Biology and Physiology of the LDL Receptor

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University of Utah

Disclosures

- Cerenis – grant for clinical trial
- NIH – research grants

Classic LDL binding studies

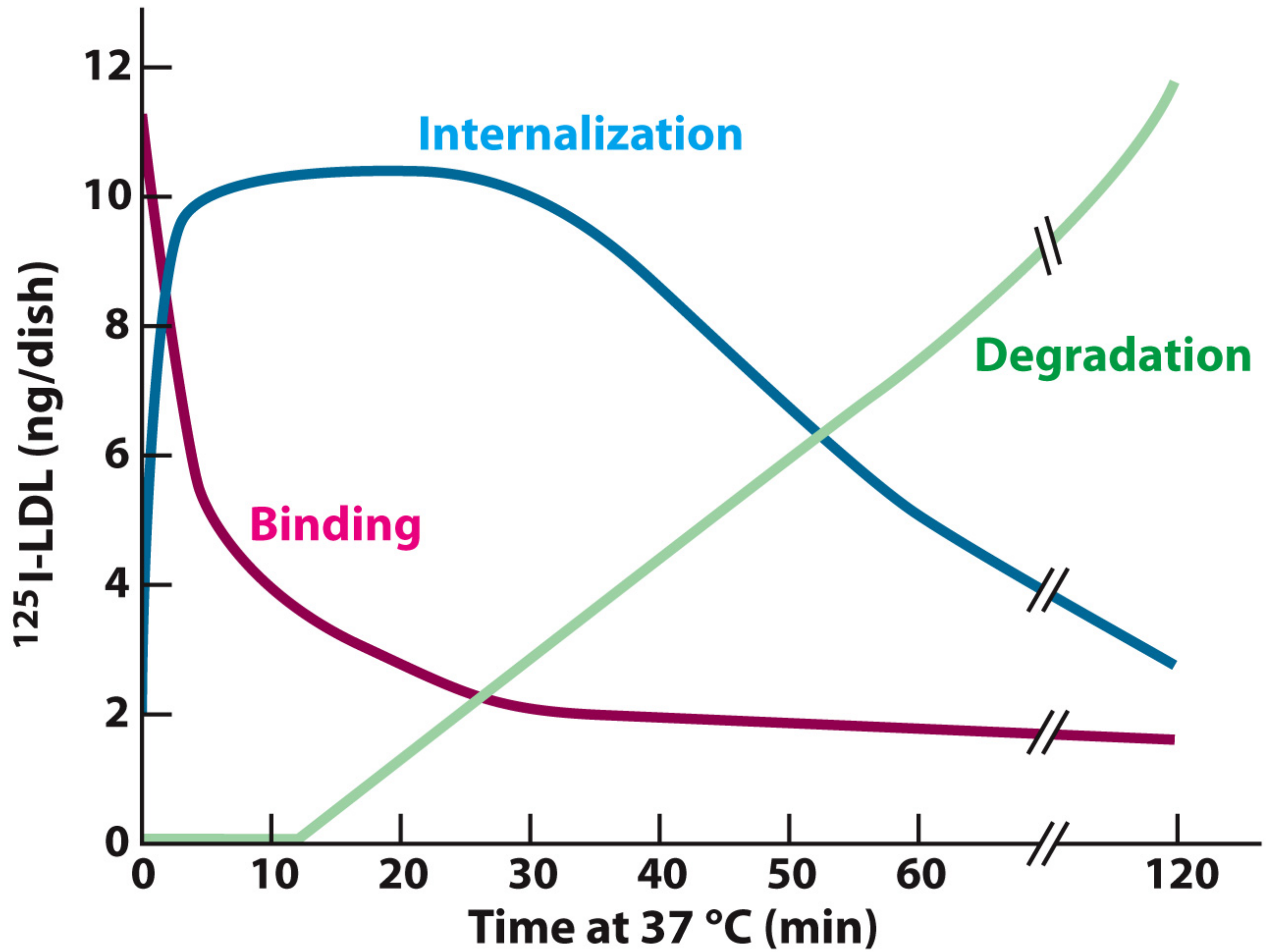
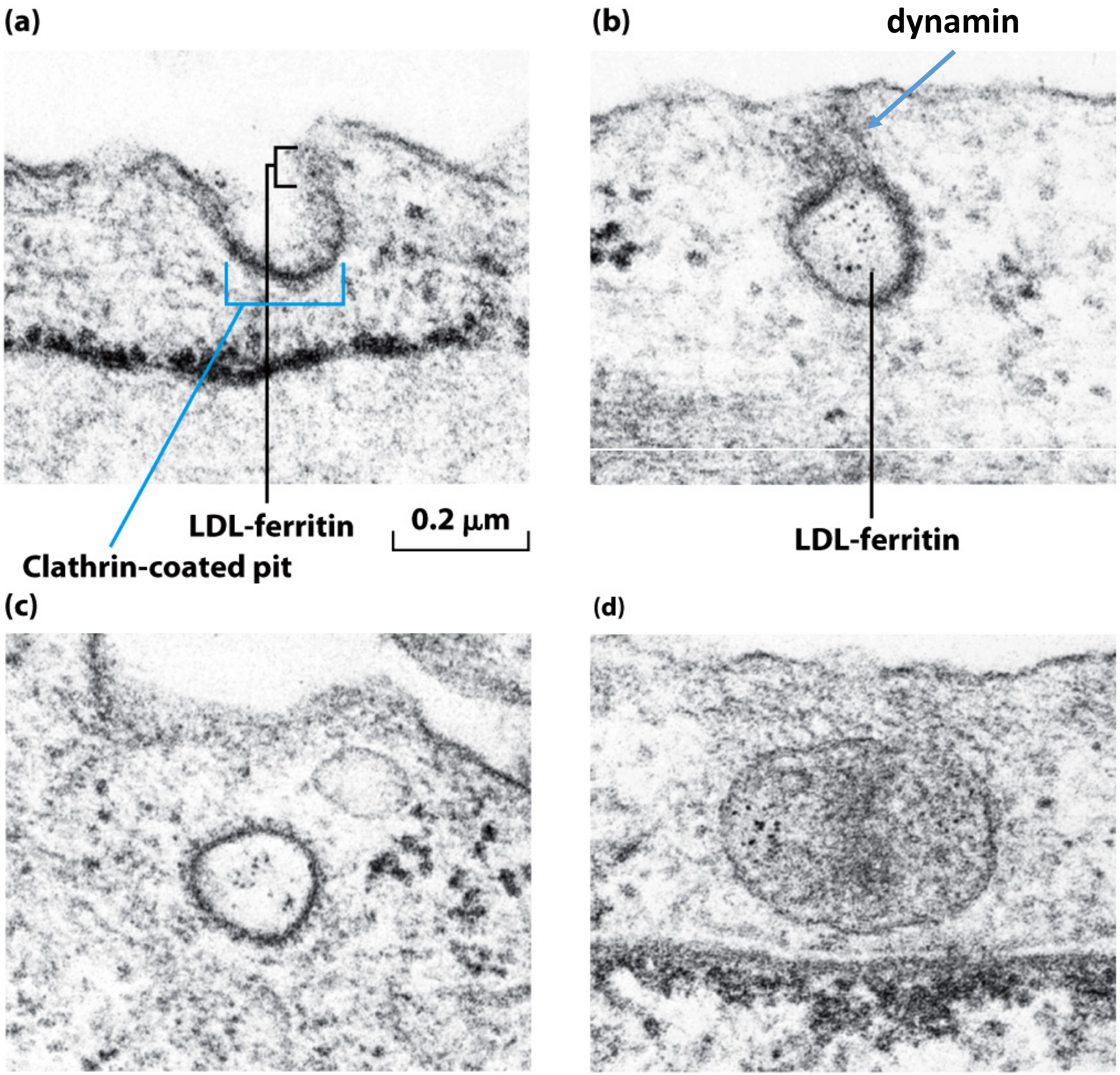


Figure 14-28
Molecular Cell Biology, Eighth Edition

Cultured normal human skin fibroblasts – incubated with ^{125}I-LDL for 2 hours at 4°C (pulse)
LDL binds to surface LDL receptors; not endocytosed. Then increase temperature to 37°C.

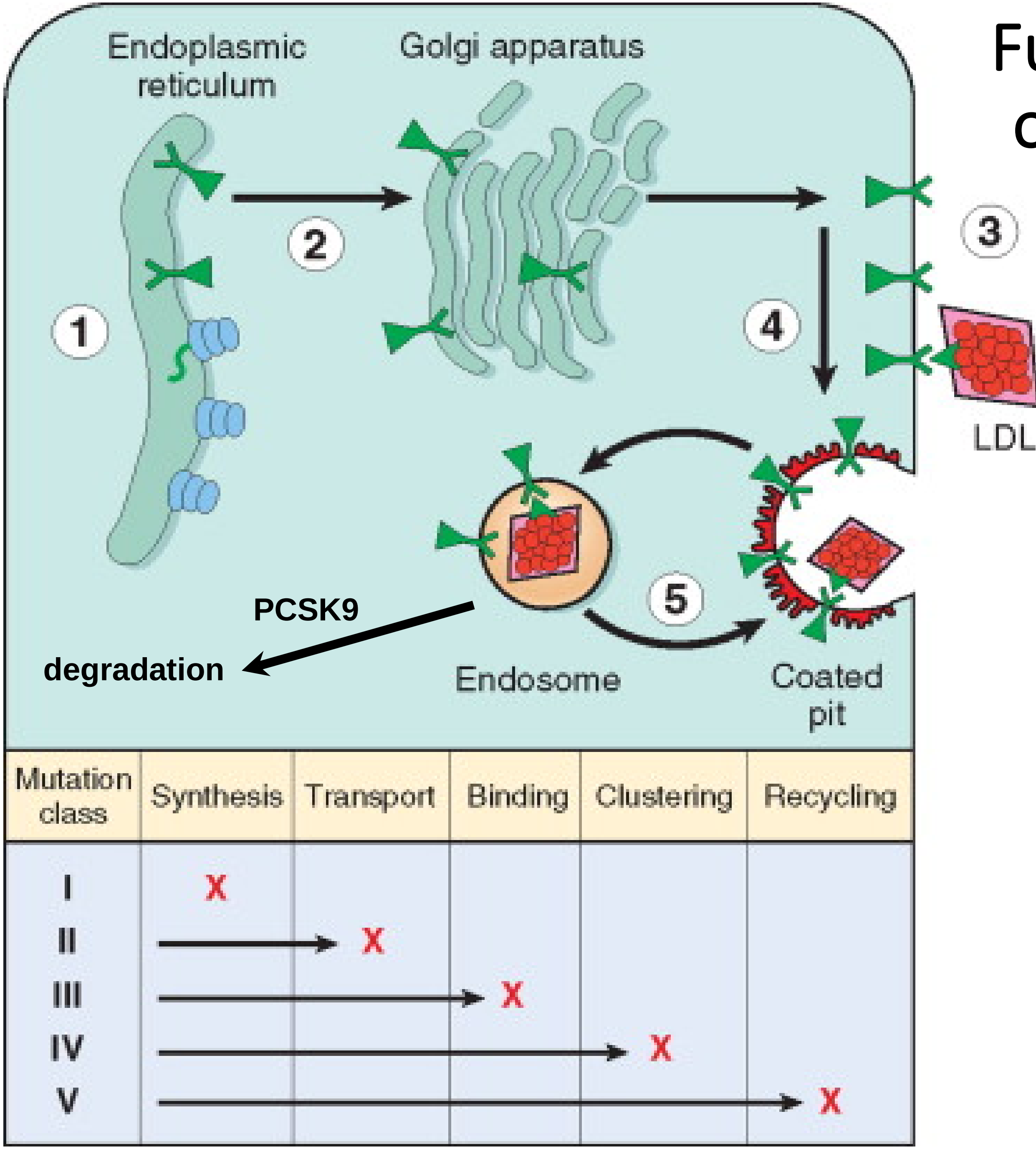


Clathrin-coated vesicle with LDL-LDLR complexes

Figure 14-26
Molecular Cell Biology, Eighth Edition

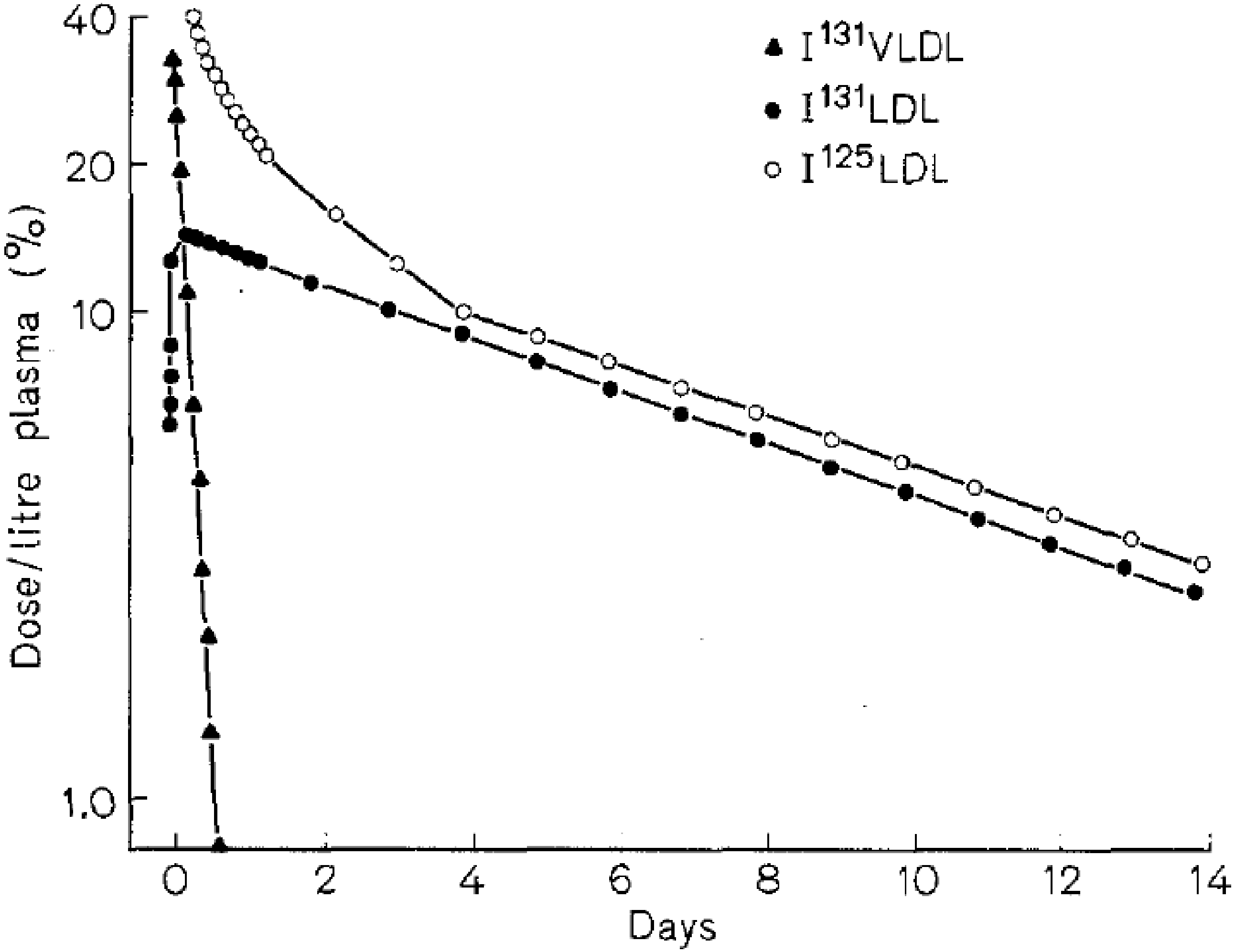
LDL-LDLR complexes delivered to endosome (6 minutes after warming)

Functional classes of *LDLR* mutants



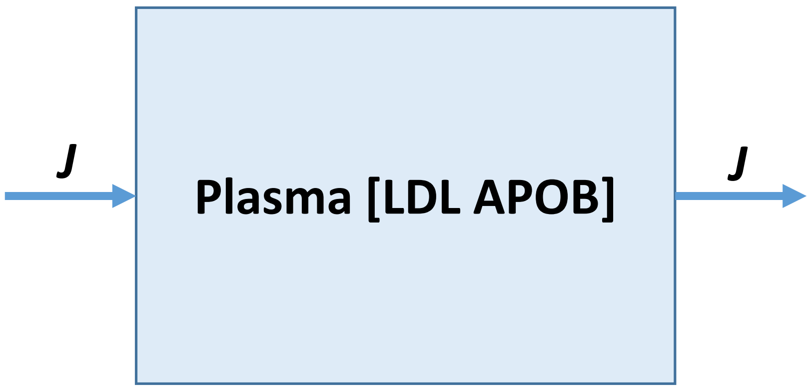
Kumar: Robbins and Cotran Pathologic Basis of Disease, Professional Edition , 8th ed.

Adapted from
Hobbs HH, et al.
Annu Rev Genet 1990;24:133



Plasma VLDL B-apoprotein and LDL radioactivity curves following the simultaneous injection of I^{131} VLDL and I^{125} LDL to a normal subject

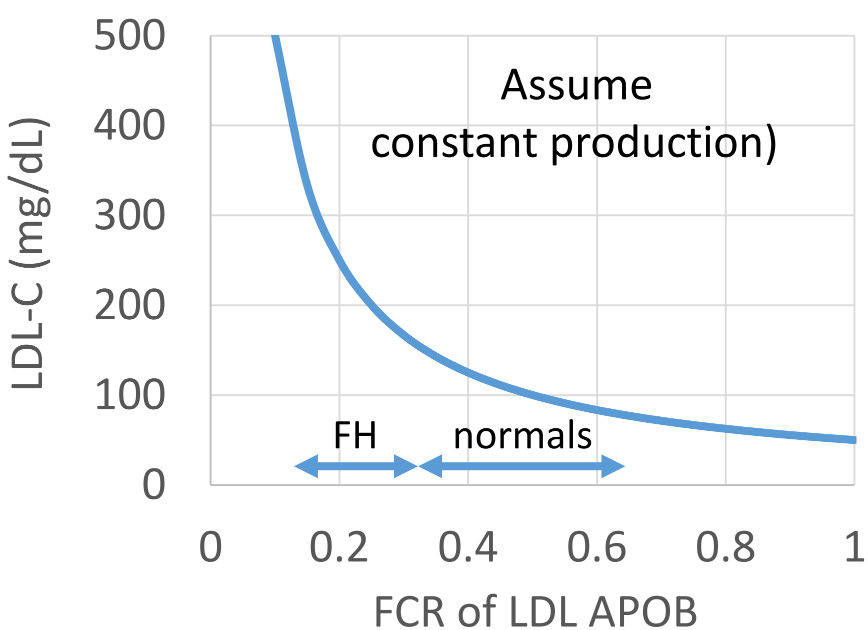
Kissebah AH, et al. Diabetologia 1976; 12:501

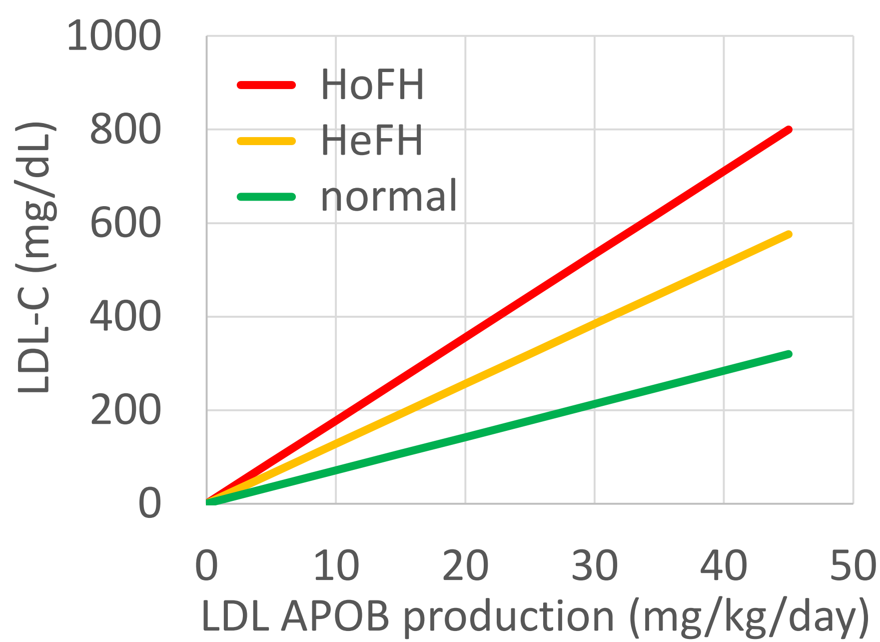


$$J = \mathrm{k}\,[\mathrm{LDL\ APOB}](\mathrm{plasma\ volume})$$

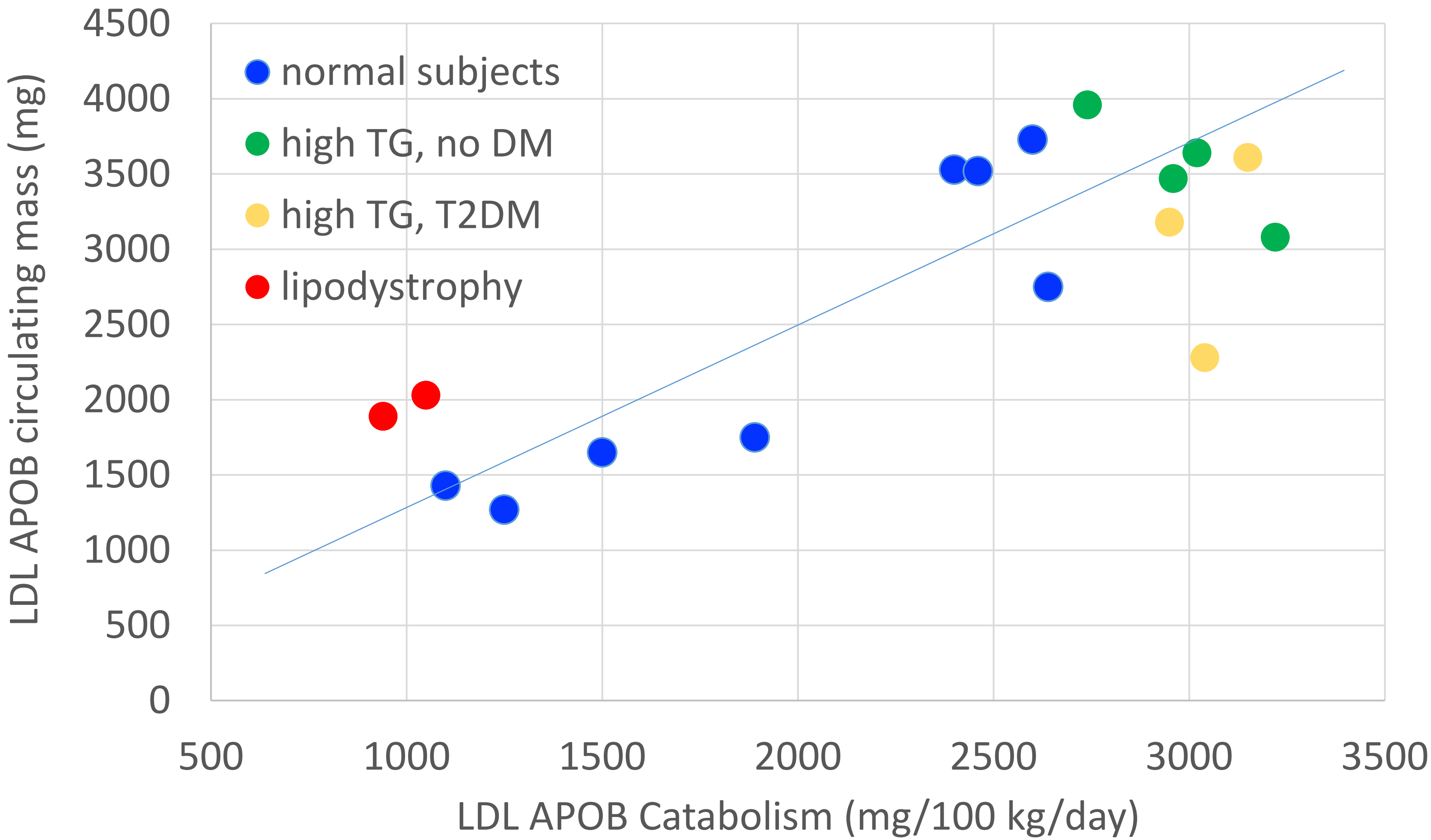
$$FCR = \frac{J}{[\mathrm{LDL\ APOB}]\ (\mathrm{plasma\ volume})}$$

- LDL plasma concentration is determined by production rate and fractional catabolic rate (FCR)
- Typical FCRs (Bilheimer DW, JCI 1979)
  - HoFH 0.18
  - HeFH 0.29
  - Normal 0.45
- About 70% of LDL flux in humans is due to hepatic LDLR activity (Dietschy JM, et al. J Lipid Res 1993; 34:1637)



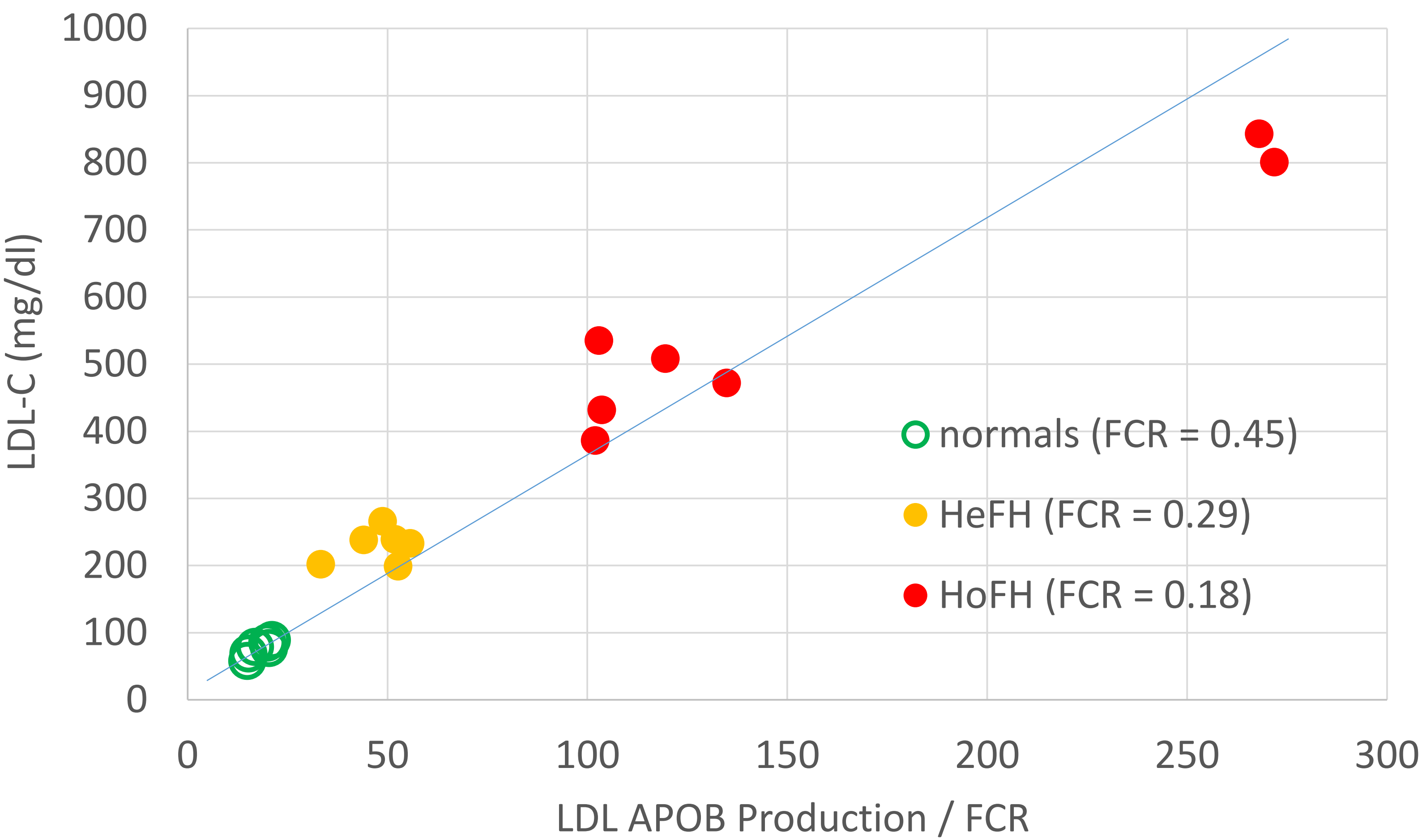


Kinetic studies of VLDL and LDL in subjects with normal FCR



adapted from Kissebah AH, et al. Diabetologia 1976; 12:501

Kinetic studies of VLDL and LDL in normals, HeFH, and HoFH

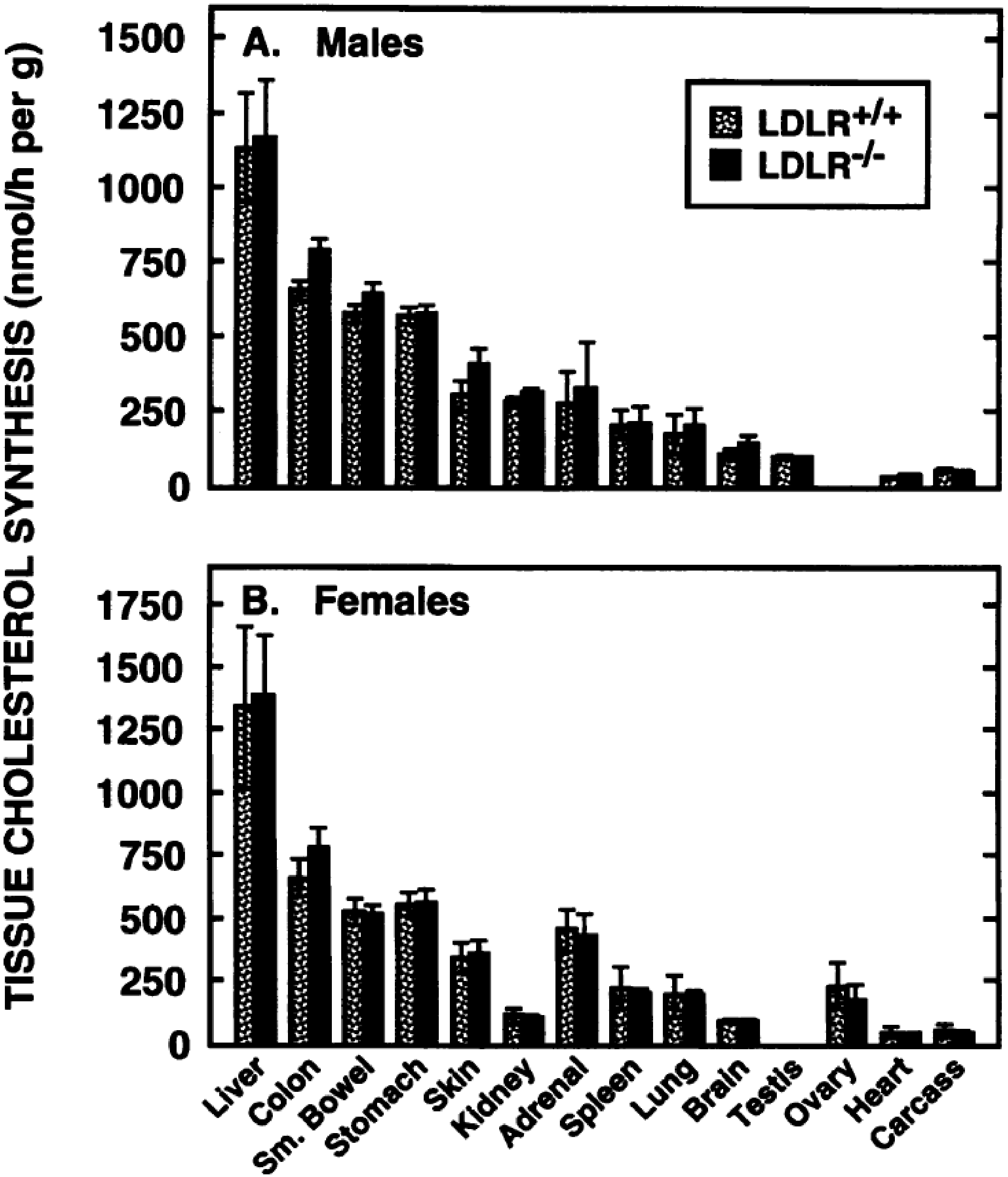


adapted from Bilheimer DW, et al. JCI 1979; 64:524

Comparing LDLR$^{+/+}$ with LDLR$^{-/-}$ mice (on low cholesterol diets)

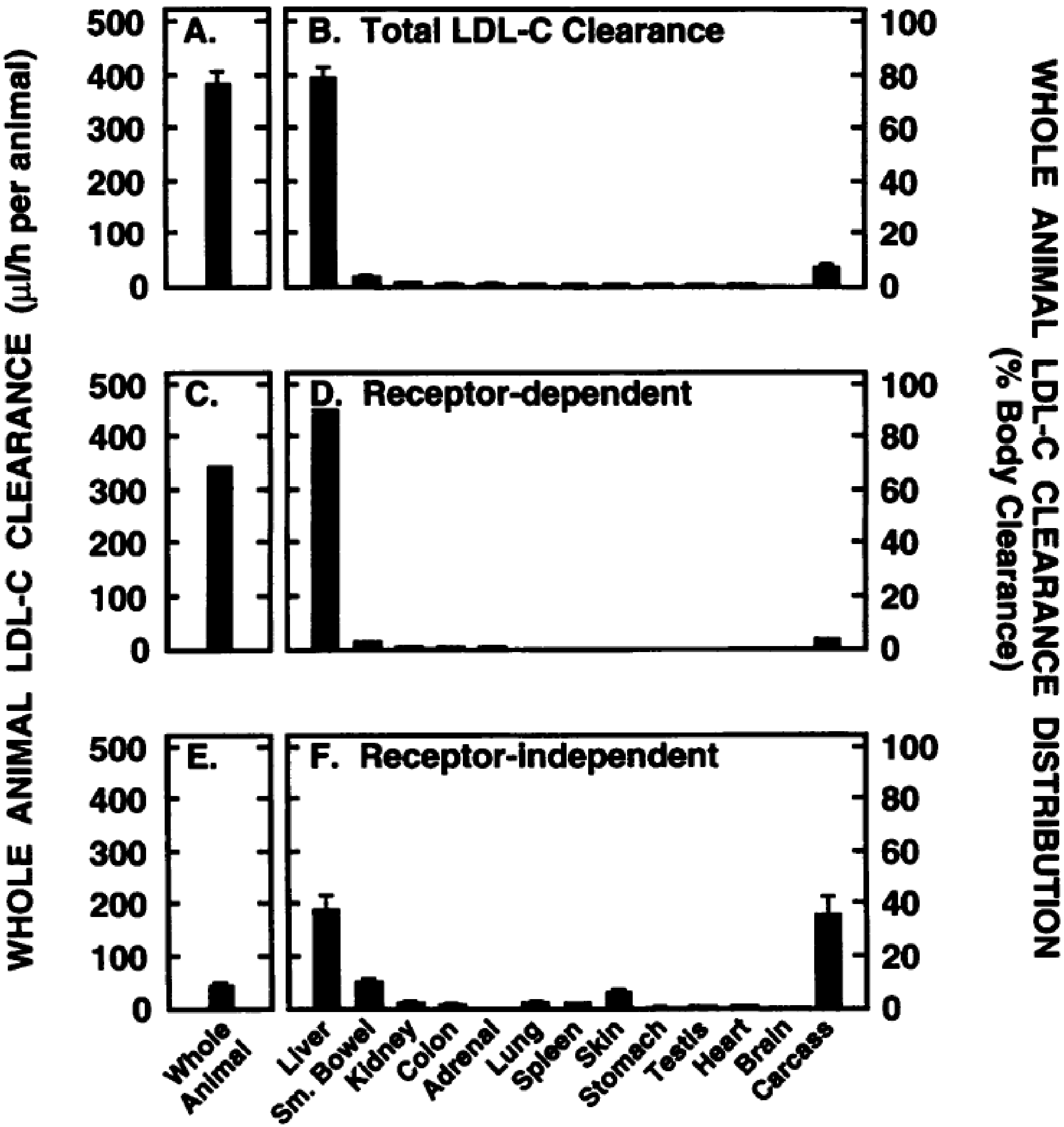
- In LDLR$^{+/+}$
  - 78% cholesterol synthesis occurred outside liver
  - FCR: 7.1 pools of LDL-C per day
  - 79% of this degradation took place in the liver.
  - **88% LDLR dependent**
  - 91% of whole body LDLR-dependent transport in the liver
  - 38% of whole body LDLR-independent uptake in the liver
- In LDLR$^{-/-}$
  - LDL-C production rate increased 1.7-fold
  - LDL-C turnover (FCR) decreased from 7.1 to 0.88 pools/d,
  - plasma LDL-C level increased 14-fold, from 7 to 101 mg/dl.

