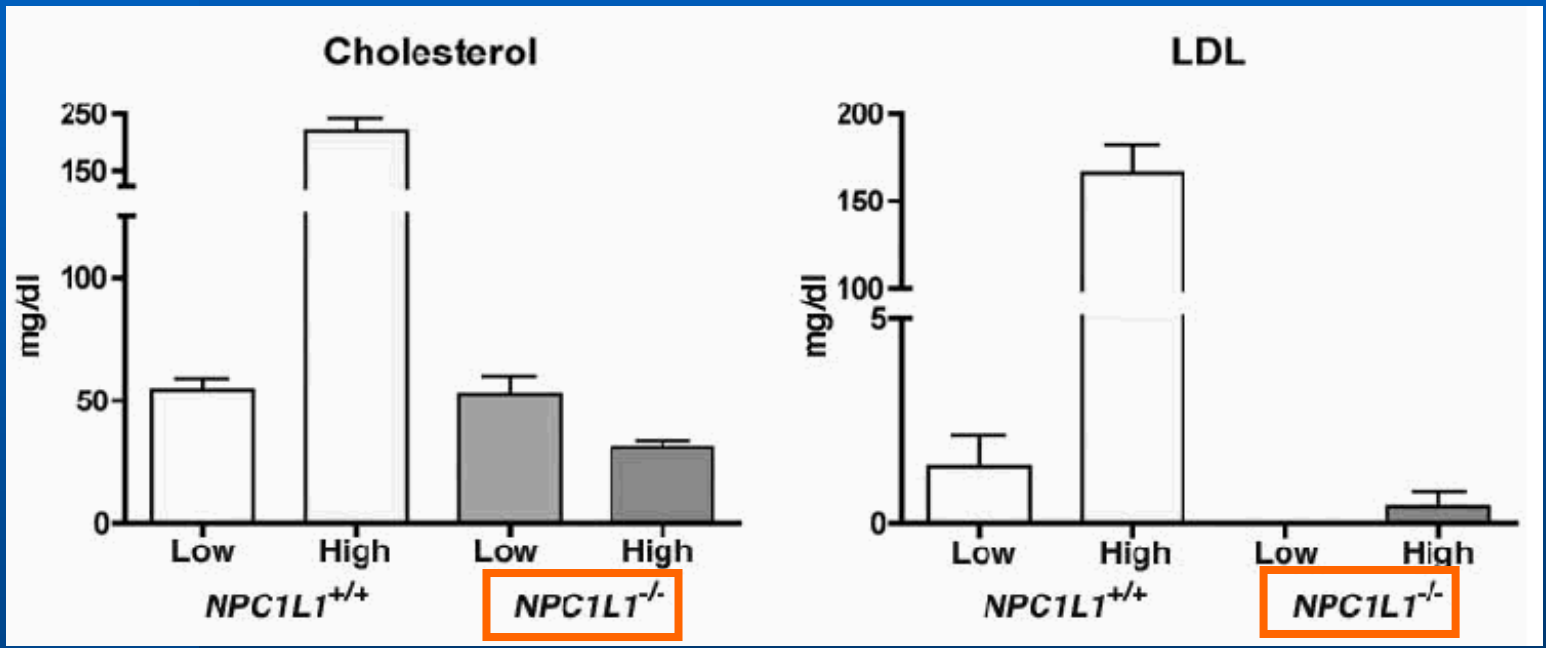
NPC1L1 controls caveolin localization



Davies *et al.* (2005) JBC 280:12710-20

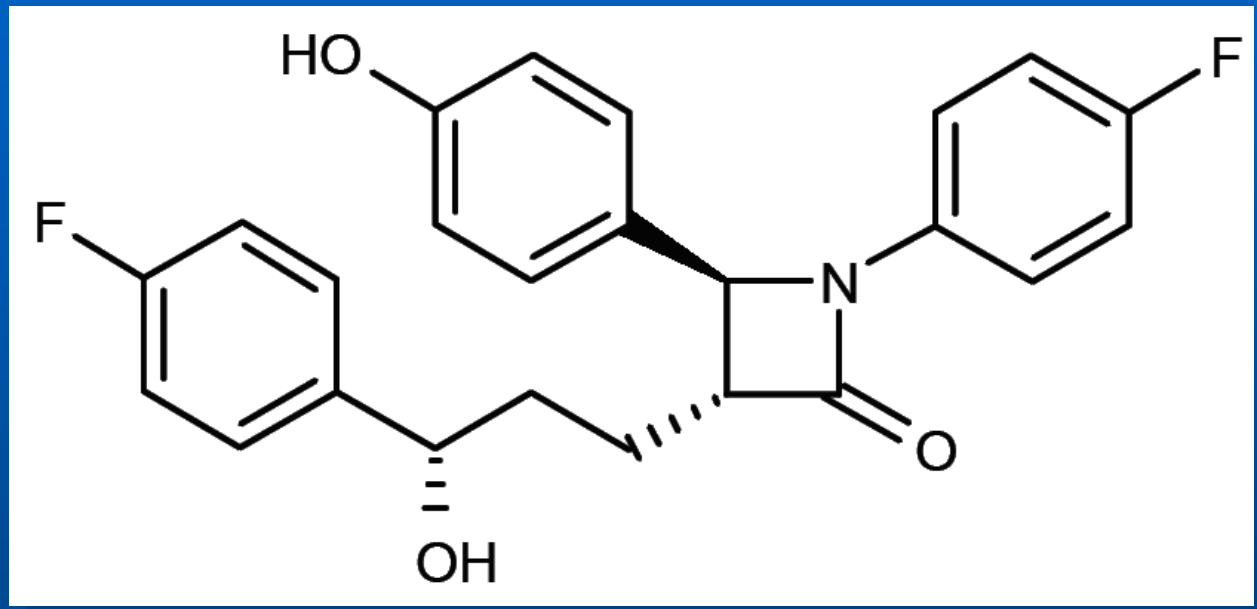
NPC1L1 as a target for treating obesity ?

NPC1L1 KO mice are protected from hypercholesterolemia induced by a high fat diet



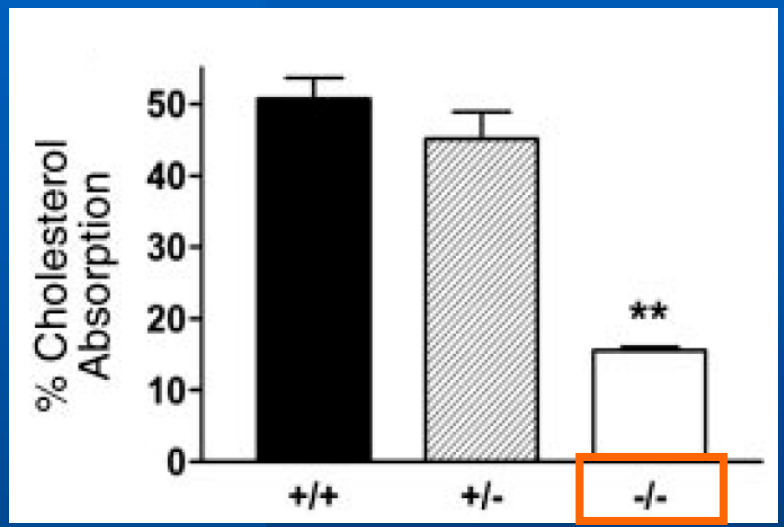
Davies *et al.* (2005) JBC 280:12710-20

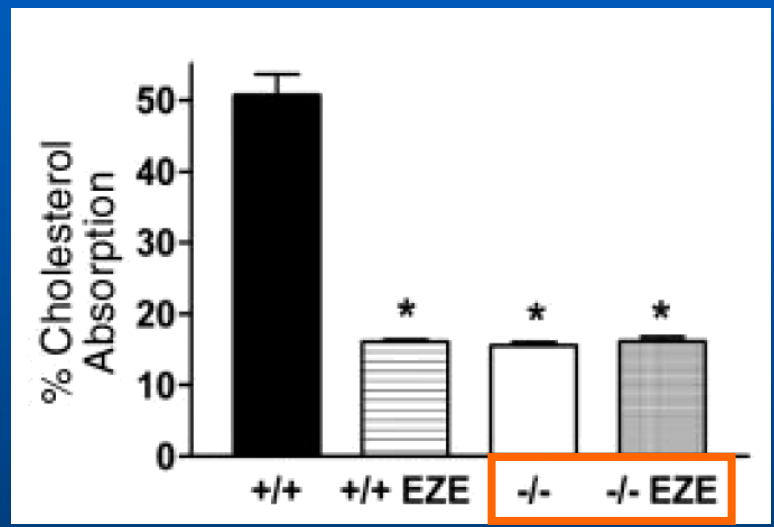
Ezetimibe as inhibitor of sterol absorption



