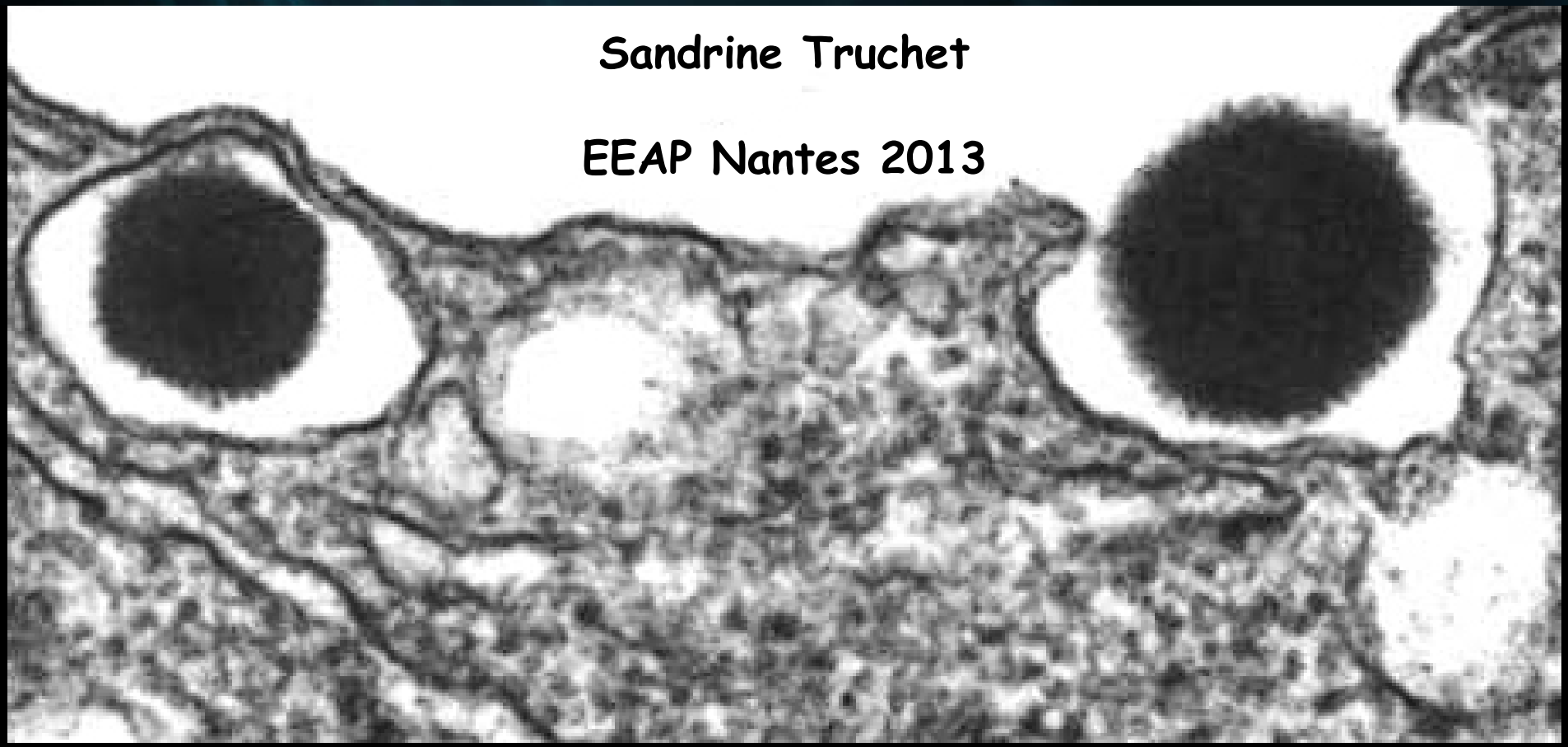
milk secretion:

role of the SNARE proteins

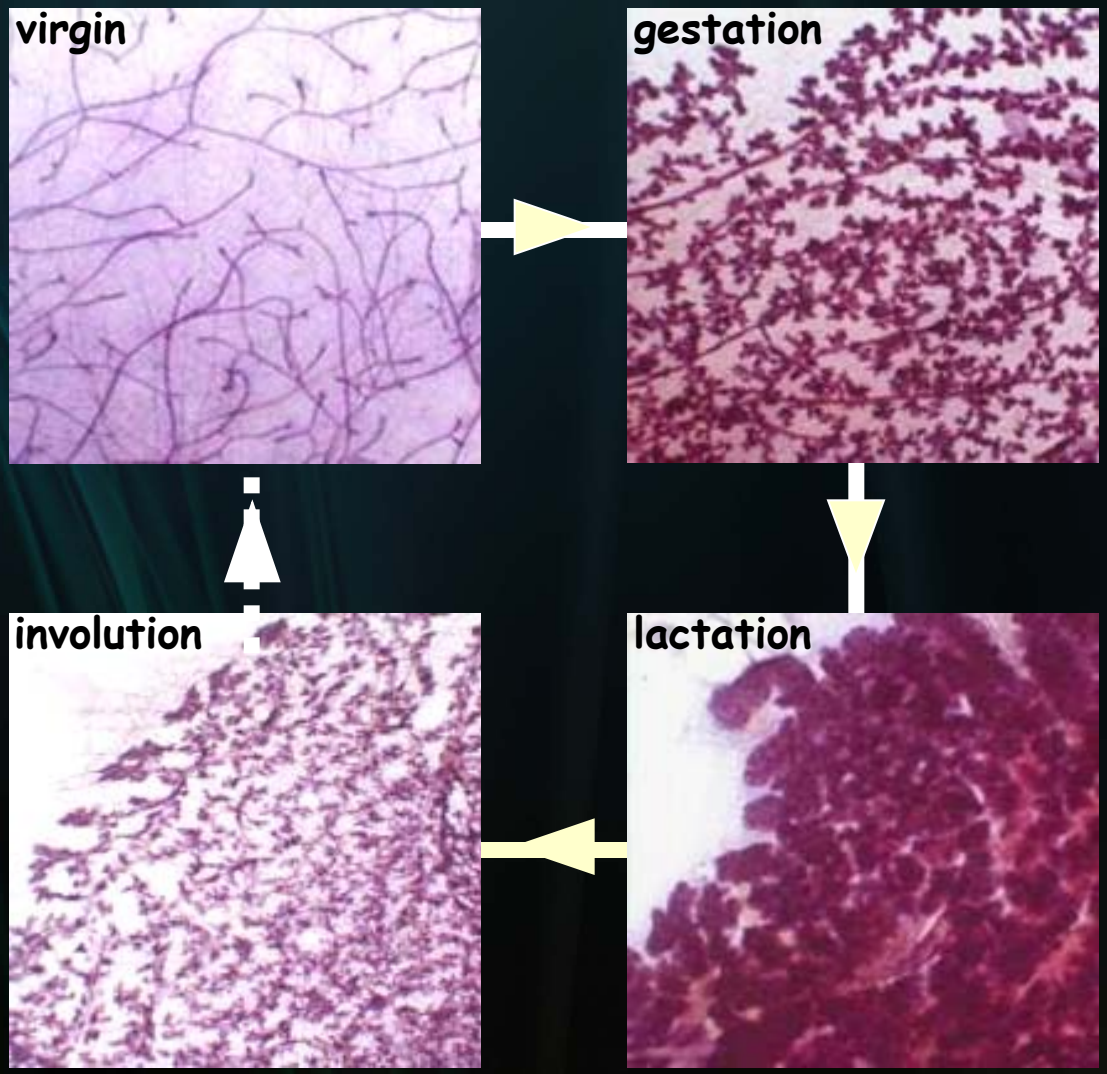
Sandrine Truchet

EEAP Nantes 2013

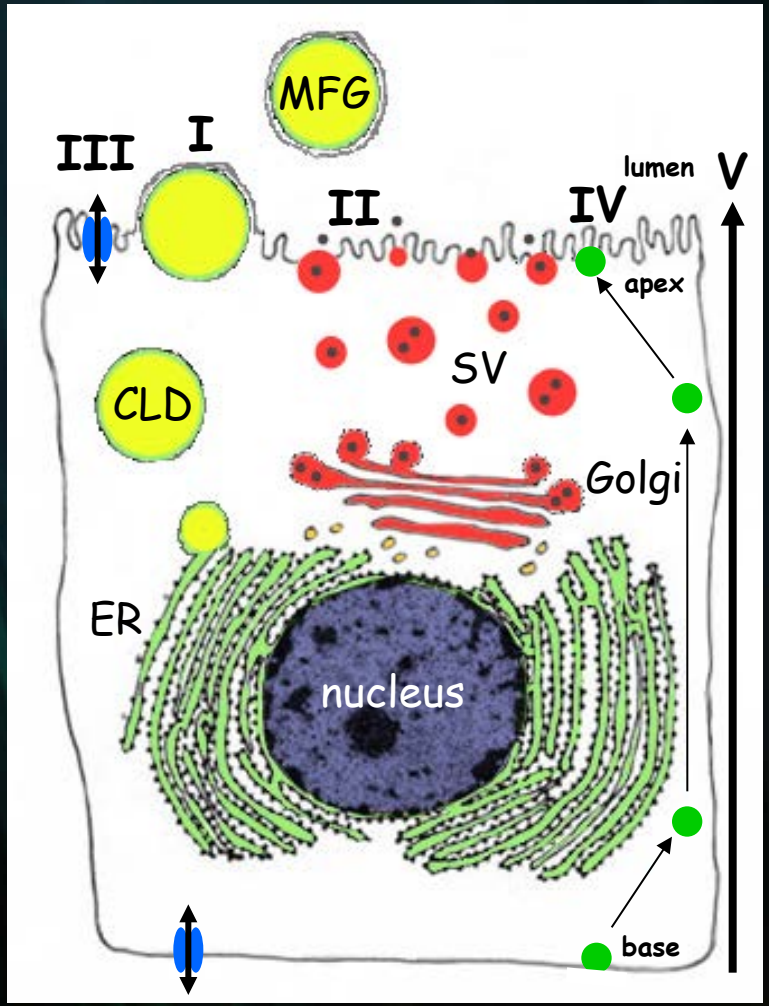


INRA, UR1196 Génomique et Physiologie de la Lactation, F-78352, Jouy-en-Josas Cedex, France

development of the mammary gland



secretory routes in the MEC



I: budding

II: exocytosis

III: transporters

IV: transcytosis

V: paracellular transport

adapted from McManaman & Neville, 2003

milk product secretion: molecular mechanisms

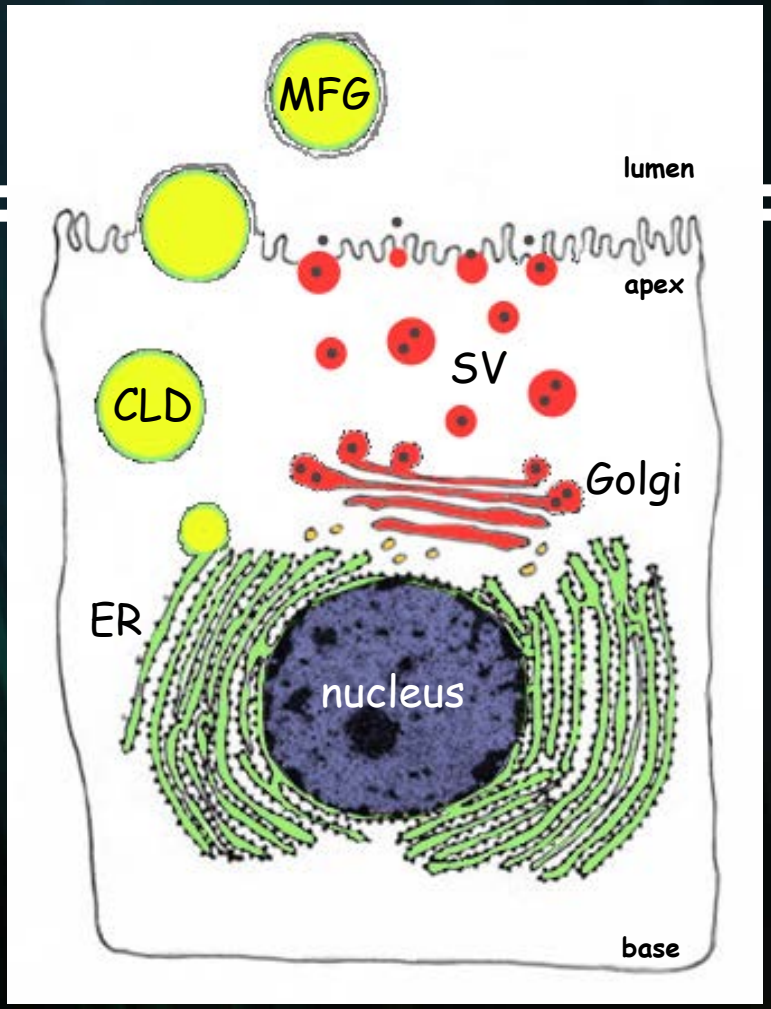
budding

exocytosis

adipophilin

xanthine oxidase

butyrophilin



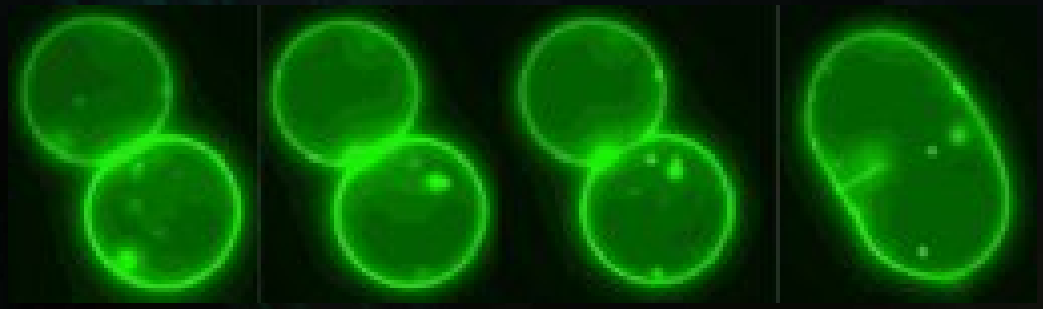
SNARE proteins?