Osono Y, et al. JCI 1995; 95:1124

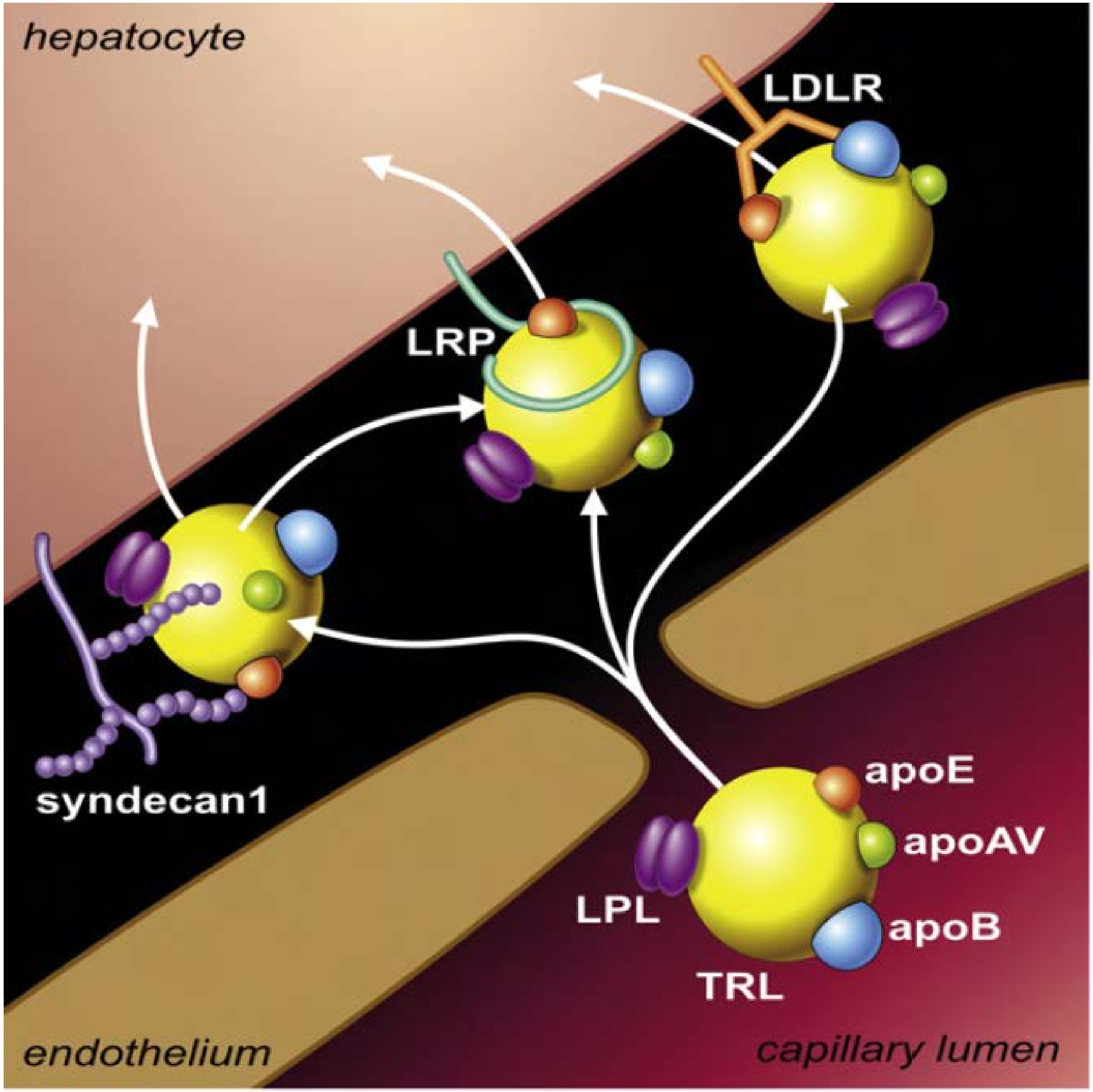


NOTE: very little change in cholesterol synthesis with LDLR KO. Most tissues synthesized their own cholesterol or compensate by taking up LDL through alternative pathways.

Osono Y, et al. JCI 1995; 95:1124

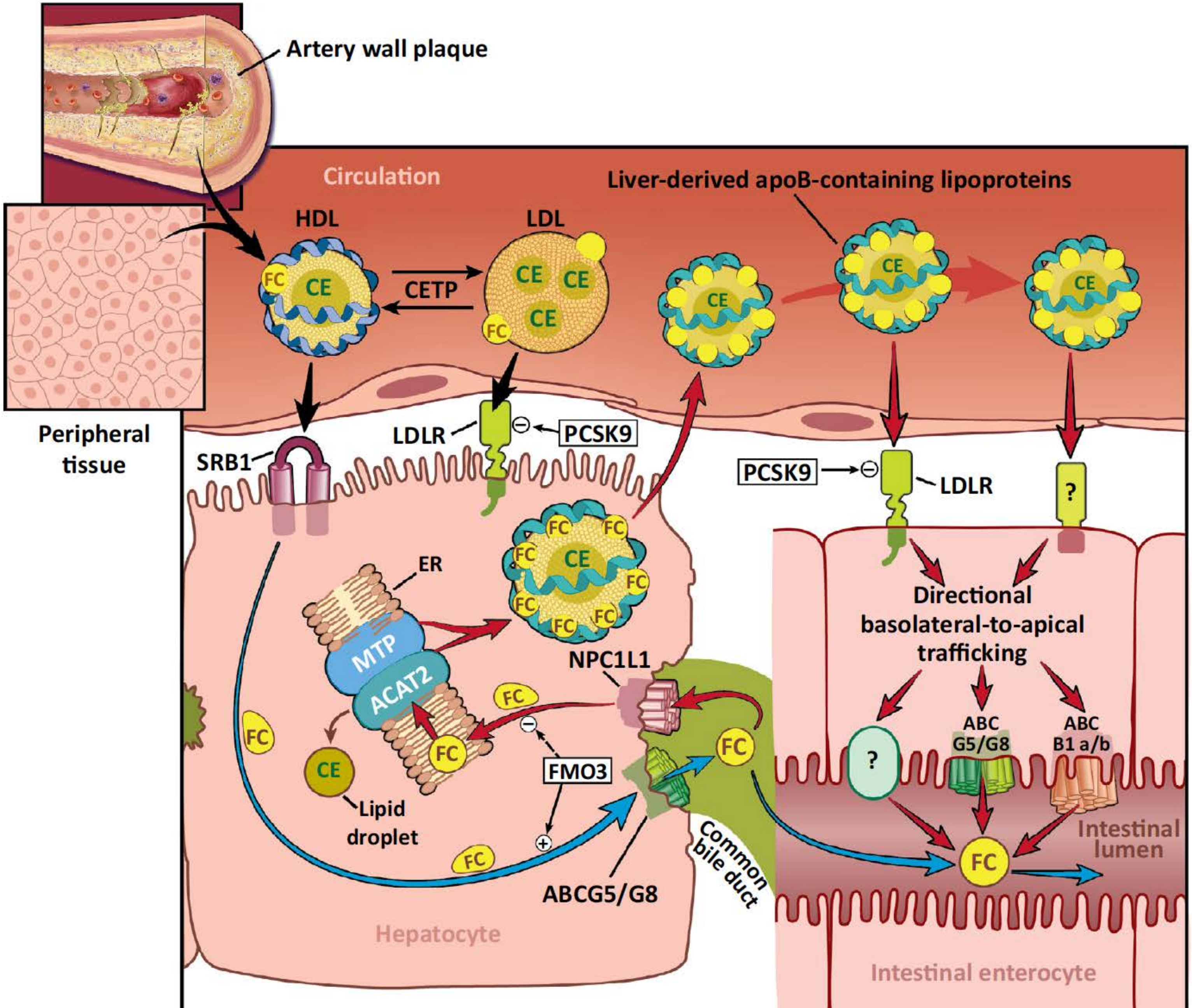


Osono Y, et al.
JCI 1995; 95:1124



- Hepatic secretion of apo E into space of Disse more effective than circulating apo E.
- LDLR internalization (normal LDLRAP1) not required for uptake.
- Syndecan 1 (SDC1) binds apo E, LPL, HL.
- SDC1 required for remnant removal.
- Sulfation of SDC1 is required for remnant removal.
- Sulfate removal by *SULF2* (markedly upregulated by diabetes) impairs remnant removal.

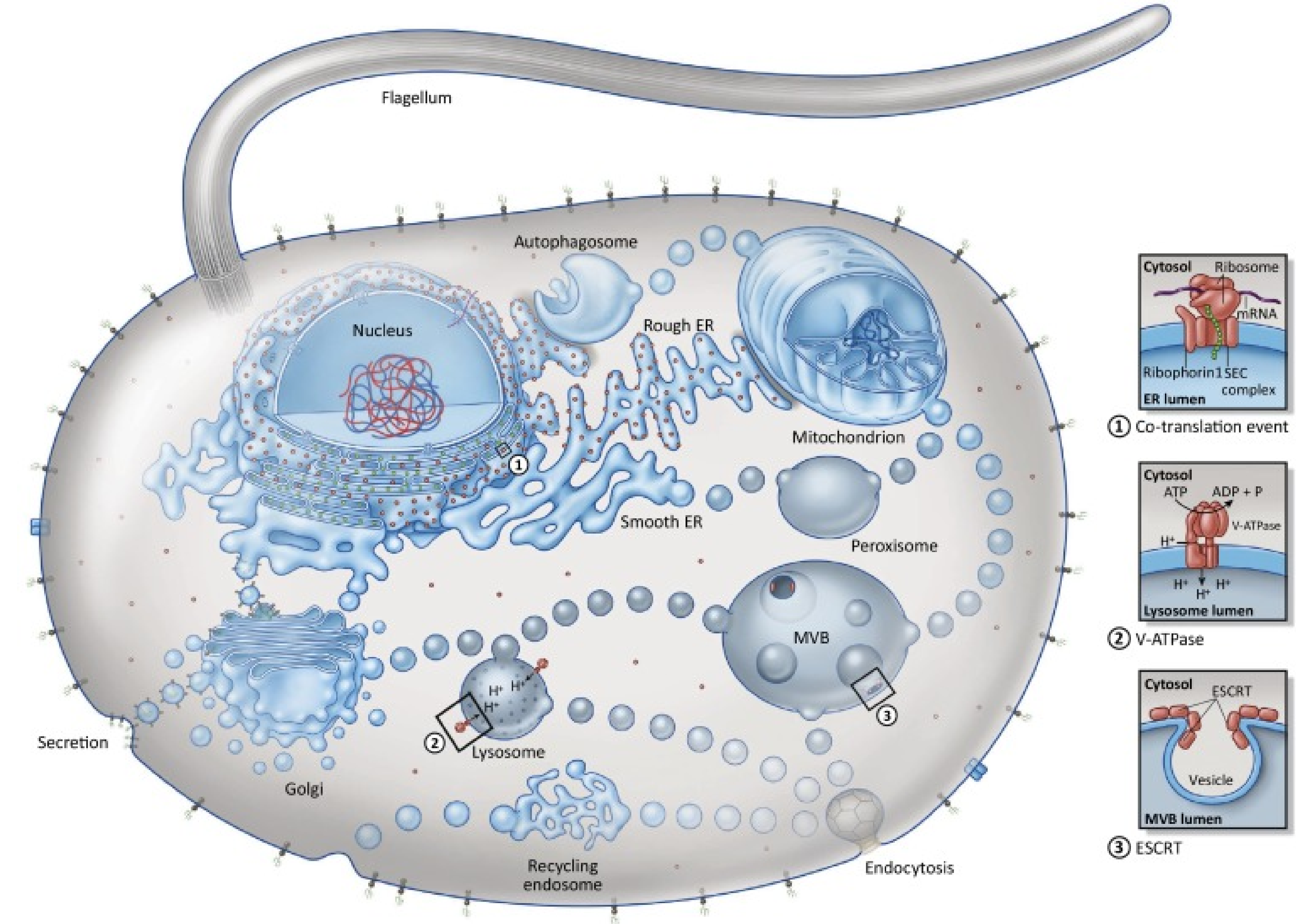
Dallinga-Thie GM, et al. Atherosclerosis 2010; 211:1



Temel RE, Brown JM. Trends Pharmacol Sci 2015;36:440

Summary of basic LDLR physiology

- LDL receptors normally mediate most LDL removal from the plasma compartment.
  - About 80% of all LDL removal is LDLR-mediated
  - About 80% of LDLR-mediated uptake is into the liver
- With normal LDL receptors, LDL levels are strongly determined by LDL production rate
  - LDLR expression can still be affected by medications (statins), saturated fat, cholesterol intake, etc.
- At a given production rate, LDL levels are inversely proportional to FCR
  - 50% reduction in LDLR activity → ~2-fold higher LDL-C
- LDLR also facilitates TRL remnant/IDL processing and mediates TICE (trans-intestinal cholesterol excretion)



Trends in Microbiology DOI: (10.1016/j.tim.2016.03.005)

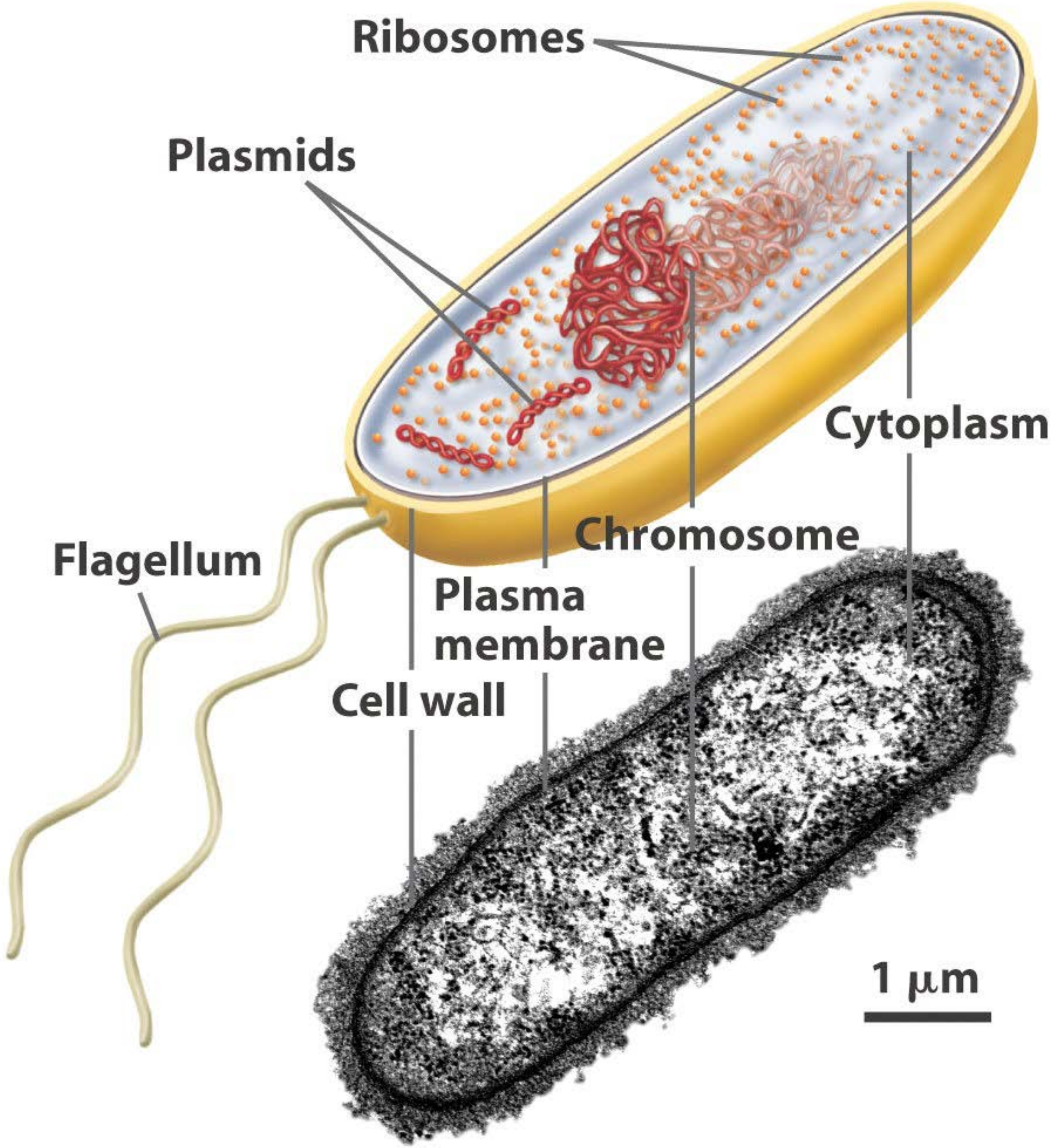
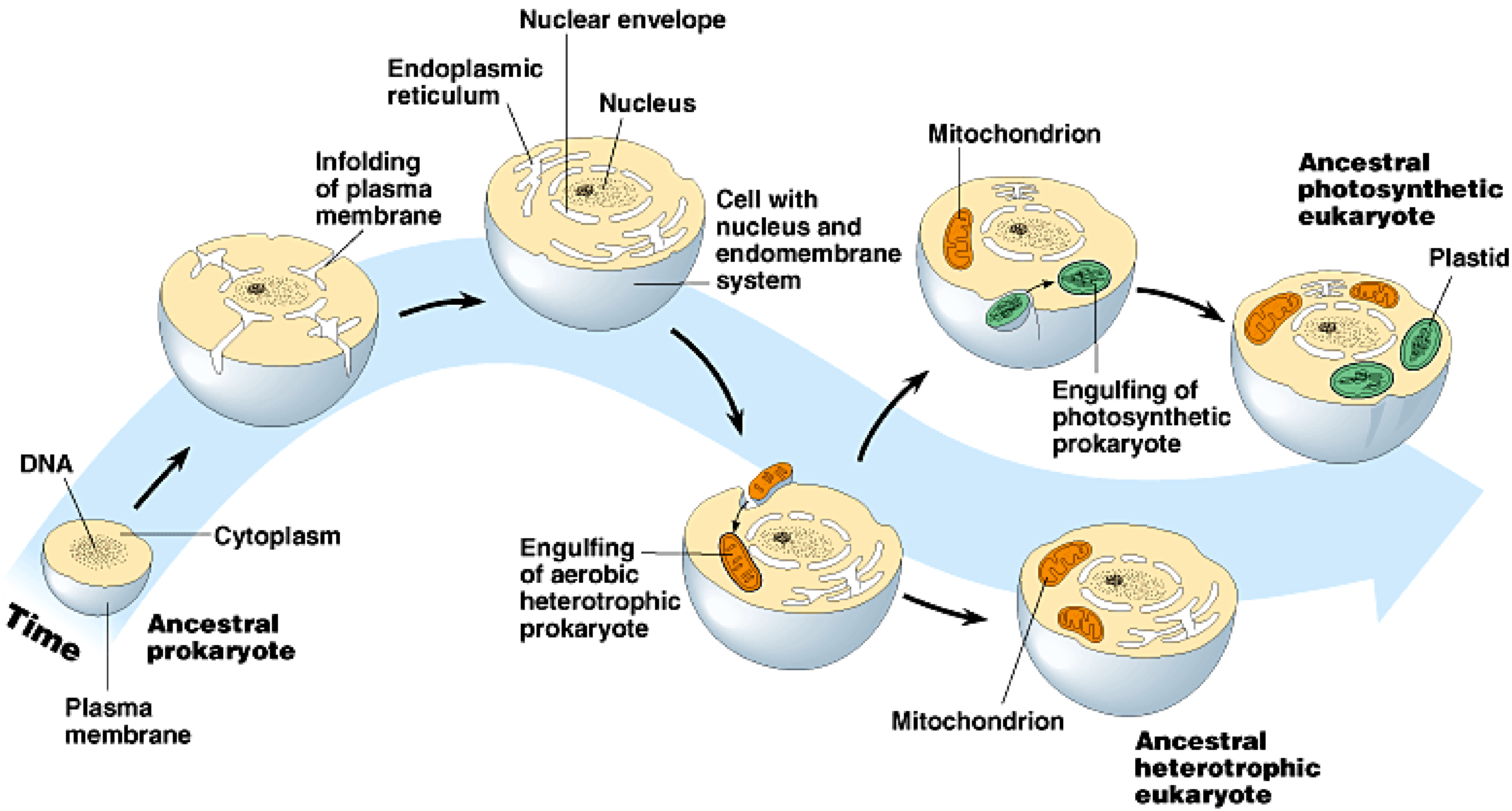
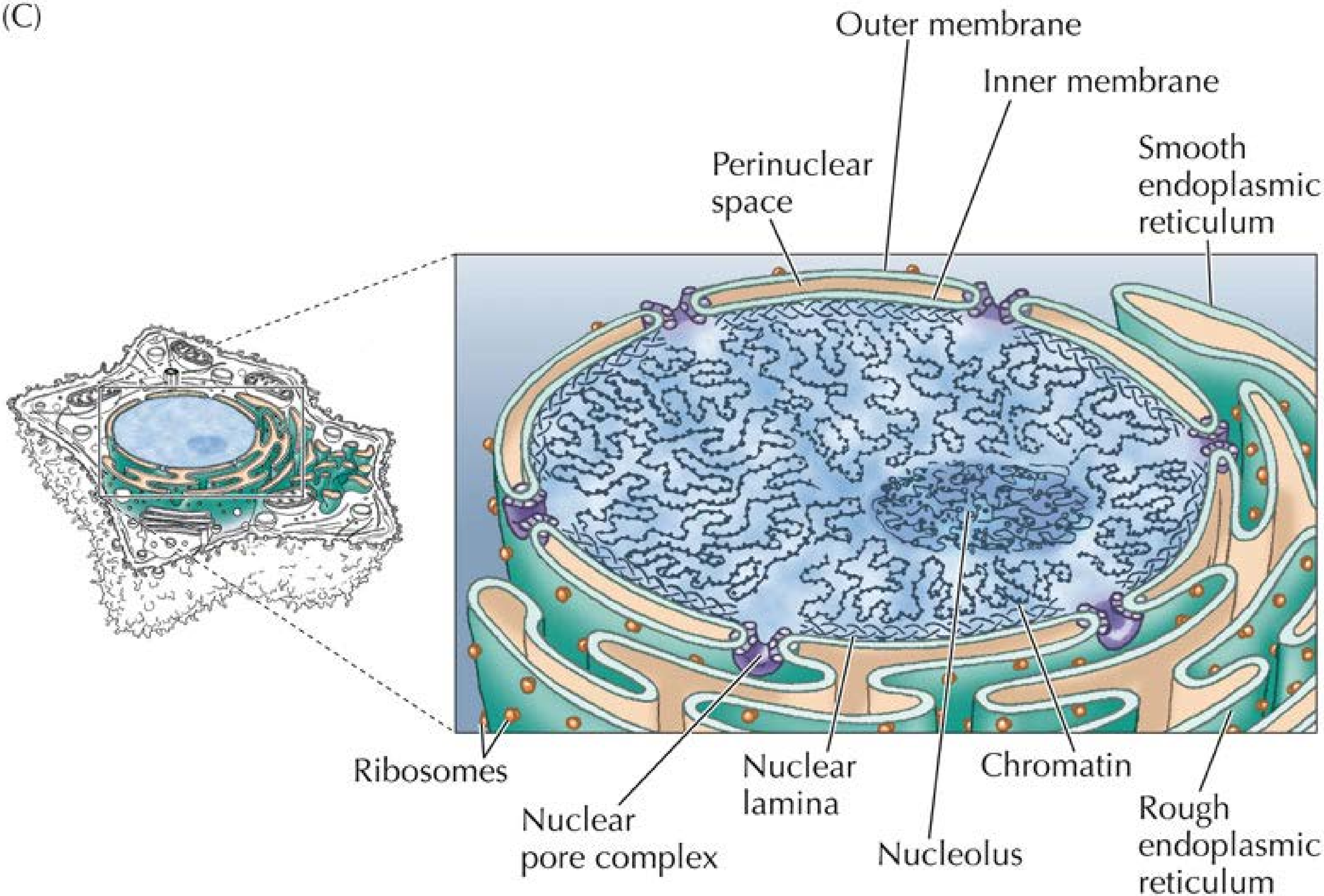


Figure 7-1 Biological Science, 2/e


Nuclear envelope
Endoplasmic reticulum
Nucleus
Infolding of plasma membrane
Cell with nucleus and endomembrane system
Mitochondrion
Ancestral photosynthetic eukaryote
Plastid
Engulfing of photosynthetic prokaryote
DNA
Cytoplasm
Time
Ancestral prokaryote
Plasma membrane
Engulfing of aerobic heterotrophic prokaryote
Mitochondrion
Ancestral heterotrophic eukaryote

(C)



THE CELL, Fourth Edition, Figure 9.1 (Part 3)