Ezetimibe, a dual target inhibitor:
>< NPC1L1

Ezetimibe is not efficient in NPC1L1 KO mice



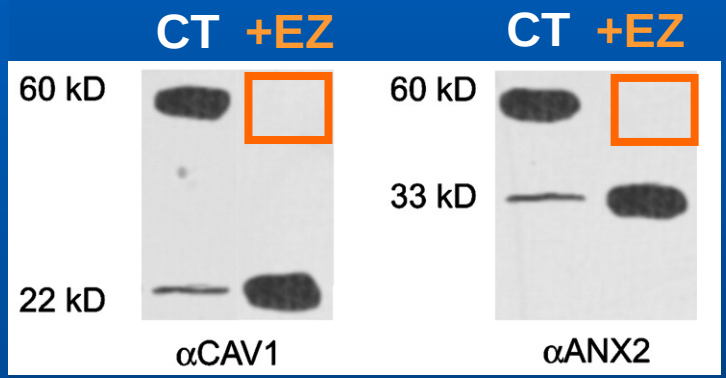


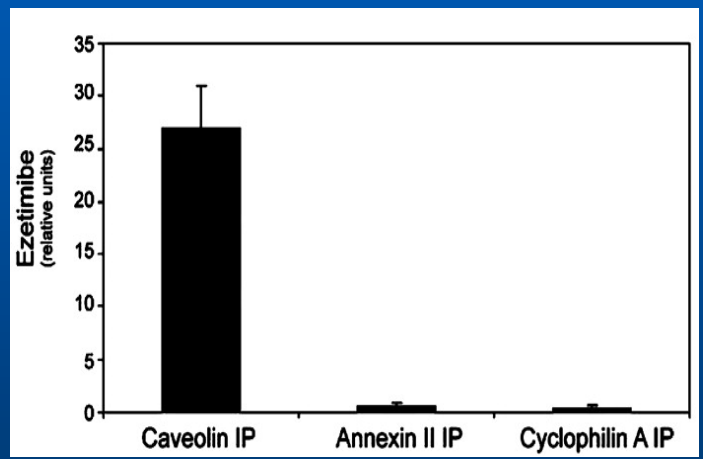
Altmann *et al.* (2004) Science 303:1201-4

Ezetimibe, a dual target inhibitor:

>< caveolin

Ezetimibe inhibits the formation of the annexin 2 – caveolin 1 complex by binding to caveolin

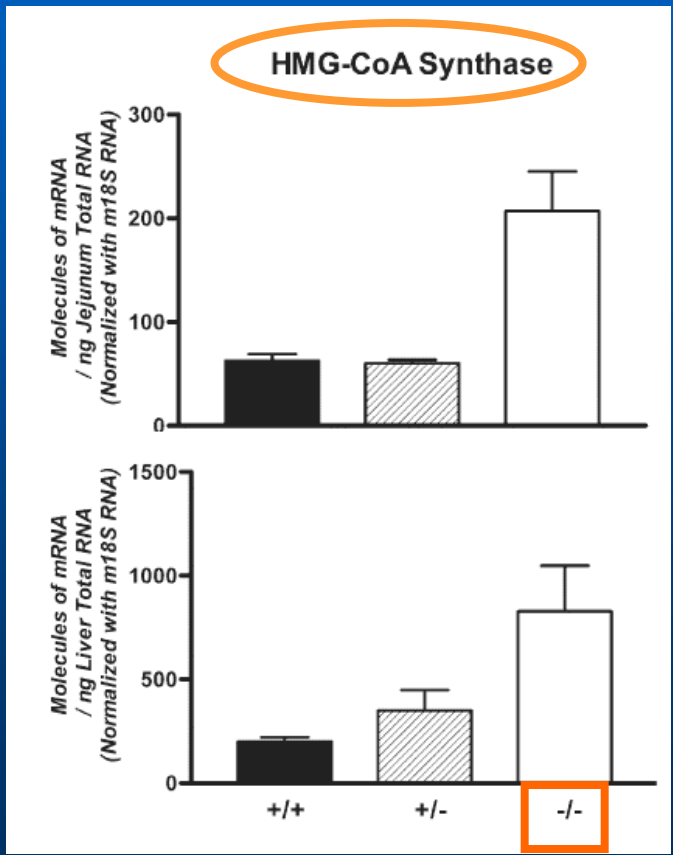




Smart *et al.* (2004) PNAS 101:3450-5

Ezetimibe in combination with statins

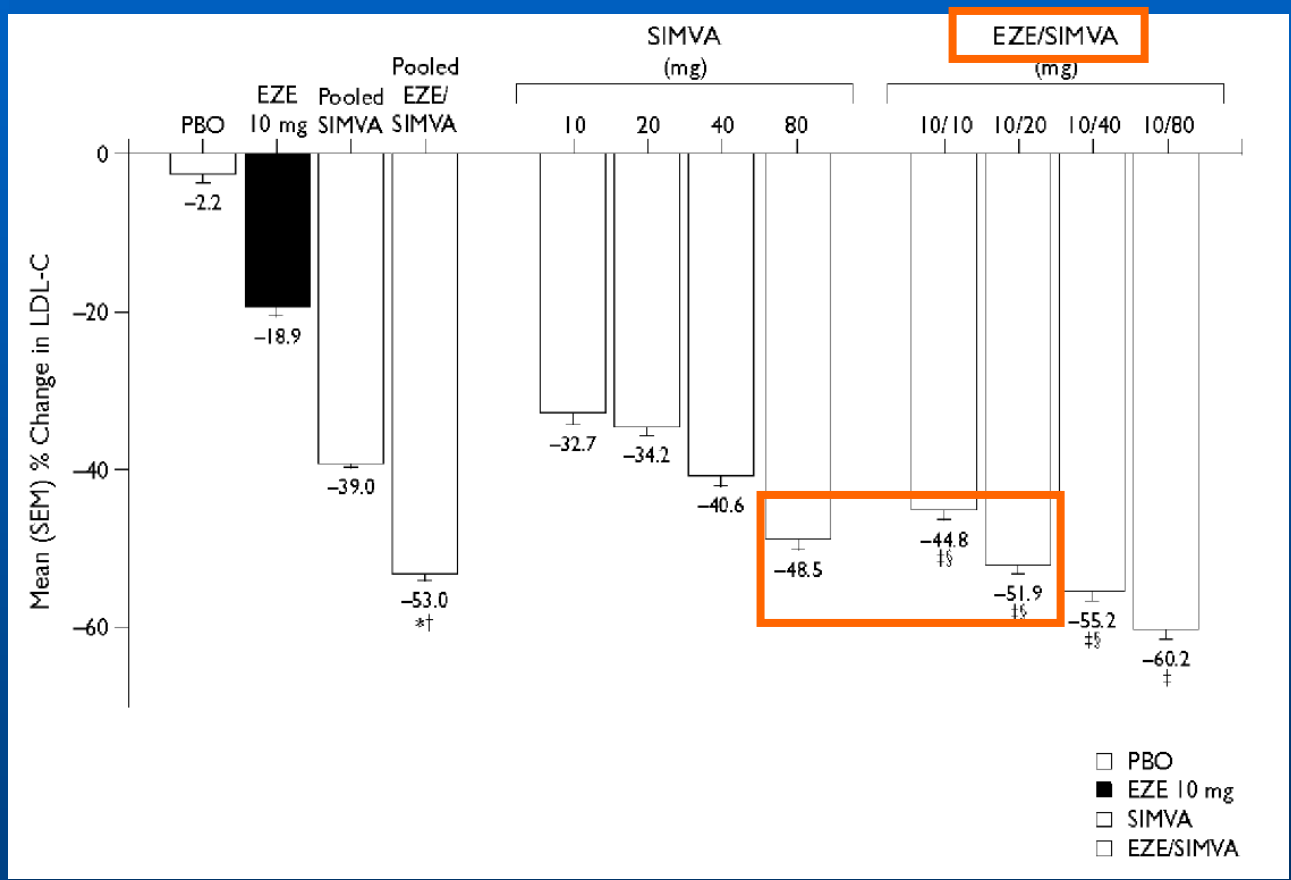
Reduction of cholesterol absorption stimulates endogeneous synthesis