adapted from McManaman & Neville, 2003

SNAREs

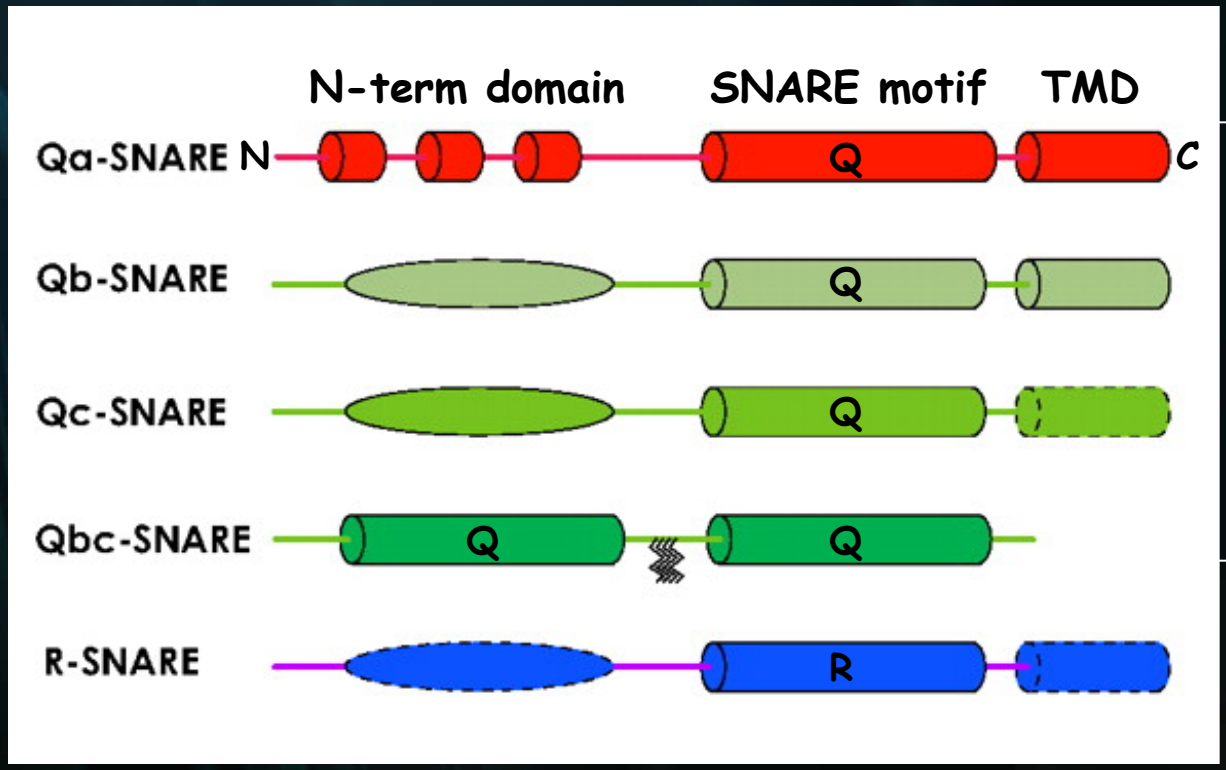
soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor

- 36 SNAREs in human
- evolutionarily conserved
- ubiquitously expressed
- involved in nearly all the membrane fusion events
- minimal core machinery for membrane fusion



SNAREs structure

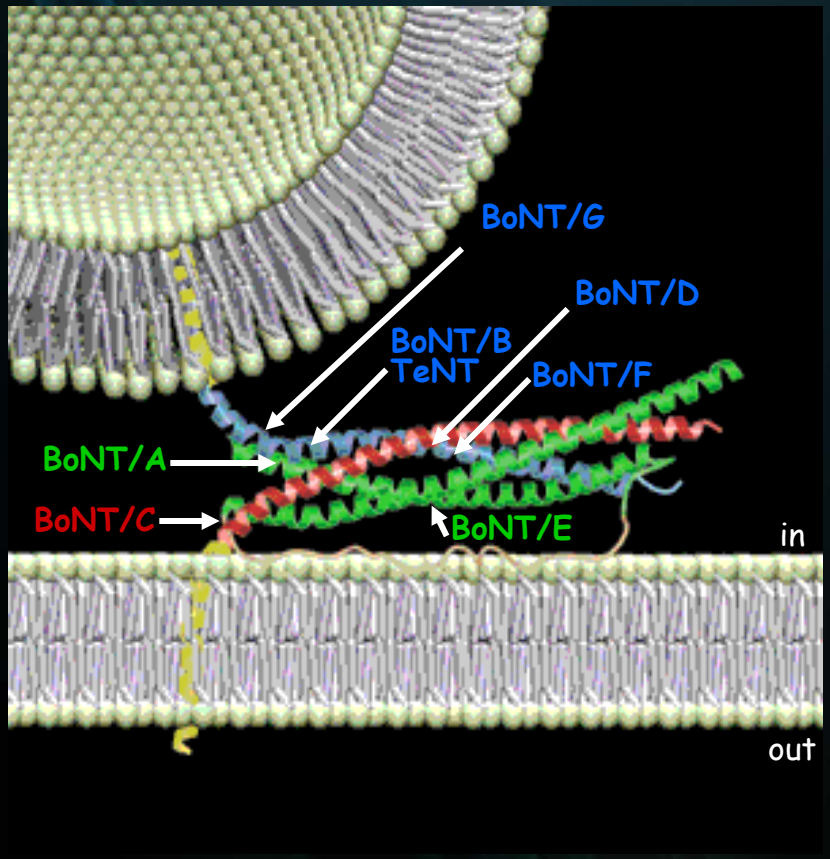
SNARE motif: 60-70 aa coiled-coil strech



Stx: syntaxin
SNAP: synaptosomal-associated protein
VAMP: vesicle-associated membrane protein

Ovsepian & Dolly, 2011

membrane fusion: SNARE complex



- intracellular traffic specificity
- vesicles docking to the target membrane
- recruitment of regulatory proteins
- membrane fusion triggering