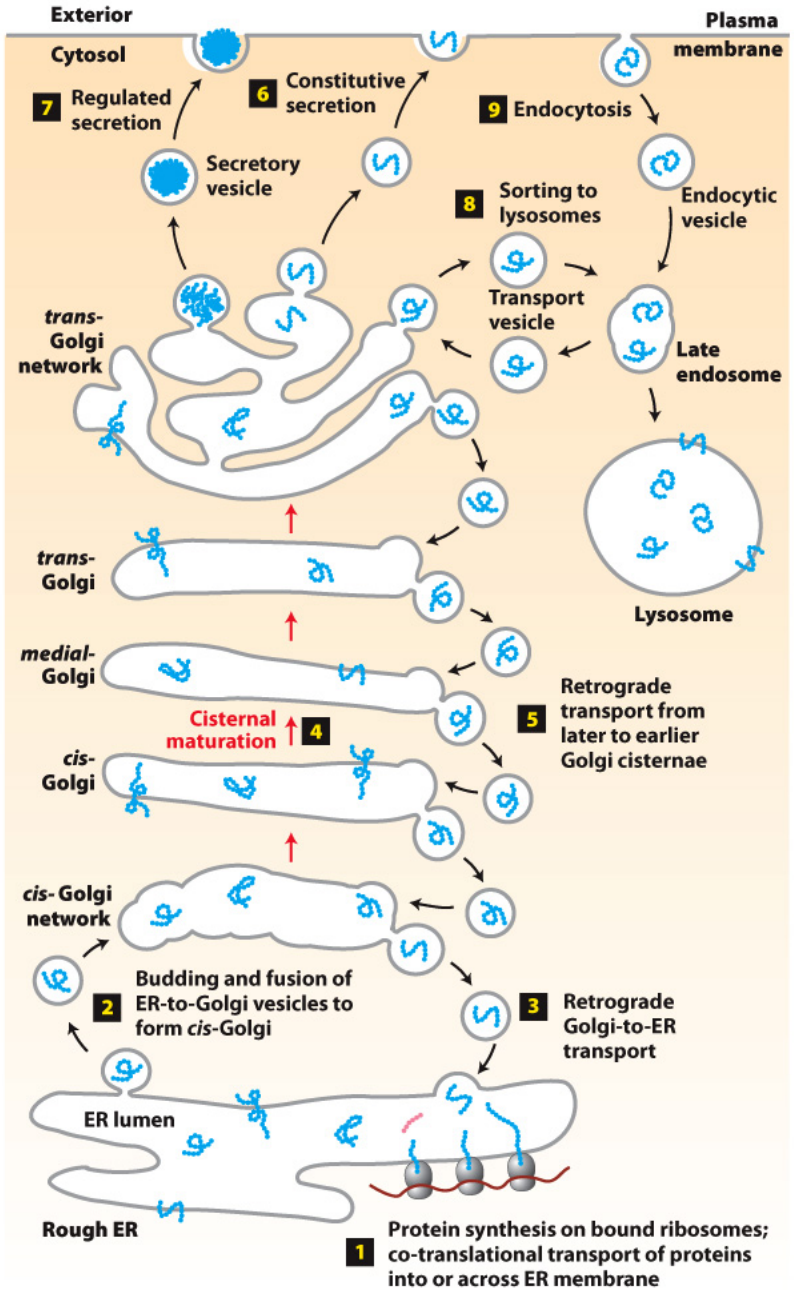
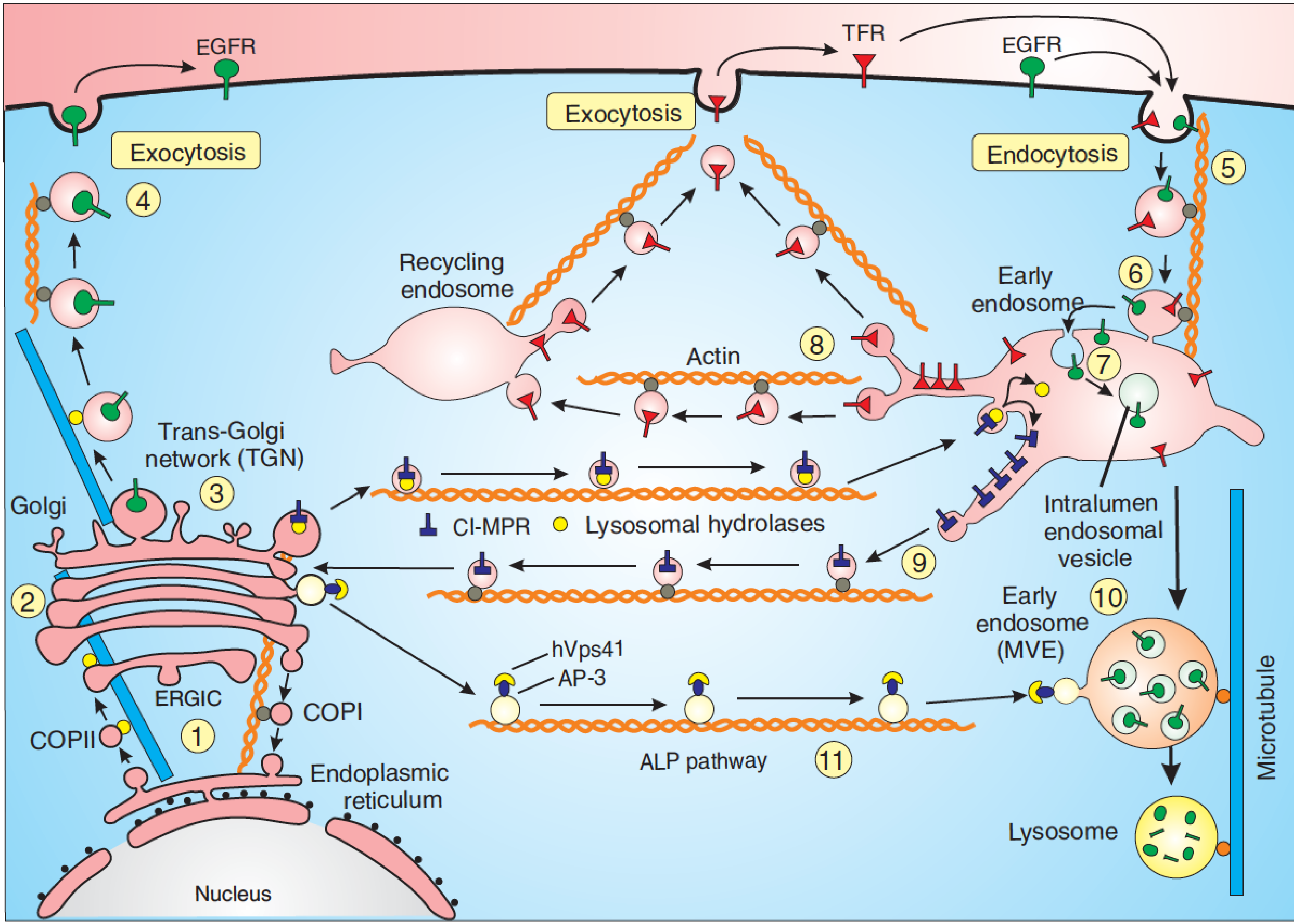
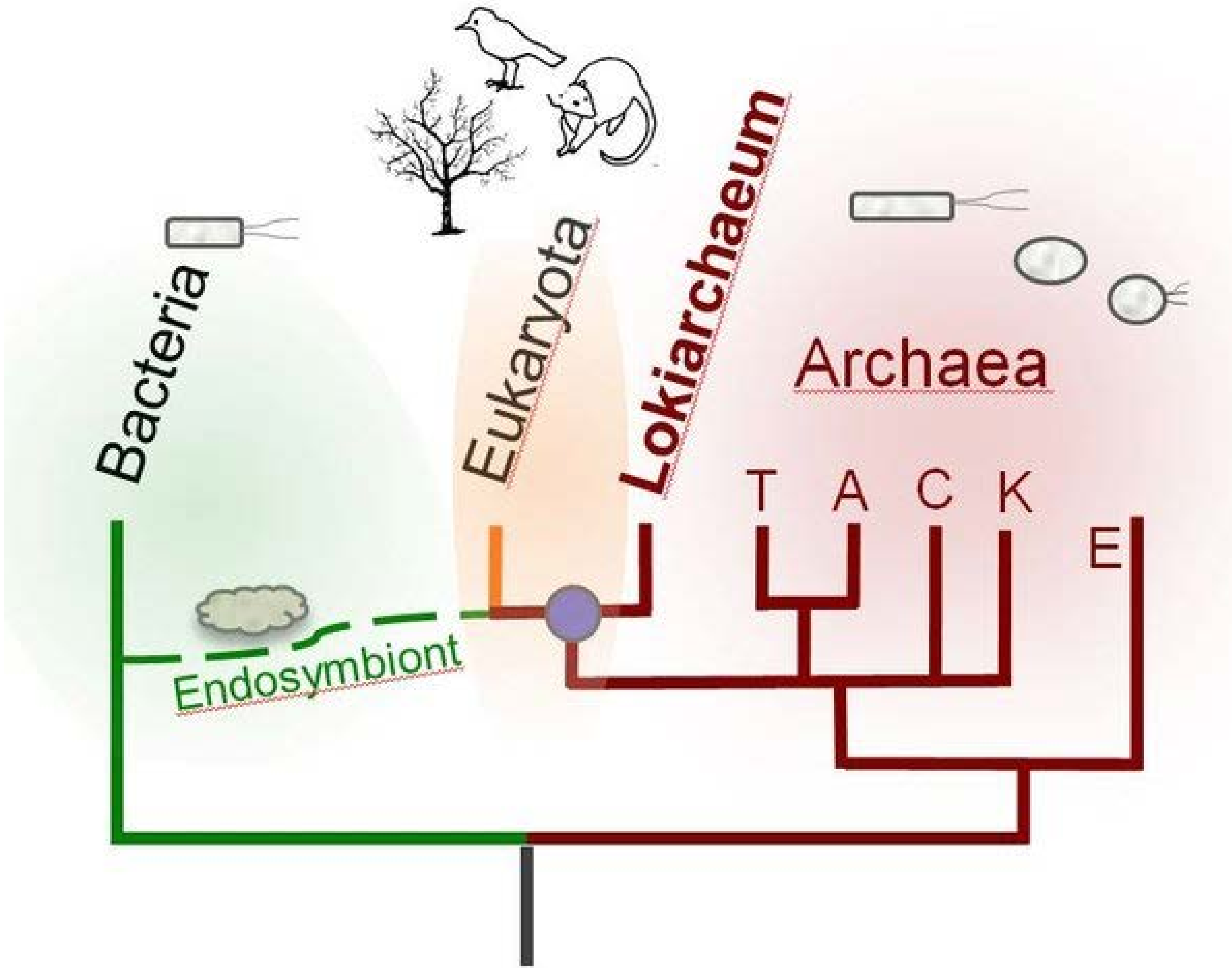


Figure 14-1

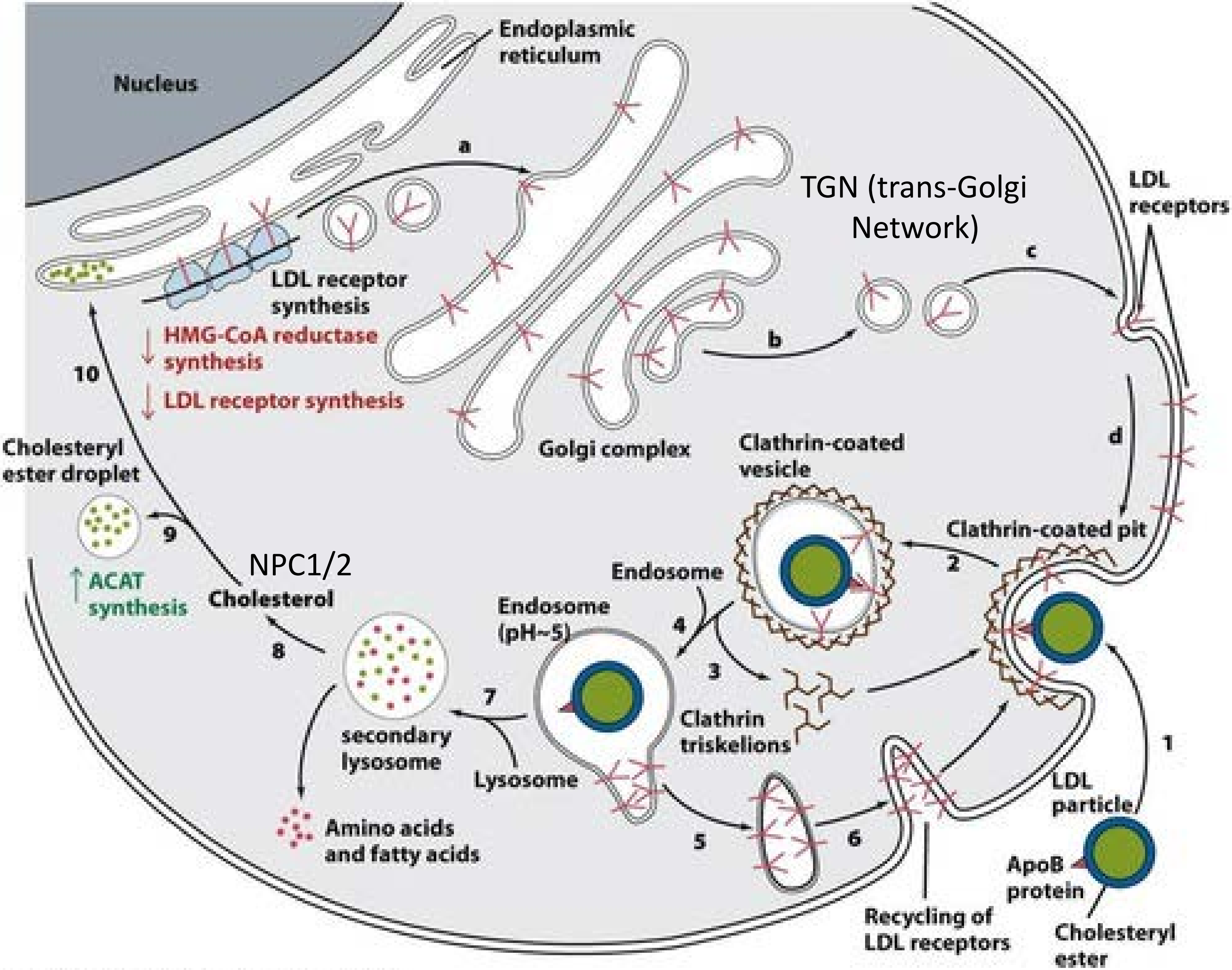
Molecular Cell Biology, Eighth Edition



Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4



https://astrobiology.nasa.gov/news/a-disputed-origin-for-eukaryotes/



Nucleus
Endoplasmic reticulum
a
LDL receptor synthesis
HMG-CoA reductase synthesis
LDL receptor synthesis
10
TGN (trans-Golgi Network)
LDL receptors
c
b
d
Golgi complex
Clathrin-coated vesicle
Cholesteryl ester droplet
9
ACAT synthesis
NPC1/2
Cholesterol
Endosome
Clathrin-coated pit
2
Endosome (pH~5)
4
3
8
7
secondary lysosome
Lysosome
Clathrin triskelions
1
LDL particle
Amino acids and fatty acids
5
6
ApoB protein
Recycling of LDL receptors
Cholesteryl ester

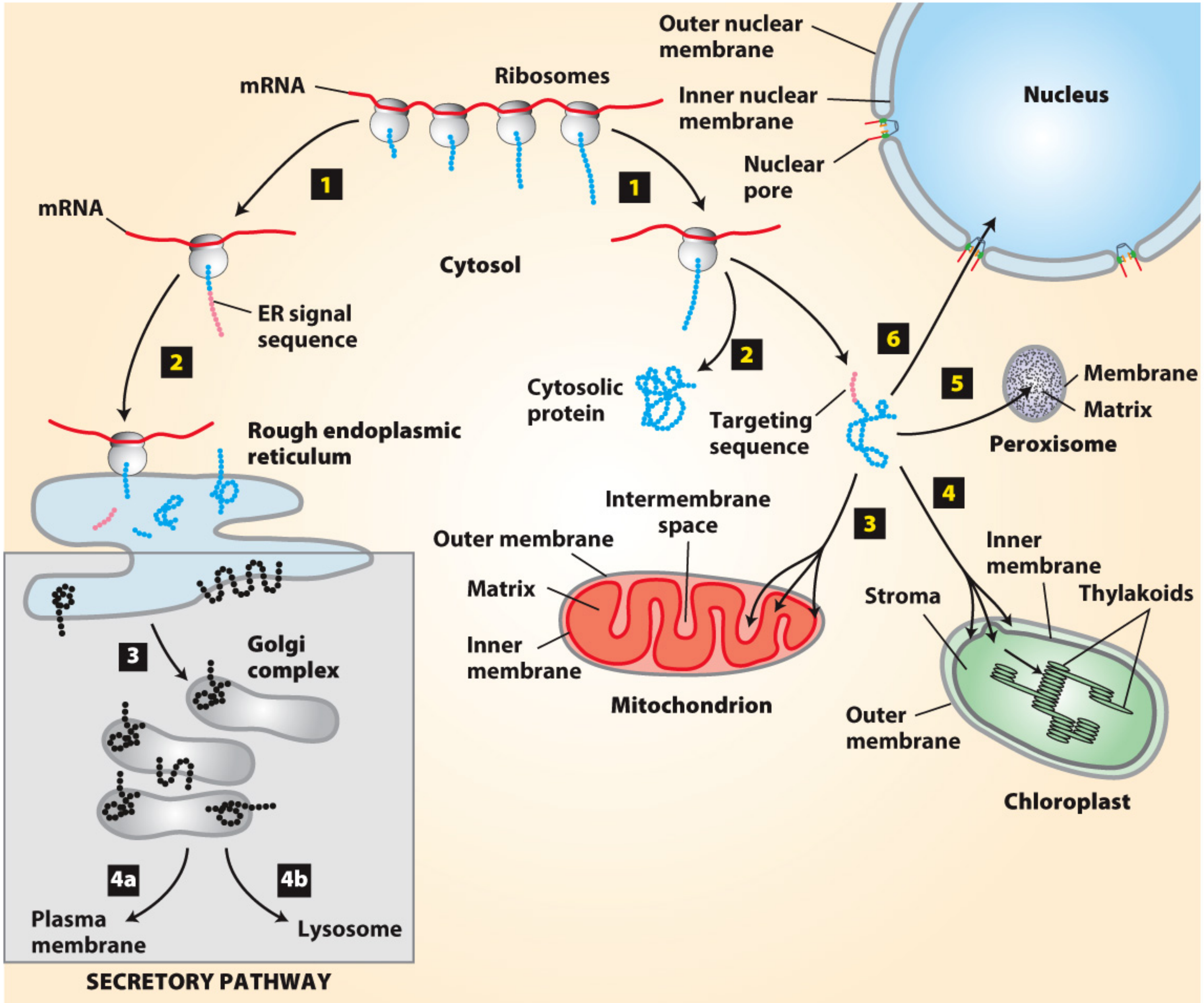


Figure 13-1
Molecular Cell Biology, Eighth Edition

SRP (signal recognition particle)

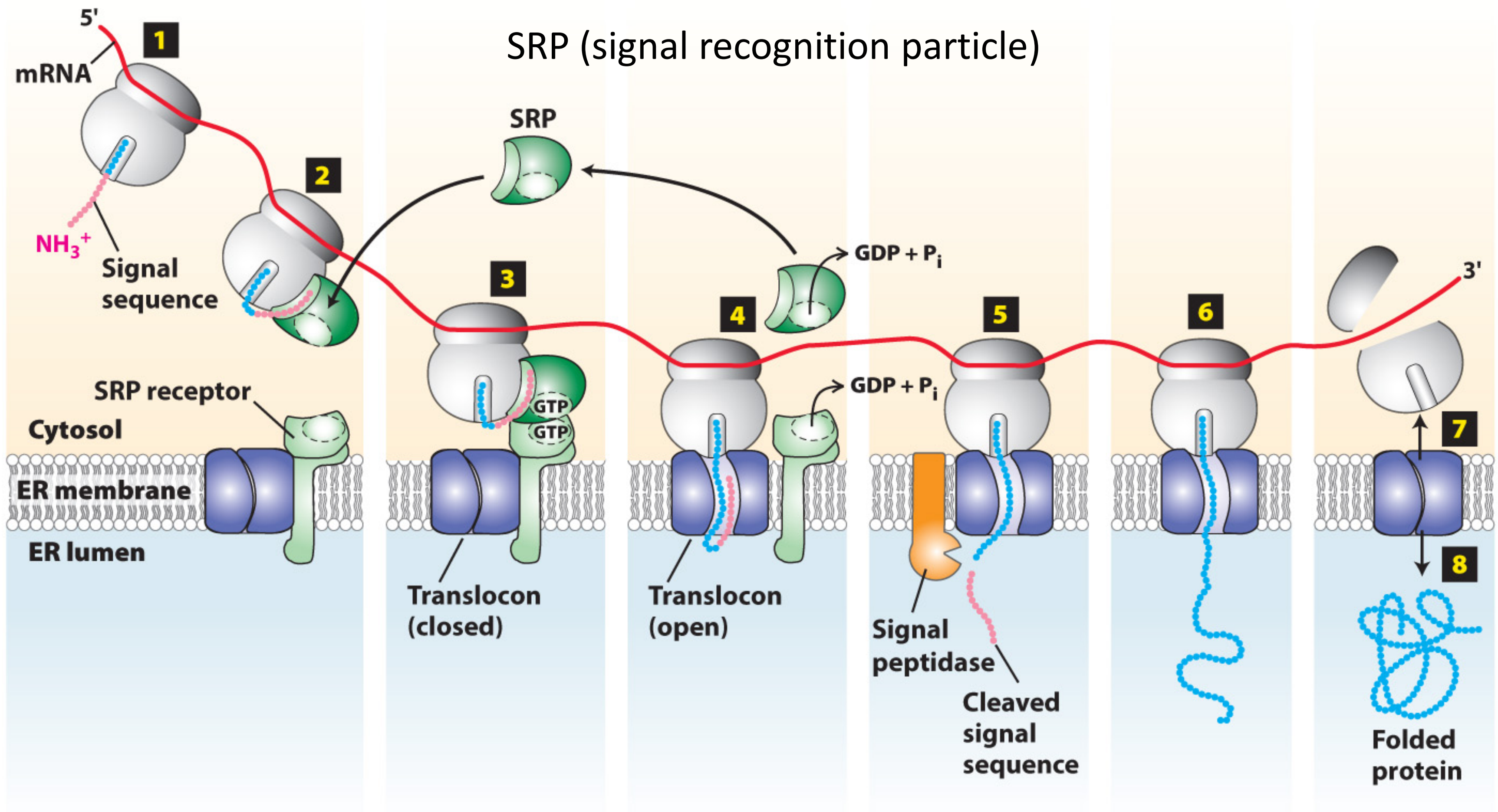


Figure 13-6
Molecular Cell Biology, Eighth Edition

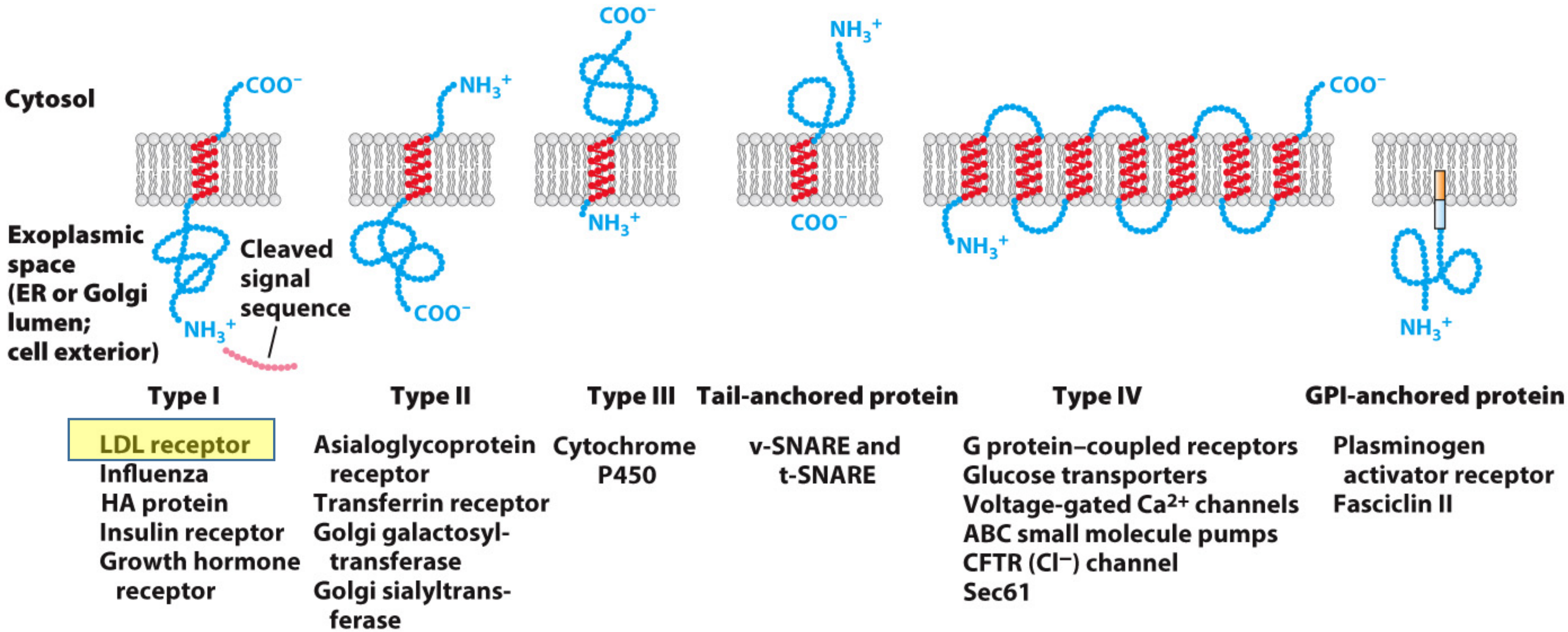
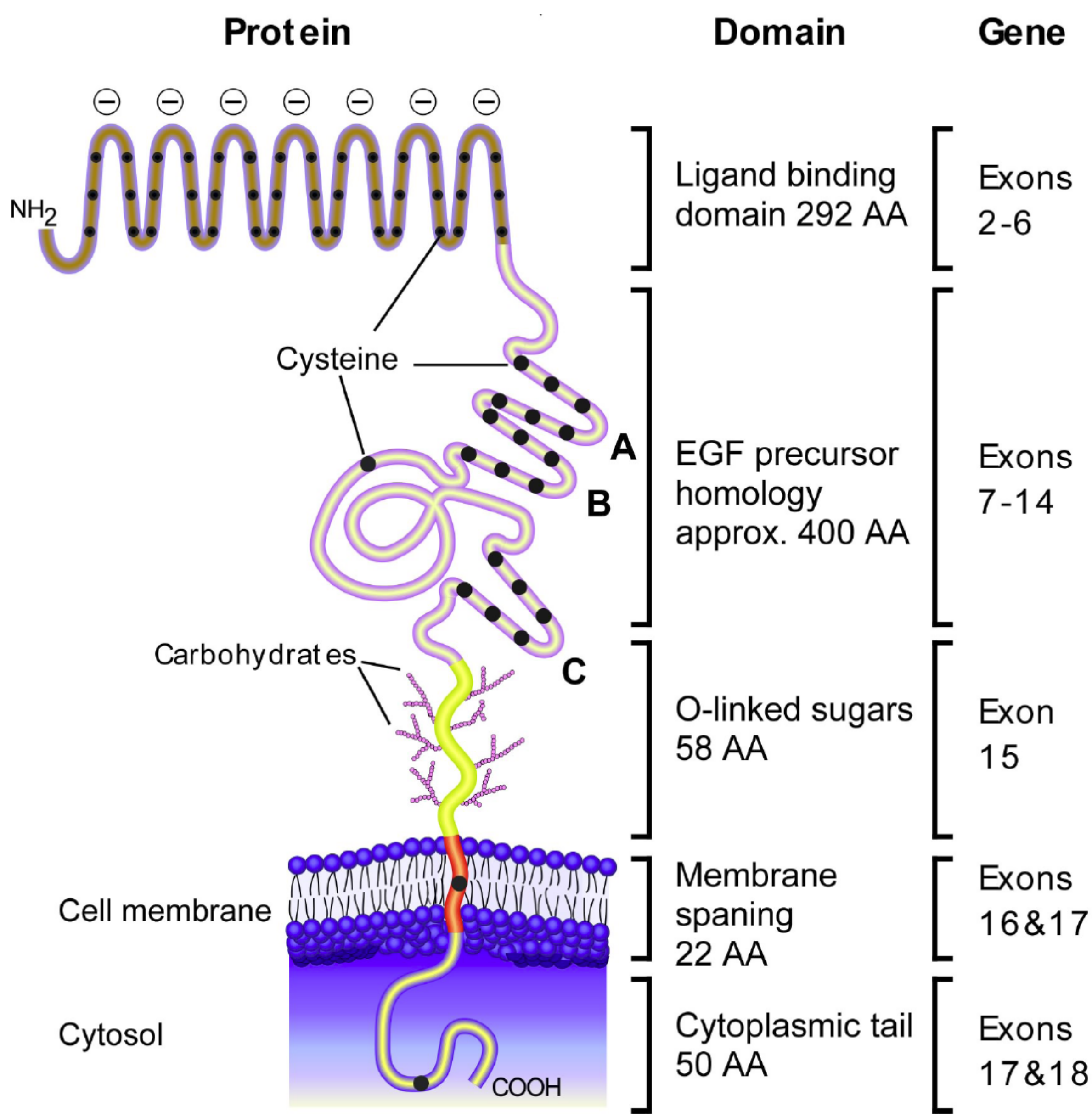


Figure 13-10
Molecular Cell Biology, Eighth Edition



Al-Allaf FA, et al. Int Arch Med 2010;3:36

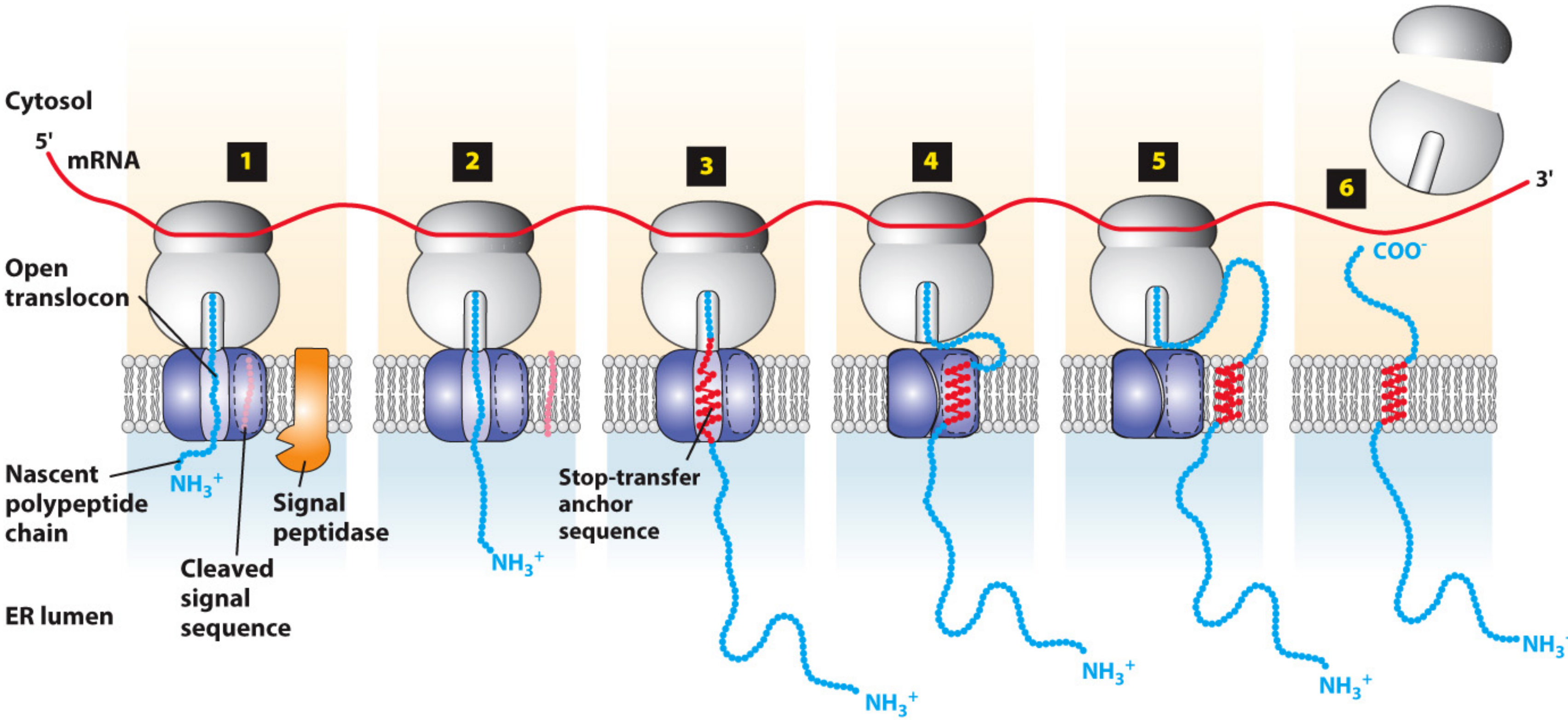


Figure 13-11
Molecular Cell Biology, Eighth Edition

Types of Class 1 (synthesis failure) LDLR mutations

- Promoter mutations (may not be as severe)
  - None known in 1990 Hobbs/Goldstein/Brown review
  - Single base pair changes causing FH are known in each of 3 SRE (SREBP binding sites), Sp1 site, TATA box
    - Khamis A, et al. Eur J Hum Genet 2015;23:790
    - http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/search.php?select_db=LDLR&srch=all
- Signal sequence (targeting to ER) (17 reported)
- Other reasons for synthesis failure (many 100s)
  - Large insertions, deletions
  - Frameshift, nonsense, splice site mutations

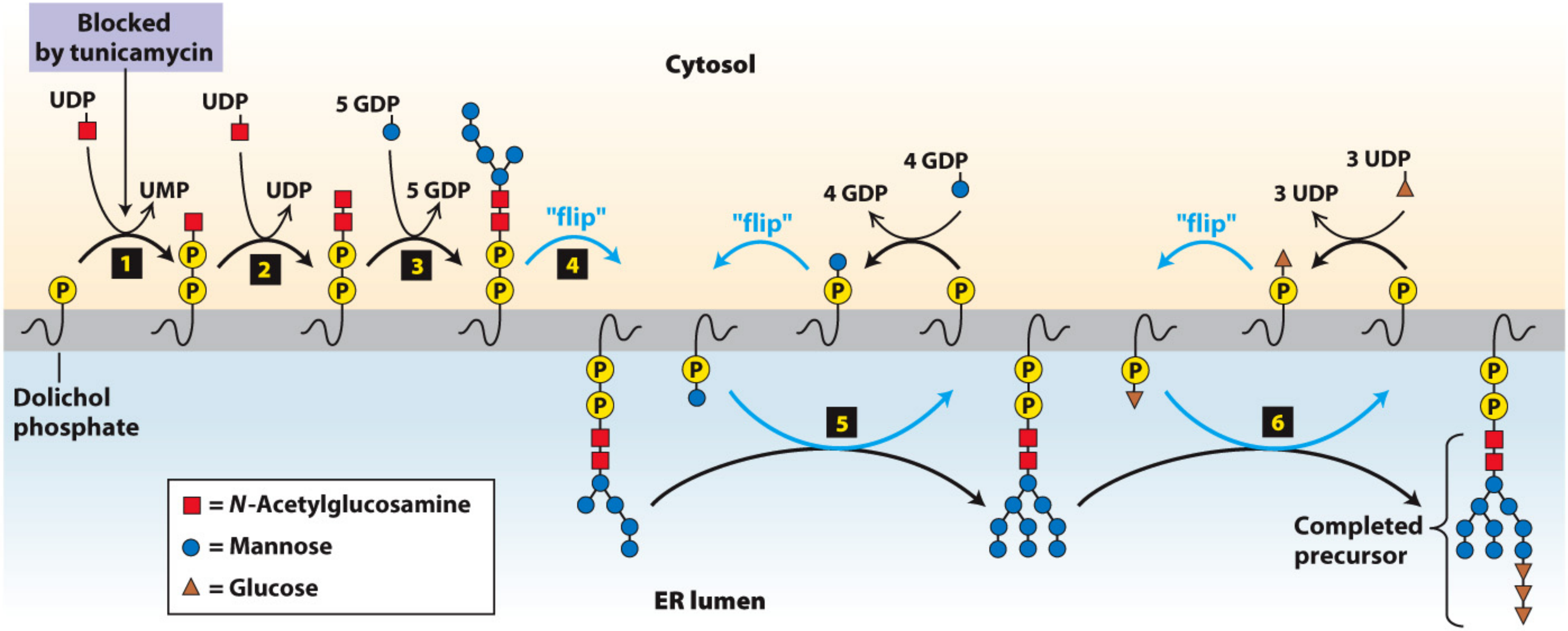


Figure 13-17
Molecular Cell Biology, Eighth Edition

Note: dolichol is an isoprenoid depleted by statins.