Smart *et al.* (2004) PNAS 101:3450-5

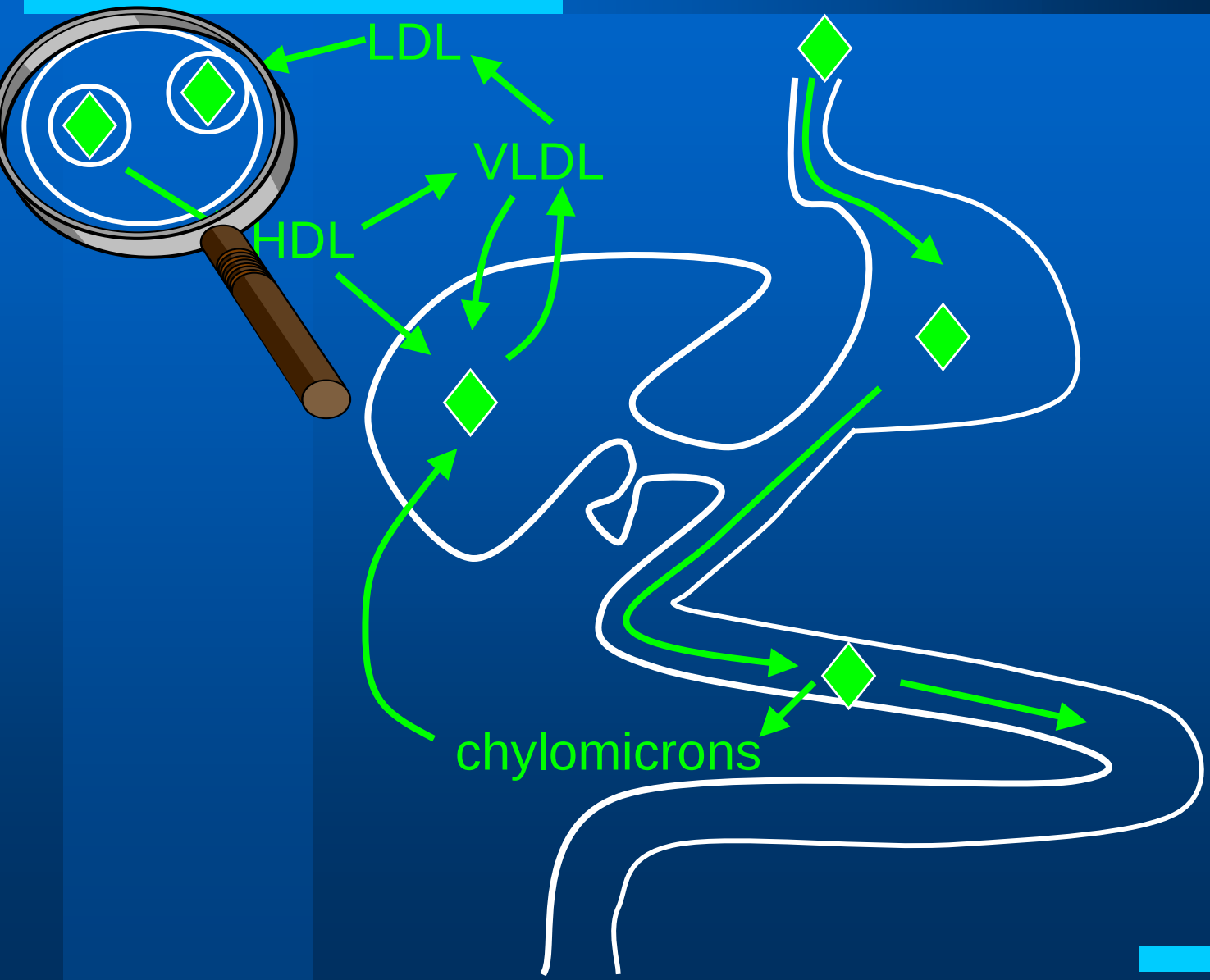
Ezetimibe approved in Europe and in USA

10 mg EZ + 10-20 mg simvastatin = 80 mg simvastatin



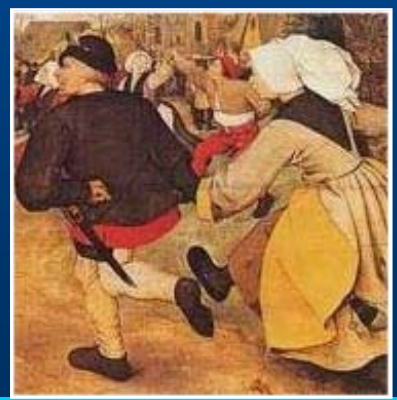
Bays *et al.* (2004) Clin. Ther. 26:1758-73

Sterol fate in the body



restoration of impaired transport

cholesterol traffic



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NPC1/2, two other RND sterol transporters

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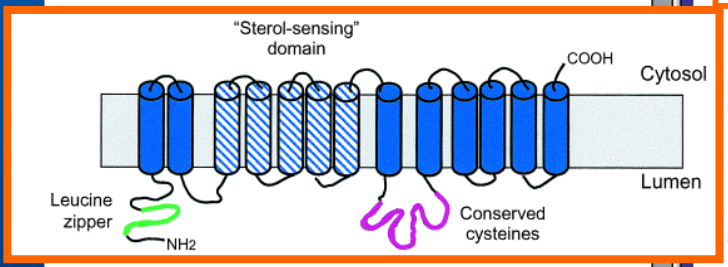

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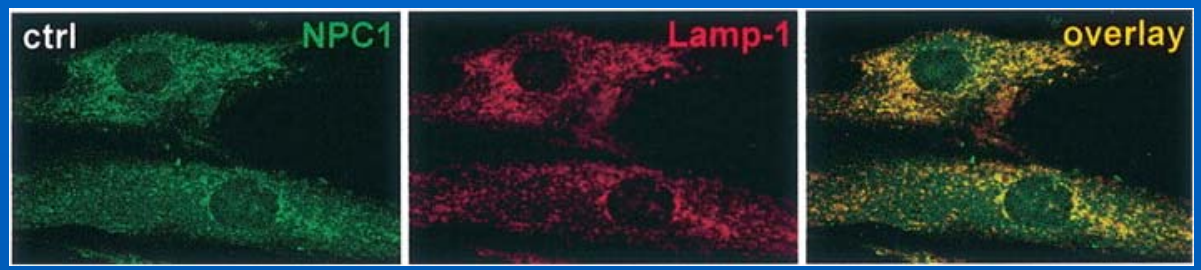
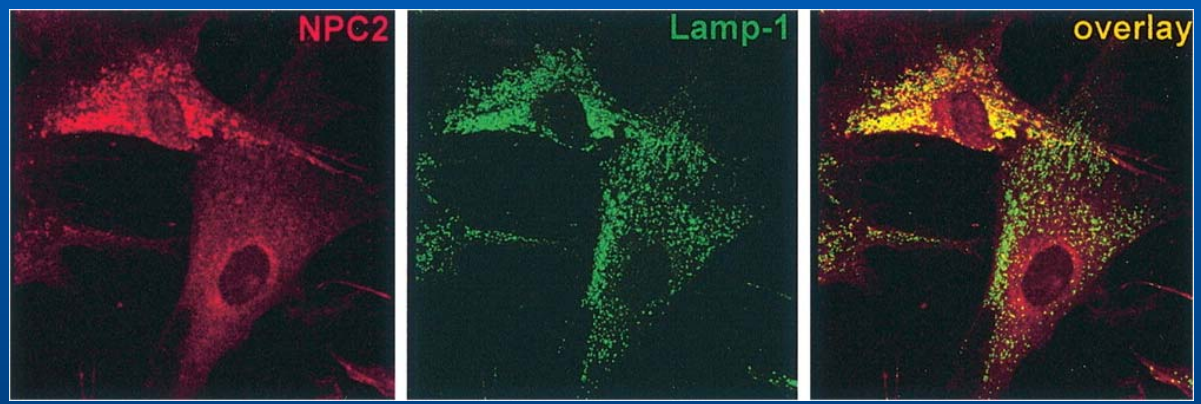
Liscum (2000) Traffic 1:218-25, Davies *et al.* (2000) Science 290:2295-98

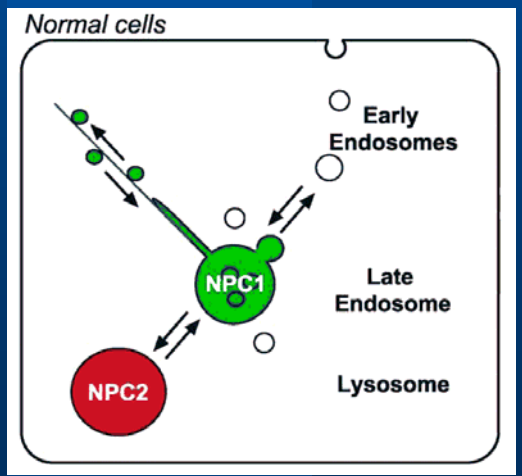
Subcellular localization of NPC1 and NPC2

NPC1
'frequent flyer'