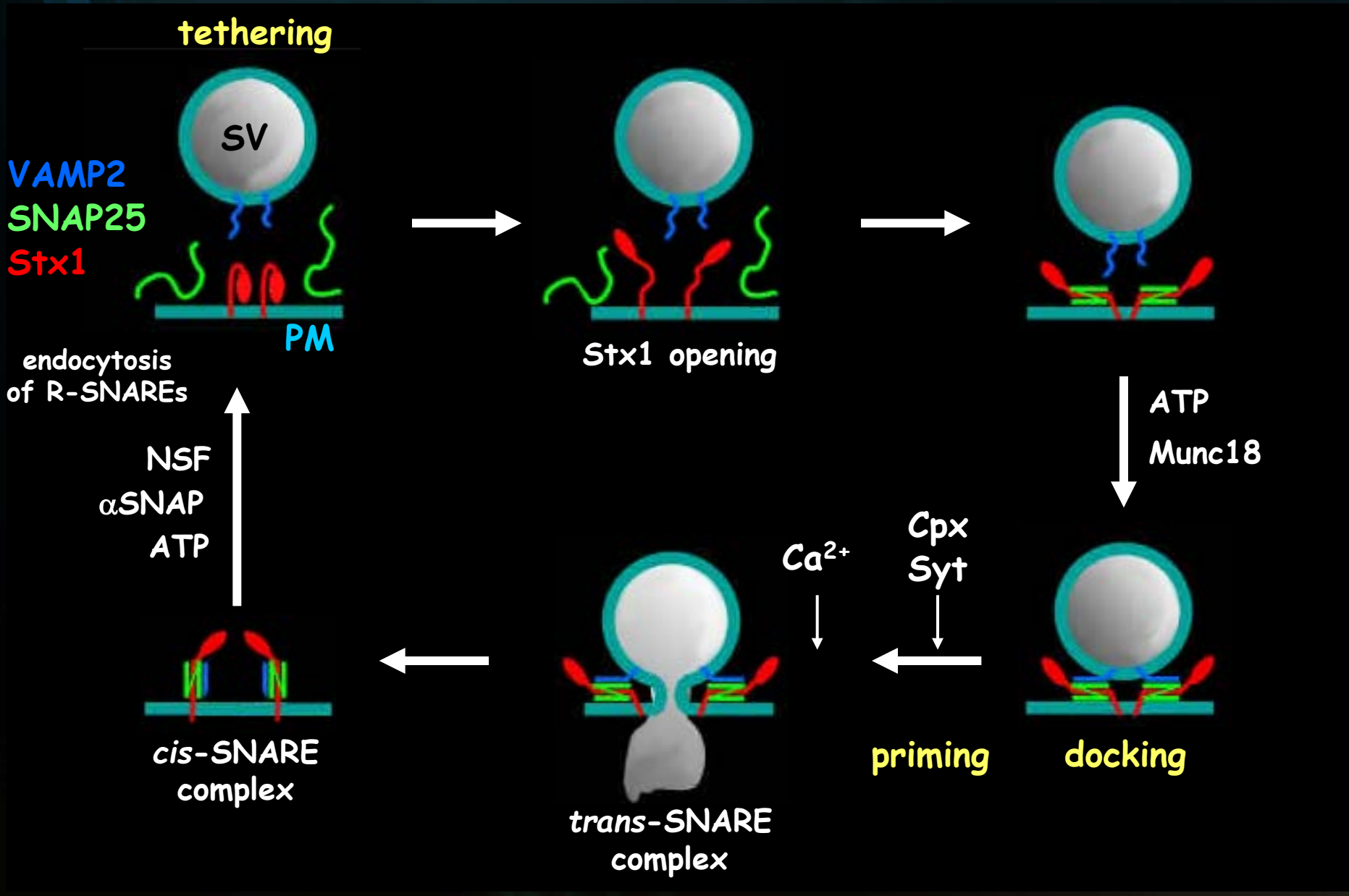
neurotransmitters secretion

VAMP2
SNAP25
Stx1

SNARE complex: Qa:Qb:Qc:R

adapted from Sutton et al., 1998

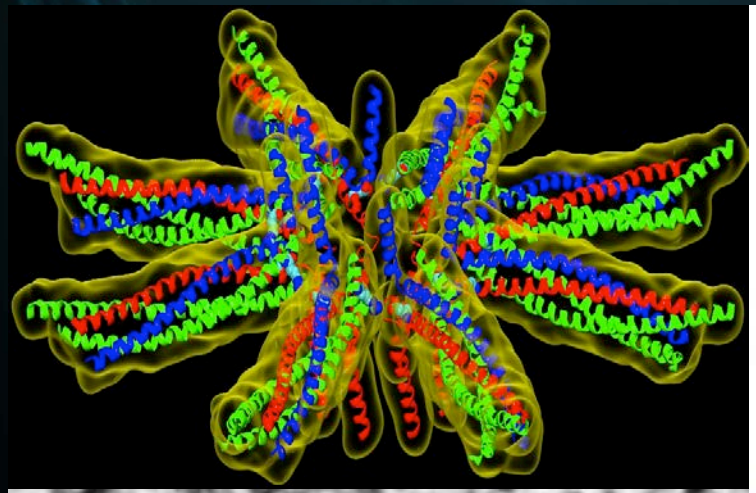
SNARE-mediated membrane fusion

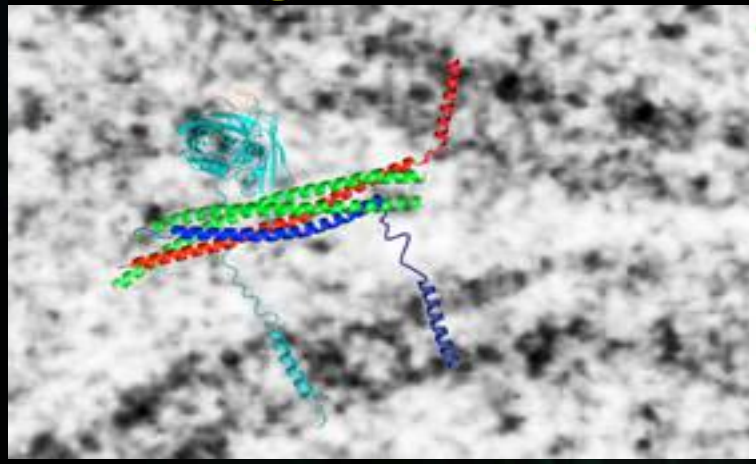


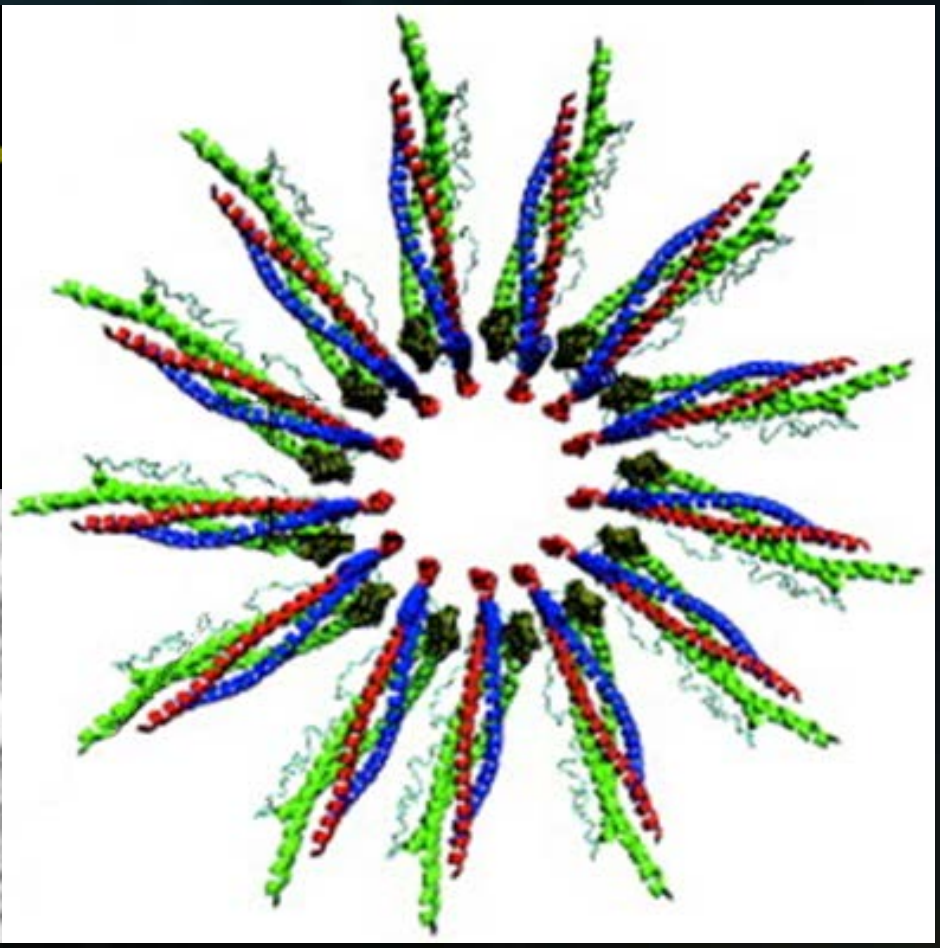
how much SNARE complexes to fuse membranes?

free energy needed to overcome the repulsive forces between 2 membranes

~ 200-400 pN.nm (~ 50-100 kBT or 2-4.10^{-19} J) Cohen & Melikyan 2004



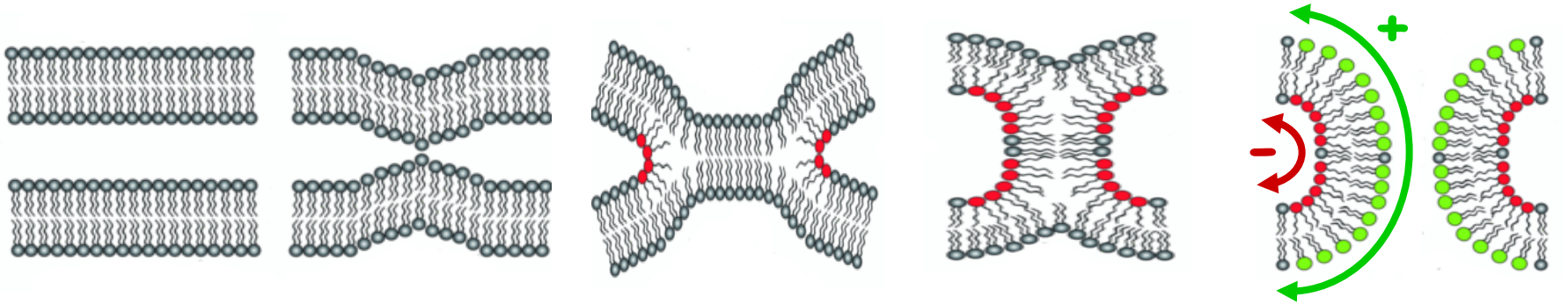




5-10 SNARE complexes (vesicle-planar membrane fusion)

Montecucco et al., 2005
Megighian et al., 2013
Brunger Lab website

how do membranes merge?



membrane protrusions

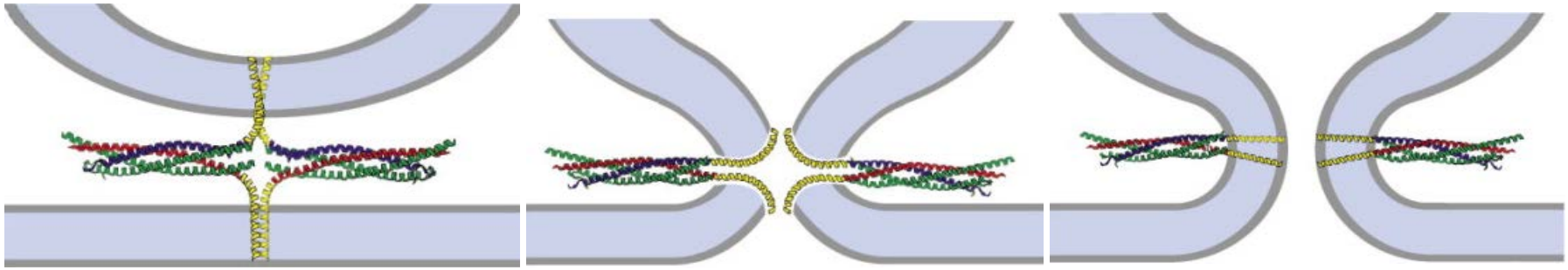
hemifusion (stalk)

expansion of the stalk -> hemifusion diaphragm

fusion pore

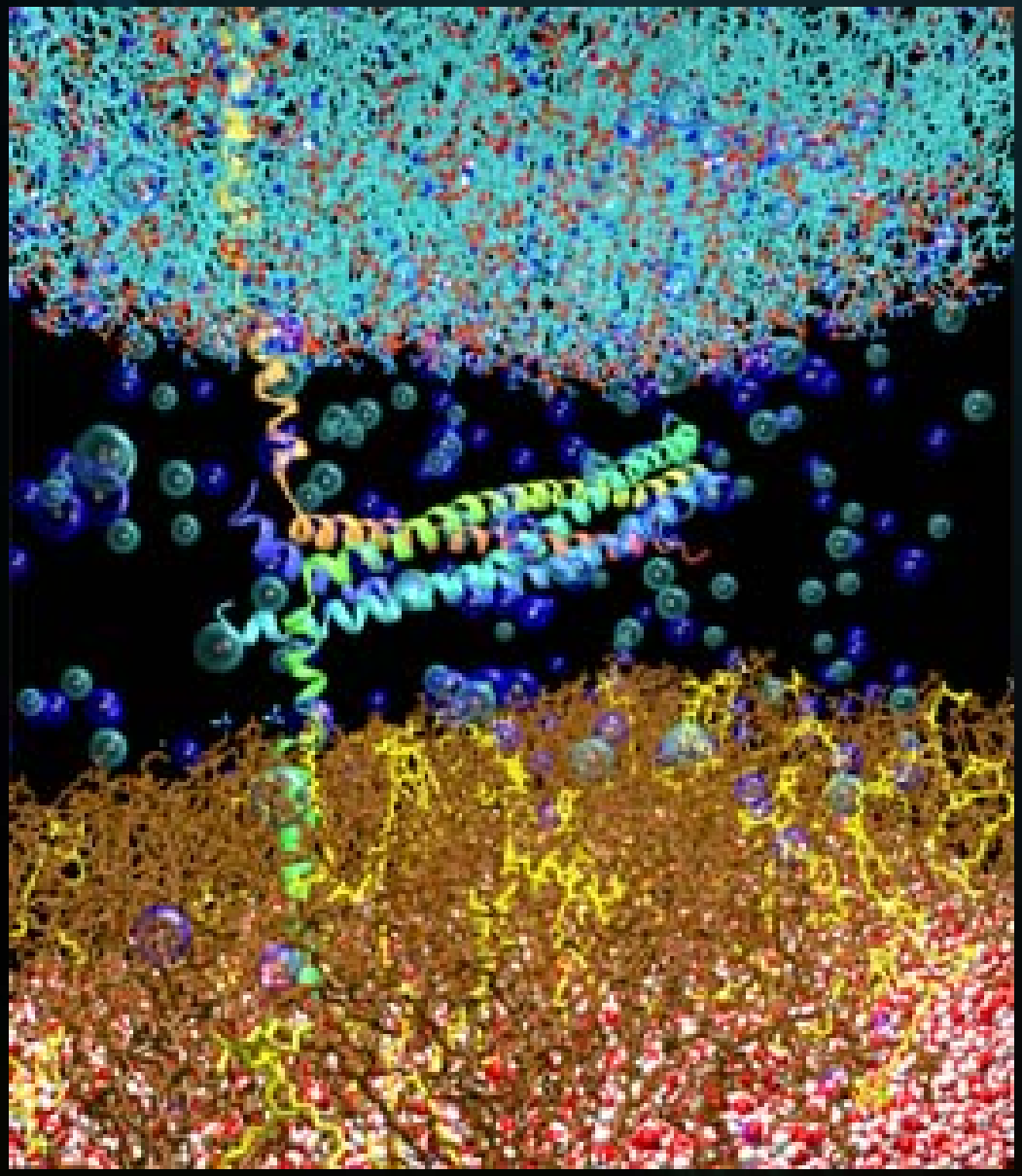
● **lipids inducing - curvature (DAG, phosphatidylethanolamine)**