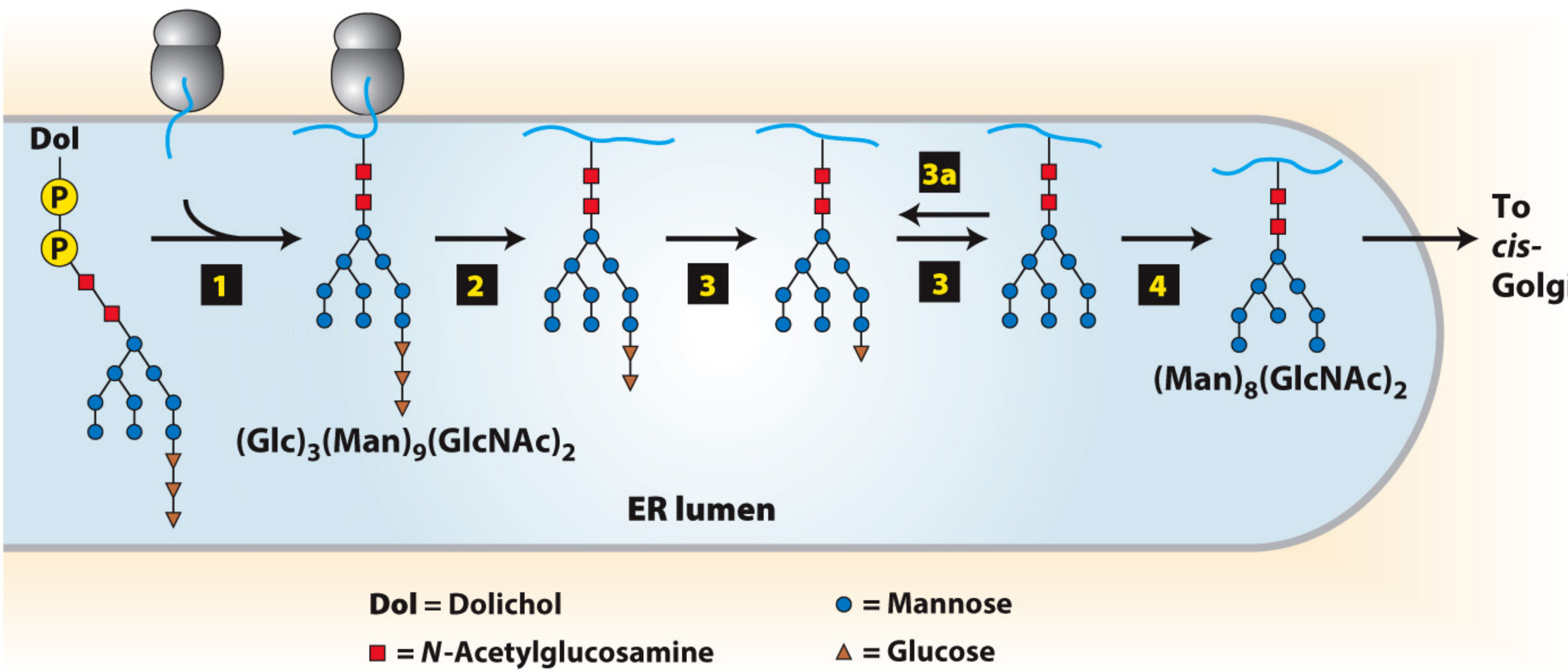


Figure 13-18
Molecular Cell Biology, Eighth Edition

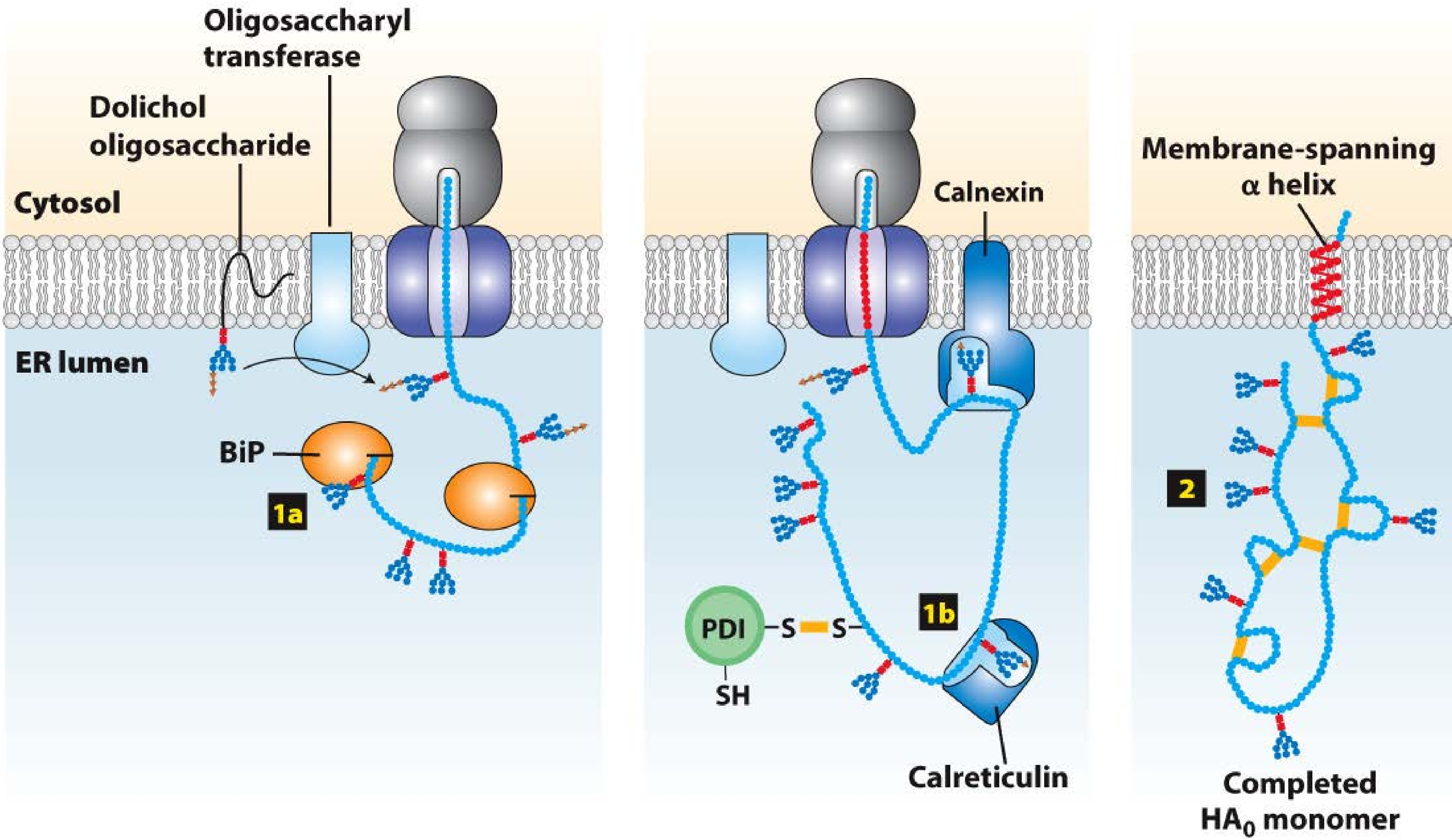
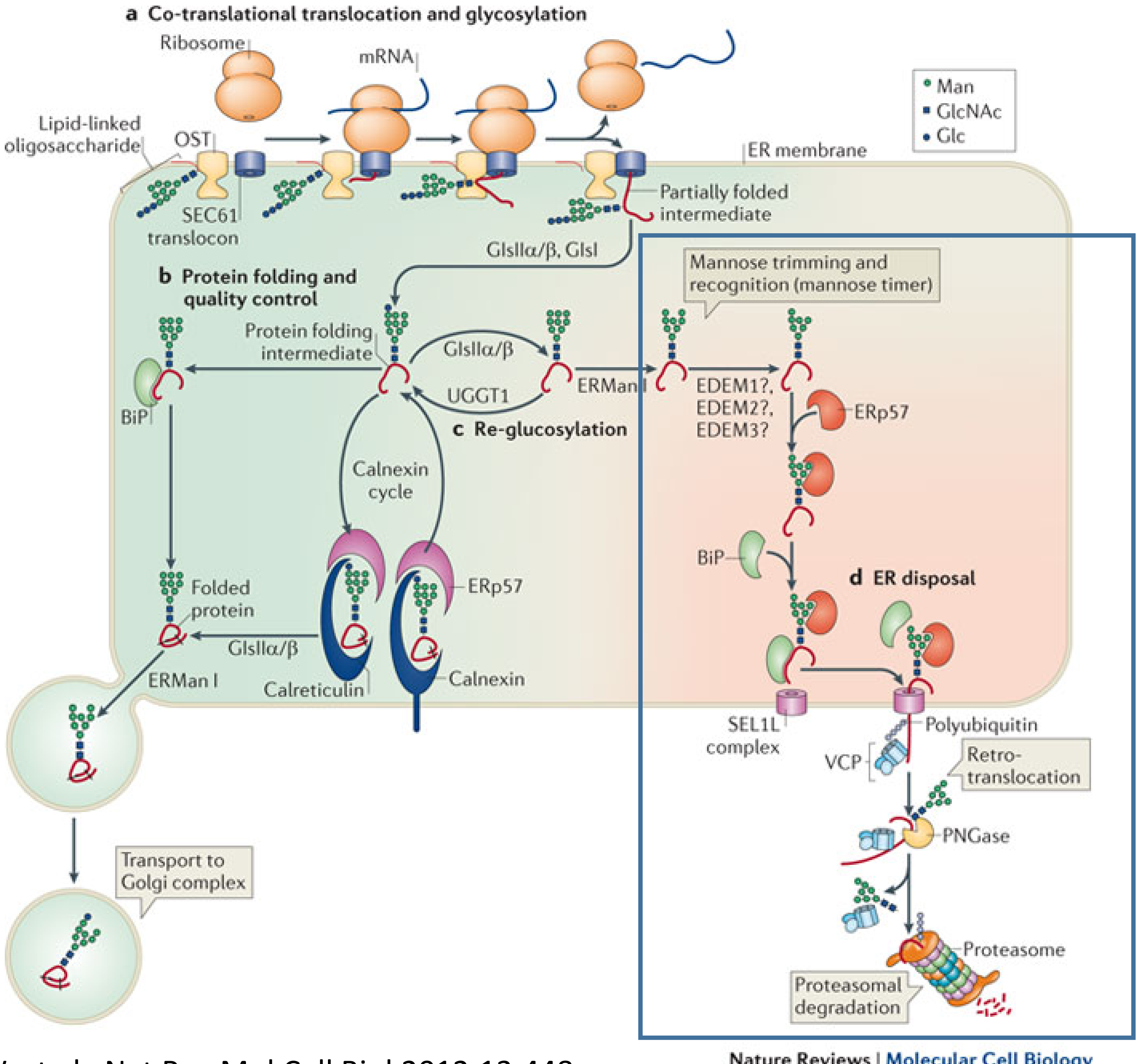


Figure 13-20a
Molecular Cell Biology, Eighth Edition

PDI (protein disulfide isomerase)

(example here is for hemagglutinin folding)



Moremen KW, et al. Nat Rev Mol Cell Biol 2012;13:448

Class 2 (misfolding) mutations of the LDLR are most common

- About 50% of LDLR mutations
  - Class 2a – no protein leaves ER
  - Class 2b – reduced protein trafficking from ER
- Lead to ER stress and unfolded protein response (UPR)
  - Chaperones BiP (Gpr78), Grp94, ERp72 (a thioredoxin which reverses initial inappropriate disulfide bonds), and calnexin bind misfolded LDLR in the ER
  - UPR triggered by sensors IRE1, PERK, ATF6
  - UPR increases chaperone transcription, increases capacity of ERAD (ER associated degradation), 26 S proteasome, ER and Golgi membranes
  - Unknown, if any, clinical significance

Sorensen S, et al. J Biol Chem 2006;281:468

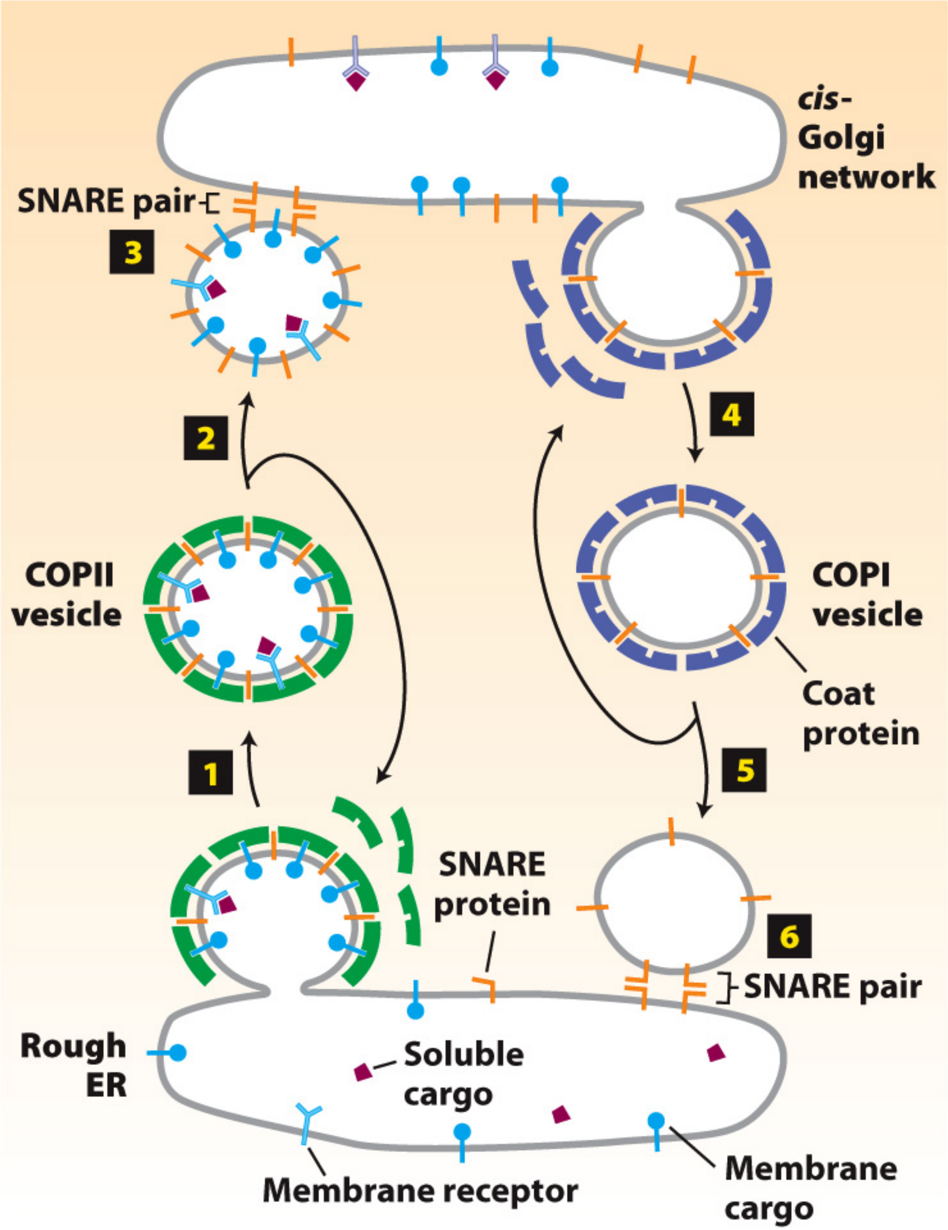


Figure 14-11
Molecular Cell Biology, Eighth Edition

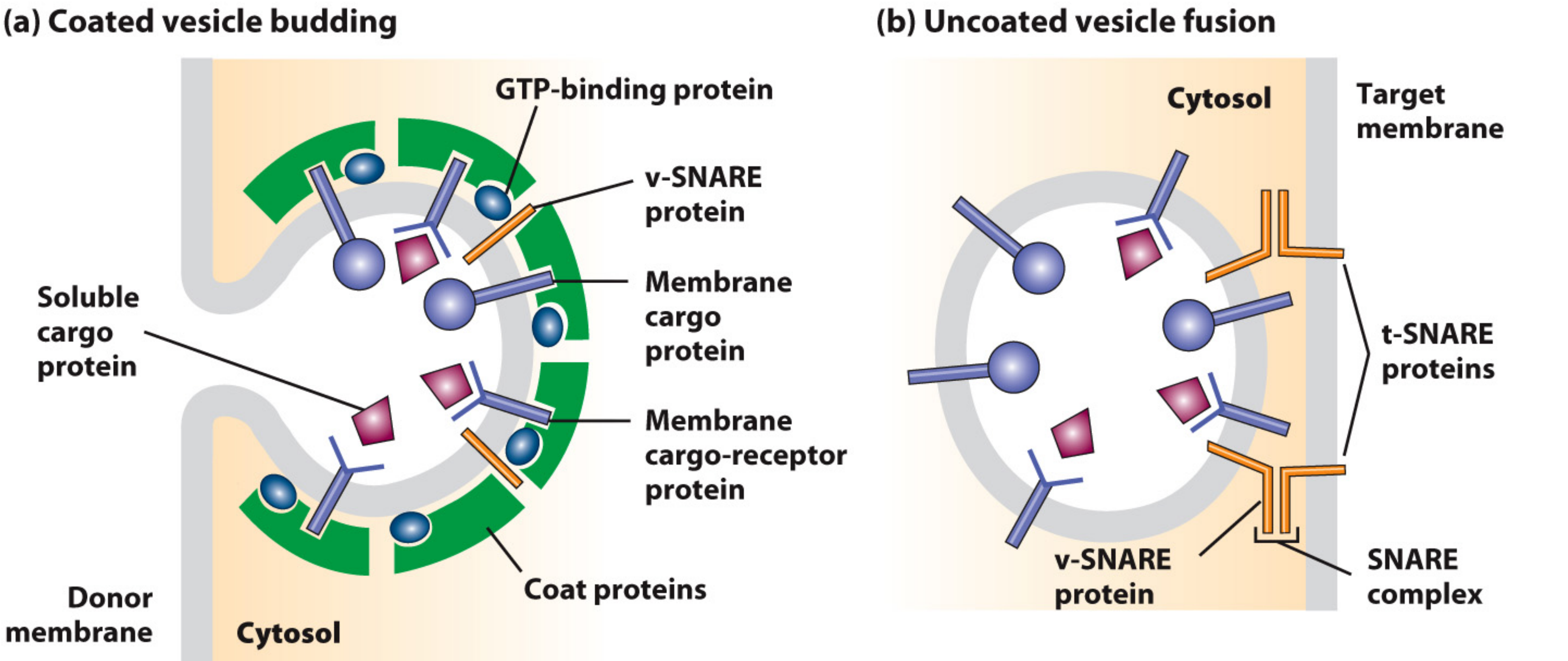


Figure 14-6
Molecular Cell Biology, Eighth Edition

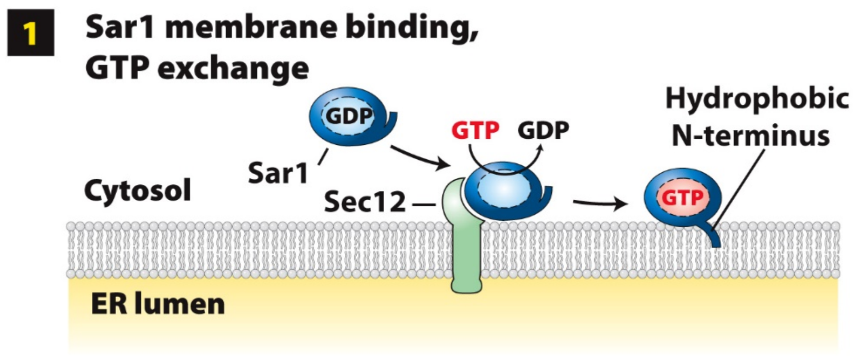


Figure 14-8 part 1
Molecular Cell Biology, Eighth Edition

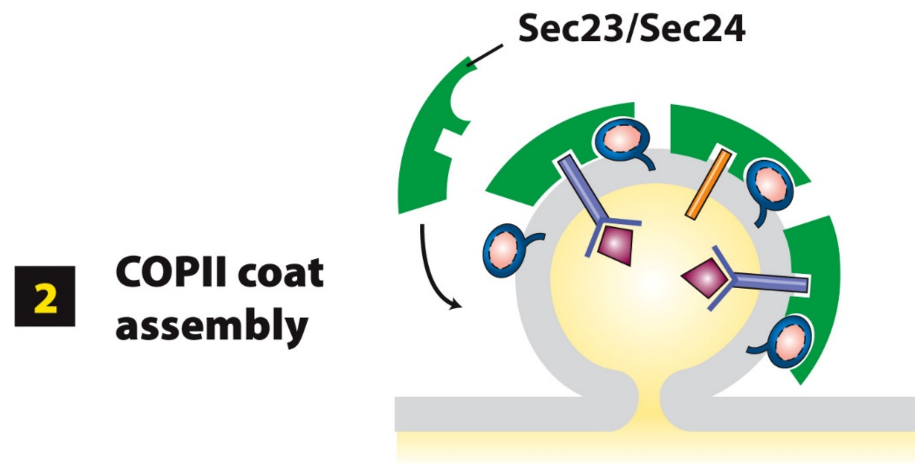


Figure 14-8 part 2
Molecular Cell Biology, Eighth Edition

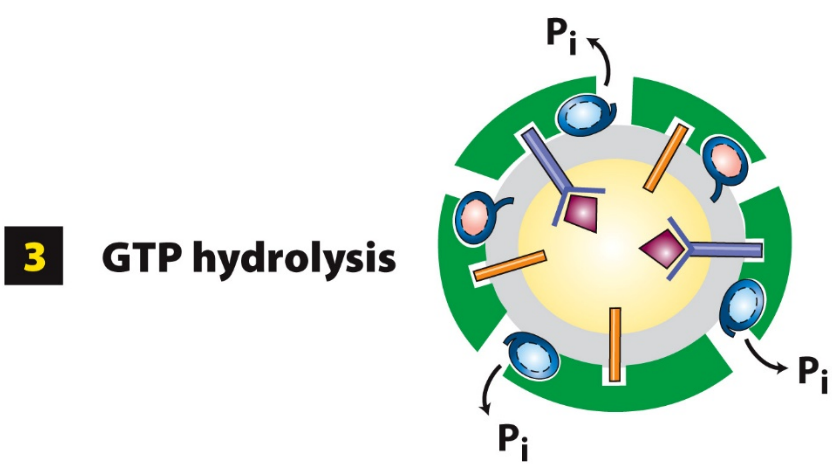


Figure 14-8 part 3
Molecular Cell Biology, Eighth Edition

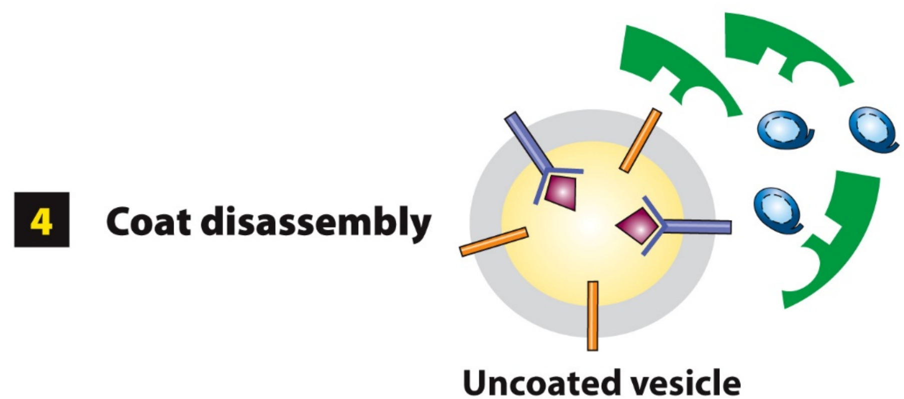


Figure 14-8 part 4
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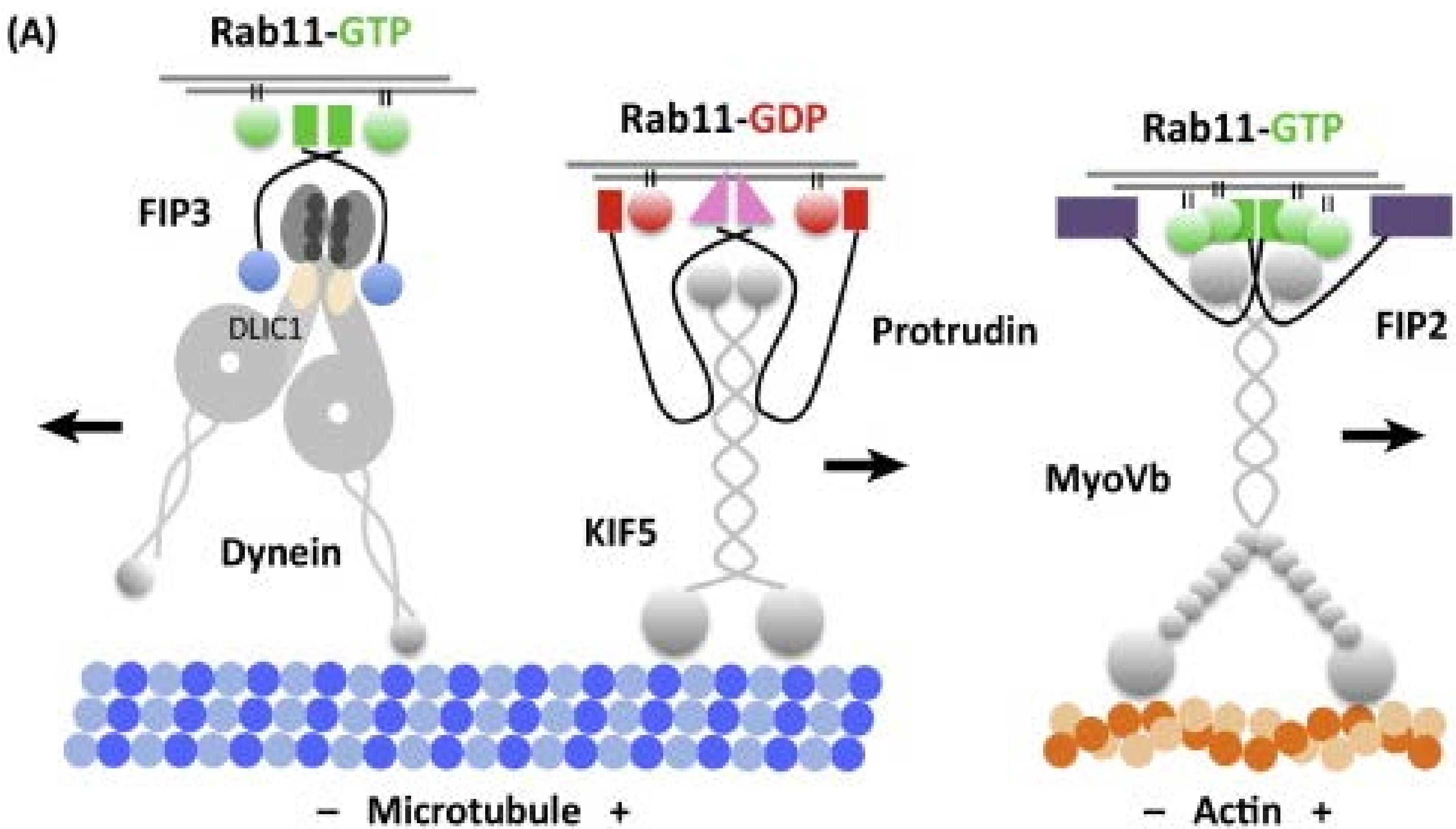
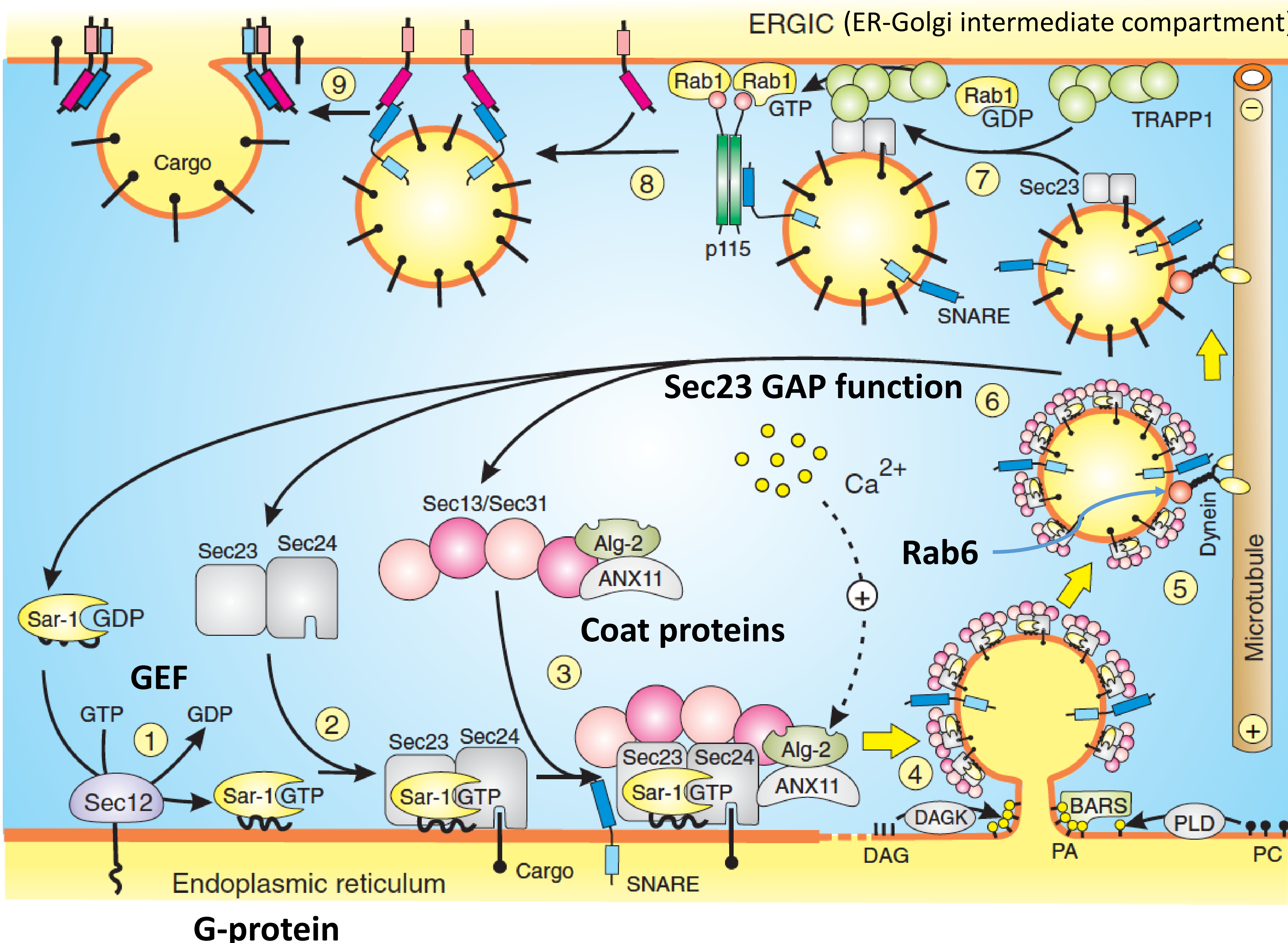
G-proteins of the Rab series recruit cellular motors and control direction of vesicular transport



