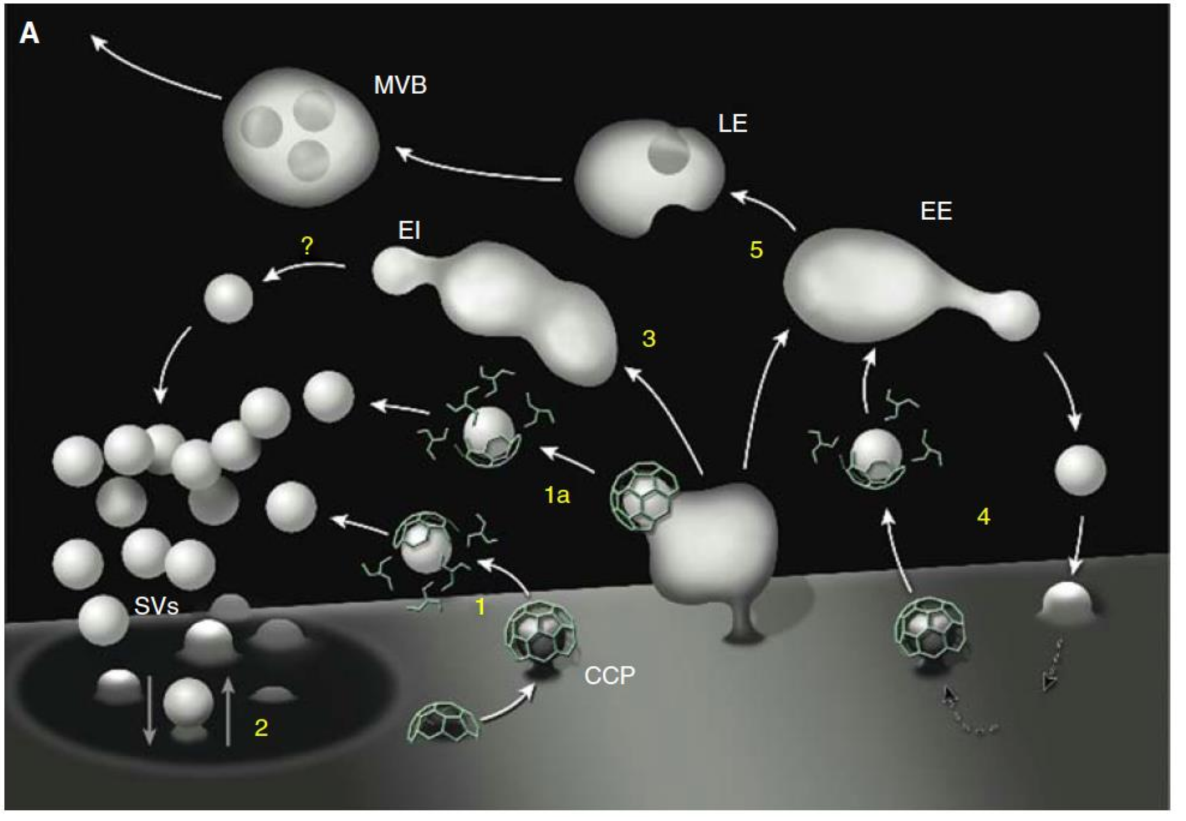
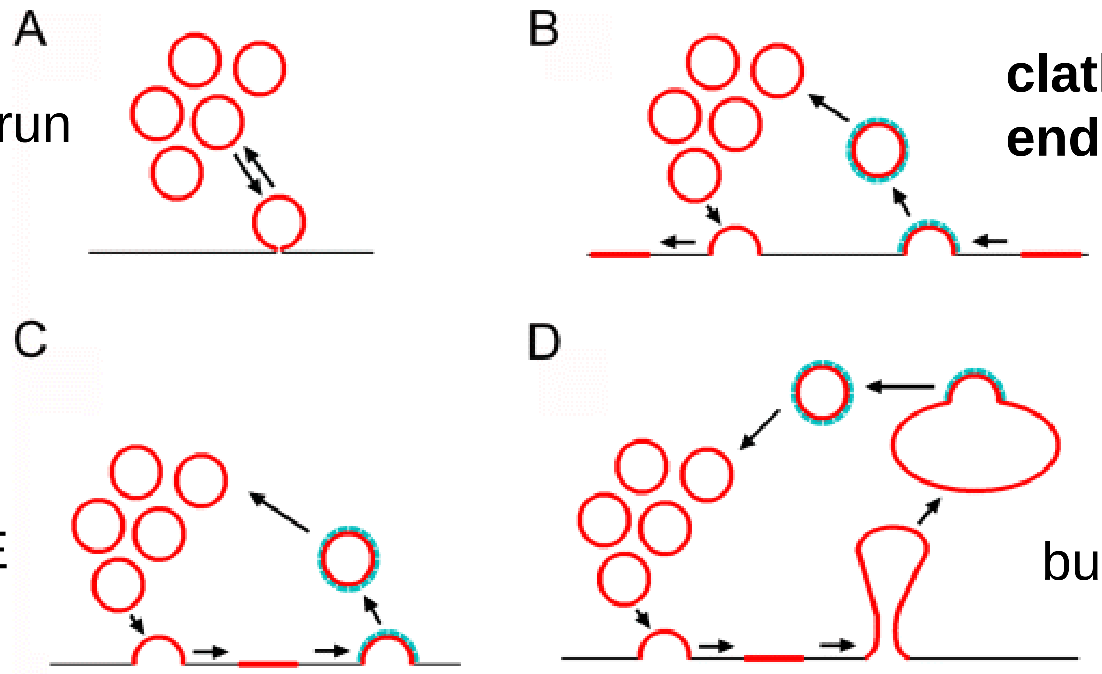
# Synaptic Vesicle Regeneration: Endocytosis