● **lipids inducing + curvature (lysophosphatidylcholine)**



adapted from Chernomordik & Kozlov 2008

if you want membranes to fuse…



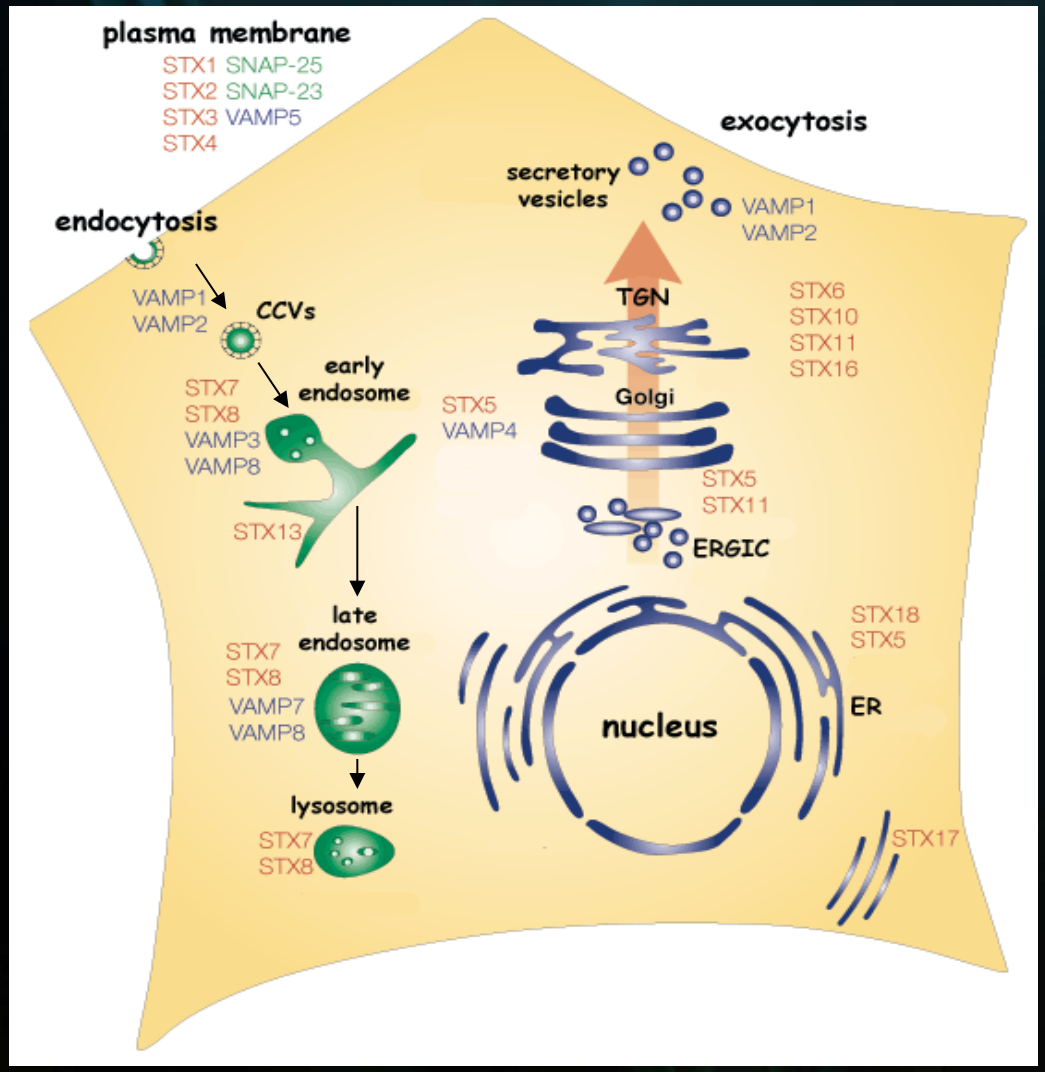
lipid environment
+
SNAREs
+
regulatory proteins

-> stability
-> kinetics
-> spatio-temporal

regulation
of the fusion pore

=> finely tuned exocytosis

SNAREs intracellular localization



> 30 mammalian SNAREs

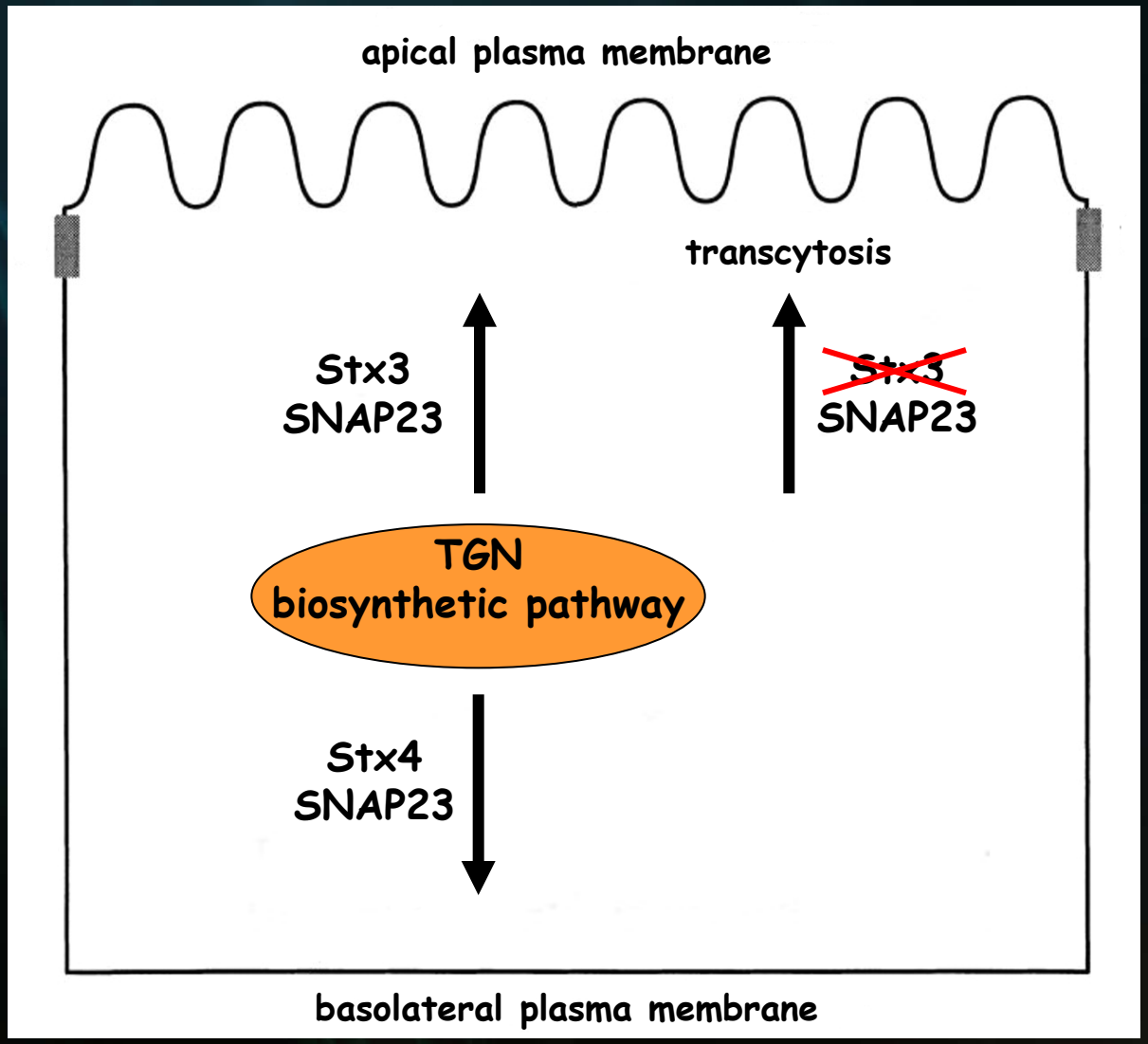
Q-SNAREs

- SNAPs: 25, 23, 29, 47
- Stx: 1 to 18
- others: Vti1a, Vti1b, Bet1, Gos28, membrin

R-SNAREs

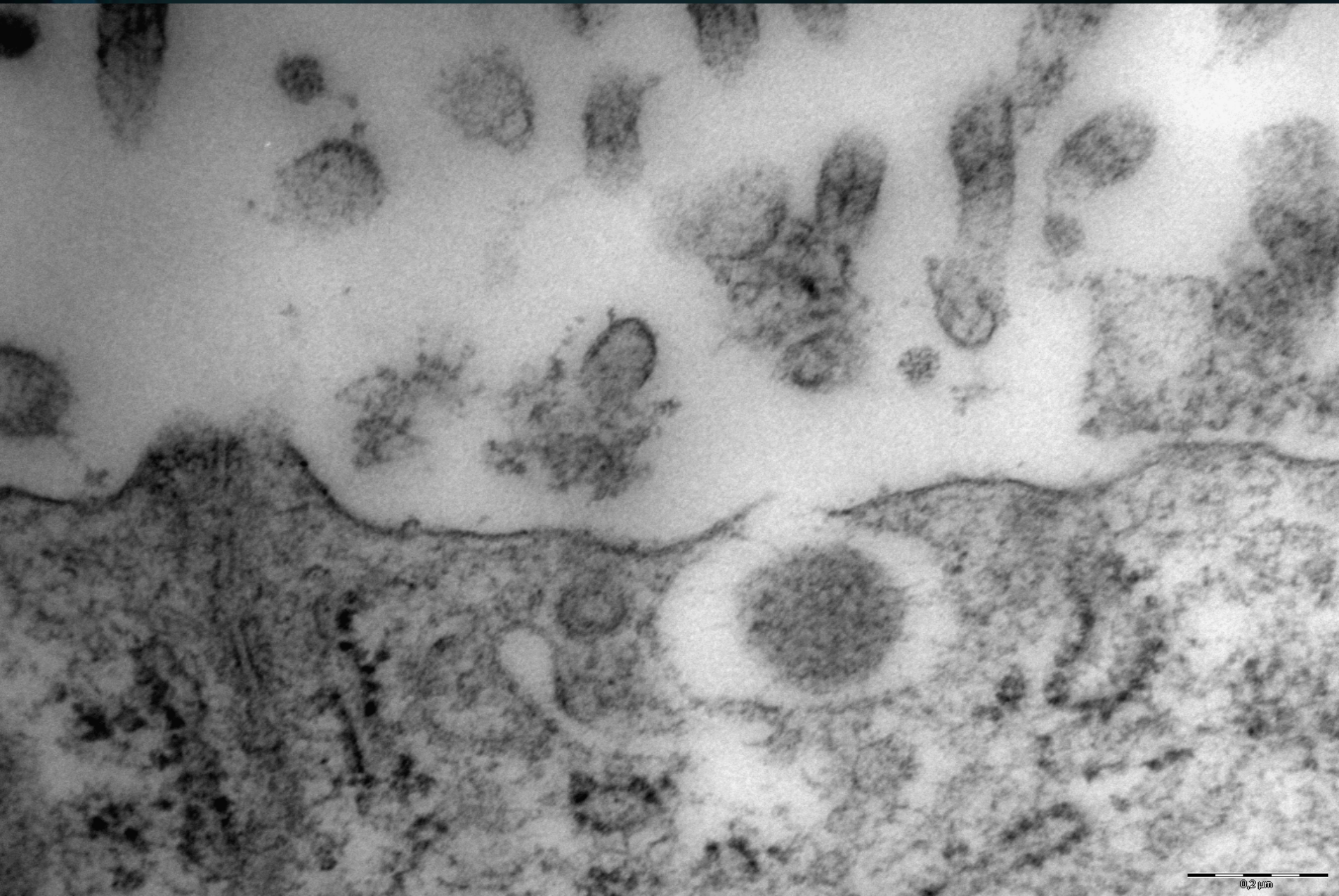
- VAMPs: 1, 2 (synaptobrévins), 3 (cellubrevin), 4, 5 (myobrevin), 7 (Ti-VAMP), 8 (endobrevin)
- others: Sec22b, Ykt6

SNAREs in polarized membrane traffic (MDCK cells)