Welz T, et al. Trends Cell Biol 2014;24:407



Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4

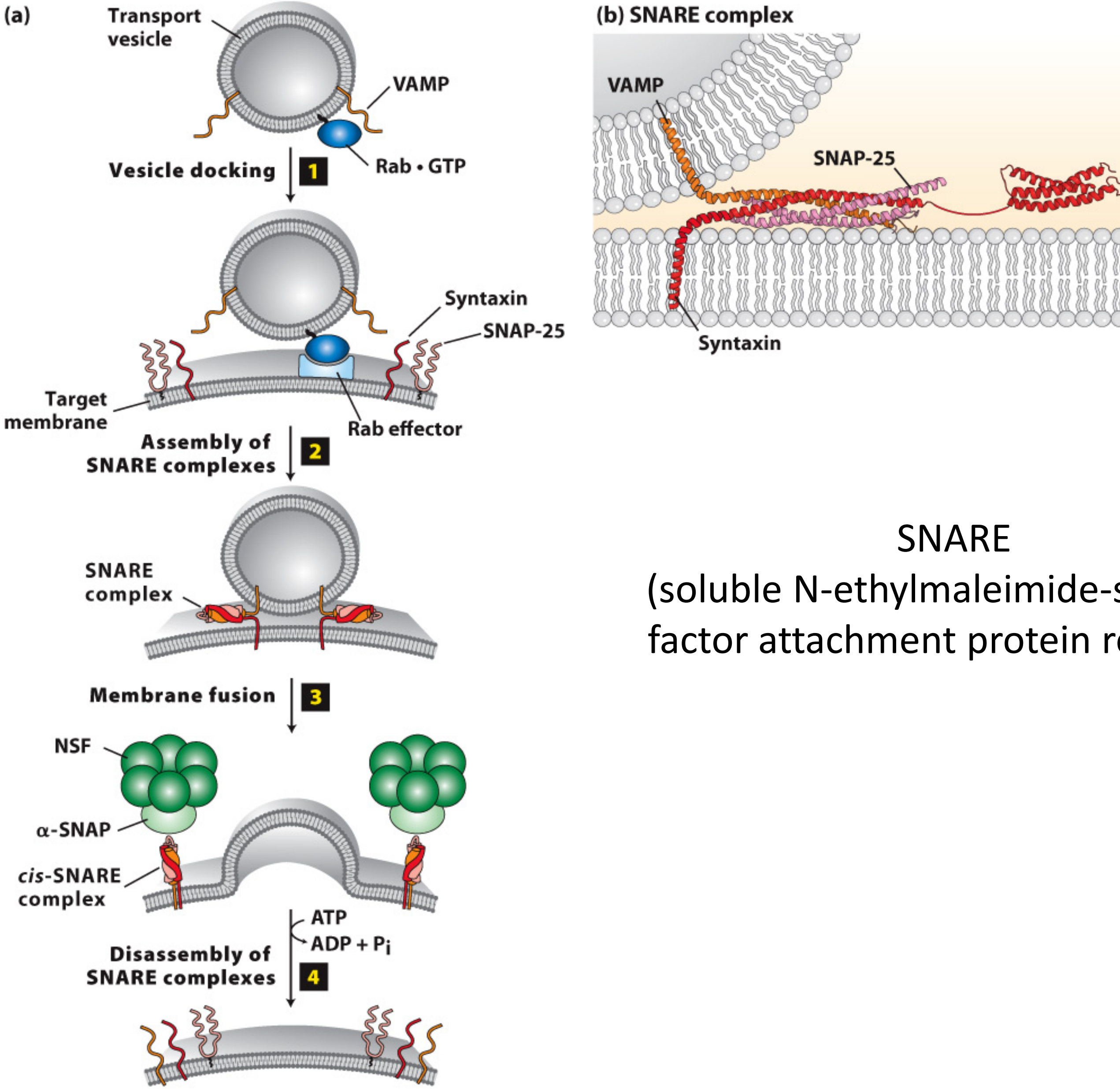
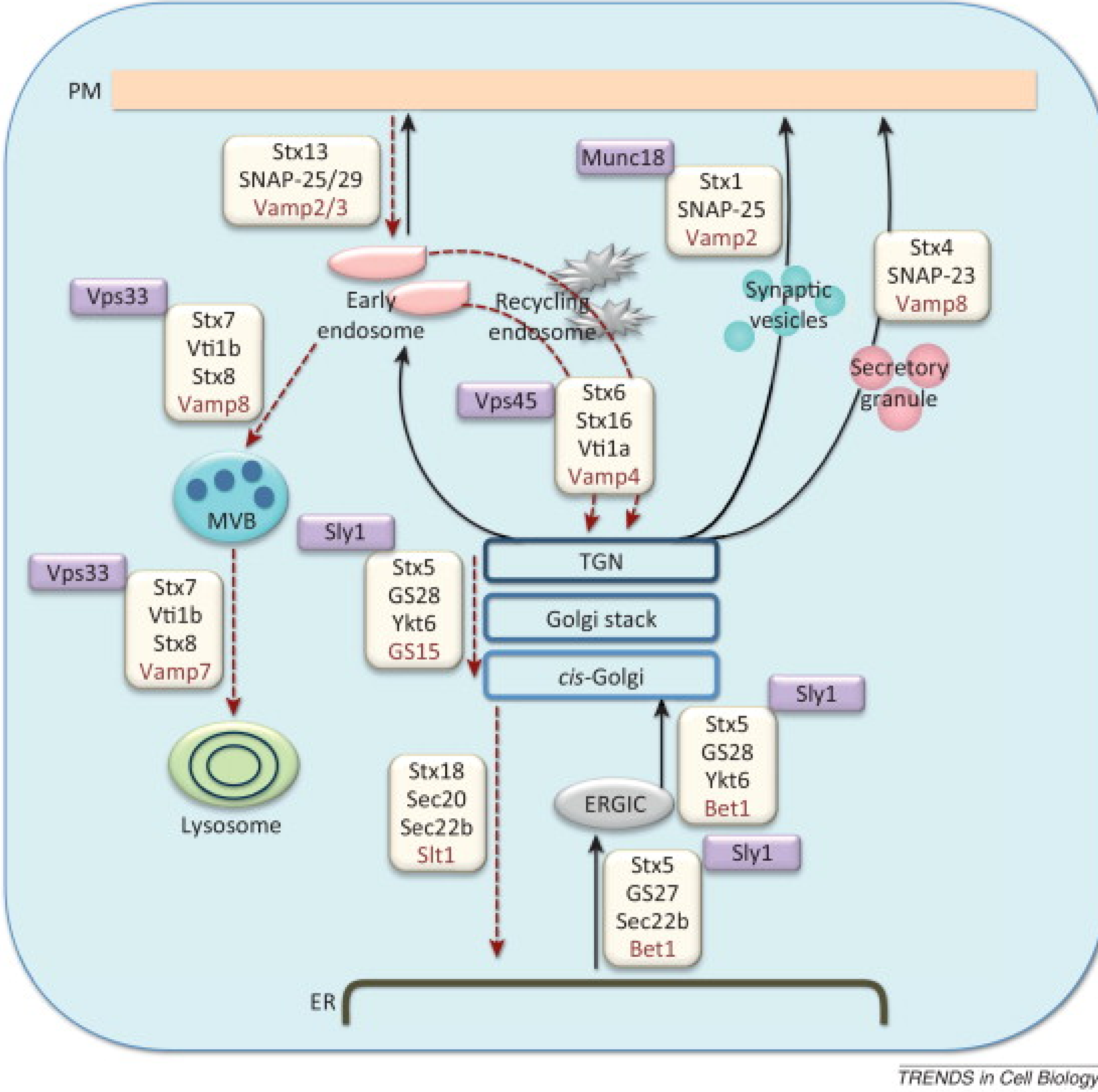


Figure 14-10
Molecular Cell Biology, Eighth Edition

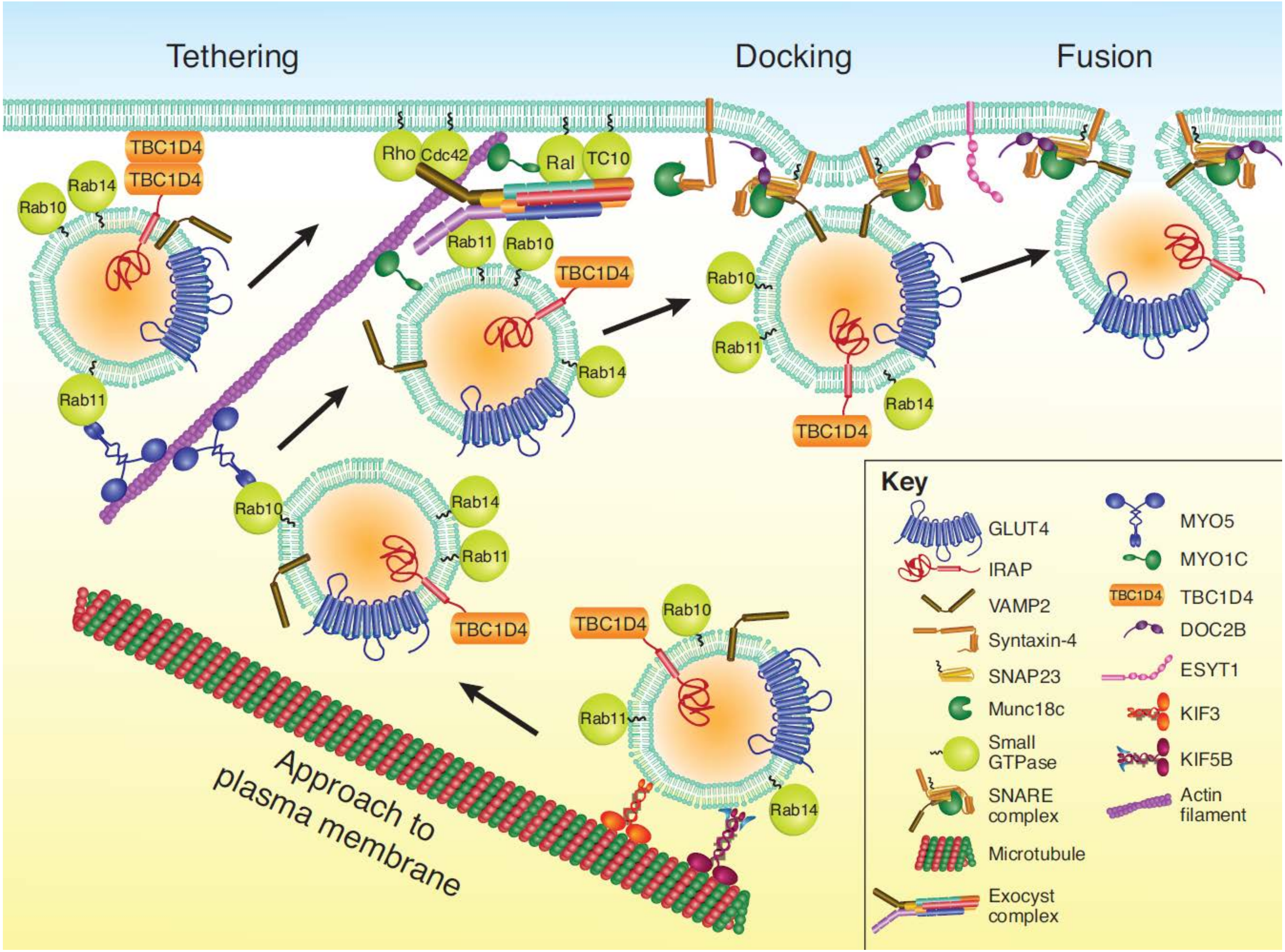
SNARE
(soluble N-ethylmaleimide-sensitive factor attachment protein receptor)

Summary of COPII vesicle cycle

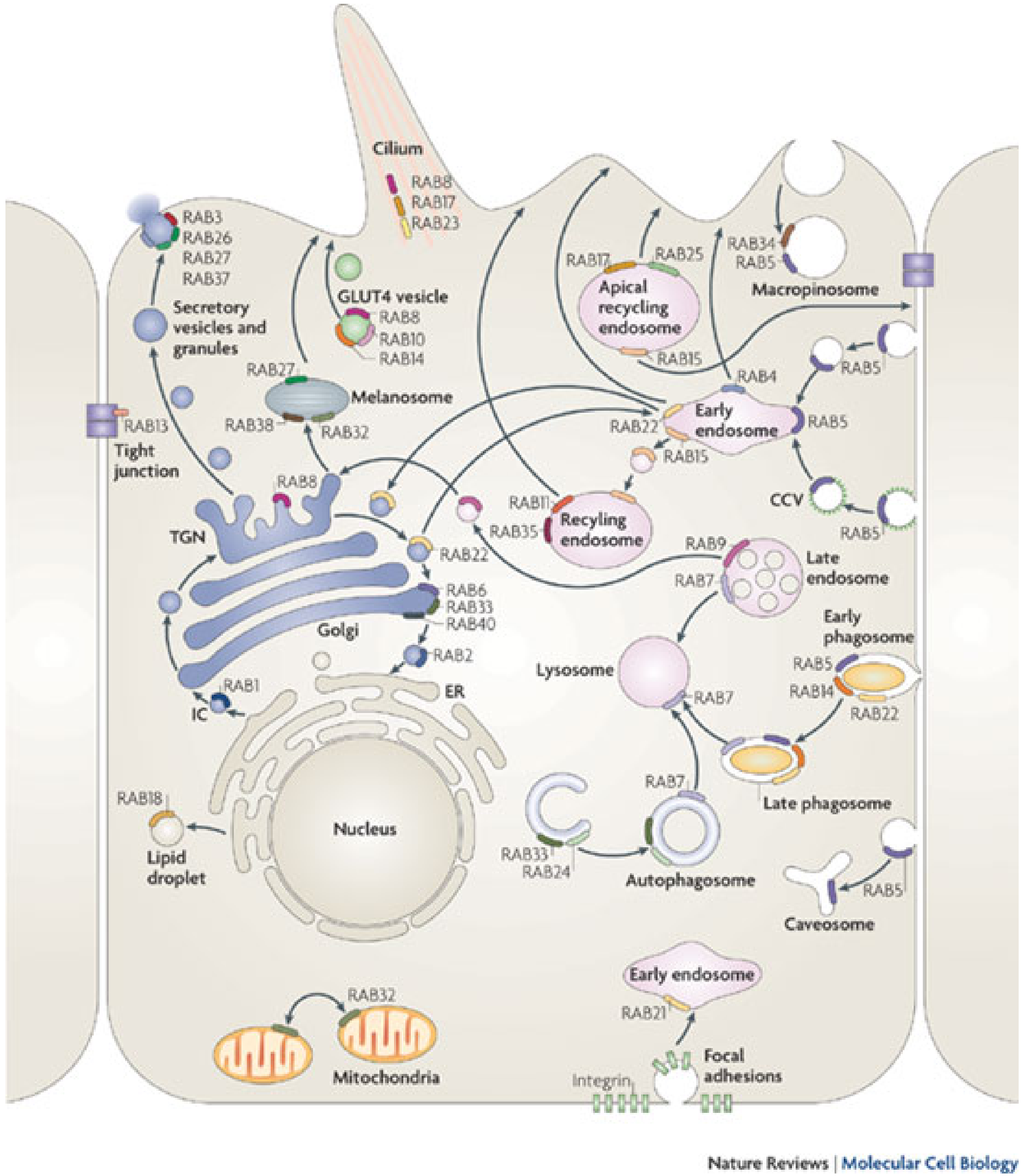
- G-protein (Sar1) activation by GEF (Sec12)
- Assembly of coat proteins on Sar1-GTP
- Sar1-GTP and coat proteins deform membrane to form vesicle
- Scission of vesicle (phosphatidic acid formation)
- Sec23 GAP → release of outer coat proteins
- GEF (TRAPP1) activates Rab1 on Golgi
- Rab1-GTP and SNARE proteins mediate fusion of COPII vesicle with Golgi



Trends in Cell Biology 2014 24, 35

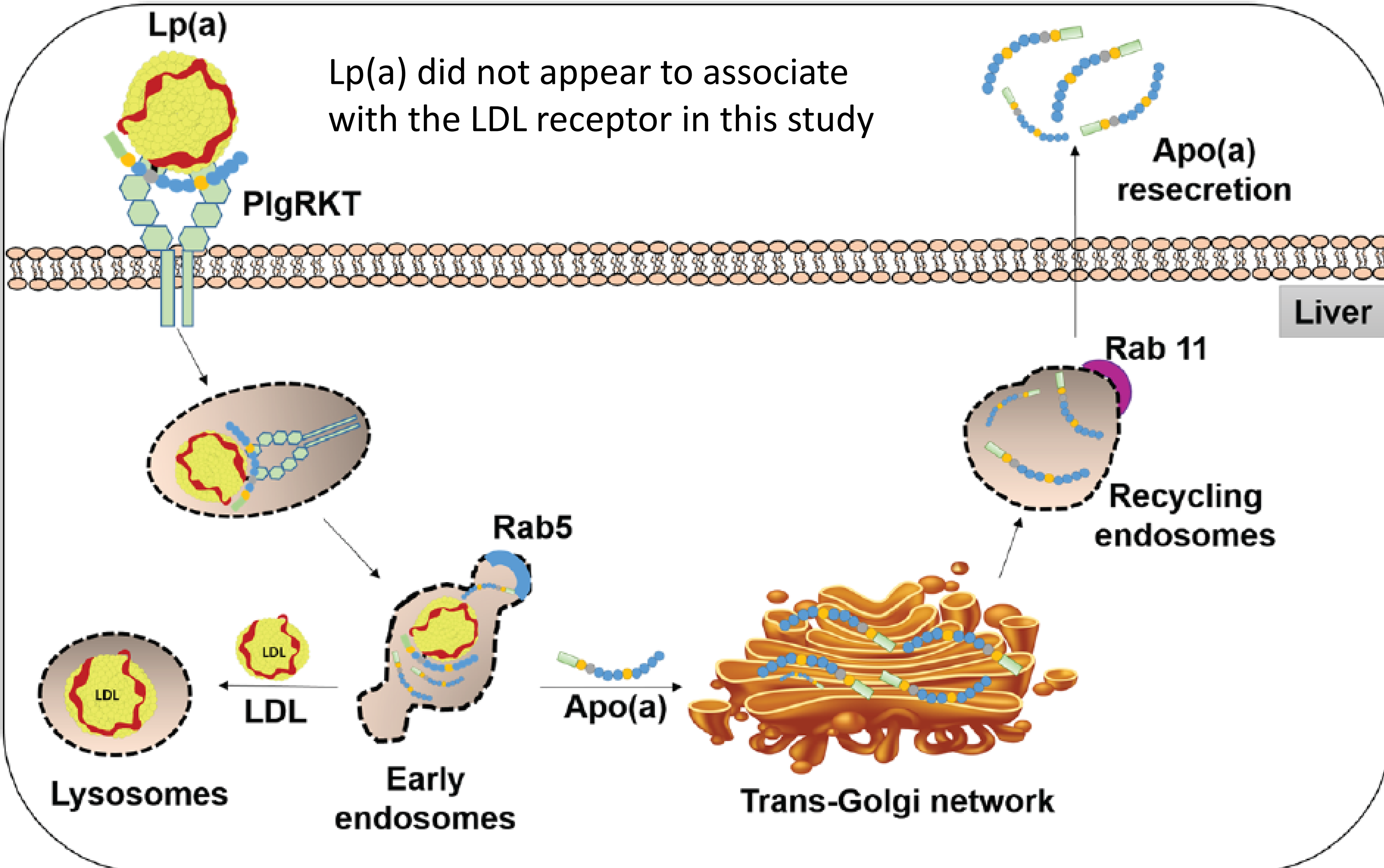


Stockli J, et al. J Cell Sci 2011 124 4147



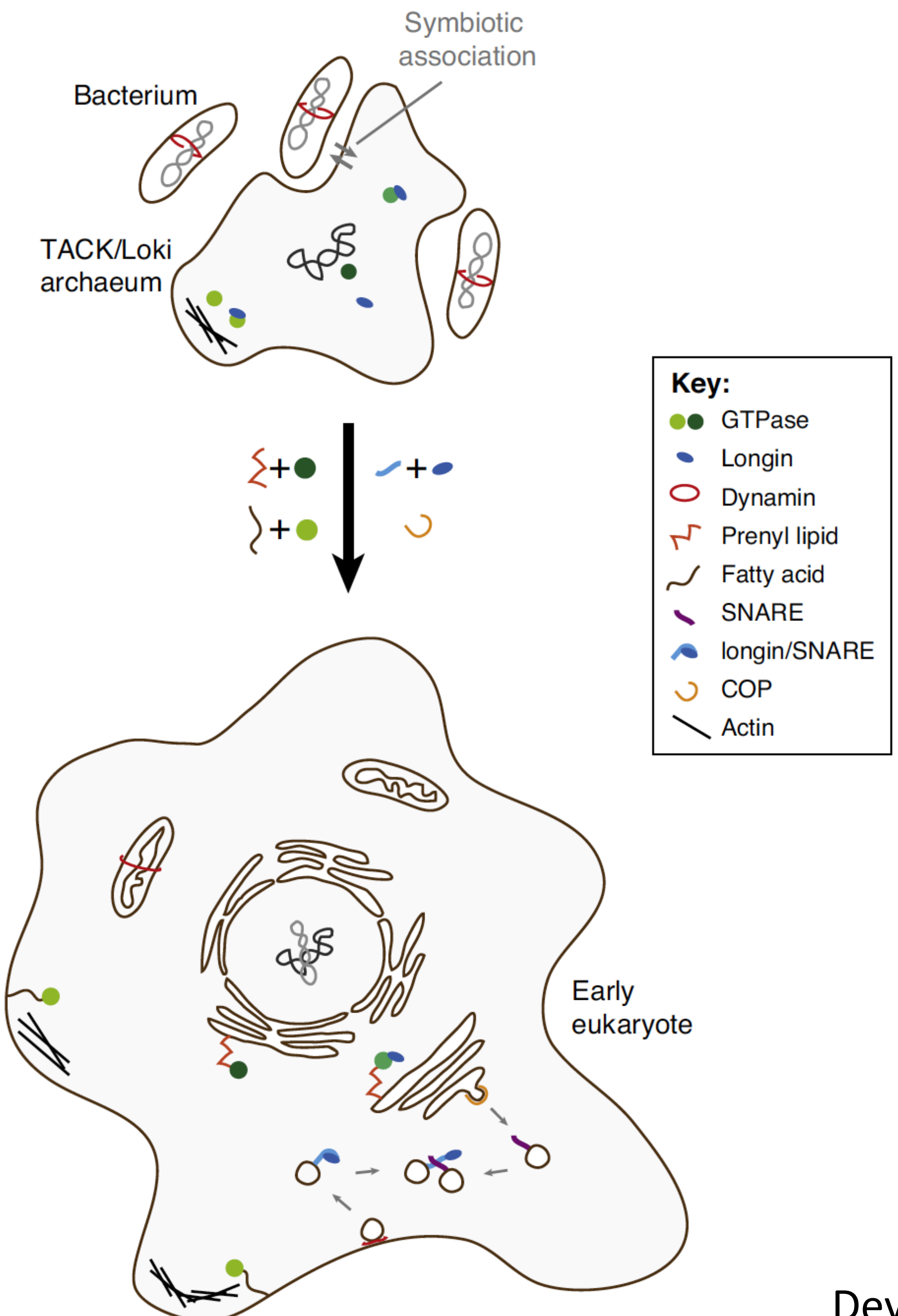
Note: Rab1 prenylation is inhibited by statins. Implicated in statin-induced myopathy.

Stenmark H. Nat Rev Mol Cell Biol 2009;10:513

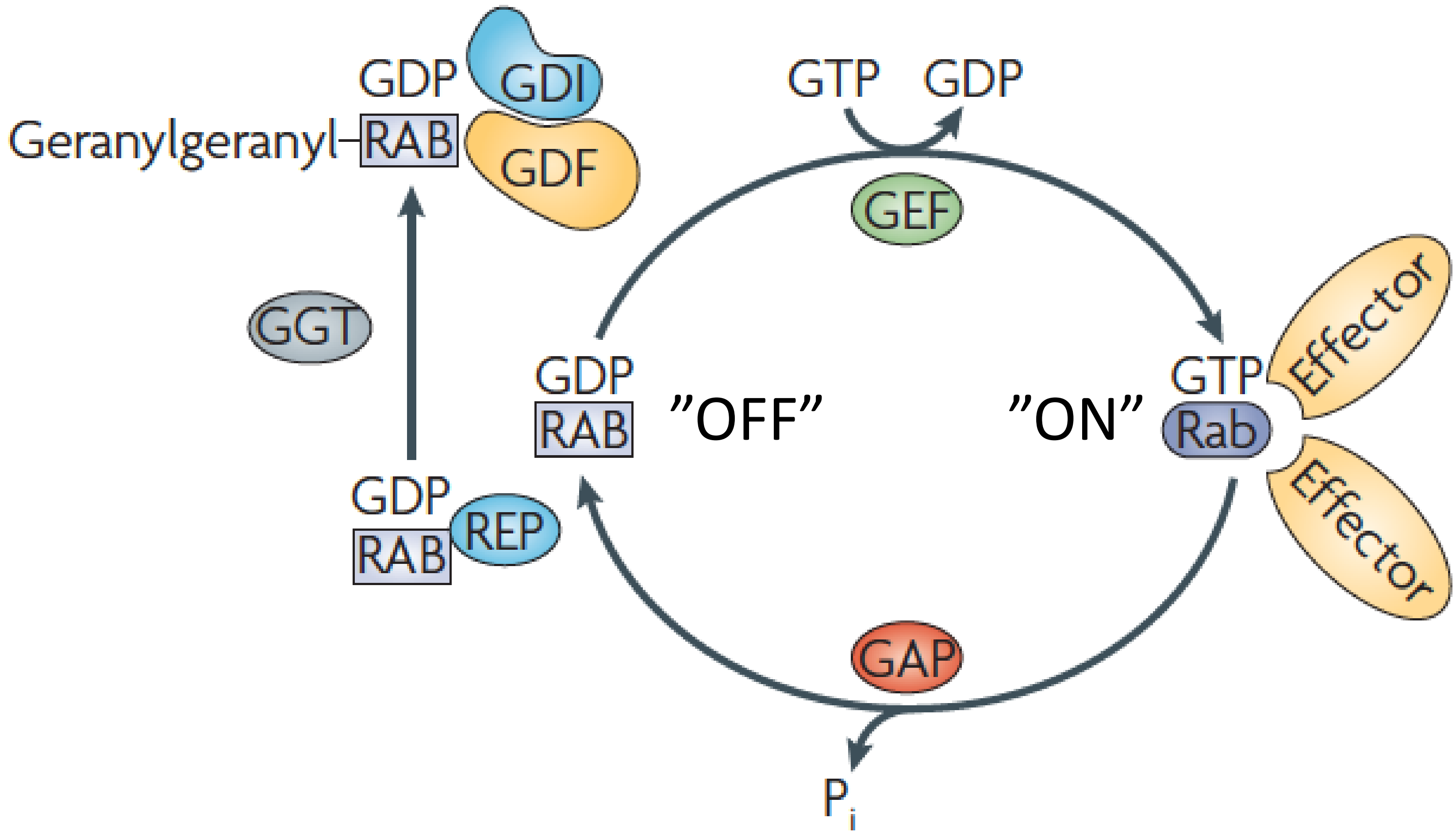


Sharma M, et al. Circ Res 2017;120:1091

Loki archaeum have GTPases (G-proteins)

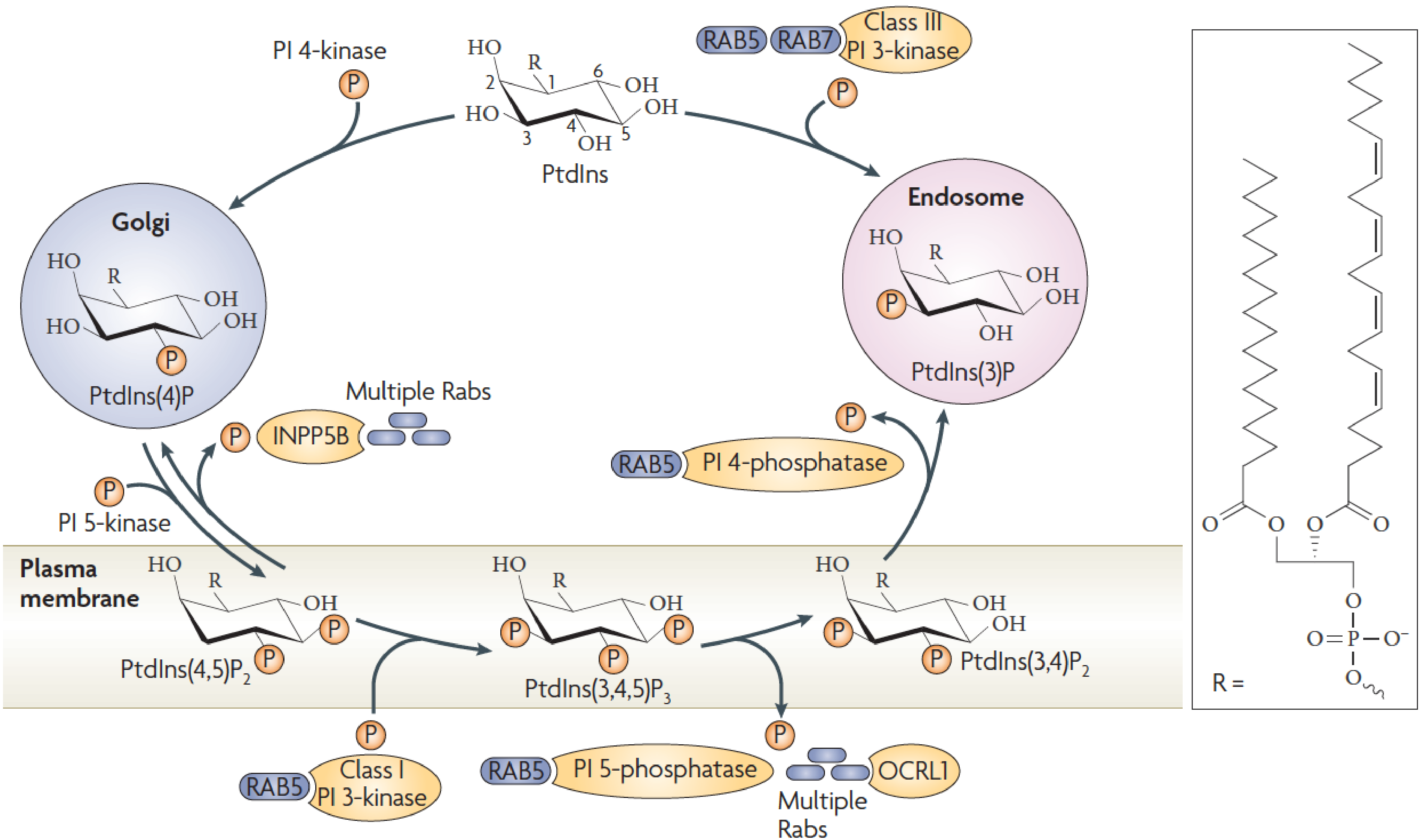


Dey G, et al. Trends in Cell Biology 2016;26:476

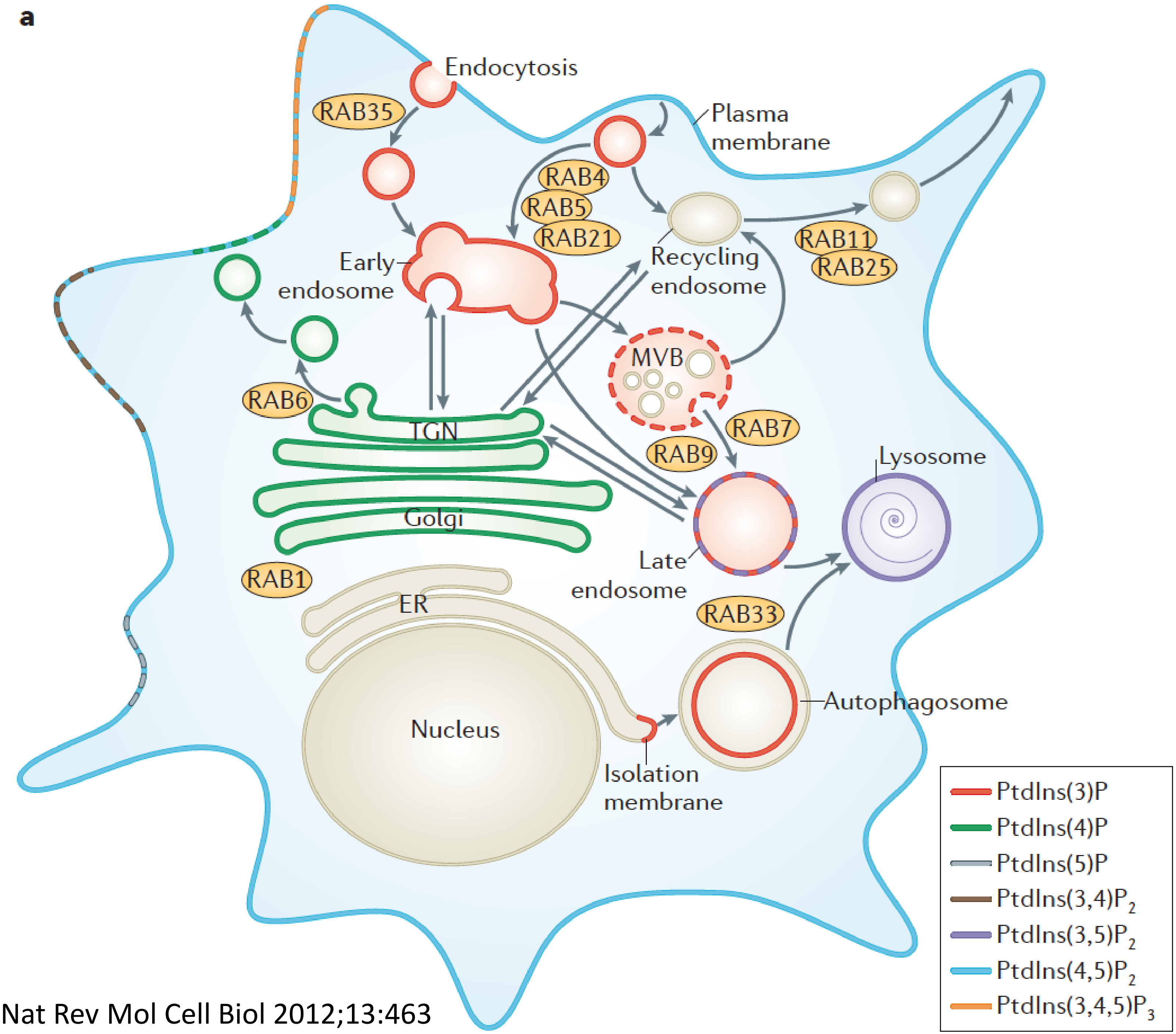


GEF guanine nucleotide exchange factor (activates G proteins)
GAP GTPase activating protein (shuts off G proteins)
GDI GDP dissociation inhibitor (binds and inhibits by displacement from membrane)
GDF GDI displacement factor (allows activation by movement to membrane)
REP Rab escort protein
GGT geranylgeranyl transferase