NPC2
late endosomes/lysosomes resident



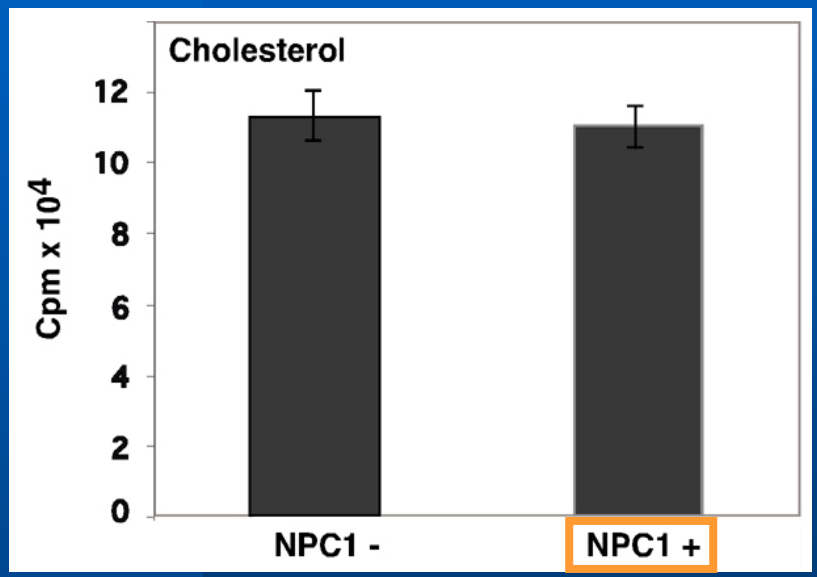


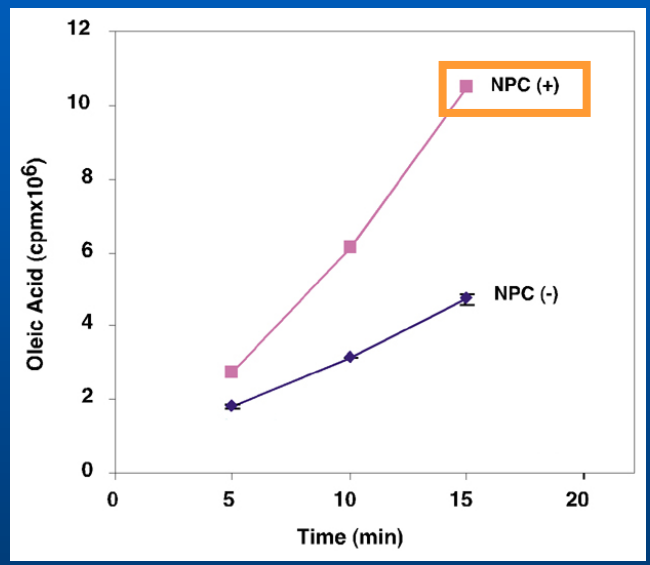
Liscum & Sturley (2004) BBA 1685:22-7

Blom *et al.* (2003) Hum. Mol. Genet. 12:257–72

NPC1 and NPC2 as lipid transporters

NPC1 expressed in *E. coli* transports oleic acid and other lipids but NOT cholesterol !



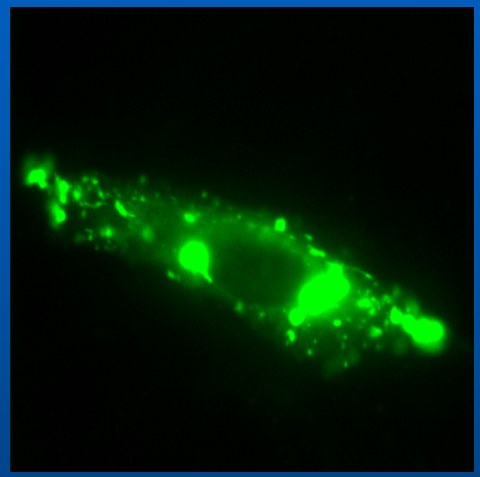


Davies *et al.* (2000) Science 290:2295-8

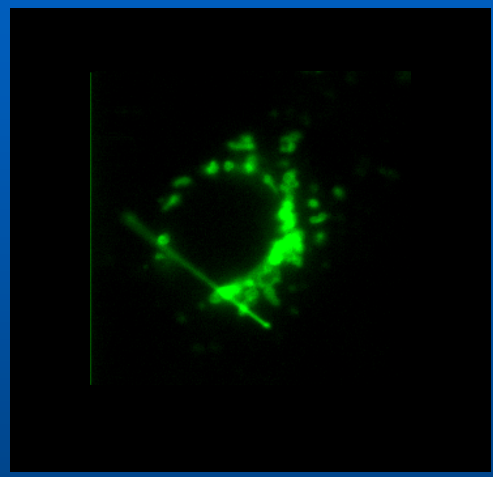
Shuttle role of NPC1 is impaired in Niemann-Pick disease

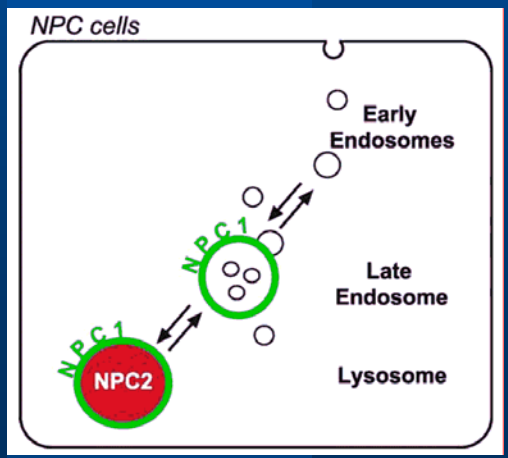
NPC1 trafficking

normal



pathologic





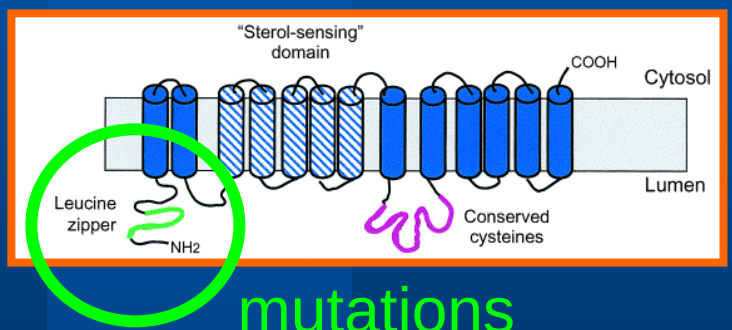
Liscum & Sturley (2004) BBA 1685:22-7

Zhang *et al.* (2004) PNAS 98:4466-71

Niemann – Pick disease

Mutations in NPC cause mislocalization of NPC1

…inducing a lipid ‘traffic jam’



mutations in the lysosomal targeting signal

Blanchette-Mackie (2000) BBA 1486:171-83; Blom *et al.* (2003) Hum. Mol. Genet. 12:257–72

Niemann – Pick disease

accumulation of cholesterol and polar lipids in lysosomes

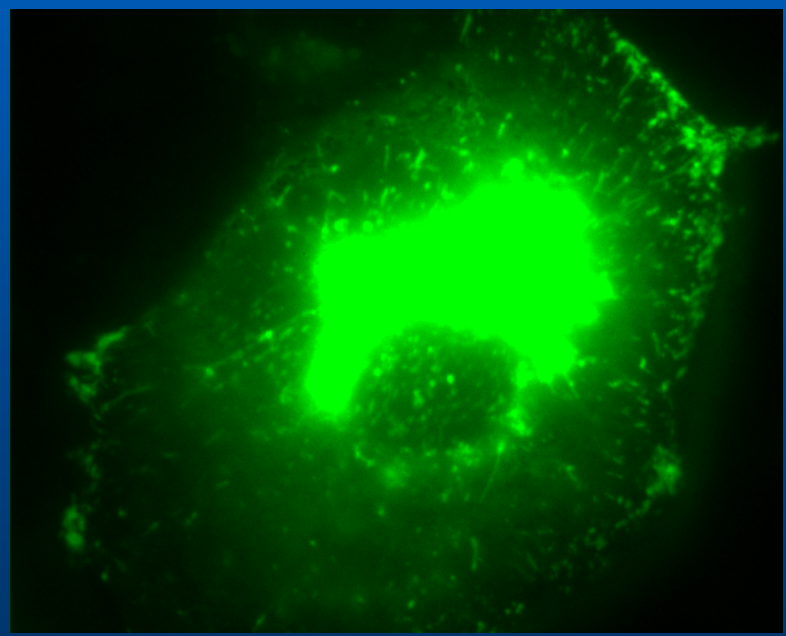
Cells		Age at diagnosis (years)	Total lipids (μg/mg cell protein)	Cholesterol (μg/mg cell protein)	Gb3[a] (μg/mg cell protein)	Cholesterol (% of total lipids)
Normal controls (n=10)	Range	1–63	340–450	37–54	<1.6–10.9	9–12
	Mean±S.D.	11.2±19.6	368±50.2	40.5±7.81	6.18±4.12	11.1±1.07
Niemann-Pick type C						
	No. 1	0.25	775	155	6	20
	No. 2	0.5	850	193	10	23
	No. 3	2.8	660	140	12	21
	No. 4	2.9	801	157	15	20
	No. 5	3.8	1117	199	14.5	18

Blanchette-Mackie (2000) BBA 1486:171-83; Harzer *et al.* (2001) Clin Chim Acta 305:65-73

Niemann – Pick disease: therapeutic strategies

gene therapy - NPC:

- restoration of normal traffic in *in vitro* models of Niemann-Pick disease



- no efficient vector for gene delivery to the brain

Zhang *et al.* (2004) PNAS 98:4466-71

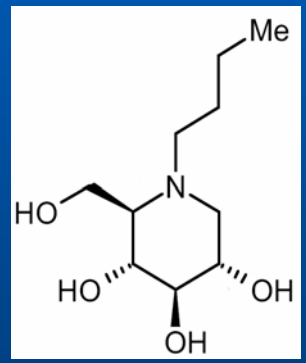
Niemann – Pick disease: therapeutic strategies

accumulation of cholesterol and polar lipids in lysosomes

statins:

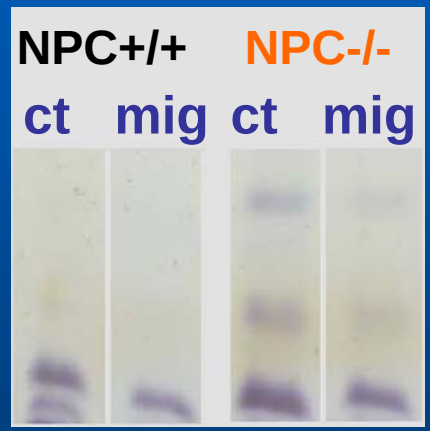
- ↓ cholesterol
- BUT no clinical benefit !

miglustat

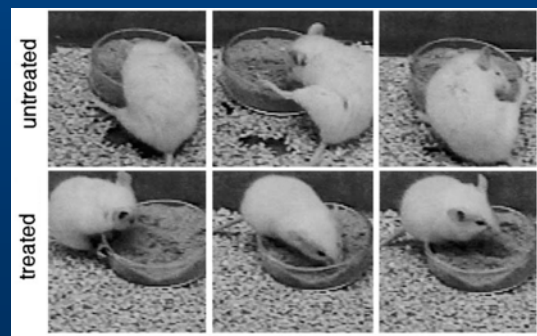


inhibitor of glucosyl-ceramide synthase

↓ gangliosides

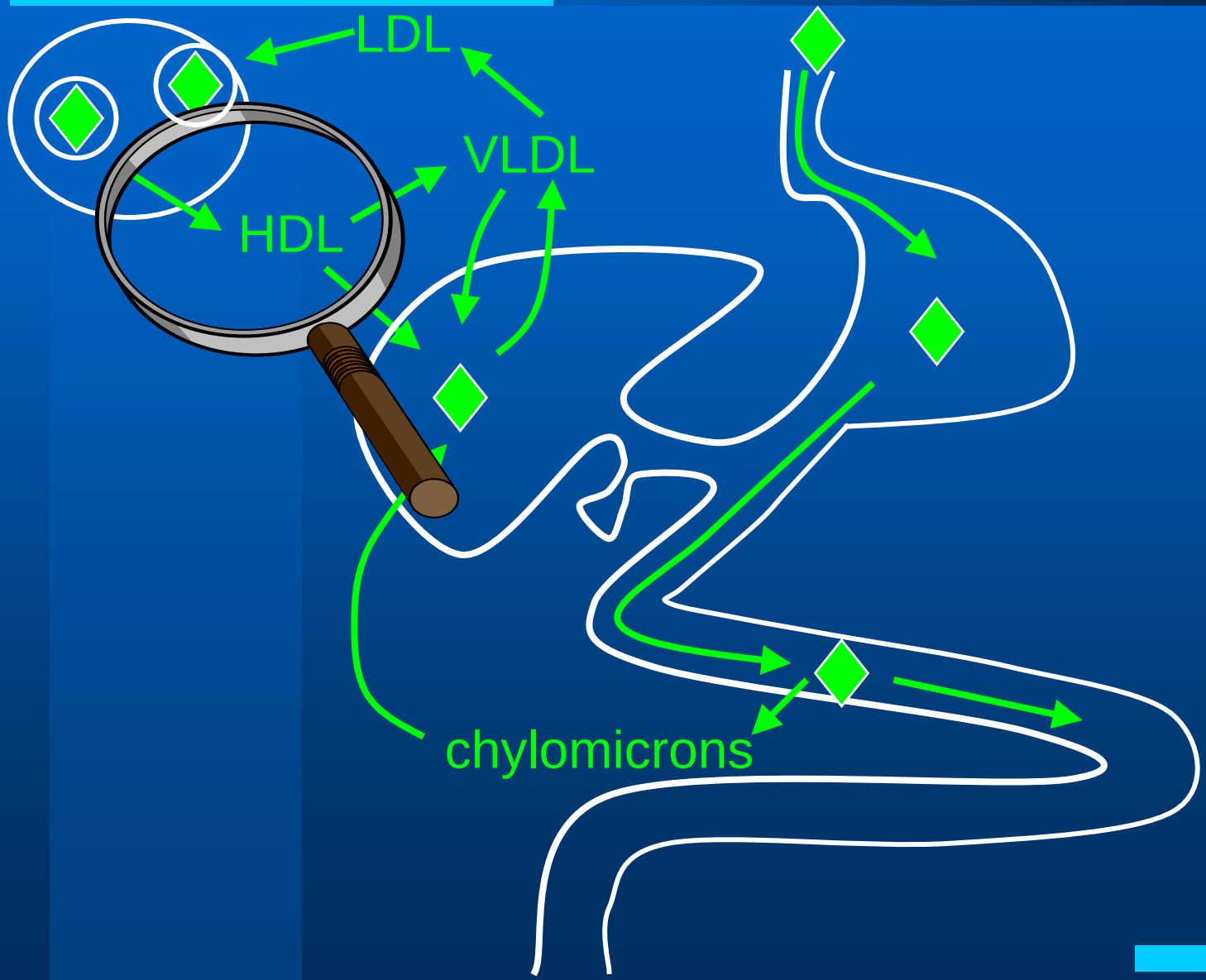


FDA and EMEA approved for Gaucher disease; tested for Niemann-Pick disease



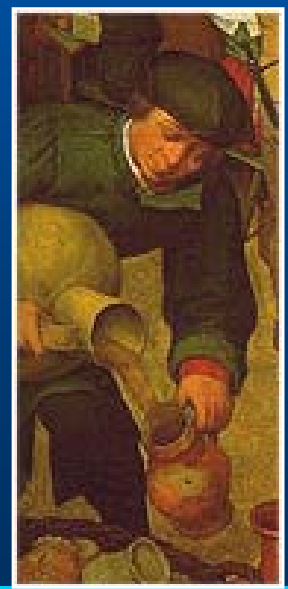
Patterson & Platt (2004) BBA 1685:77-82, Zervas *et al.* (2001) Curr Biol. 11:1283-7

Sterol fate in the body



stimulation of favorable transport

cholesterol efflux

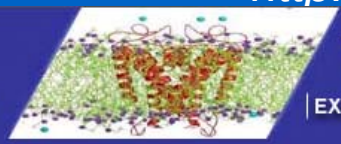


adapted from Hegele & Robinson (2005) Cur. Drug Tragets 5:31-7

ABCA1, a ABC sterol transporter

http://www-biology.ucsd.edu/~msaier/transport/

TCDB UCSD



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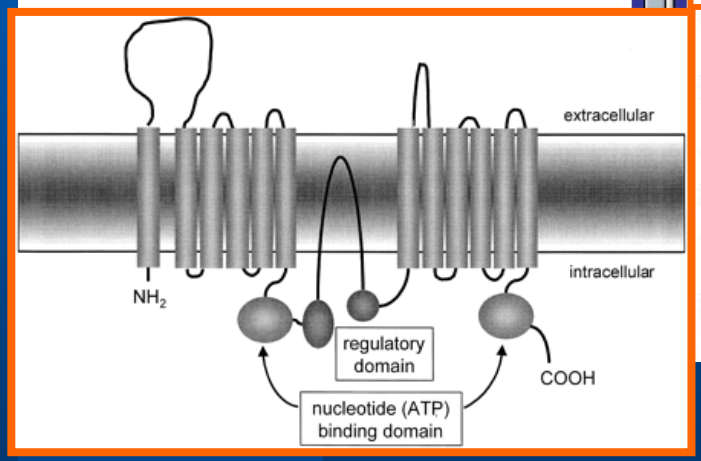
3.A.1. The ATP-binding Cassette (ABC) Superfamily