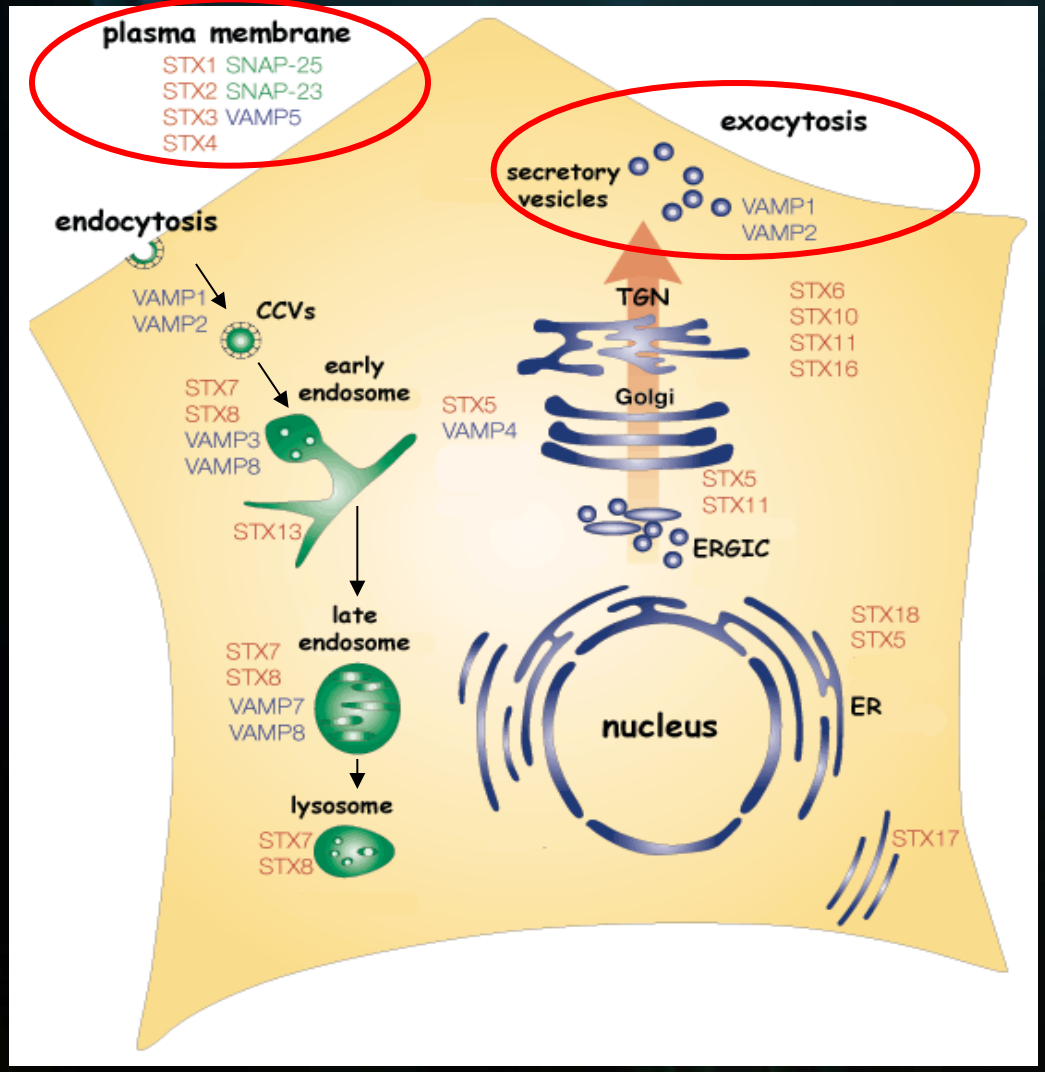


Low et al., 1998

SNAREs and milk secretion



SNAREs intracellular localization



> 30 mammalian SNAREs

Q-SNAREs

- SNAPs: 25, 23, 29, 47
- Stx: 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 16, 17, 18
- others: Vti1a, Vti1b, Bet1, Gos28, membrin

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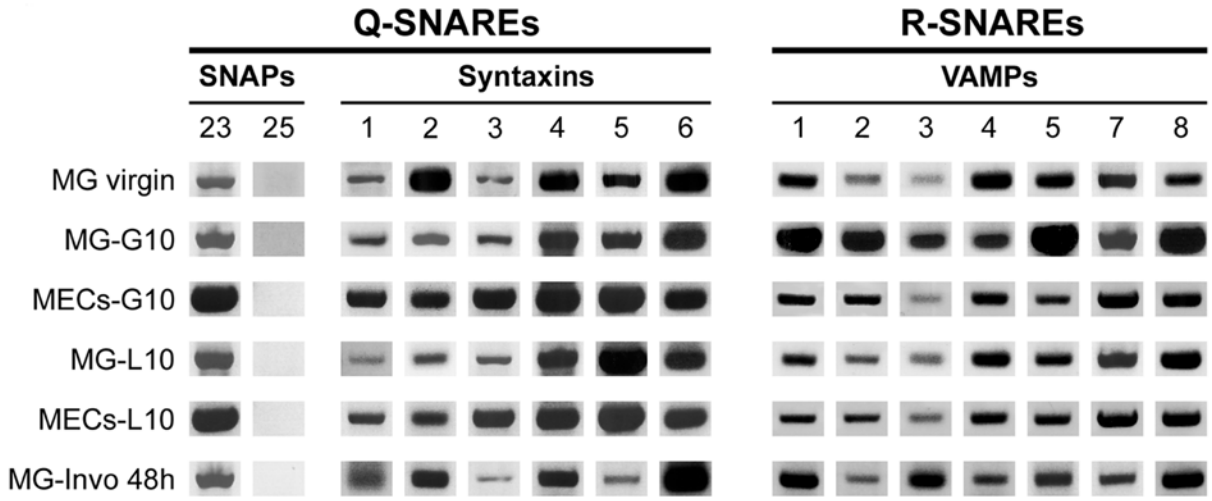
- VAMPs: 1, 2 (synaptobrévins), 3 (cellubrevin), 4, 5 (myobrevin), 7 (Ti-VAMP), 8 (endobrevin)

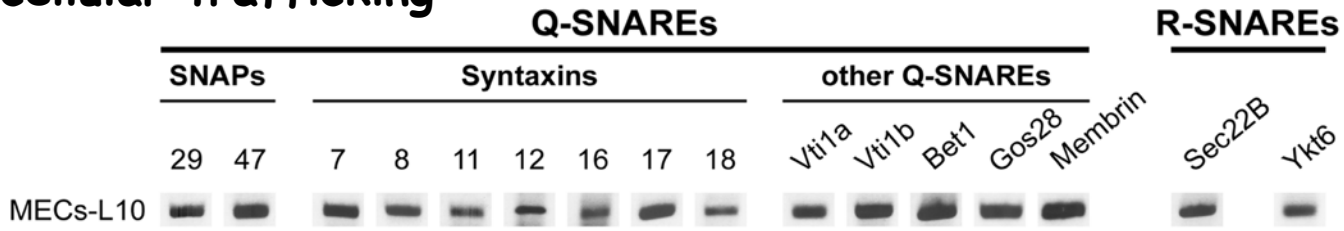
others: Sec22b, Ykt6

SNAREs expression in the mammary gland
(RT-PCR)

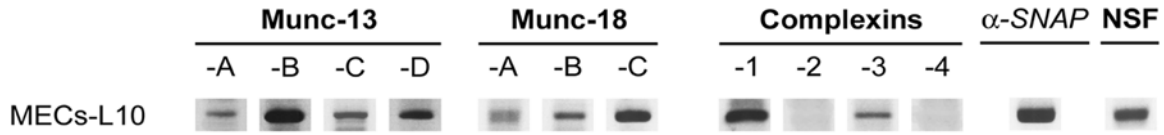
secretion of caseins



intracellular trafficking

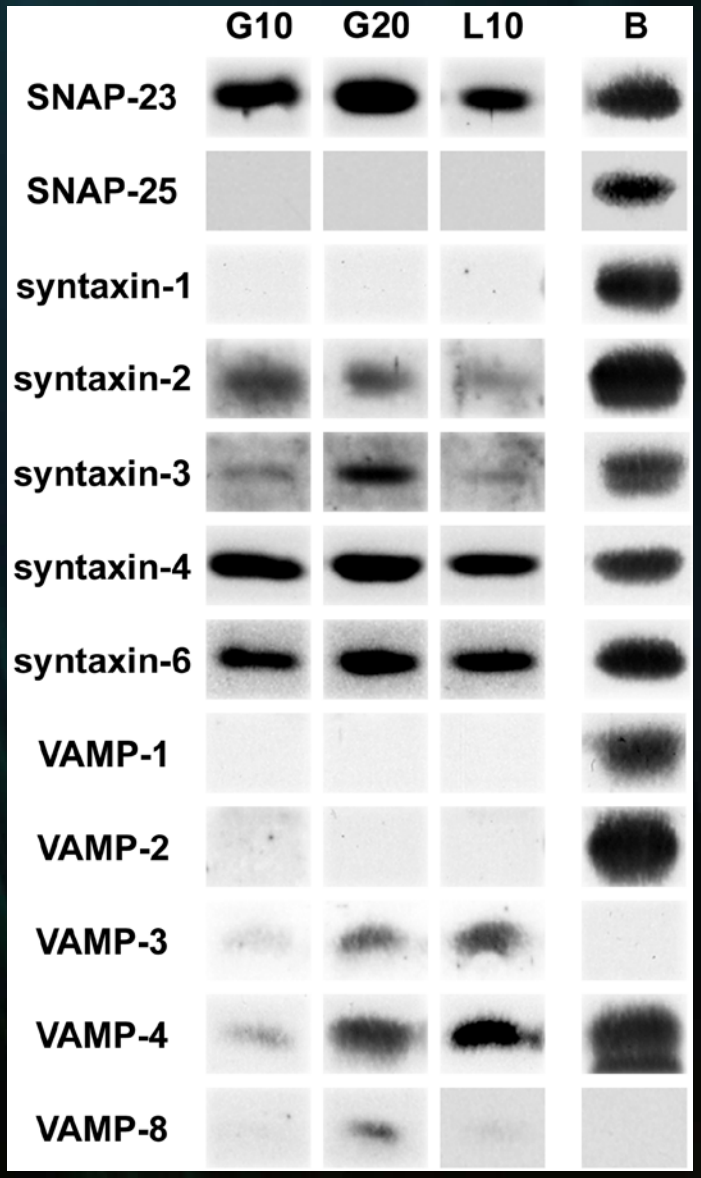


regulatory proteins



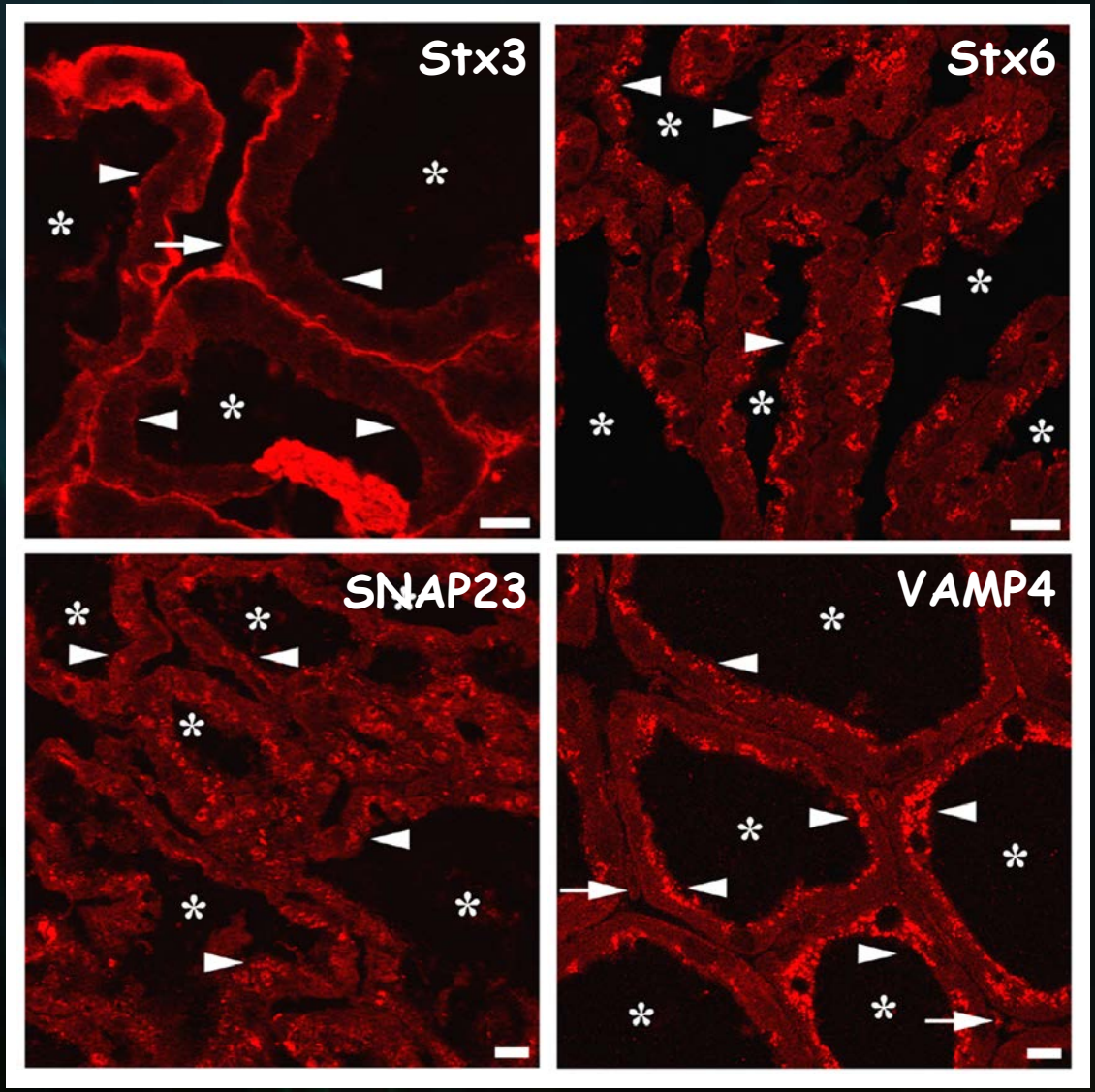
Chat et al., 2011

SNAREs expression in MECs
(Western blot)