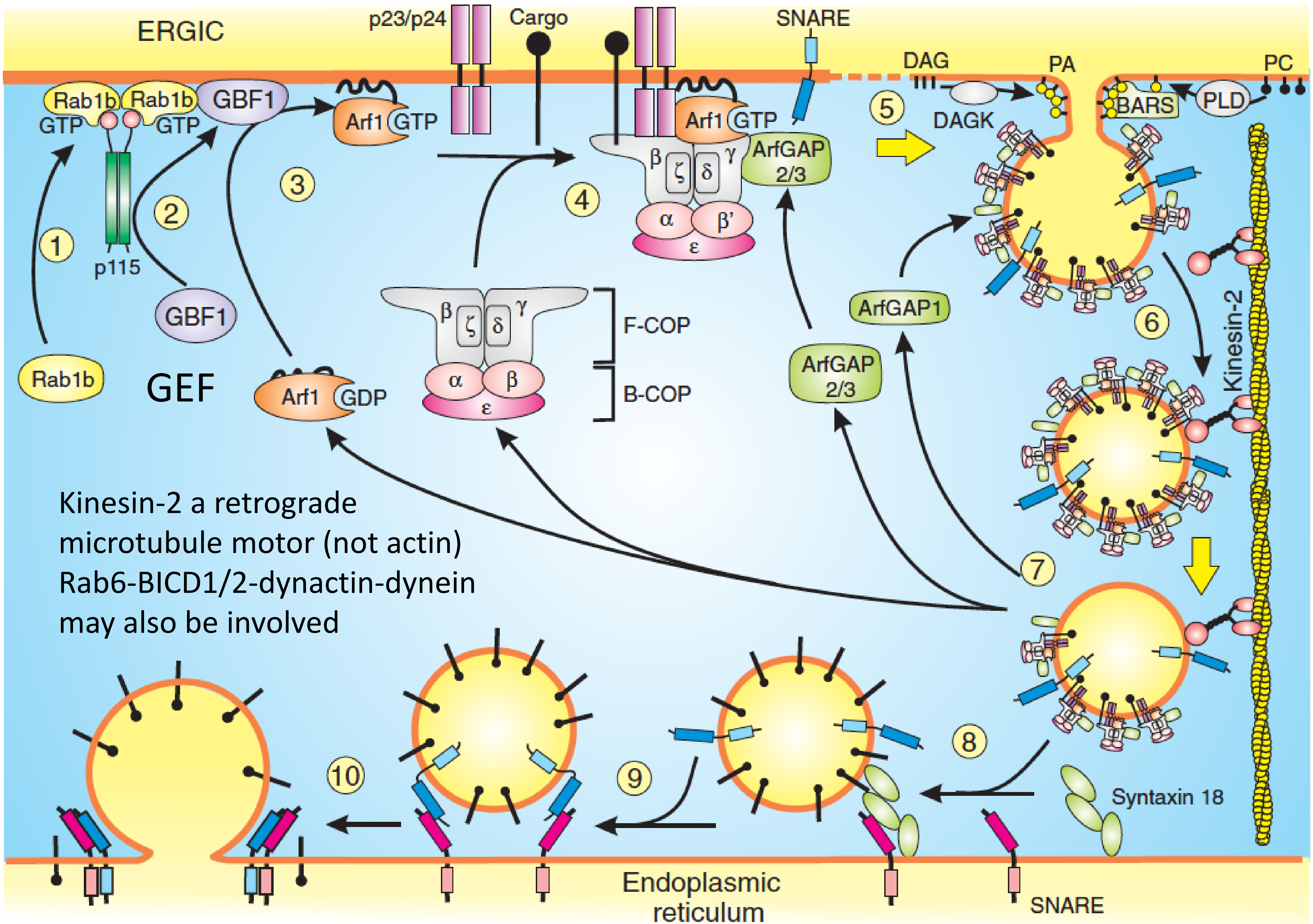
Stenmark H. Nat Rev Mol Cell Biol 2009;10:513



Stenmark H. Nat Rev Mol Cell Biol 2009;10:513



Jean S. Nat Rev Mol Cell Biol 2012;13:463



Kinesin-2 a retrograde microtubule motor (not actin)
Rab6-BICD1/2-dynactin-dynein may also be involved

Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4

Summary of COPI vesicle cycle

- G-protein (Rab1b + golgin p115) activate a GEF (GBF1) which activates another G-protein (Arf1)
- Arf1-GTP mediates assembly of coat proteins and cargo proteins
- Arf1-GTP and coat proteins deform membrane to form vesicle. SNARES, ArfGAPs (1/2/3) added.
- Scission of vesicle (phosphatidic acid formation)
- ArfGAPs → release of outer coat proteins
- kinesin-2 a retrograde microtubule motor as is dynein (which is recruited through Rab6)
- SNARE proteins mediate fusion of COPI vesicle with ER

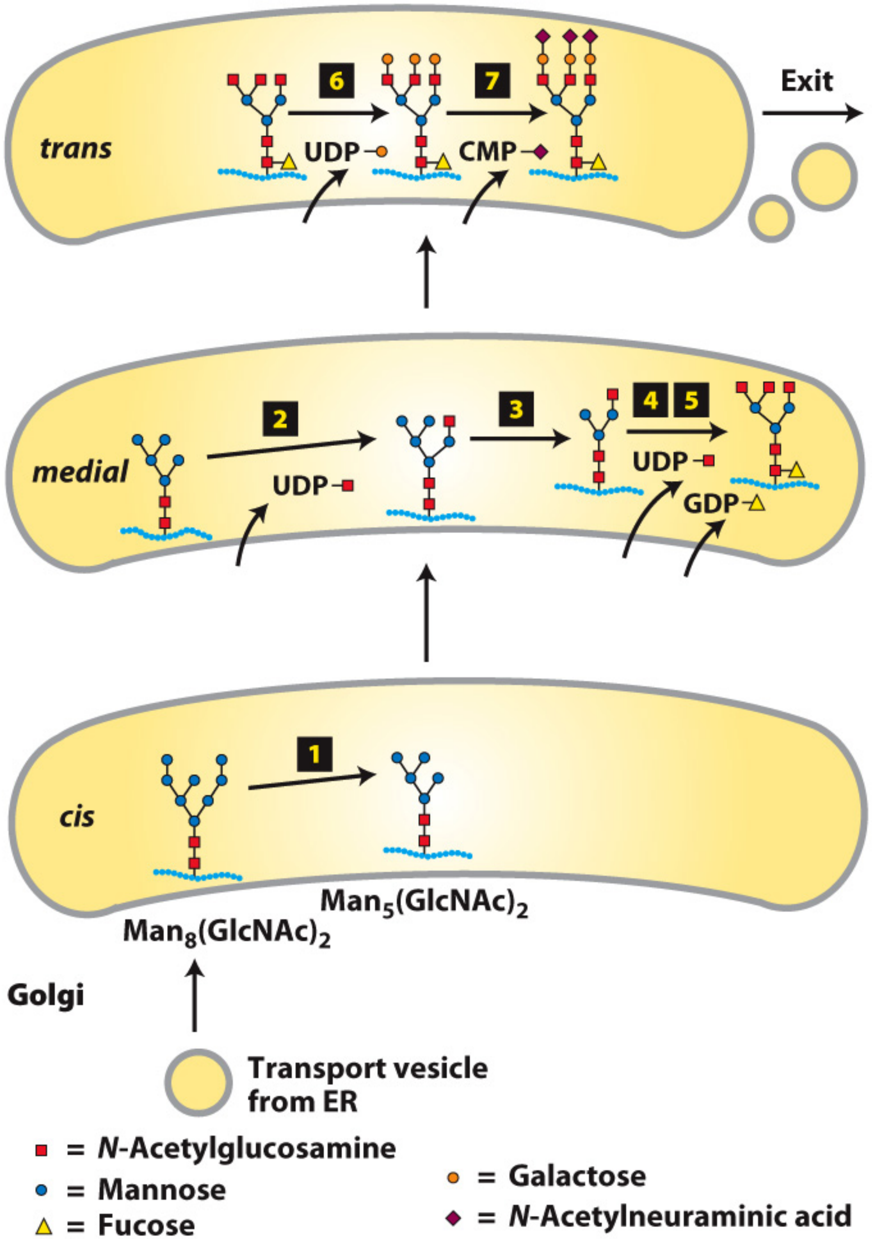
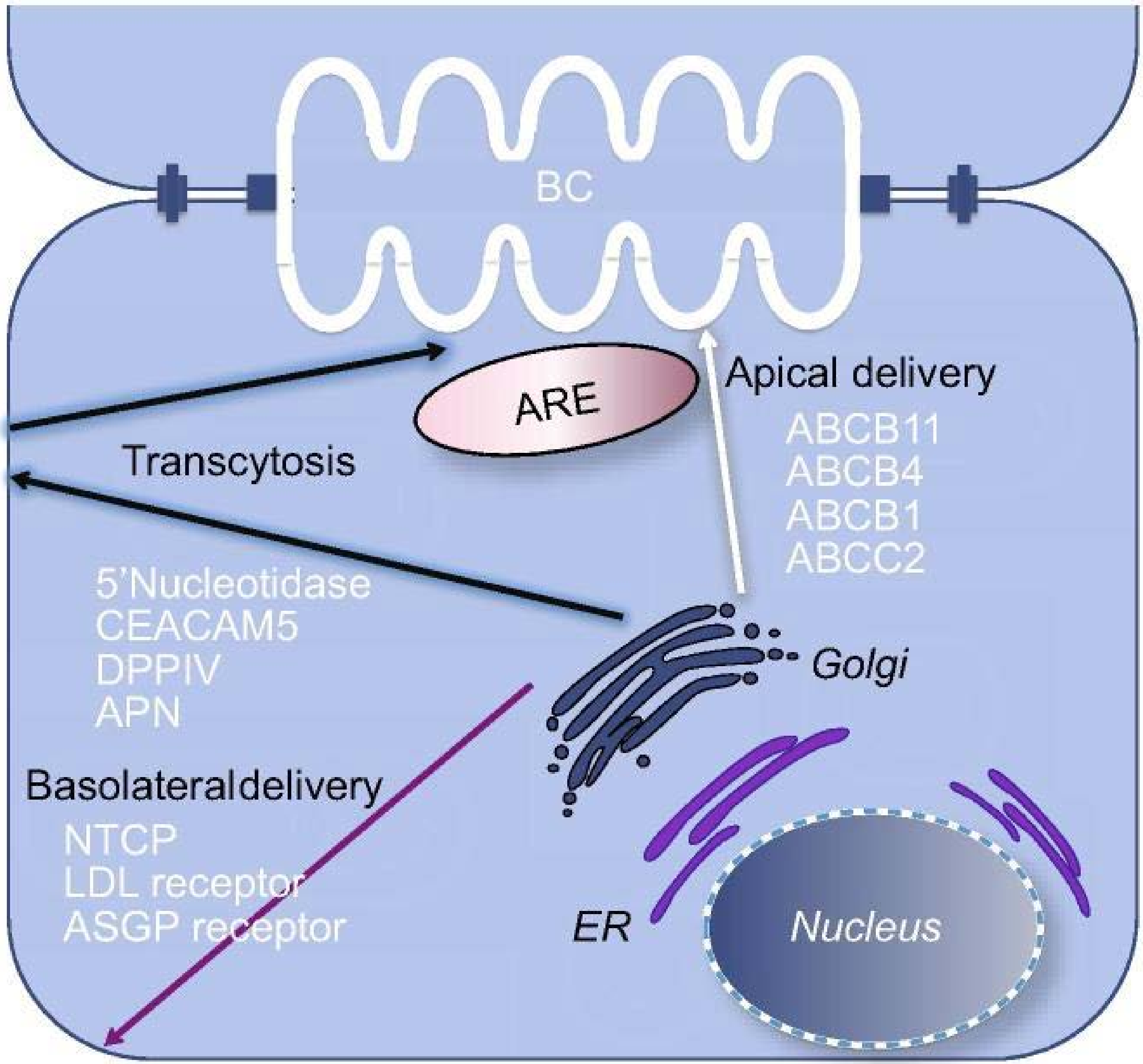


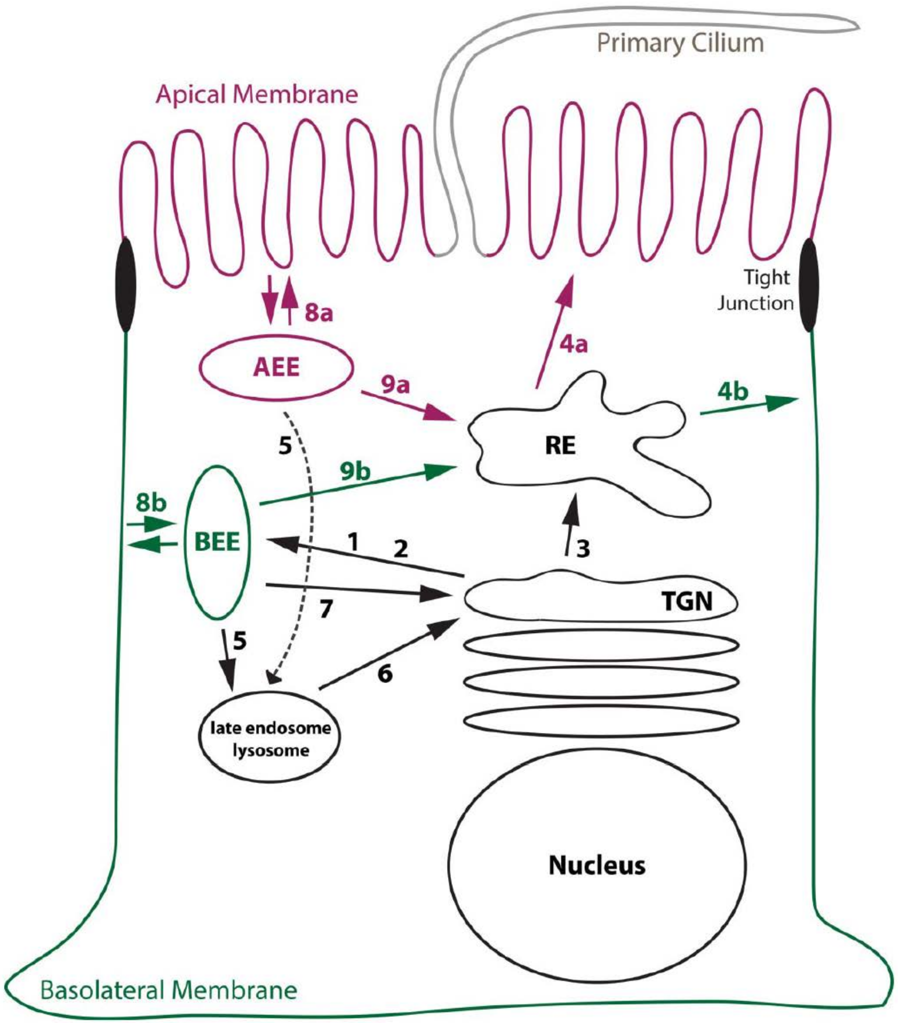
Figure 14-14
Molecular Cell Biology, Eighth Edition

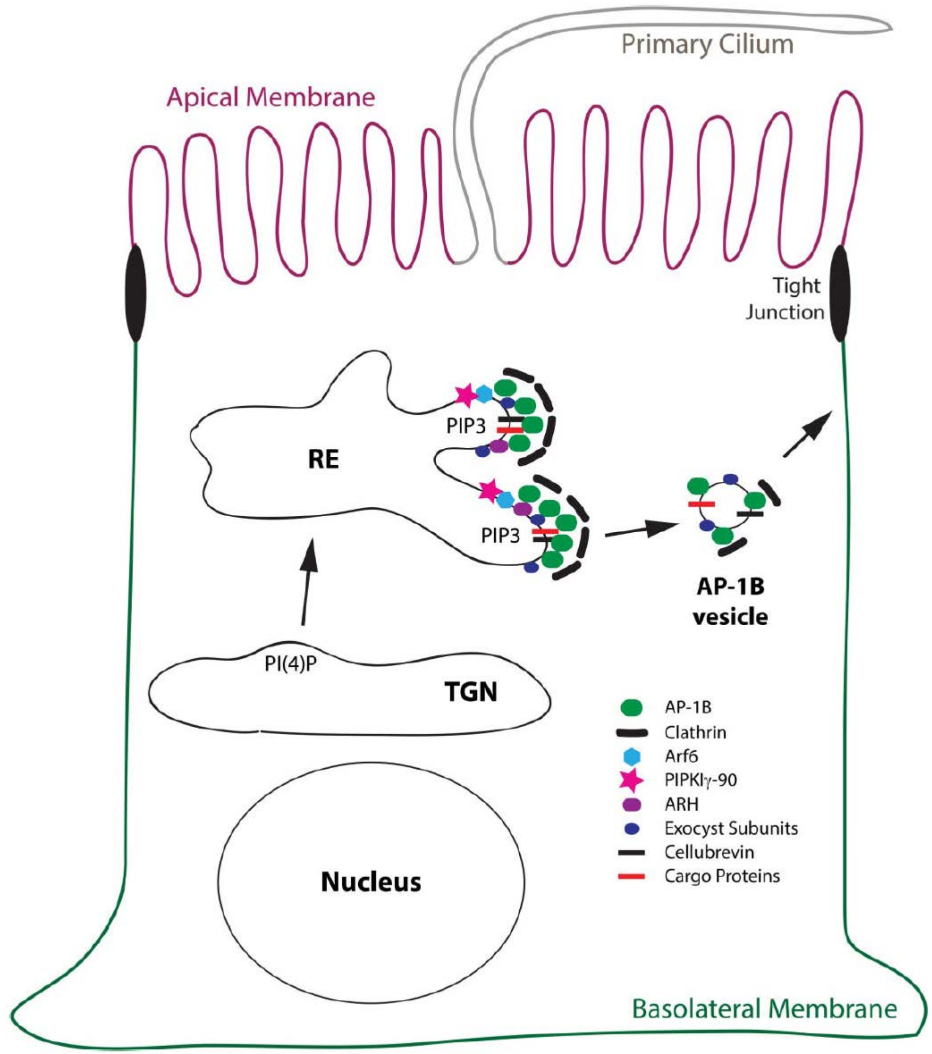
Addition of a mannose-6-P directs trafficking of lysosomal enzymes by specific mannose-6-P receptors.



Hepatocytes are polarized *epithelial* cells! Gissen P, Arias IM. J Hepatol 2015;63:1023

Clathrin, AP-1B, PIP3, Arf6, other factors help maintain cell polarity





AEE – apical early endosomes; BEE – basolateral early endosomes
RE – recycling endosome; TGN – trans-Golgi network

Folsch H. Cellular Logistics 2015;5:e1074331

Class 4 Mutants – Disrupt ARH AP-1B and basolateral sorting or AP-2 endocytosis

FH Bari: Tyr828Cys (18) | 27 | (34) FH Turku: Gly844Asp

LDLR– KNWRLKNINSINFDNPVYQKTTEDEVHICHNQDGYSYPSRQMVSLEDDVA-*COO-*

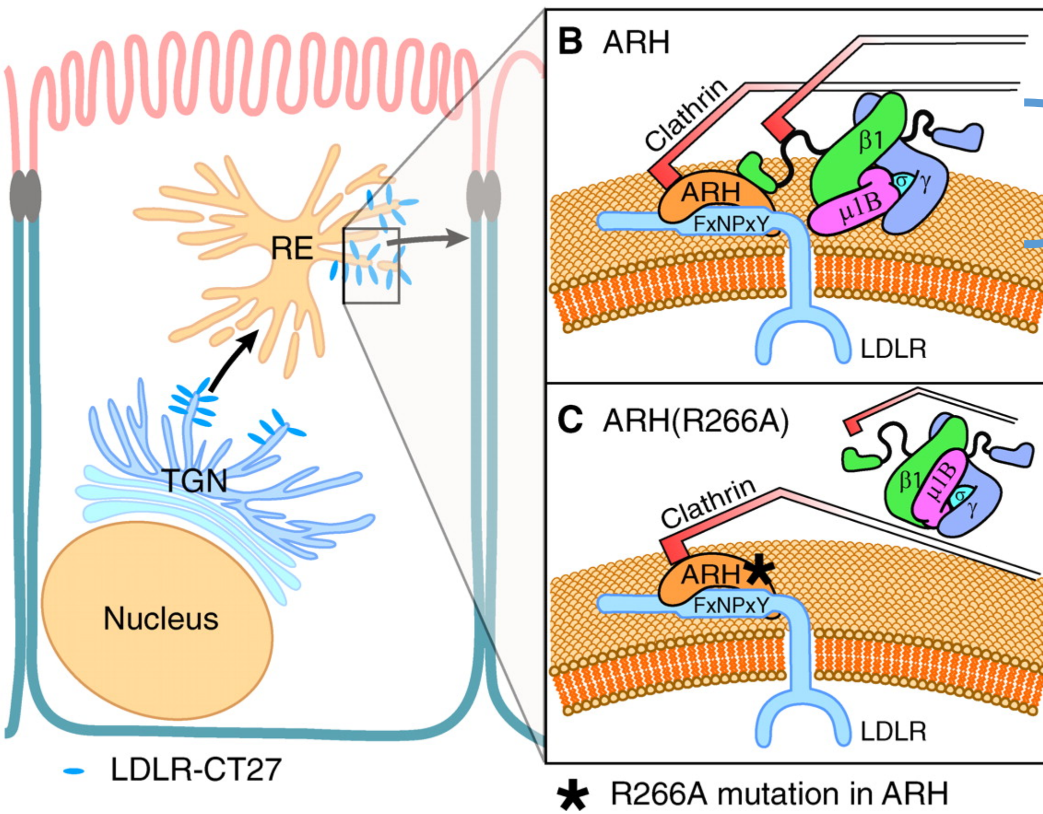
proximal determinant AP-1B-dependent | **distal determinant NOT AP-1B-dependent**

CCP signal   CCP = clathrin-coated pit – only basolateral

Sequence NPVY(A sn-Pro-Val-Tyr) totally conserved in LDLR from six species.

RE = recycling endosomes. RE subdomains maintain apical vs basolateral sorting.

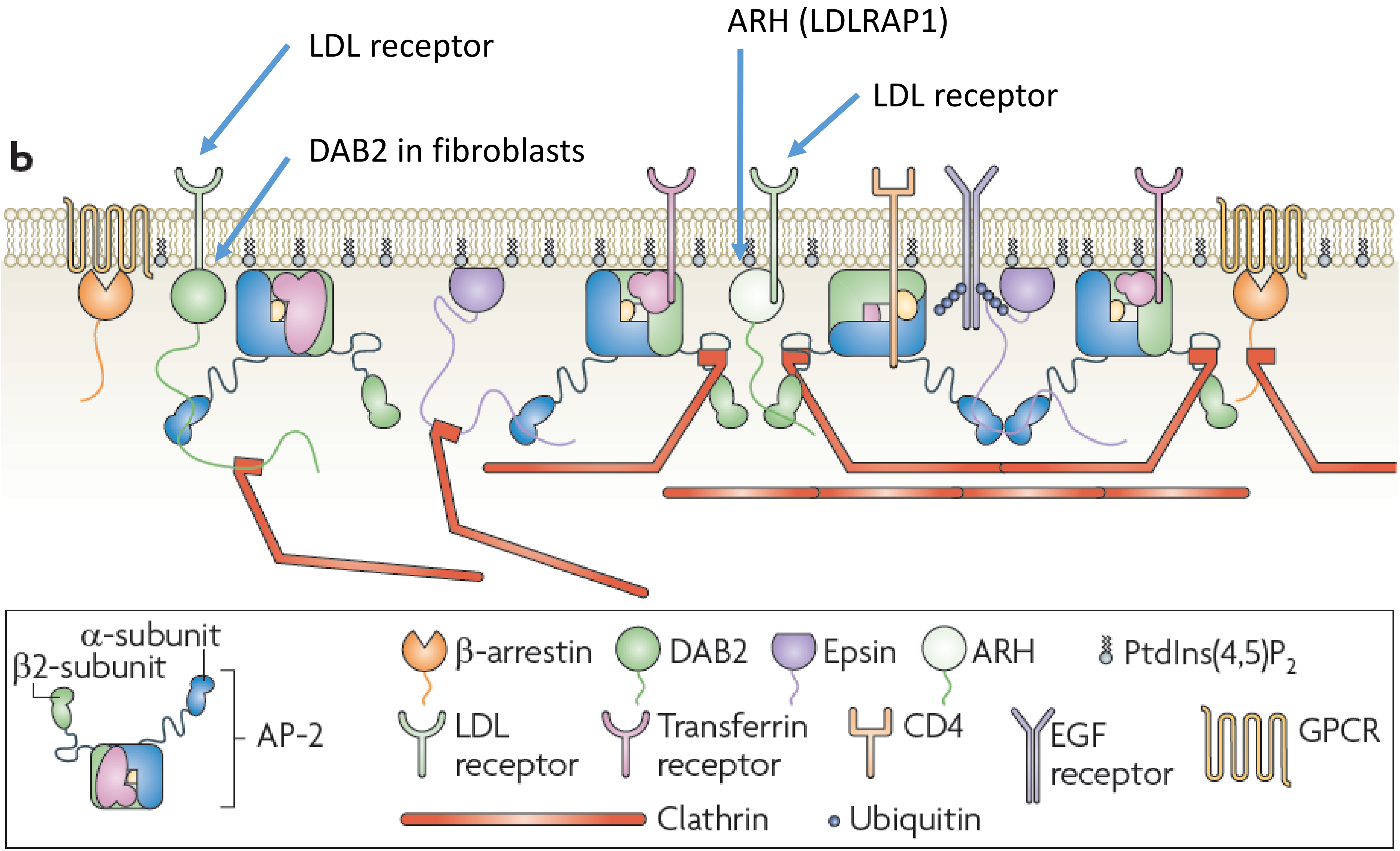
Clathrin is required to maintain cell polarity. (Deborde, et al. Nature 2008)



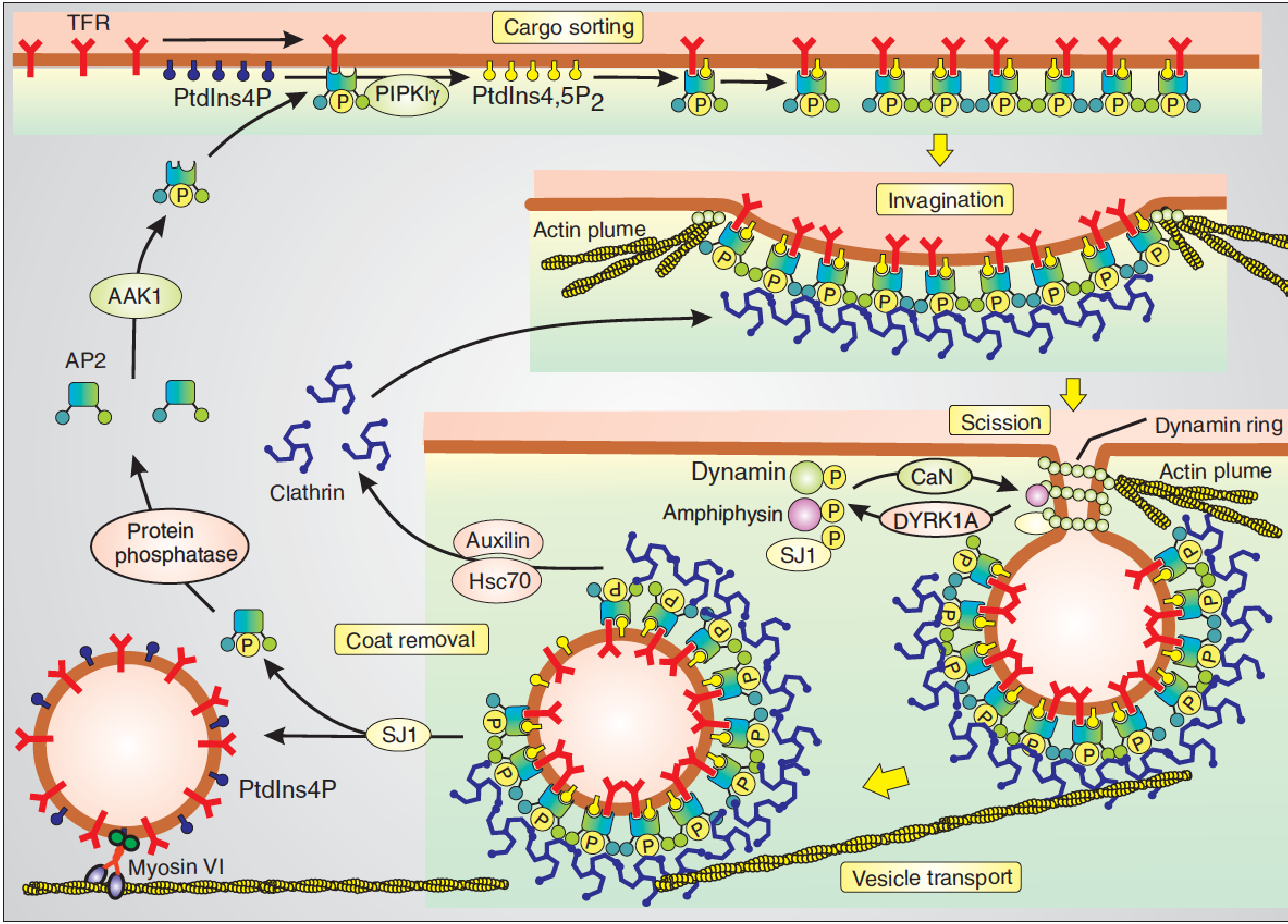
Heterotetrameric AP-1B complex.