View Proteins Read Description

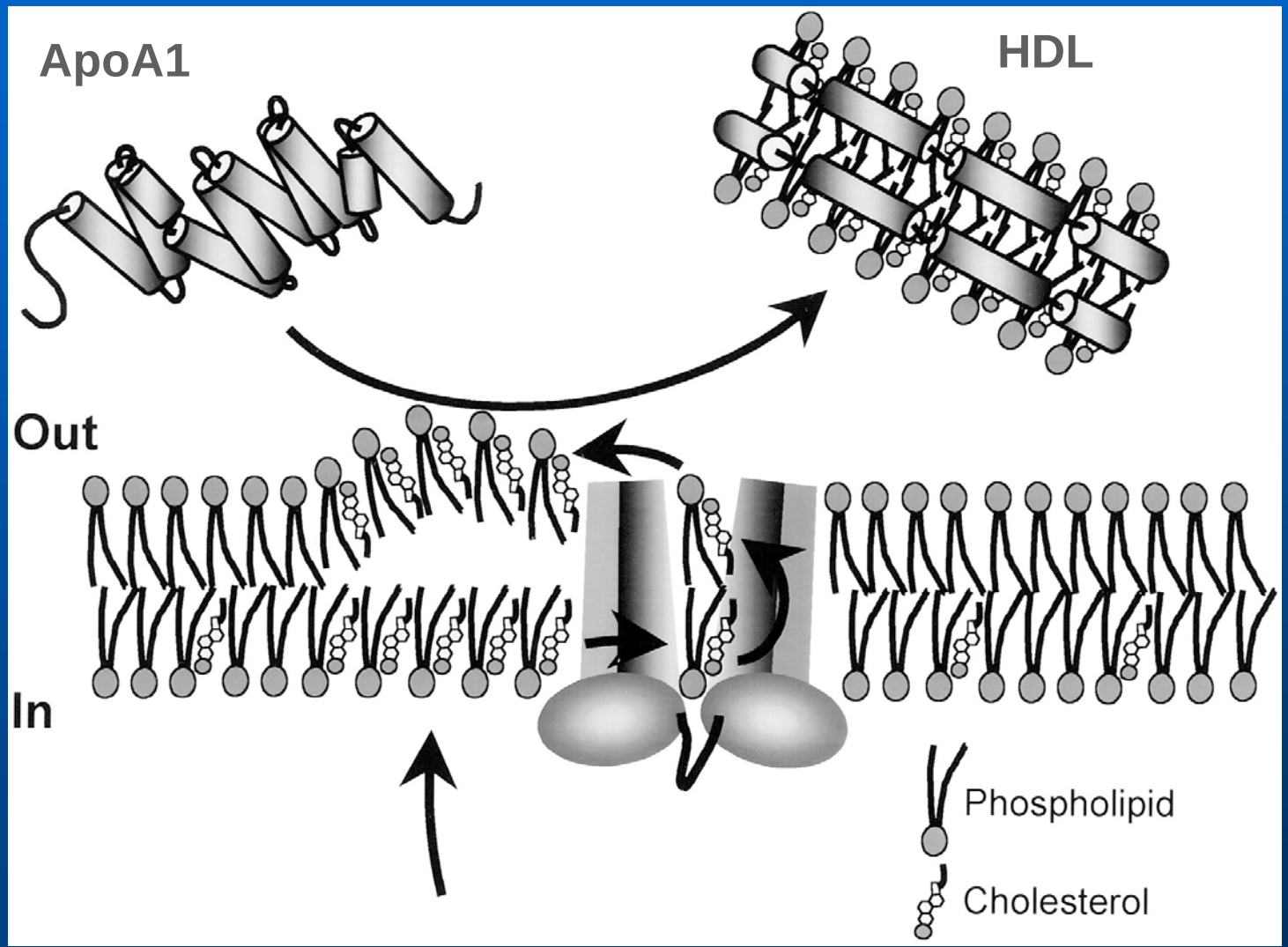


3.A.1.211. The Cholesterol/Phospholipid/Retinal (CPR) Flippase Family (ABCA)

3.A.1.211.1	The cholesterol/phospholipid flippase, ABC1 (called ABCA1 in humans; Tangier disease proteins; 2261 aas; sp: O95477)	Animals and plants	ABC1 of *Mus musculus*
3.A.1.211.2	The retinal-specific ABC transporter (RIM protein, ABCR or ABCA4) (Stargardt's disease protein) in the rod outer segment. May flip retinal in the membrane bilayer.	Animals	RIM protein (ABCR) of *Homo sapiens*
3.A.1.211.3	Multidrug resistance pump, ABCA2 (ABC2)	Animals	ABCA2 of *Homo sapiens*
3.A.1.211.4	The *aced* cell death 7 (ced-7) protein (translocates molecules that mediate adhesion between dying and engulfing embryonic cells during programmed death).	Animals	Ced-7 of *Caenorhabditis elegans* (P34358)

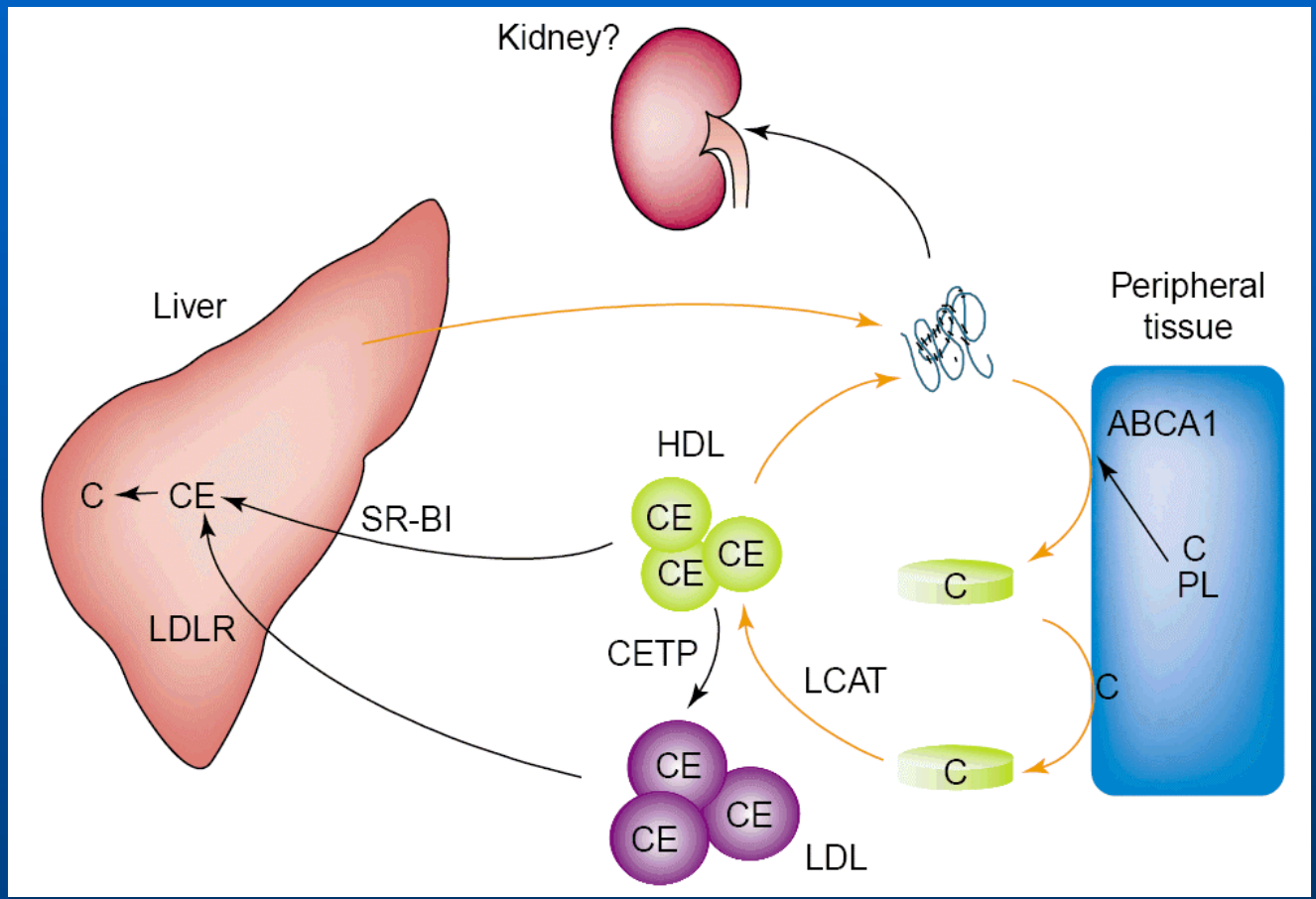
Oram & Lawn (2001) J. Lipid Res. 42:1173-9