Chat et al., 2011

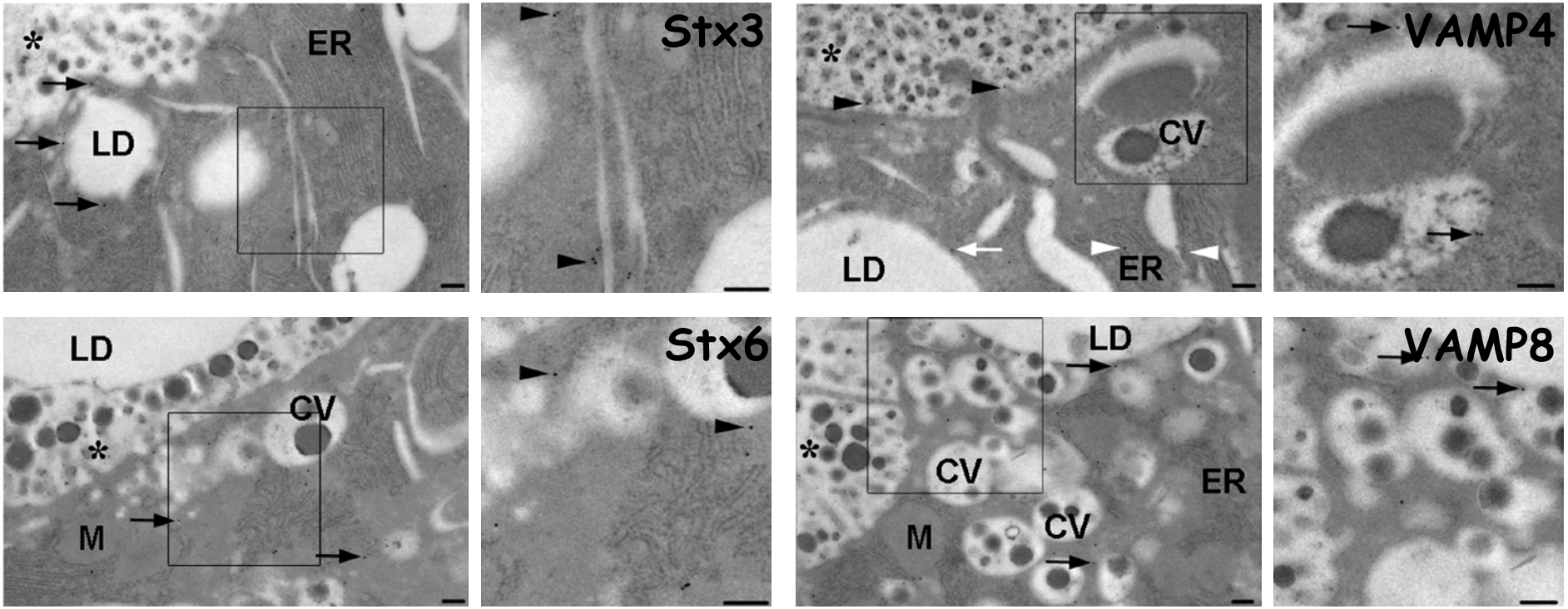
SNAREs localization in the mammary gland (L10)



asterisks = lumens, scale bar = 20 μm

Chat et al., 2011

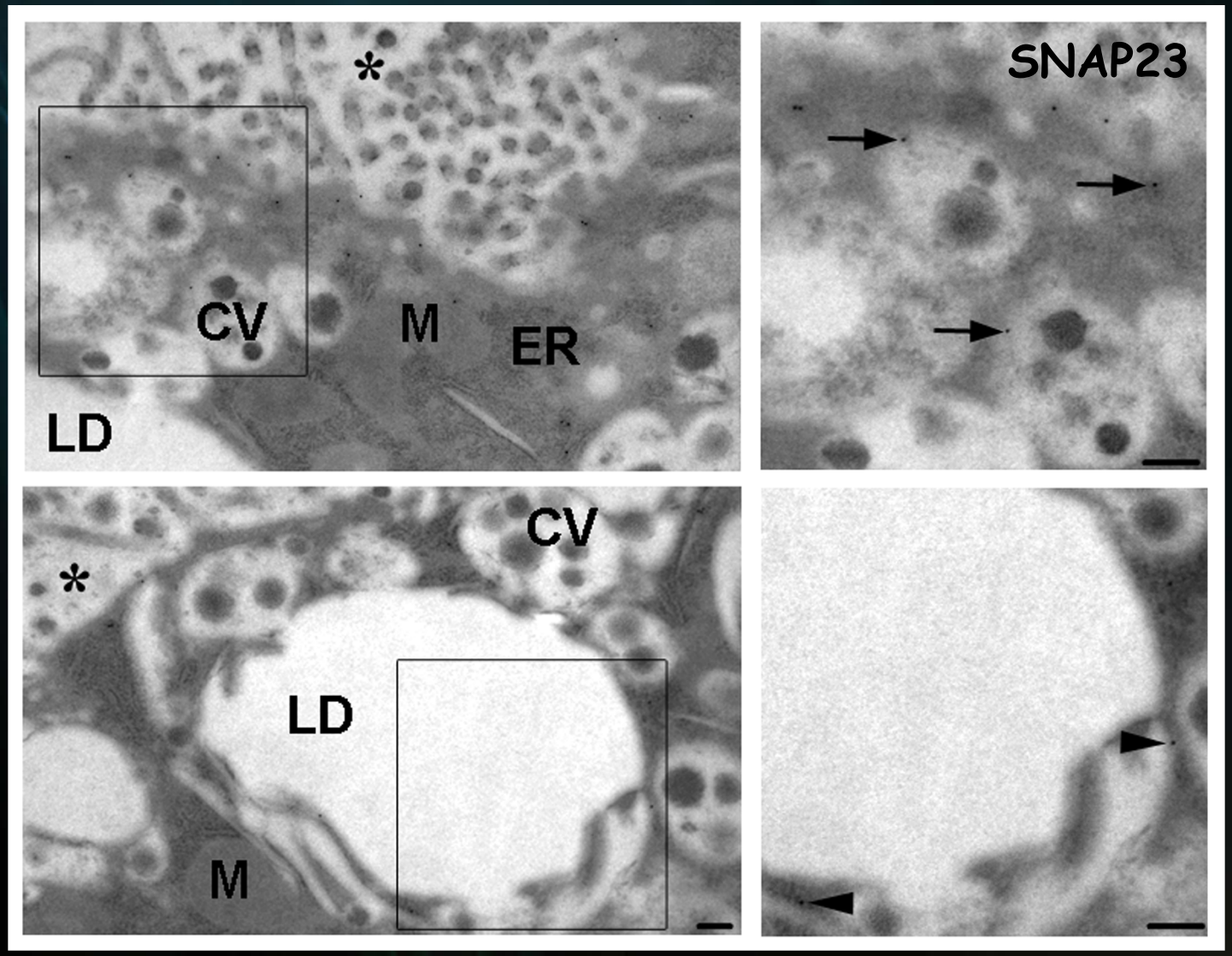
SNAREs localization in MECs (L10)



asterisks = lumens, scale bar = 200 nm

Chat et al., 2011

SNAREs localization in MECs (L10)



asterisks = lumens, scale bar = 200 nm

Chat et al., 2011

SNAP23 interacting partners (L10)

mouse mammary gland (L10)

↓

purified MECs

↓

cell membranes

↓

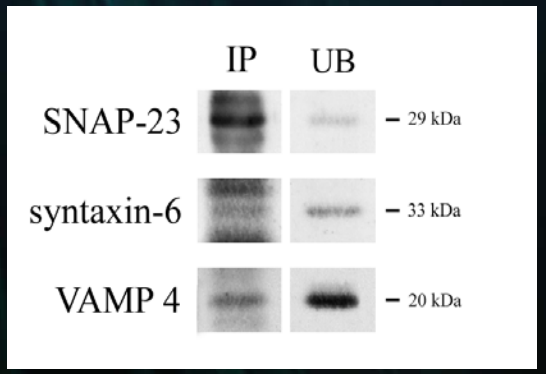
IP anti-SNAP23

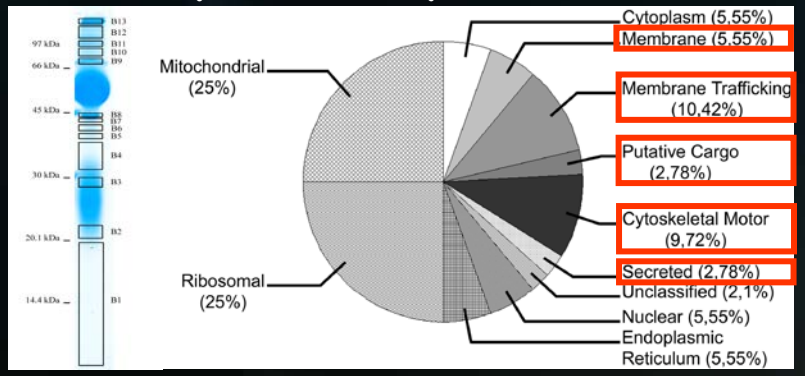
↓

SDS-PAGE (1D)

↙ Western blot

↘ mass spectrometry (LC-MS/MS)