AP-1B sorts proteins to basolateral membrane with further regulation by Arf6 (a G-protein) and specific phospho-inositides.

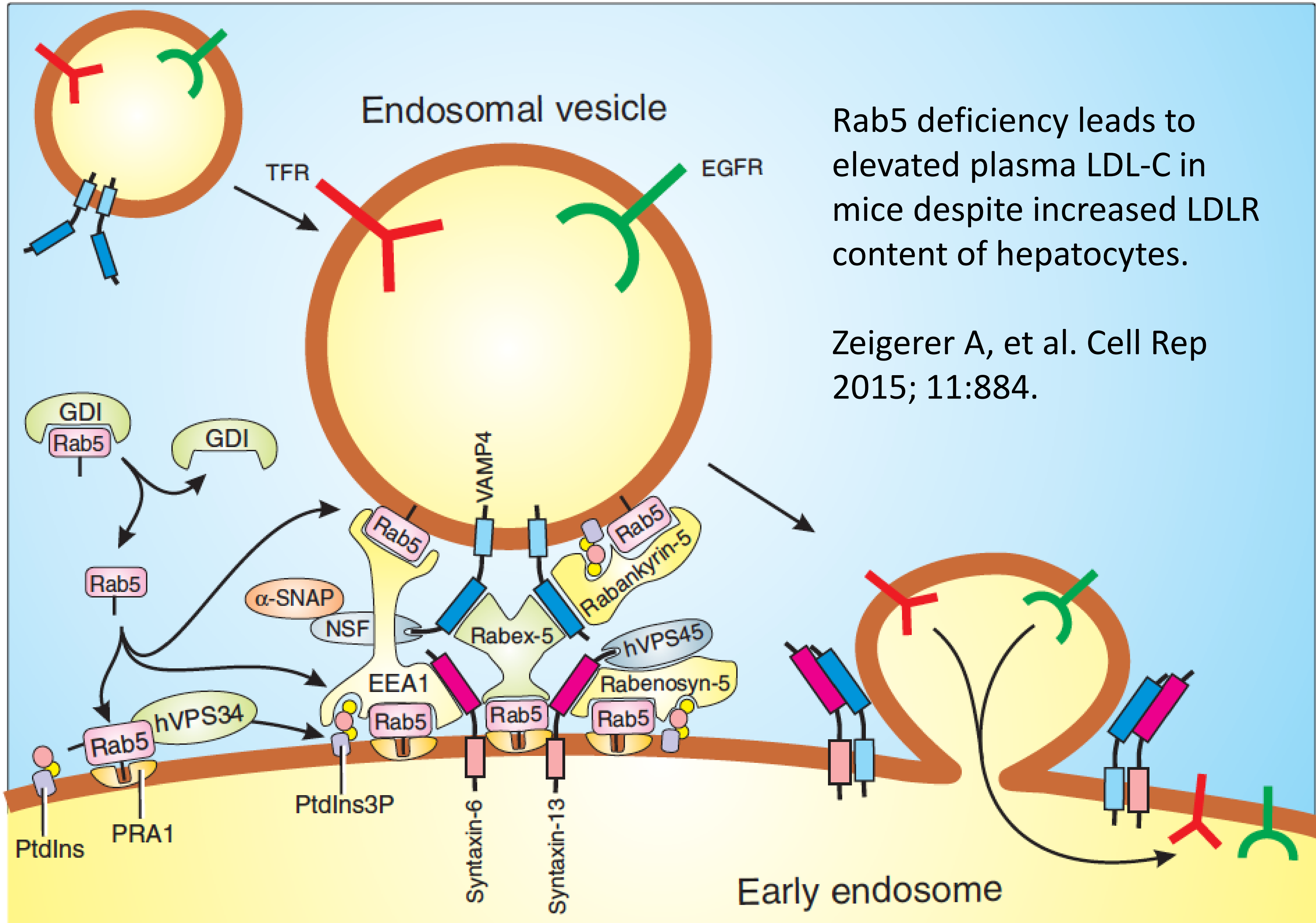
Kang RS, Fölsch H. J Cell Biol 2011;193:51



Traub, LM. Nat Rev Mol Cell Biol 2009;10:583



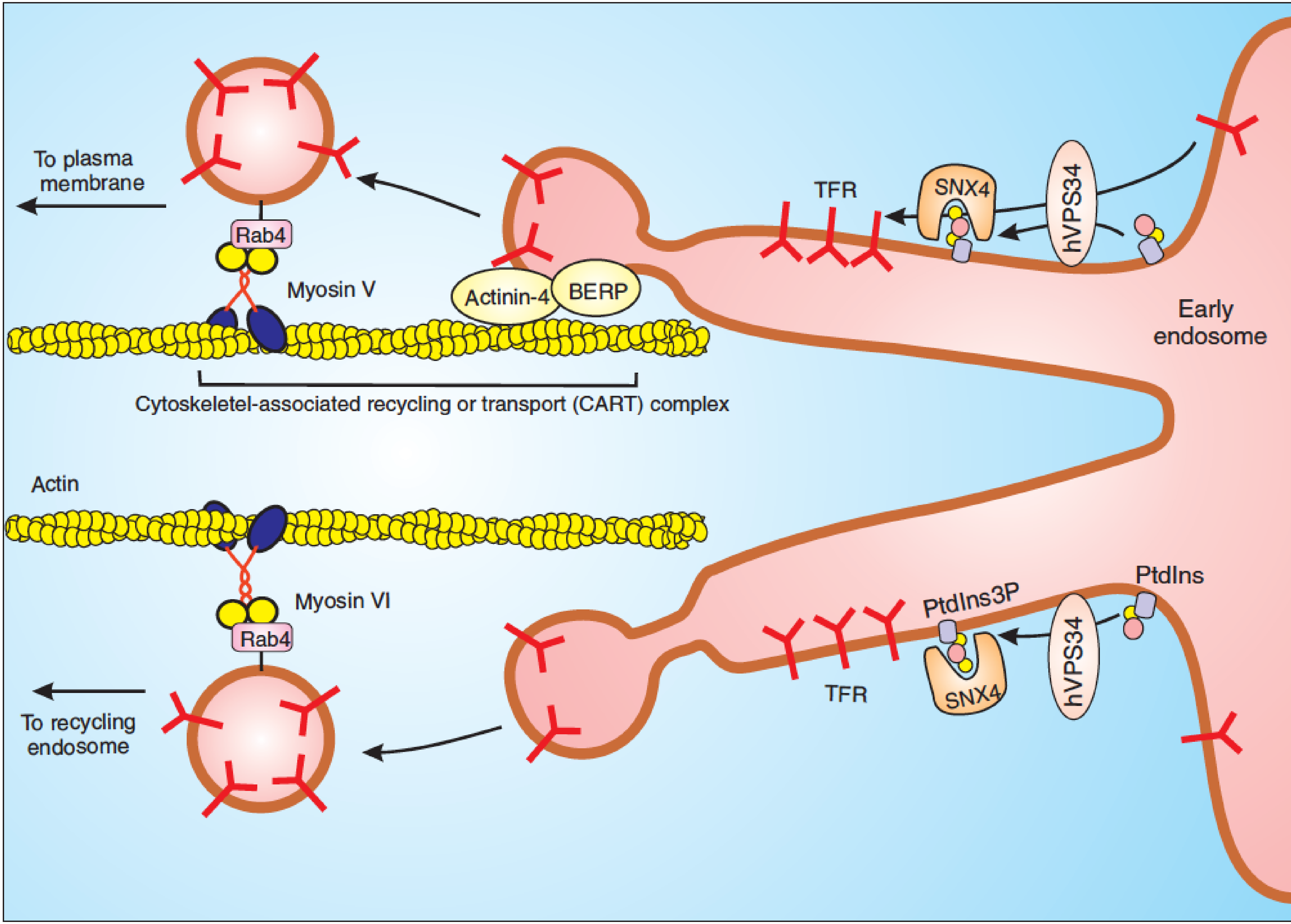
Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4



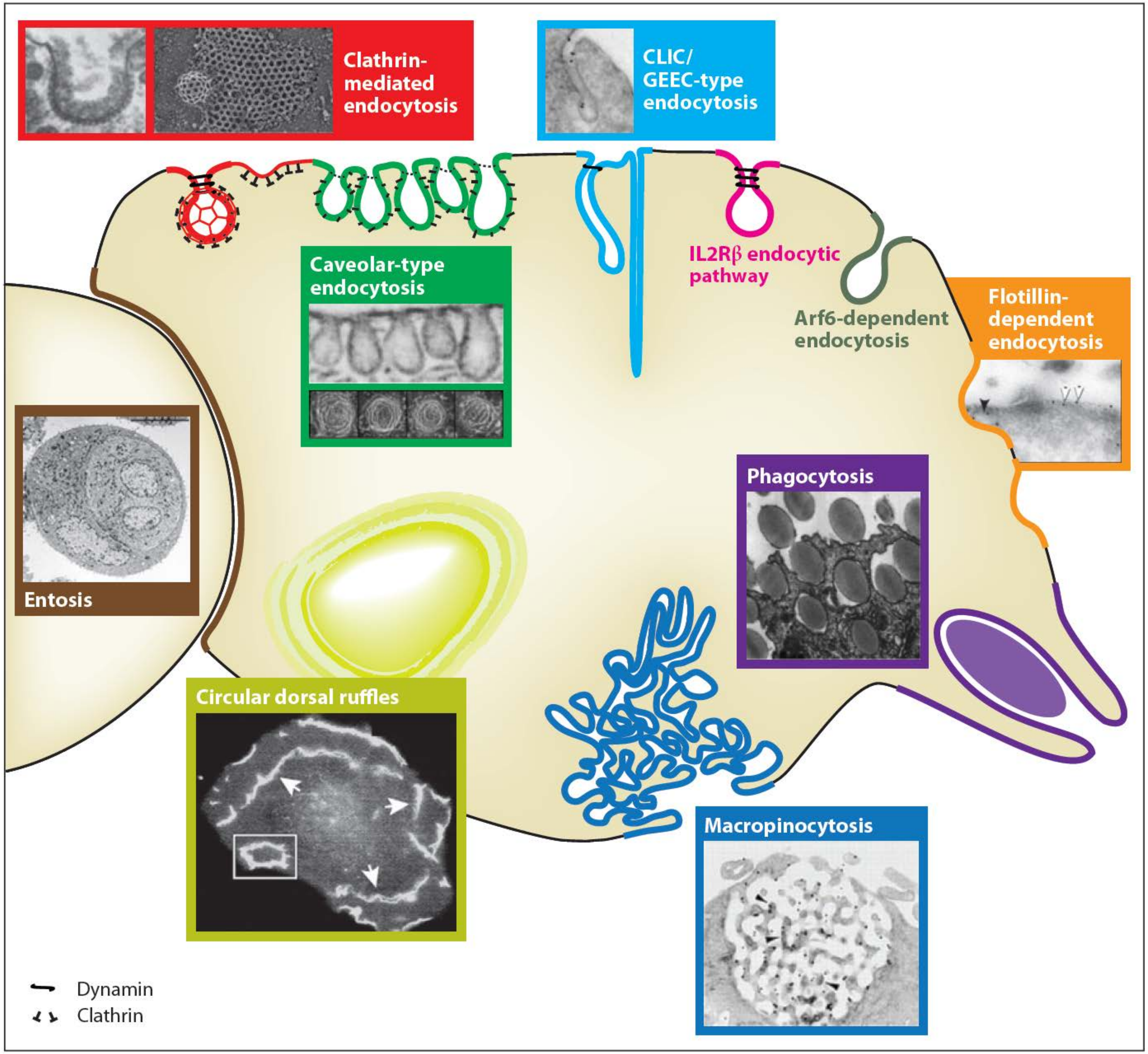
Rab5 deficiency leads to elevated plasma LDL-C in mice despite increased LDLR content of hepatocytes.

Zeigerer A, et al. Cell Rep 2015; 11:884.

Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4



Berridge MJ www.cellsignalsignalingbiology.org 2014, Module 4



We will concern ourselves with just clathrin-mediated endocytosis!

Doherty GJ, McMahon HT. Annu Rev Biochem 2009; 78:31.1

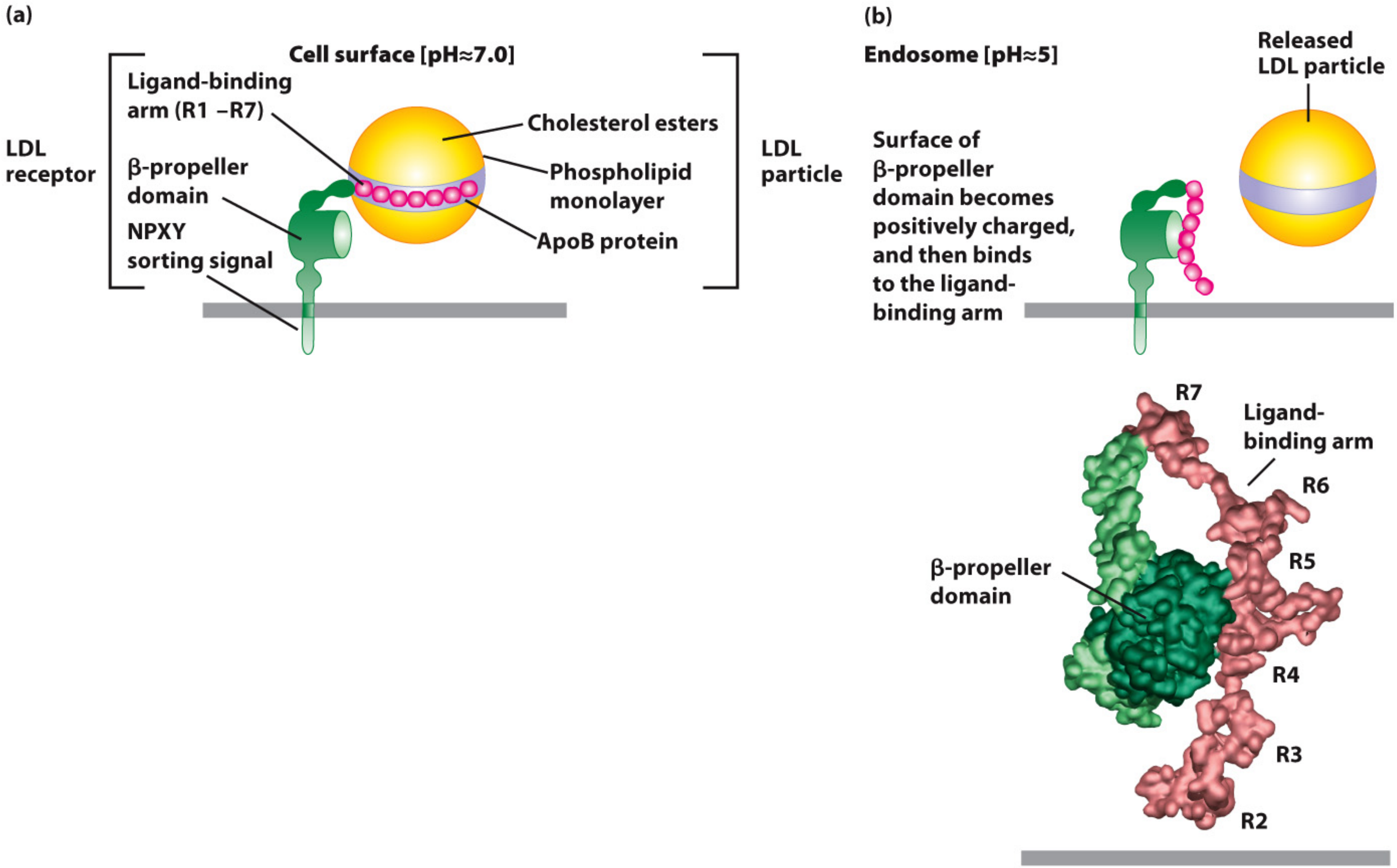


Figure 14-30
Molecular Cell Biology, Eighth Edition

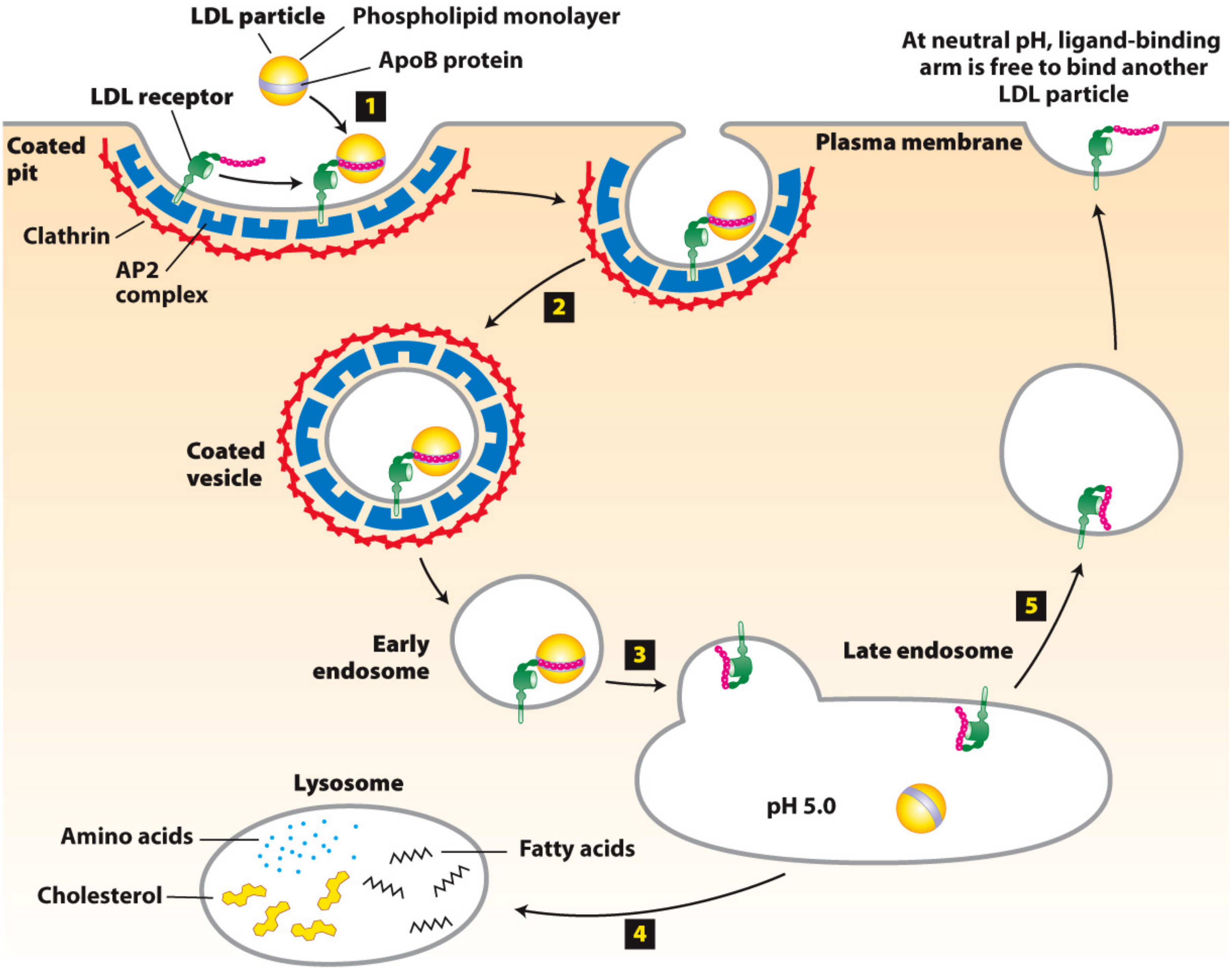
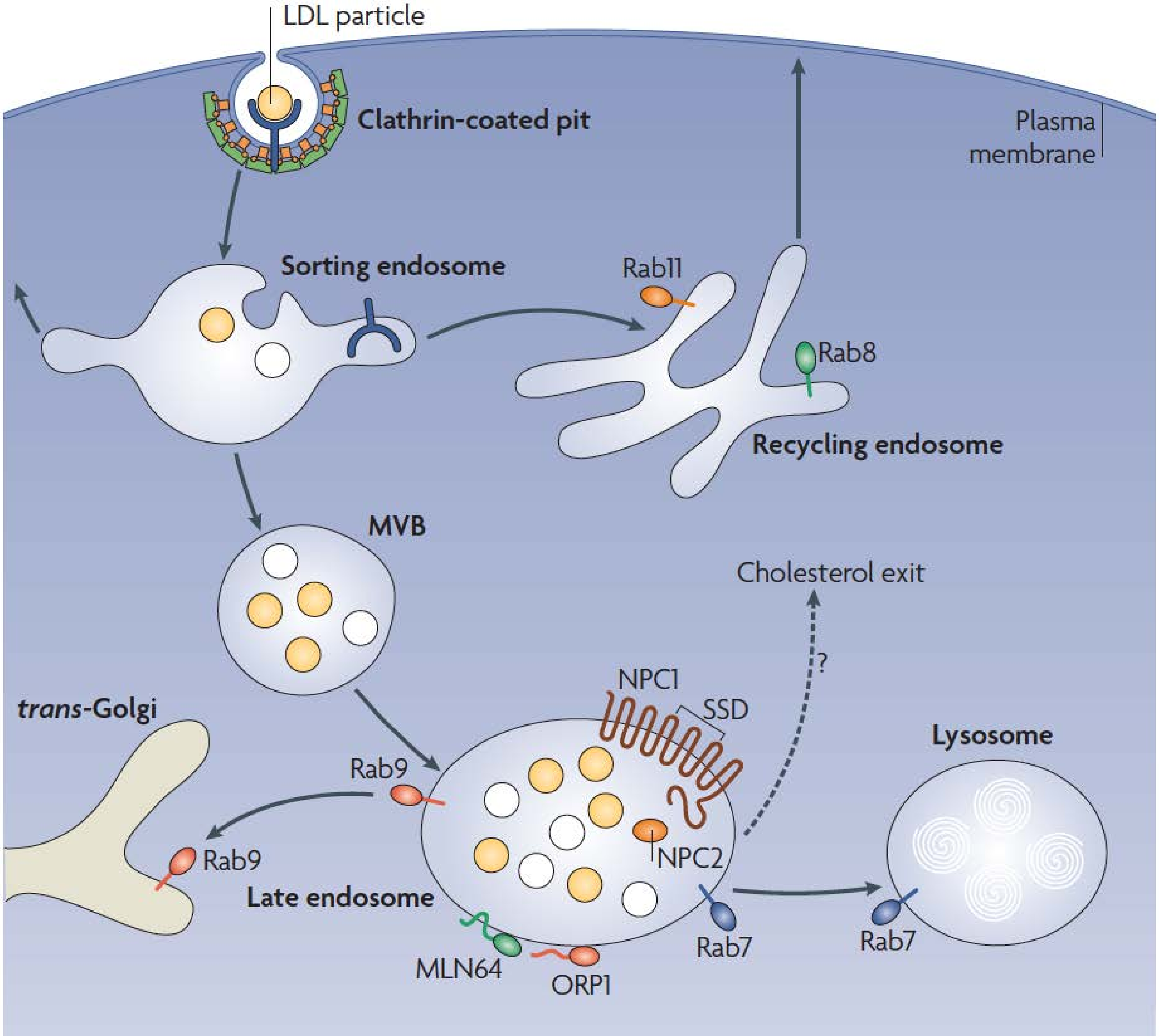


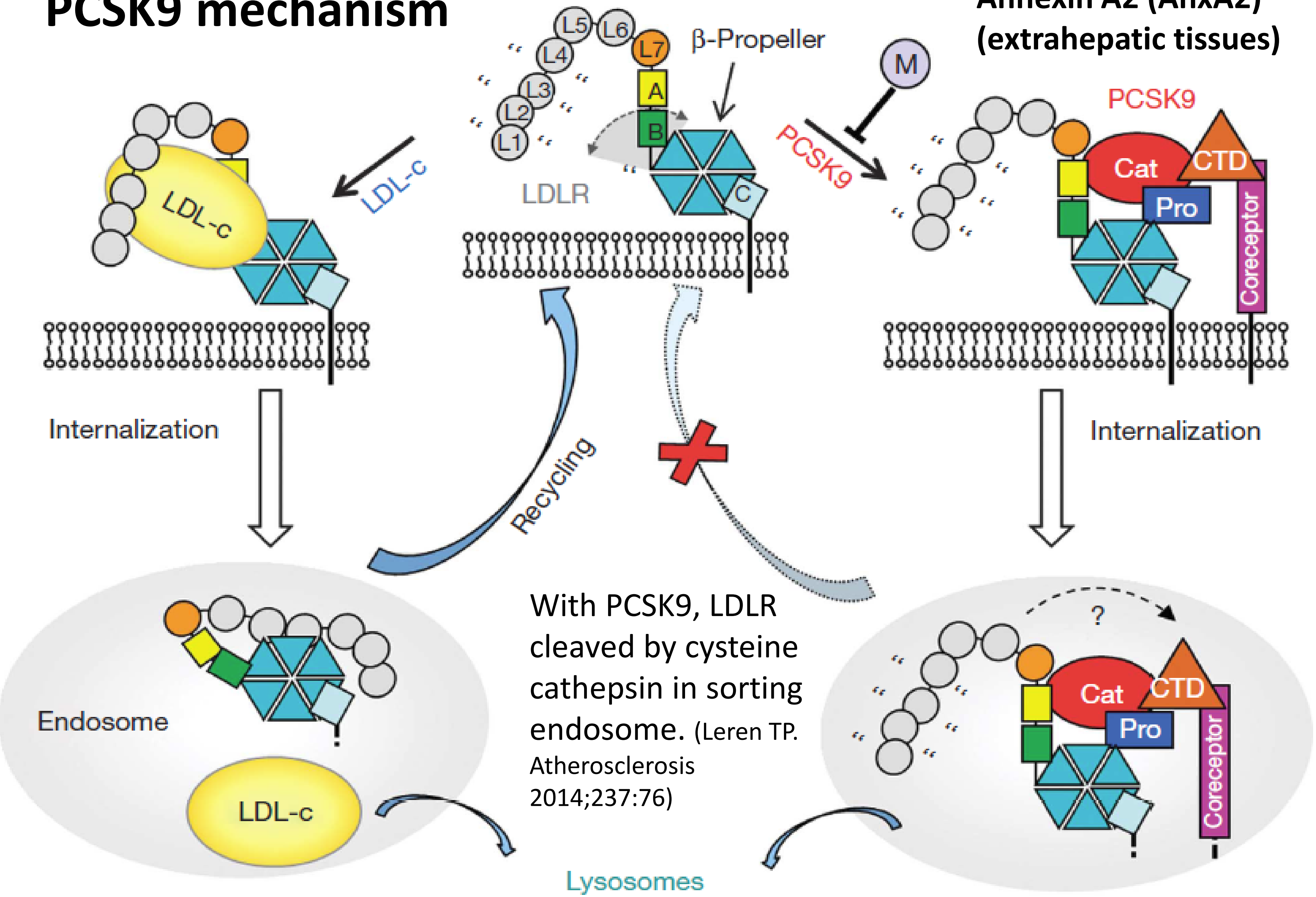
Figure 14-29
Molecular Cell Biology, Eighth Edition



Ikonen, E. Nature Rev Mol Cell Biol 2008

PCSK9 mechanism

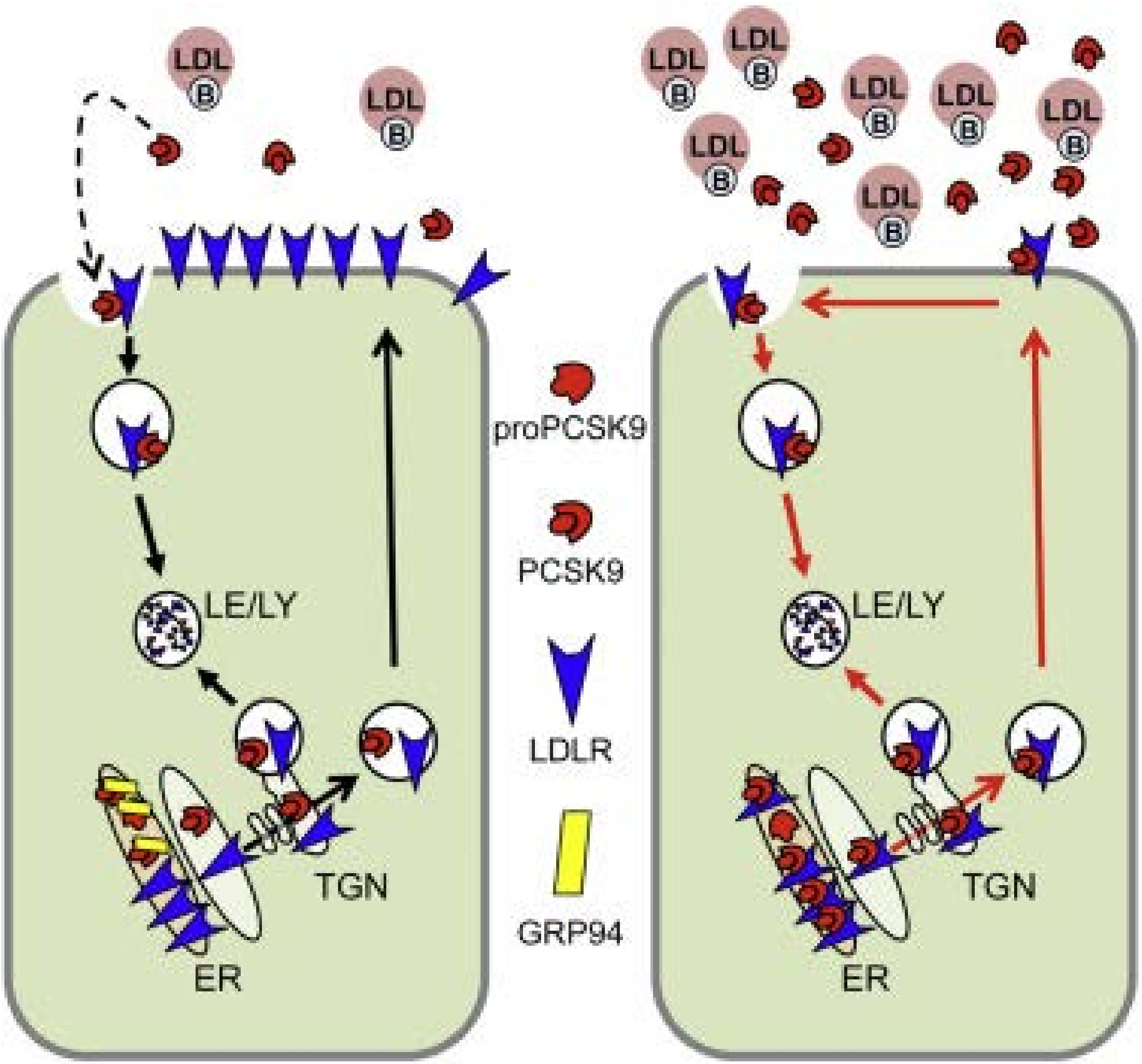
Annexin A2 (AnxA2) (extrahepatic tissues)



With PCSK9, LDLR cleaved by cysteine cathepsin in sorting endosome. (Leren TP. Atherosclerosis 2014;237:76)