ABCA1 as lipid transporter



Oram *et al*. (2002) Arterioscler Thromb Vasc Biol. 23:720-7

ABCA1 involved in reverse cholesterol transport

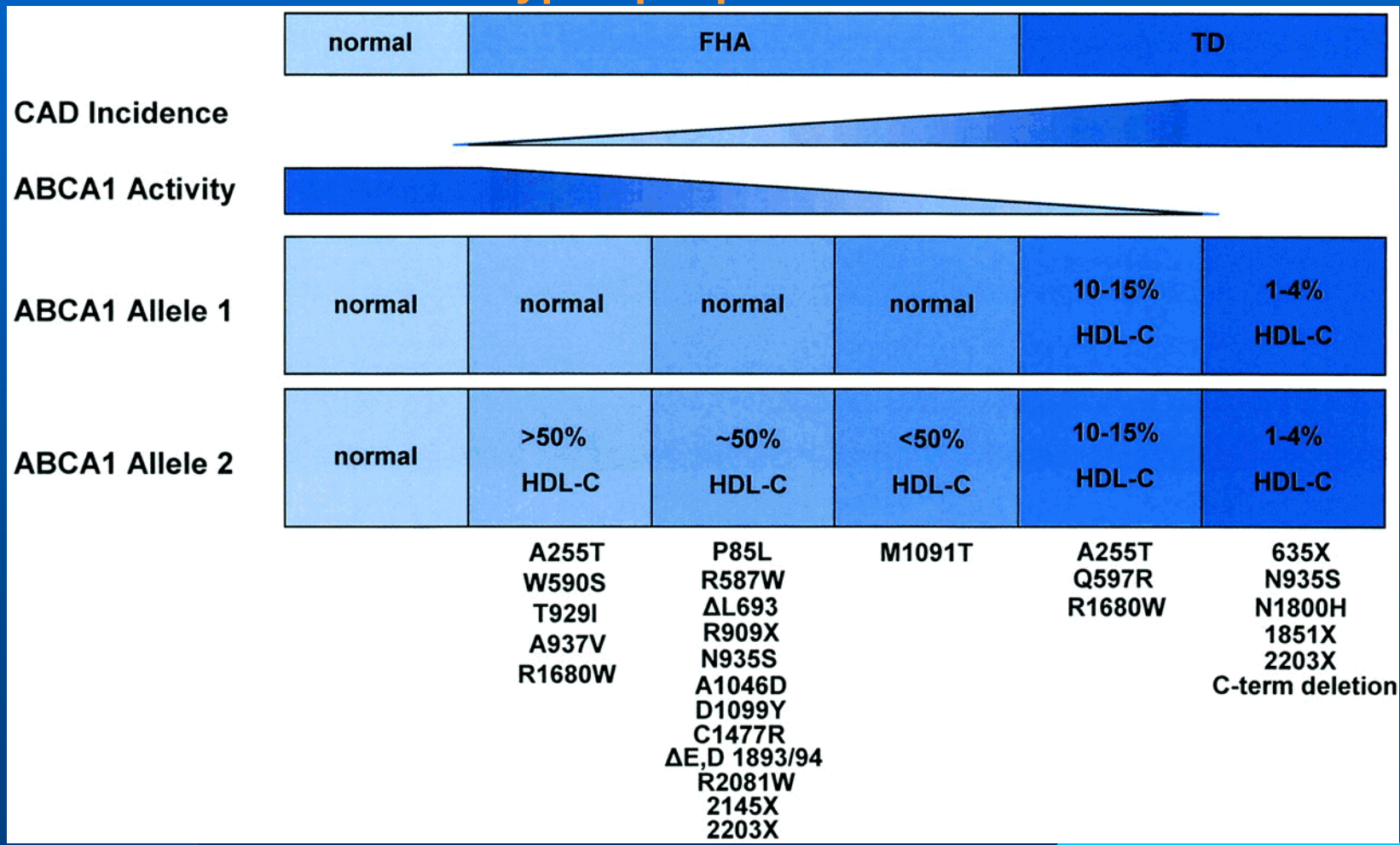


Oram (2002) Trends Mol. Med. 87:168-73

ABCA1 activity protects against atheromatosis

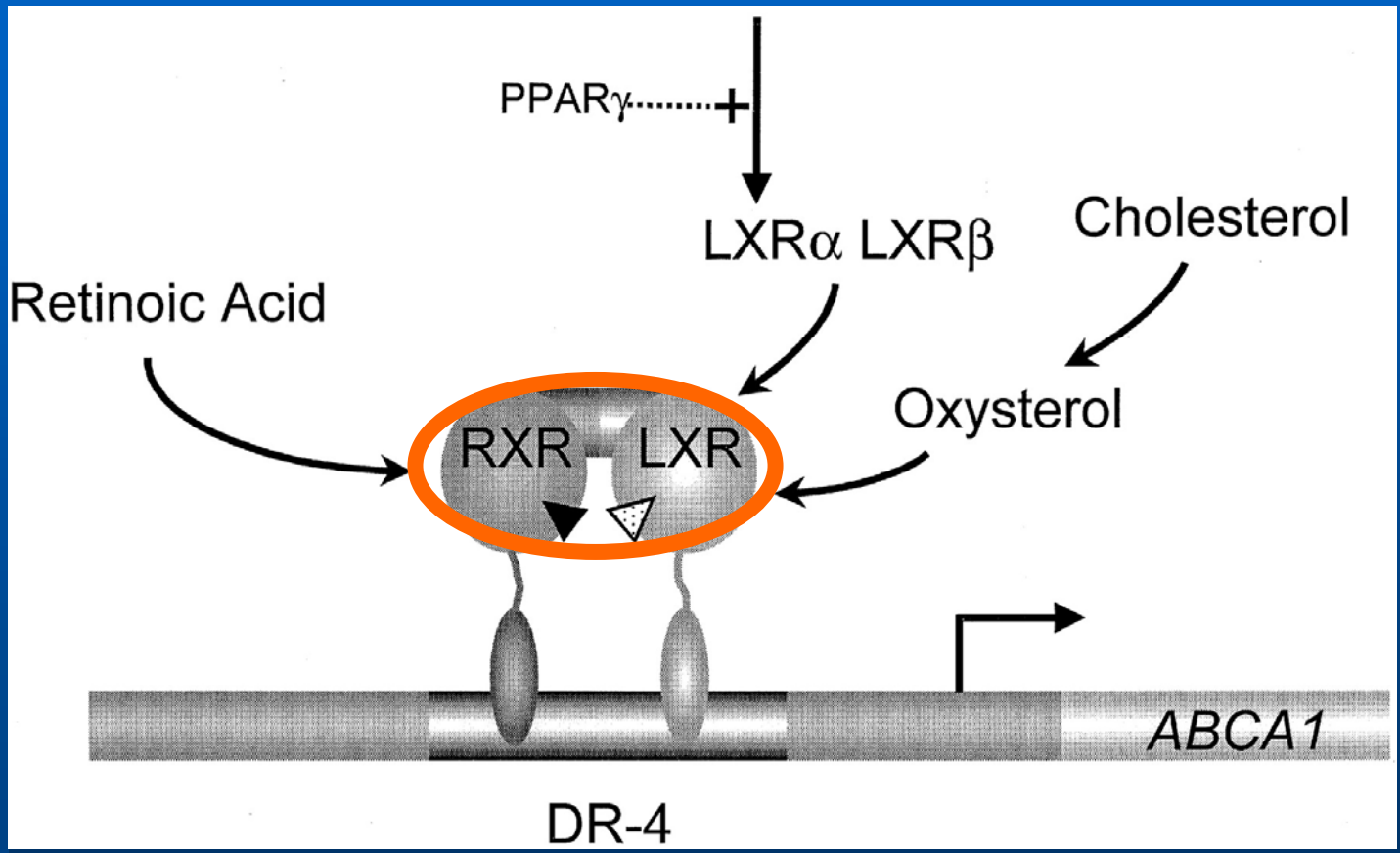
familial hypoalphaproteinemia

Tangier disease



Singaraja *et al*. (2003) Arterioscler Thromb Vasc Biol. 23:1322-32

Regulation of ABCA1 expression

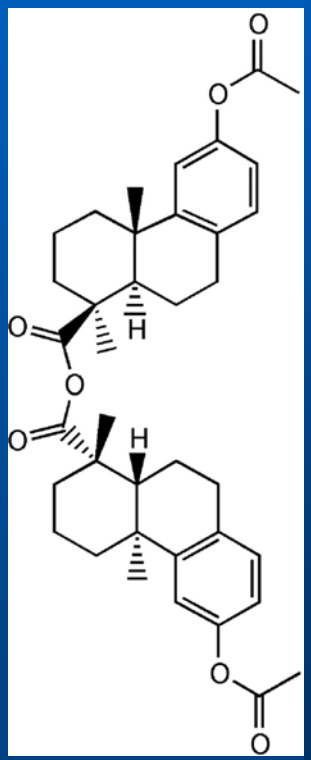


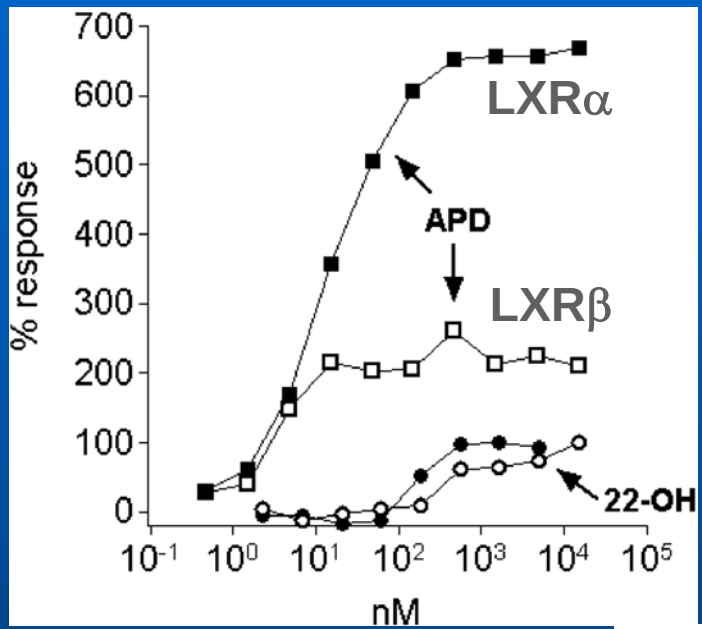
Retinoid X receptor; Liver X receptor

Oram & Lawn (2001) J. Lipid Res. 42:1173-9

LXR agonists more potent than oxysterols

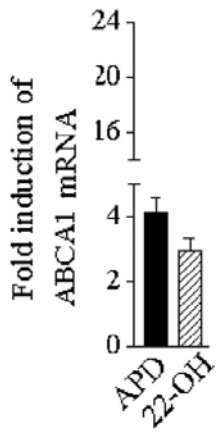
APD
acetyl-podocarpic dimer

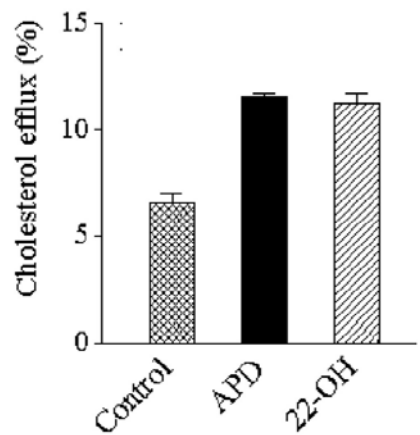




activation of LXRα (and β)

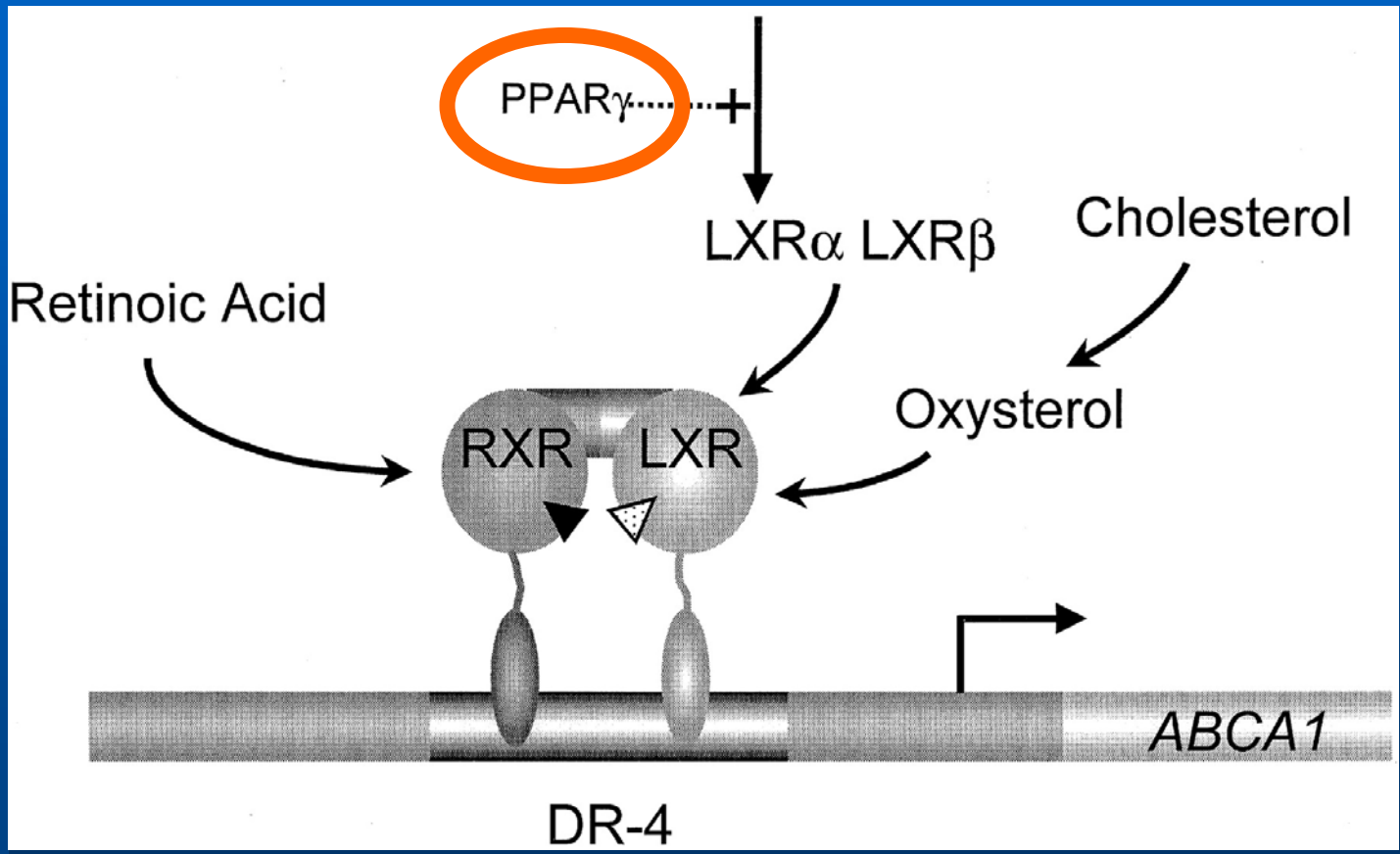
induction of ABCA1 and ↑ cholesterol efflux





Sparrow *et al*. (2002) J Biol Chem. 277:10021-7

Regulation of ABCA1 expression



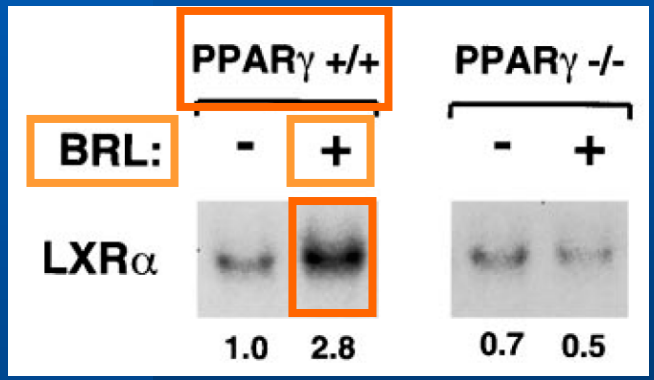
peroxisome proliferator activated receptor