Q-SNAREs: Stx6 — Stx7, 12

R-SNAREs: VAMP4 — VAMP3, 8, Sec22b

=> various SNARE complexes with different localizations and/or functions

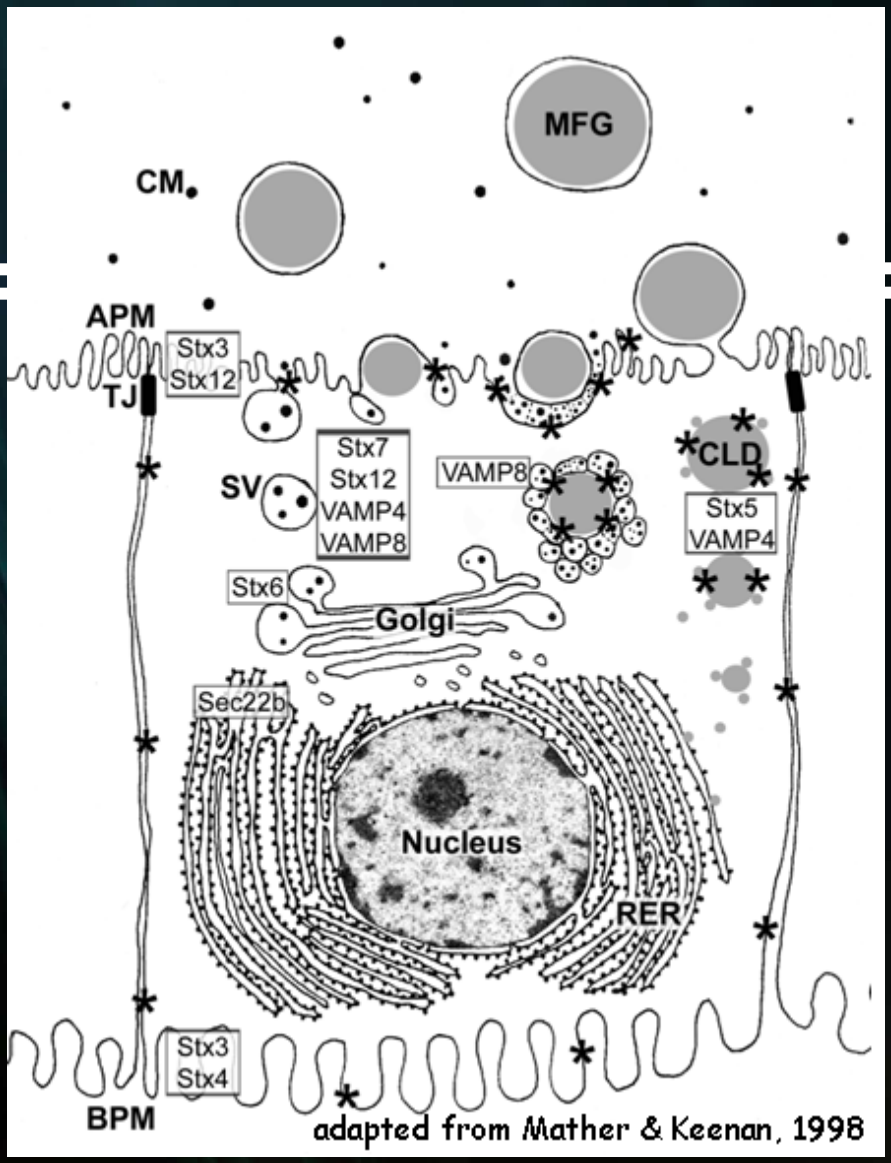
Chat et al., 2011

SNAREs and milk product secretion

budding

adipophilin
xanthine oxidase
butyrophilin

CLDs fusion
Stx5
SNAP23
VAMP4

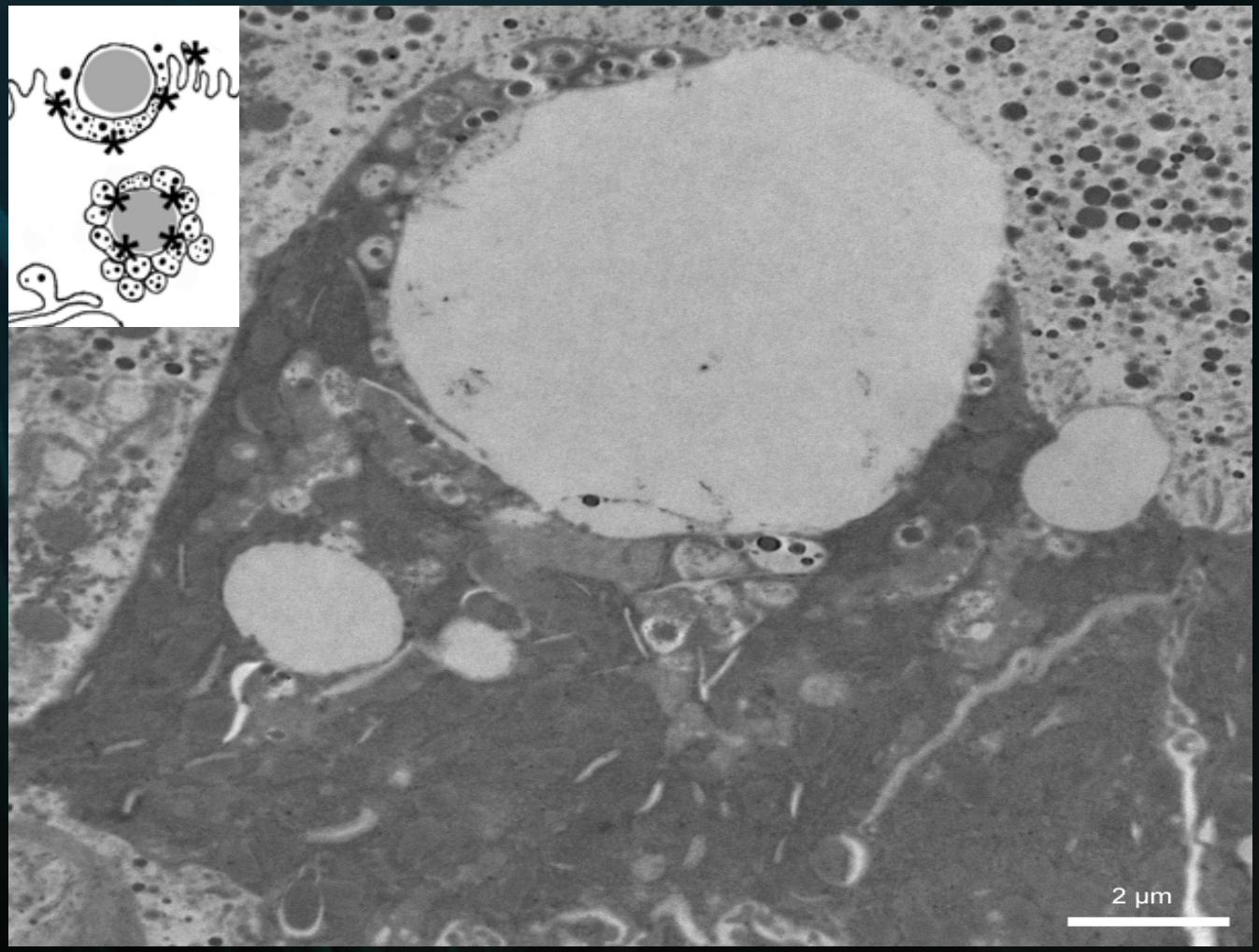


exocytosis

Stx6, 7, 12
SNAP23
VAMP3, 4, 8

Chat et al., 2011
Boström et al., 2011
Truchet et al., submitted

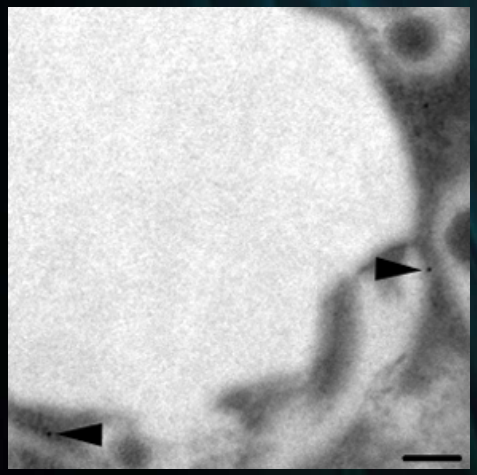
coupling of milk product secretion



=> regulatory keypoints ?

coupling of milk product secretion

SNAP23

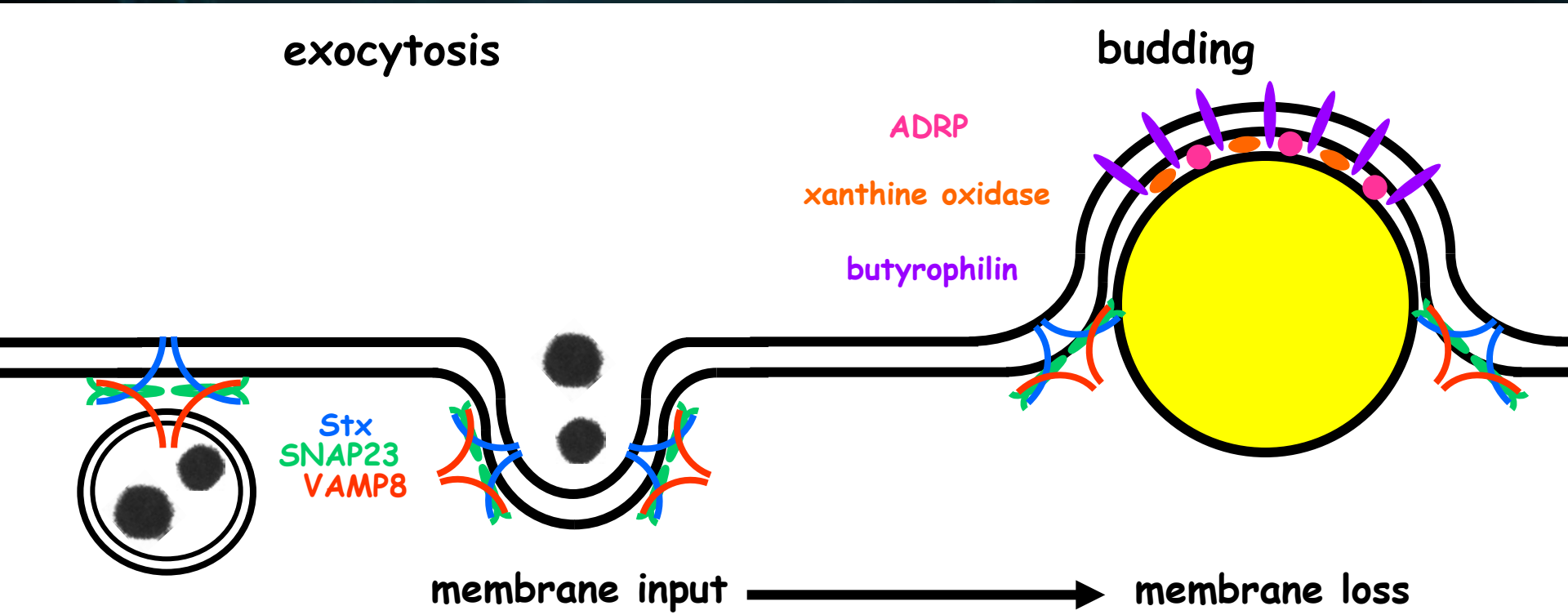


- involved in intracellular growth of CLDs — Boström et al., 2007
- associated with (proteomics)
  - CLDs — Boström et al., 2007
  - MFGs — Reinhardt & Lippolis, 2008; Honvo-Houéto, in preparation
- at the interface between CLDs and SVs — Chat et al., 2011

SNAP23:

a common regulatory keypoint for caseins and MFGs secretion ?

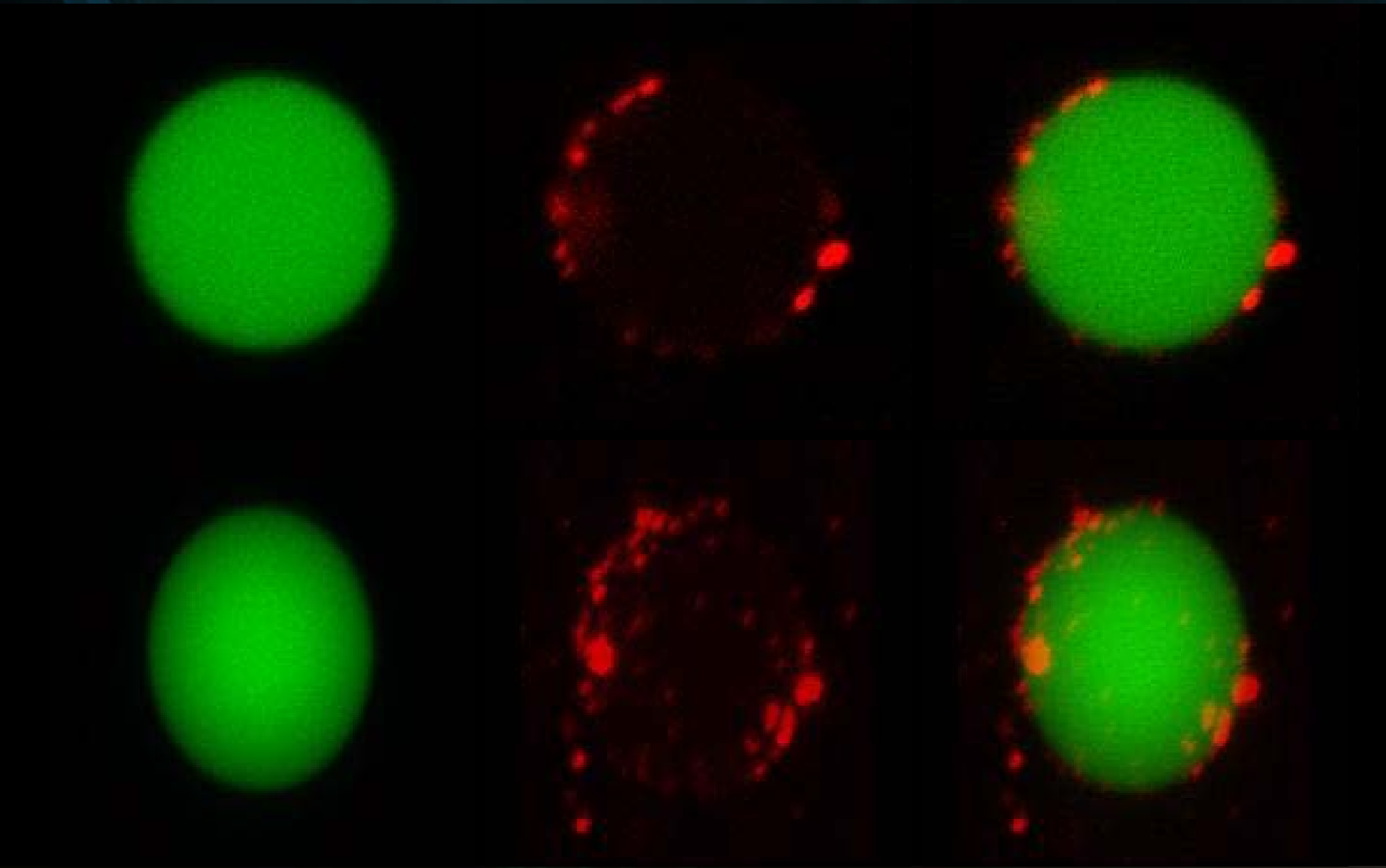
coupling of milk product secretion and apical membrane homeostasis



apical membrane homeostasis:

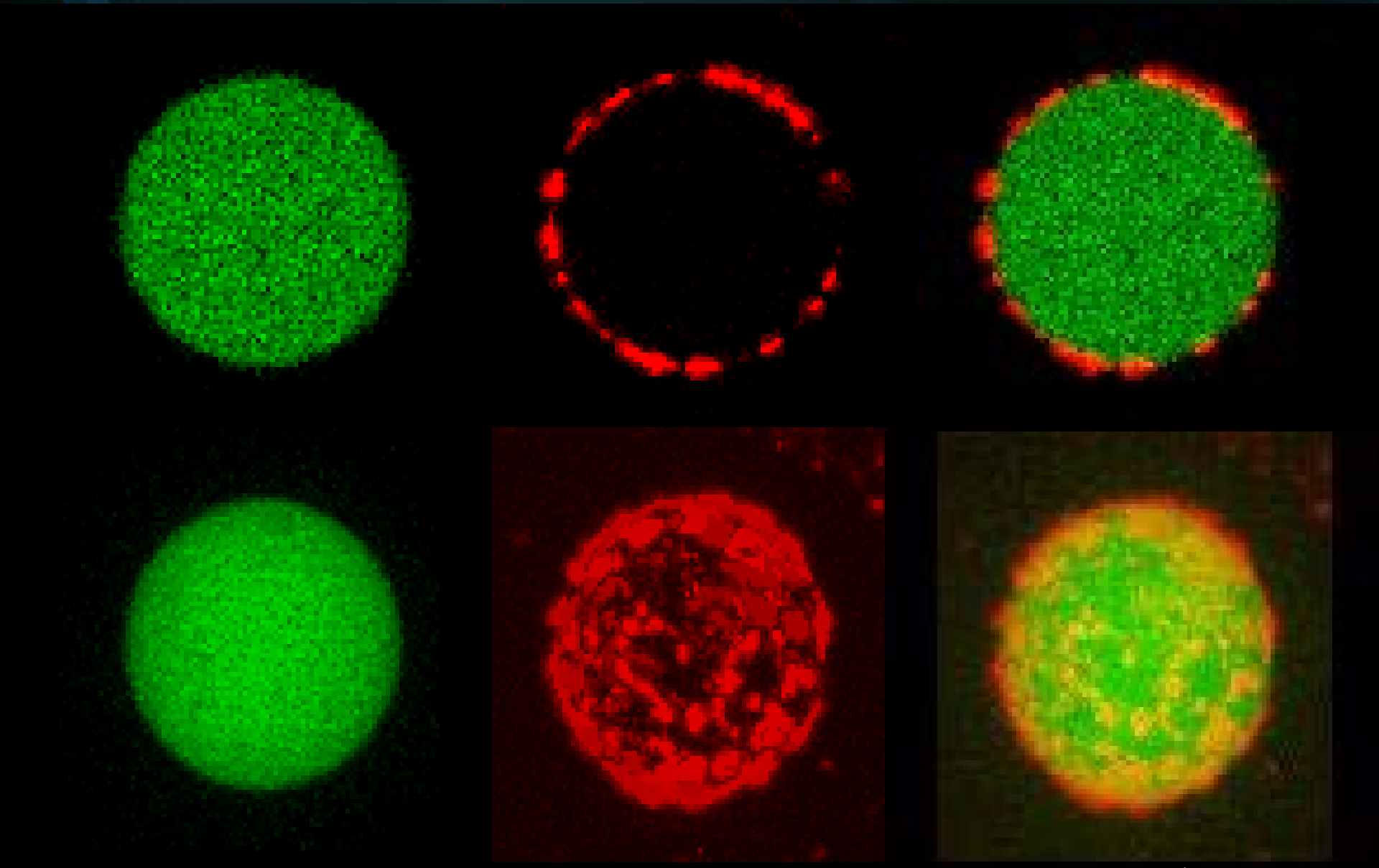
SV membrane reuse for MFG budding?

SNAP23 on mouse MFGs



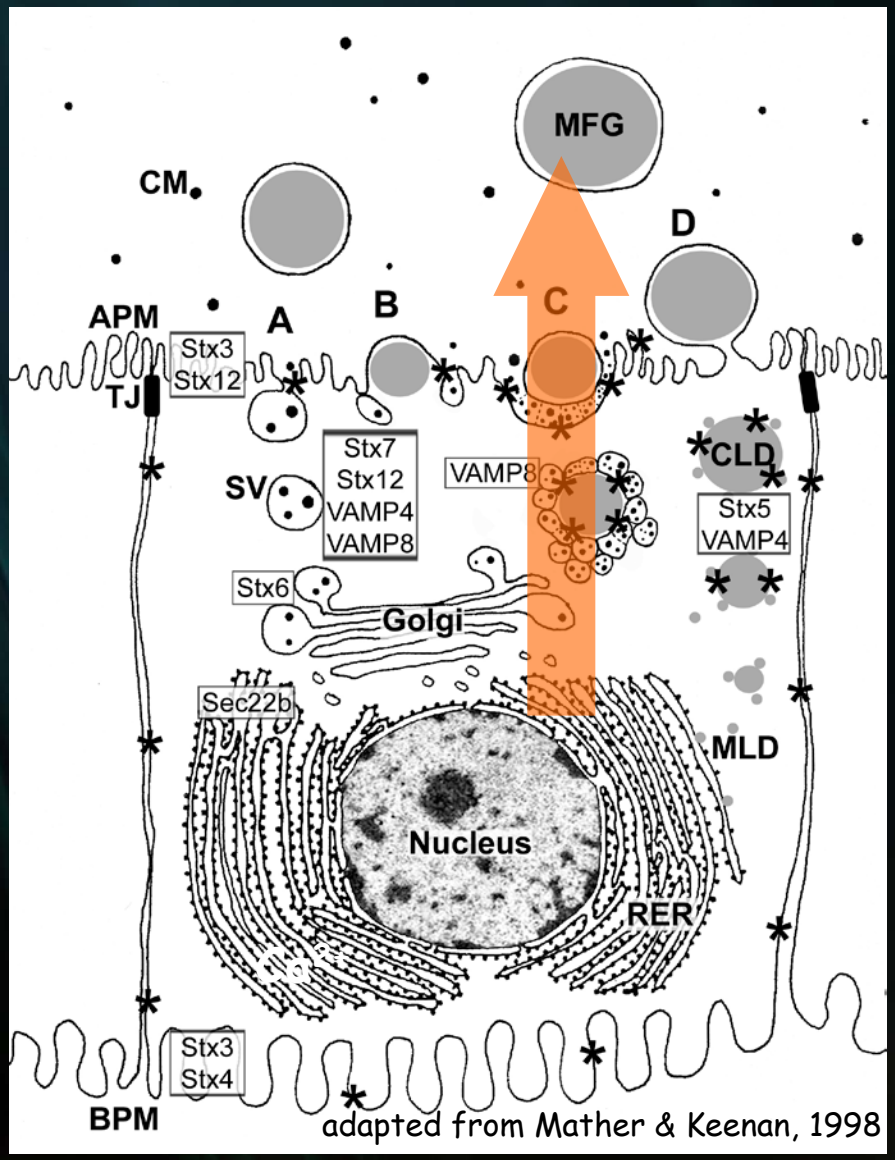
Honvo-Houéto, in preparation

VAMP8 on mouse MFGs



Honvo-Houéto, in preparation

secretagogue effect of PRL



acceleration of casein transport and secretion

↑

AA

↑

PLA2

↑

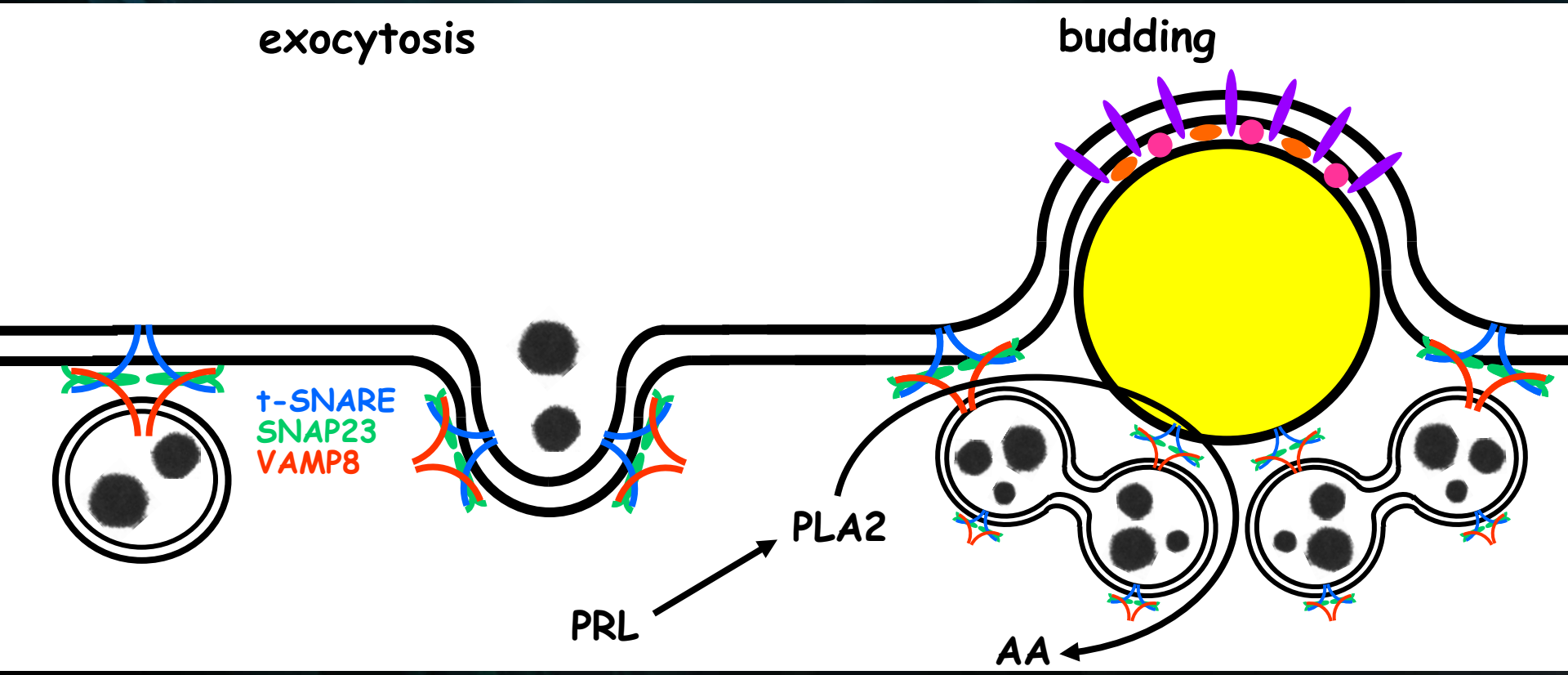
endocytosis

↑

PRL/PRL-R

Ollivier-Bousquet, 1978
Truchet et al., submitted

a possible scenario…



SNAREs as effectors of the hormonally-regulated,
spatio-temporally coordinated
milk product secretion

perspectives

- functional role of SNAREs
- different SNARE complexes
- constitutive vs. regulated secretion
- effectors of the secretagogue effect of PRL

thanks

Sophie Chat

Edith Honvo-Houeto

Céline Henry

Sarah Layani

Clémentine Mahaut

Eric Chanat

Thank you for your attention