Seidah NG, et al. PLoS ONE 2012; 7:e41865

Surdo PL, et al. EMBO Rep 2011; 12:1300



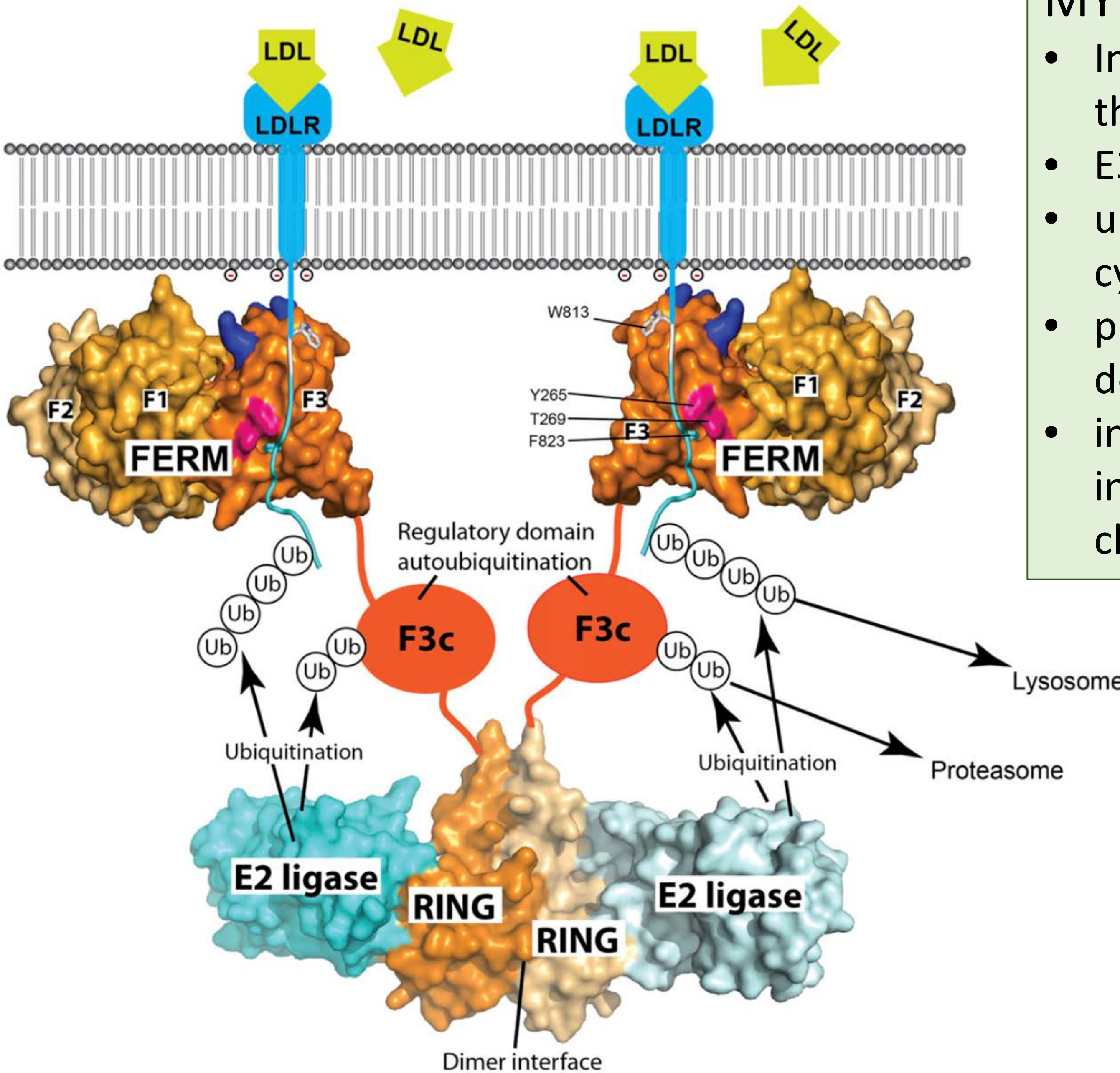
+ GRP94
(ER chaperone)

- GRP94

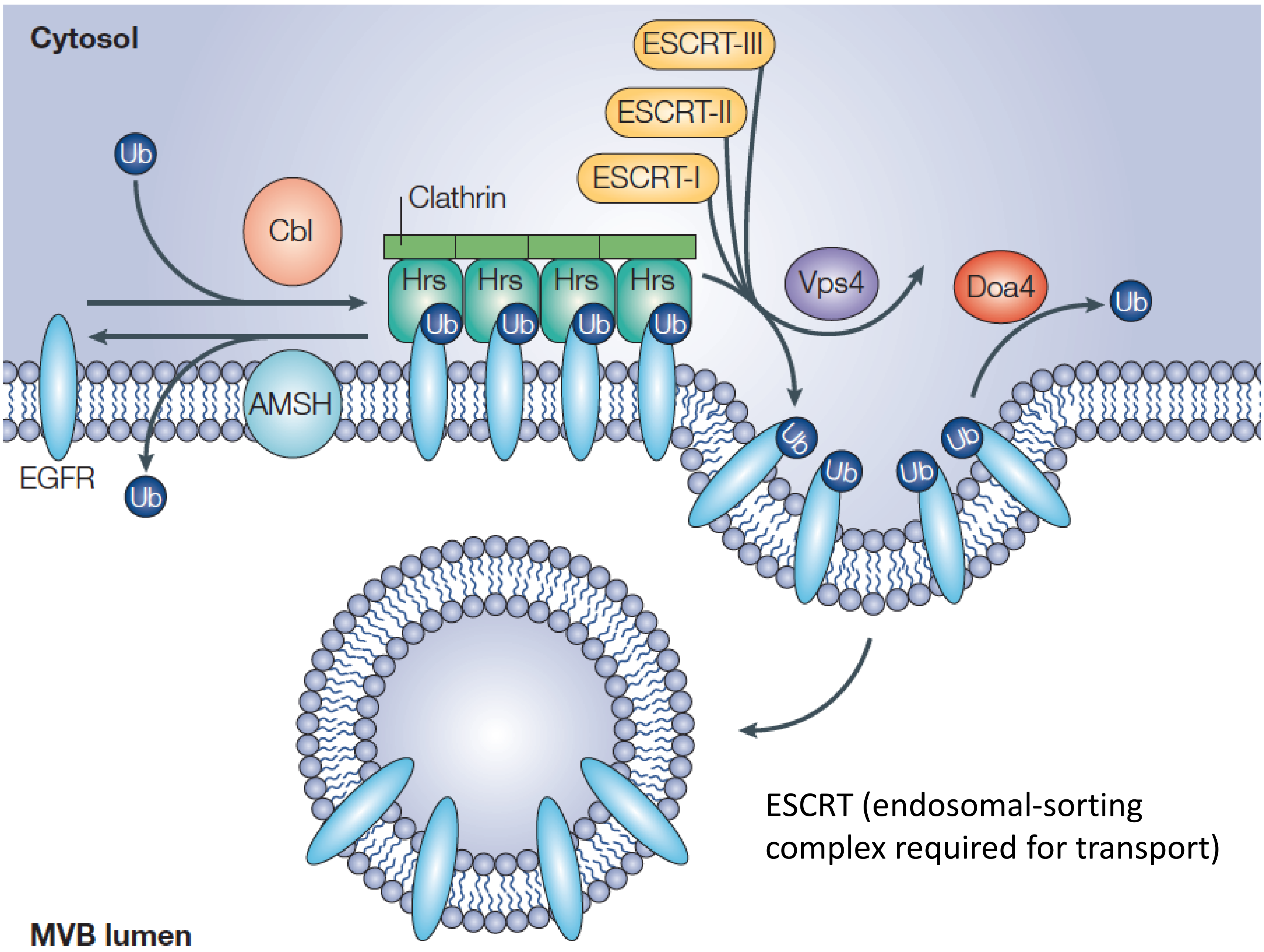
Poirier S, et al. Cell Reports 2015;13:2064

MYLIP/**IDOL**

- Inducible degrader of the LDLR
- E3 ubiquitin ligase
- ubiquitinates LDLR on cytoplasmic domain
- promote lysosomal degradation
- induced by with increased cellular cholesterol levels



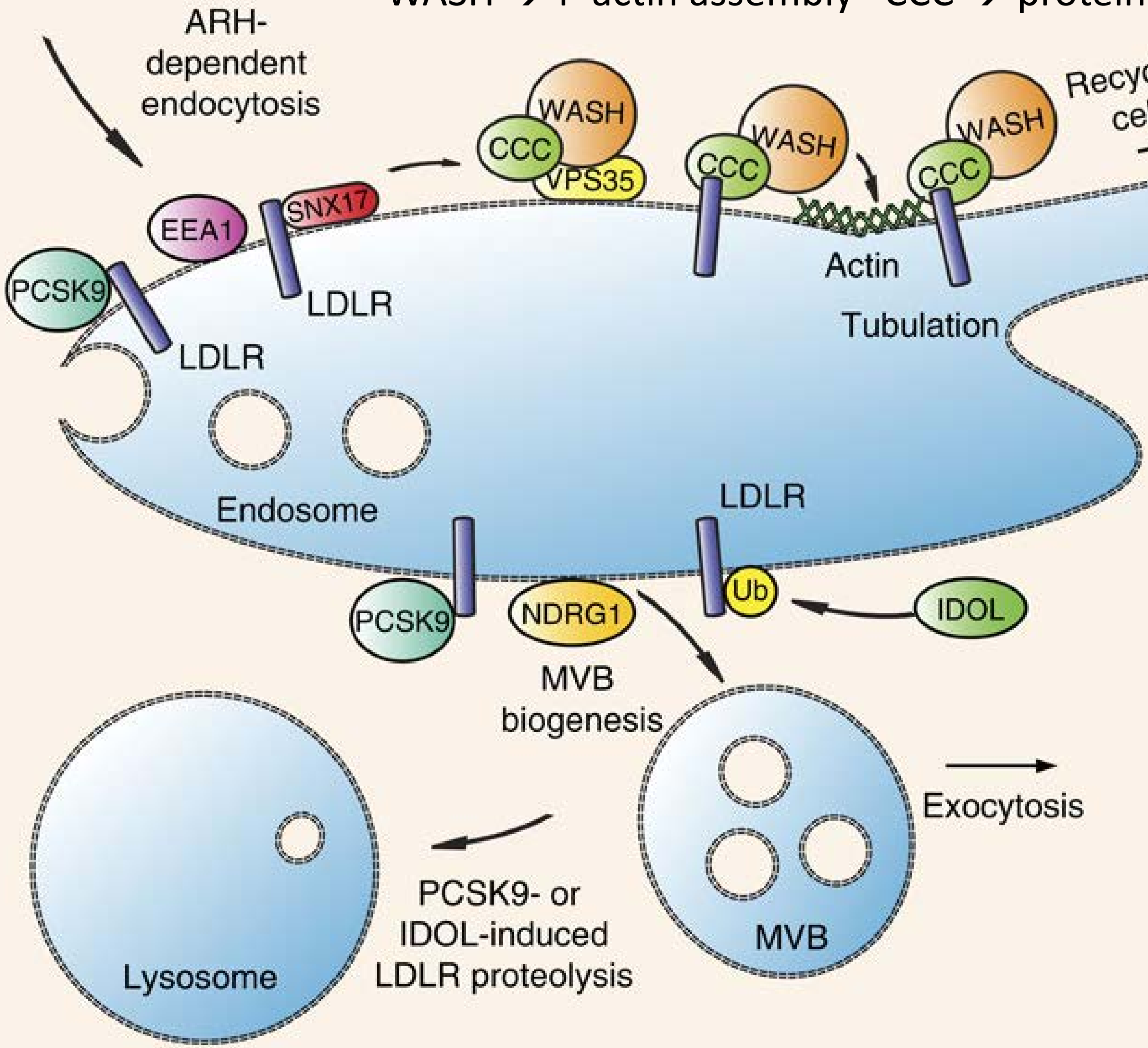
Calkin AC, et al. PNAS 2011; 108; 20107



ESCRT (endosomal-sorting complex required for transport)

Welchman RL, et al. Nat Rev Mol Cell Biol 2005;6:599

WASH → F-actin assembly   CCC → protein sorting specificity



CCC-WASH complexes involved in sorting of many proteins.

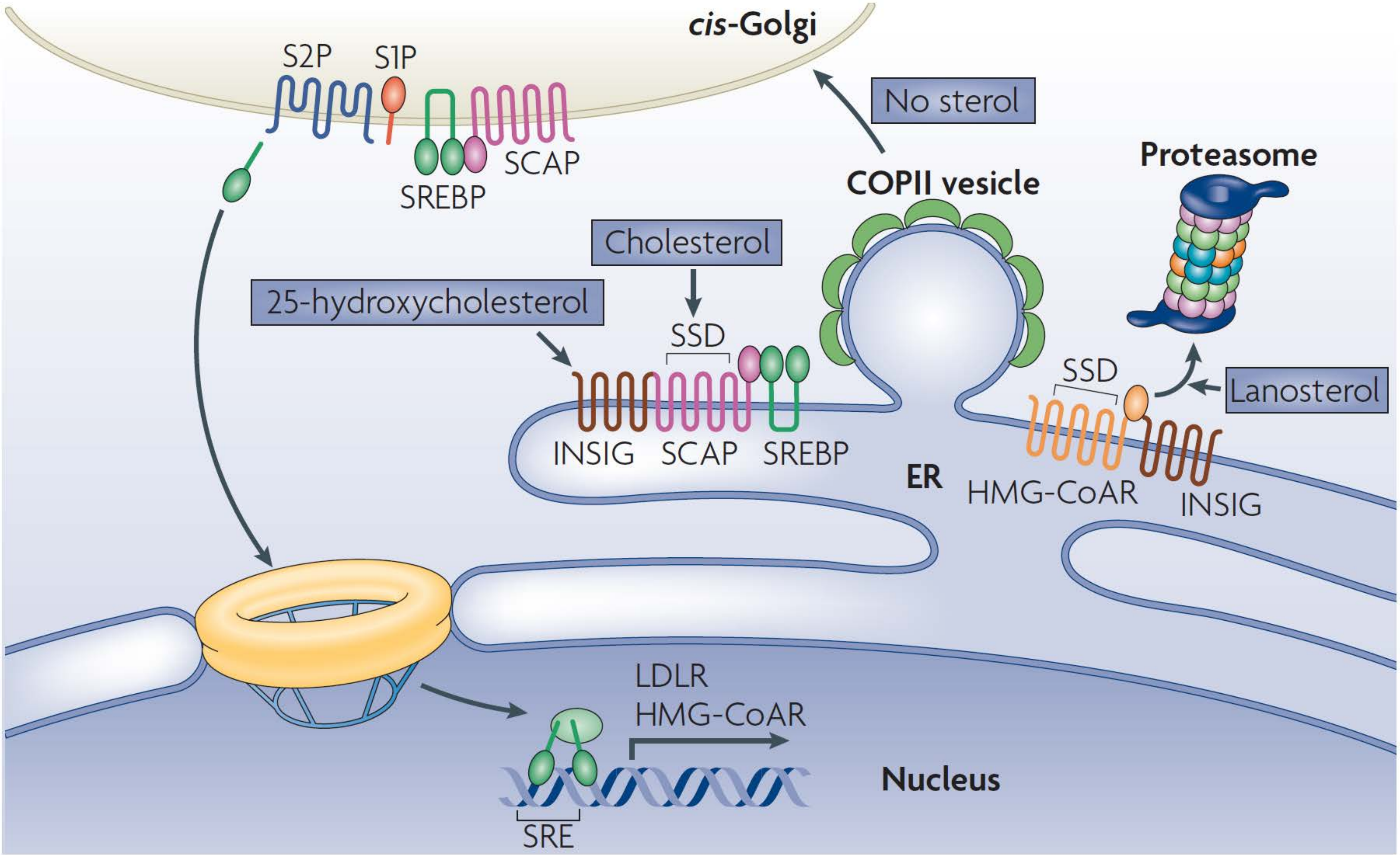
Defects in components of the CCC or WASH complexes lead to autosomal recessive intellectual disability with spastic paraplegia and elevated LDL-C (with impaired LDLR recycling).

Bartuzi P, et al. Nat Comm 2016;7:10961

Time permitting

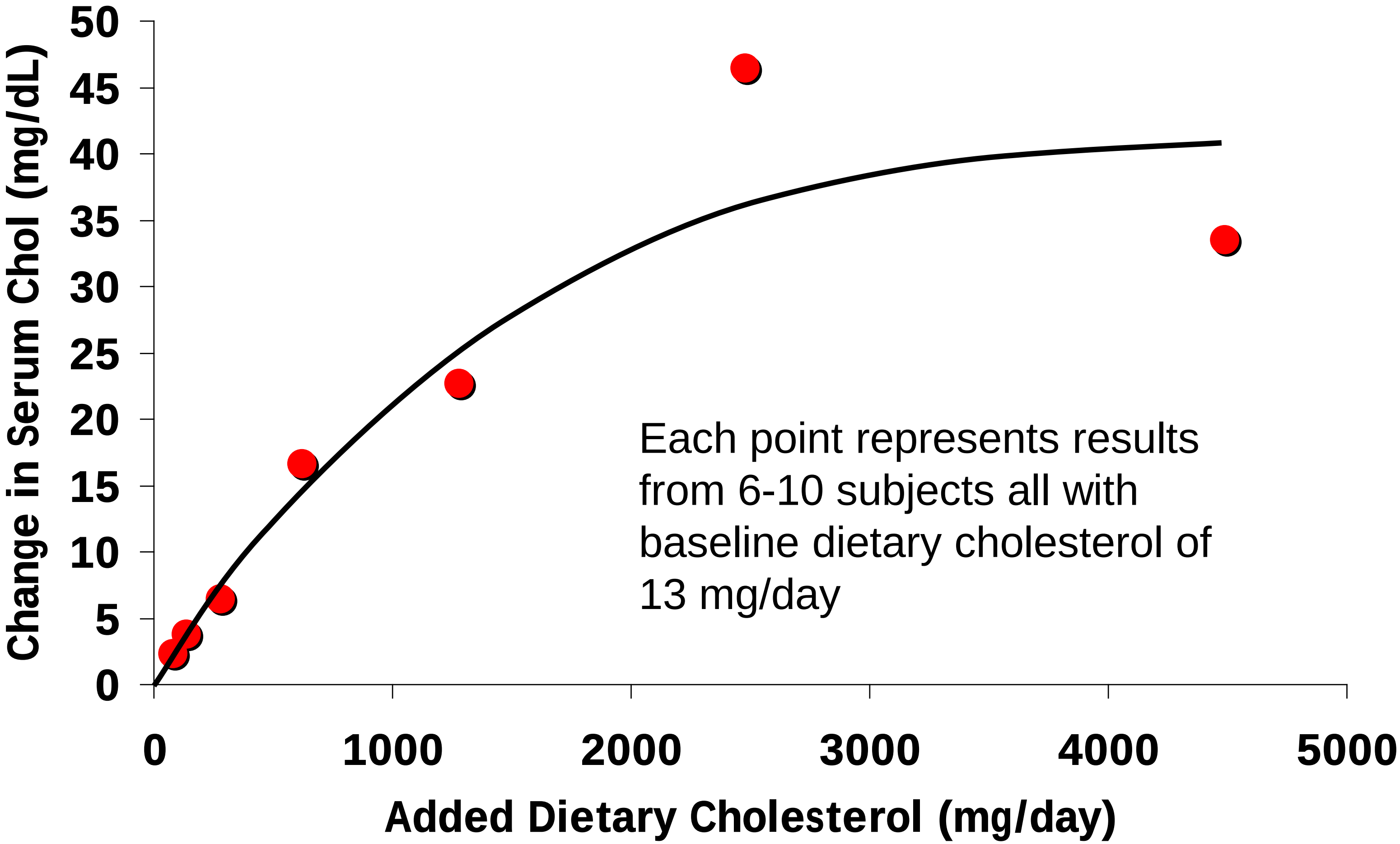
Dietary cholesterol and plasma LDL-C

Cholesterol homeostasis – SREBP-2



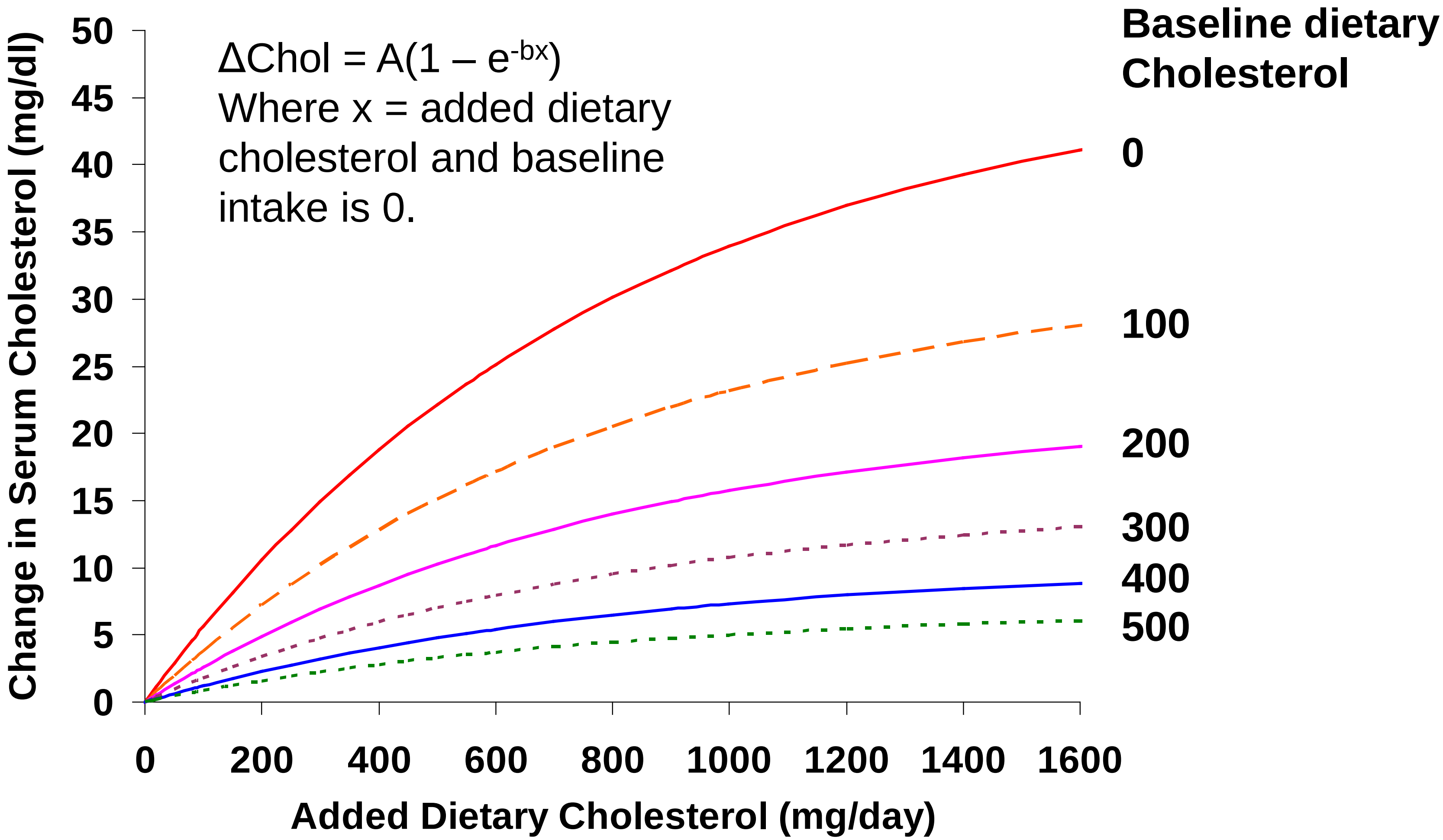
from Ikonen, E. Nature Rev Mol Cell Biol 2008 9:125 see also Brown MS, et al. J Lipid Res 2009 50 S15

Effect of cholesterol added to a defined liquid diet



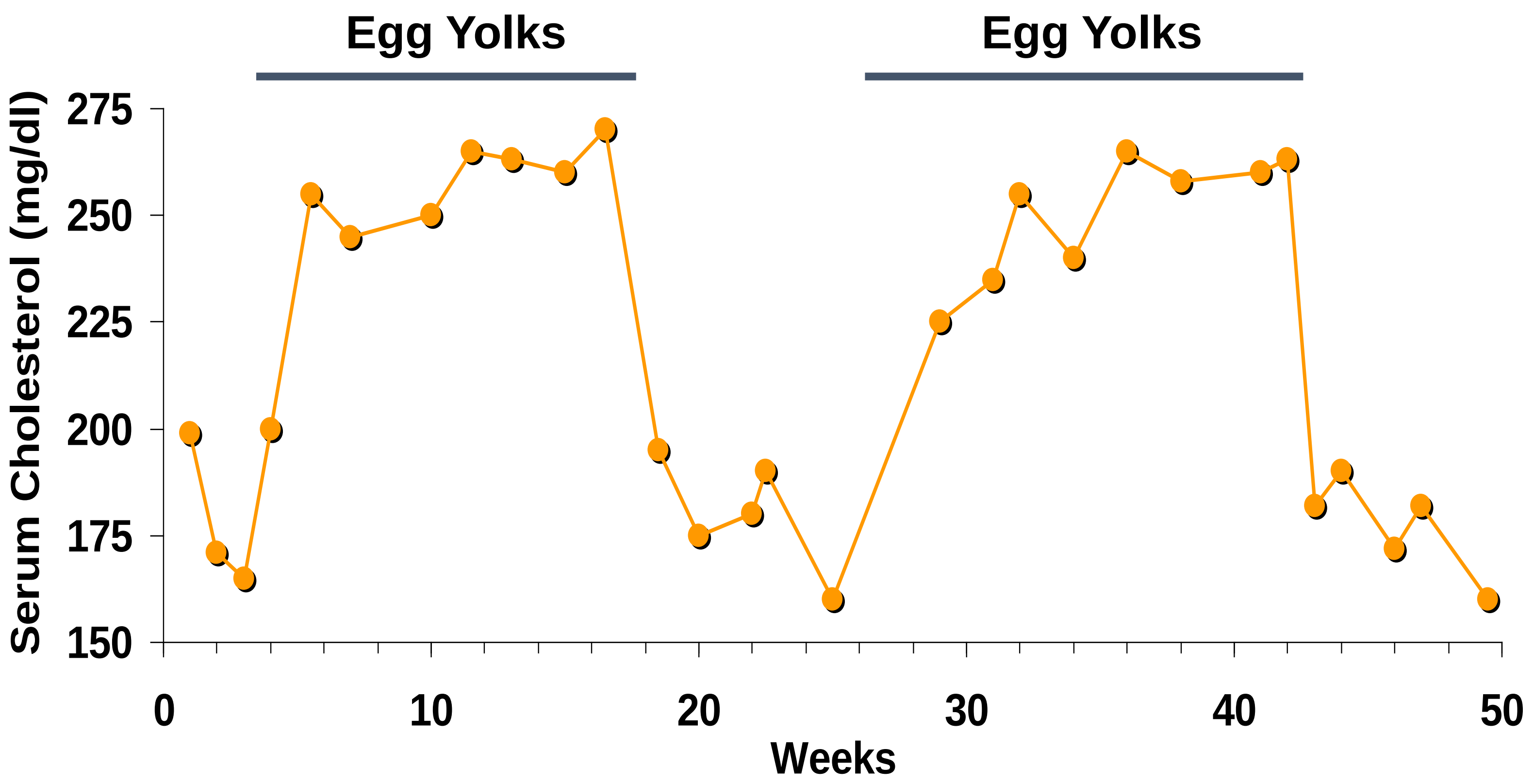
Beveridge JMR, et al. J Nutr 1960; 71:61

Effect of dietary cholesterol on serum cholesterol – a meta-analysis



Hopkins PN. Am J Clin Nutr 1992; 55:1060

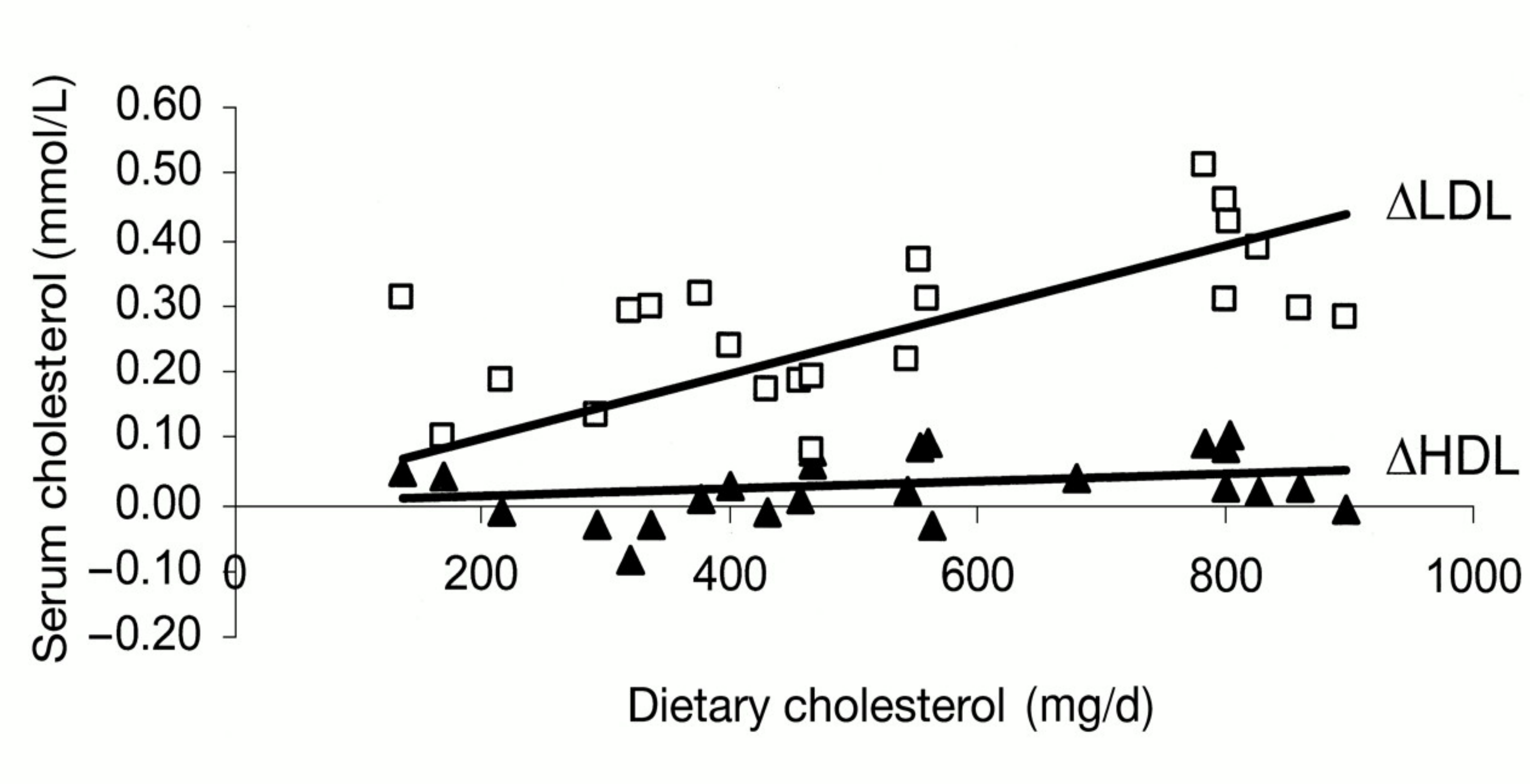
Effect of egg yolks (supplying 2400 mg cholesterol daily) in a 34 year-old man



Diet was otherwise free of cholesterol. Saturated fat 14% calories, monounsaturates 17%, and polyunsaturates 10%. Weight was kept constant.

Connor WB, et al. J Am Diet Assoc 1978; 52:281

Dietary cholesterol increases the ratio of LDL to HDL in humans



Weggemans RM, Zock PL, Katan MB. Am J Clin Nutr 2001; 73:885

How does dietary cholesterol raise serum LDL?

- Increased hepatocyte cholesterol leads to:
  - Decrease in SREBP2 (sterol response element binding protein 2) proteolytic cleavage and release
  - Down-regulation of SREBP2-responsive genes including:
    - LDL receptor
    - Cholesterol synthesis genes (like HMG-CoA reductase)
  - ↑ oxysterols, desmosterol → ↑ LXR (liver X receptor) activation
    - Increased bile acid formation from cholesterol (via CYP7A1) (MAINLY IN RODENTS, little effect in humans)
    - Increased ABCG5 and ABCG8 which transport cholesterol from hepatocytes into the bile.
    - Increase ABCA1 which transfers cholesterol to HDL
    - Increased IDOL transcription with increased LDLR ubiquitination and degradation (important effect in primates and humans, but not mice)
- Decreased LDL receptors results in lower fractional catabolic rate of LDL and increased LDL in plasma