distinguish cargo
from PM proteins
deform membrane
scission

regulation
coupling to exocytosis
speed
**multiple pathways**
**to distinct exocytic pathways?**

## multiple mechanisms



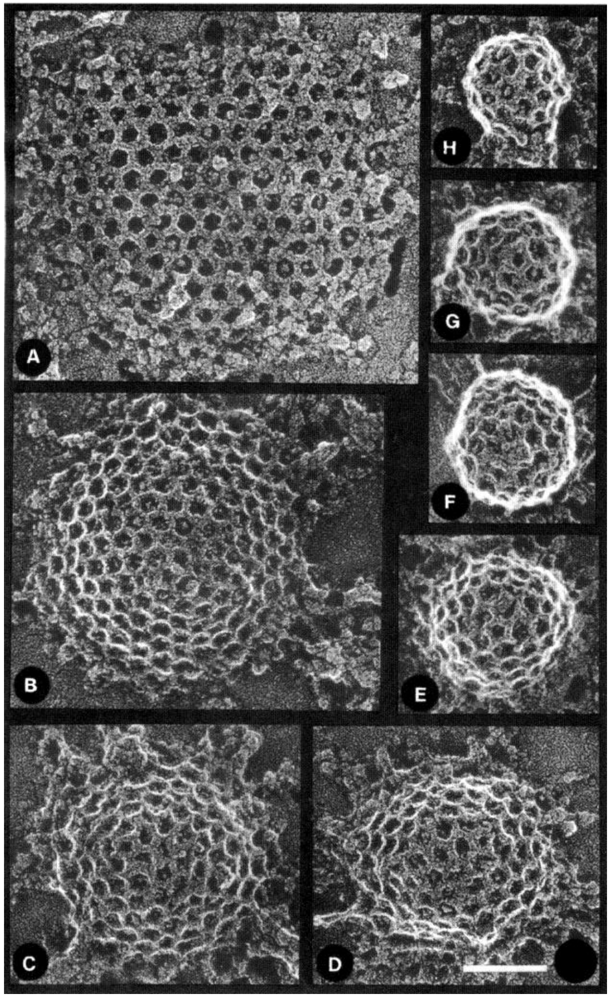

kiss-and-run