Oram & Lawn (2001) J. Lipid Res. 42:1173-9

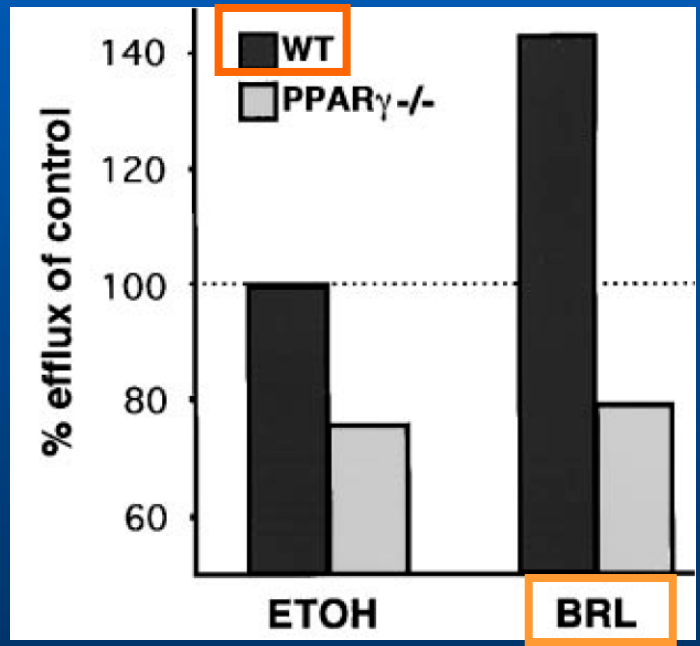
PPARγ agonists: new target for thiazolidinediones ?

BRL (rosiglitazone)

induction of LXRα mRNA expression mediated by PPARγ



increase in cholesterol efflux mediated by PPARγ



Chawla *et al*. (2001) Mol. Cell 7:161-71

Questions for future research

- **inhibition of cholesterol absorption**
  - **Deleterious consequences of inhibiting the formation of the caveolin-annexin complex ?**

    (Cohen *et al*. (2004) Physiol Rev. 84:1341-79; Kim *et al*. (2002) Front Biosci. 7:d341-8)
    - Development of inhibitors targeting exclusively NPC1L1 ?

- **restoration of NPC trafficking**
  - **Appropriate vectors for gene delivery in the brain?**
    - Viral vectors (Yenari & Sapolsky (2005) Methods Mol Med.104:75-88)

- **increase in ABCA1 expression**
  - **Other genes under the control of LXR ?**
    - Lipid metabolism, carbohydrate metabolism, energy homeostasis, inflammatory response

      (Steffensen & Gustafsson (2004) Diabetes 53 S1:S36-42)