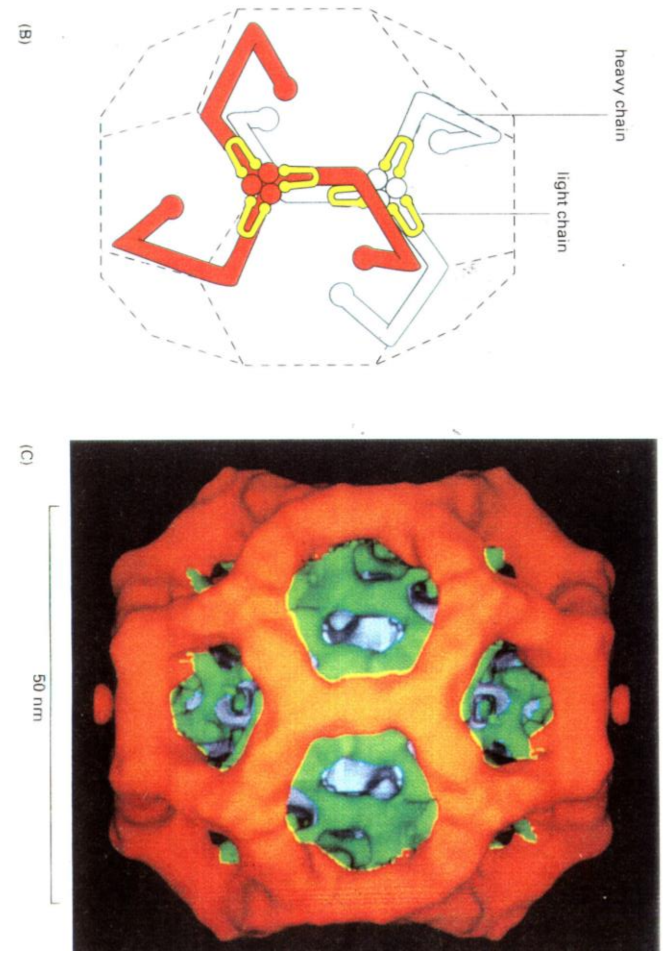
**clathrin-mediated endocytosis (CME)**

CME

bulk endocytosis

# clathrin



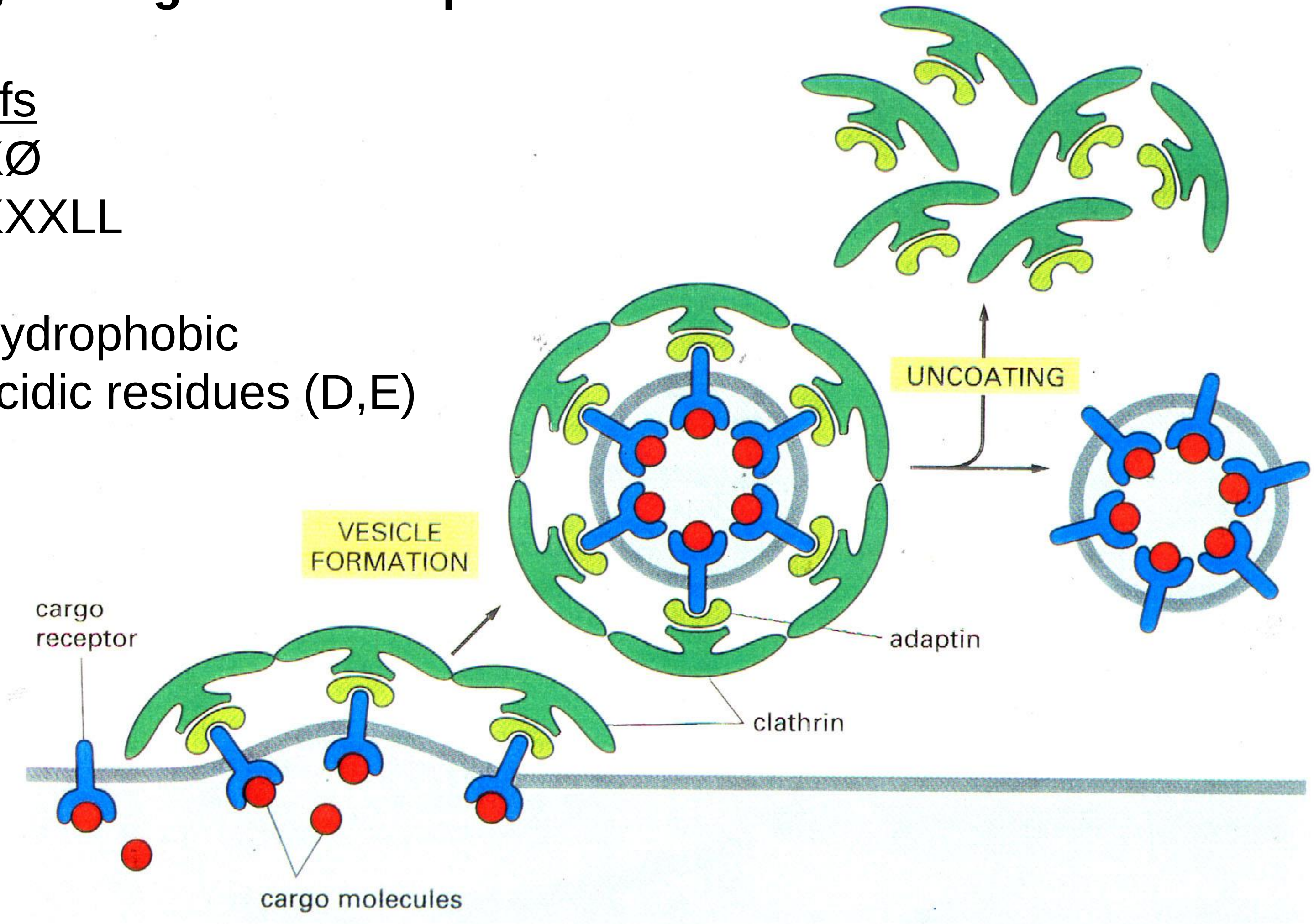




assembly of clathrin heavy chain triskelia into lattice produces invagination that may drive endocytosis

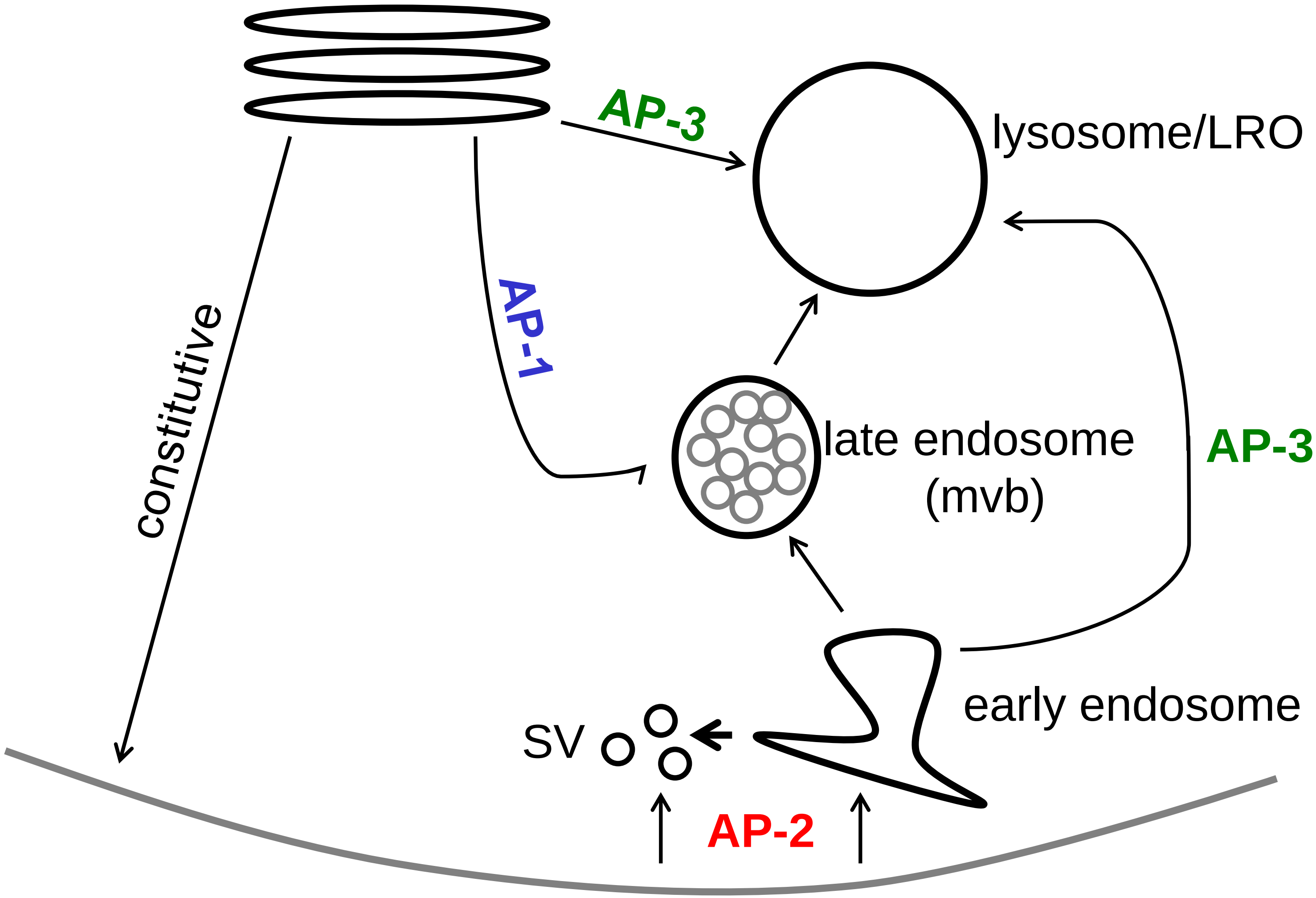
**cargo recognition: adaptors**

<u>motifs</u>
YXXØ
AAXXXLL

Ø=hydrophobic
A=acidic residues (D,E)

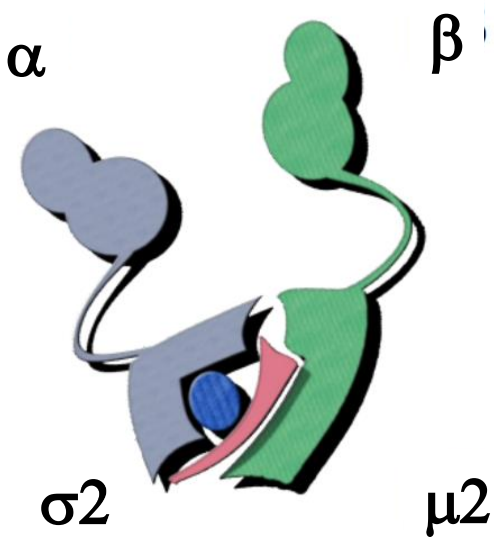



adaptor proteins bind to membrane cargo
tyrosine- or dileucine-based sorting motifs




different adaptors operate in different trafficking pathways

## AP-2




heterotetramer
α, β bind to clathrin
σ/α recognize dileucine motifs
μ recognizes tyrosine-based

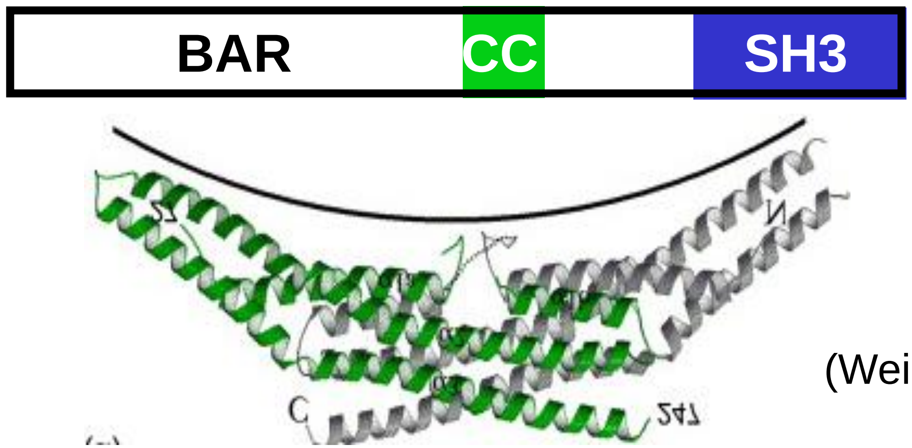
coats
AP-1,-2: clathrin
AP-3: VPS41

specialized adaptors
stonin a specialized adaptor (syt)
AP180 an adaptor for syb2

despite extensive studies,
work in *C. elegans* suggests
limited or no role for clathrin

# BAR (Bin/Amphiphysin/Rvs) domain proteins

**endophilin**

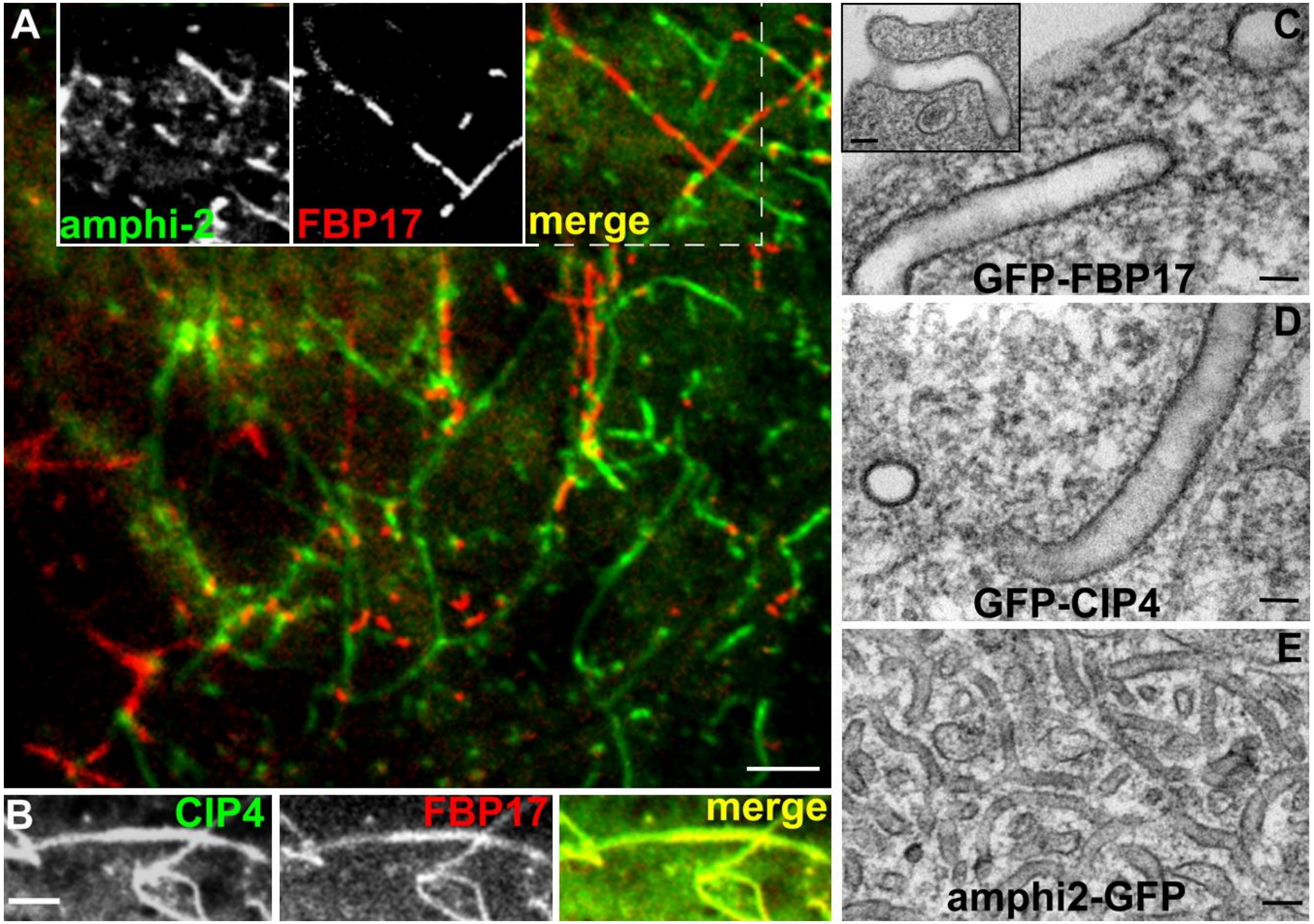



(Weissenhorn, 2004)

sense/promote membrane curvature

N-BAR higher curvature
F-BAR lower curvature
I-BAR concave

# BAR domain proteins produce tubules of different sizes

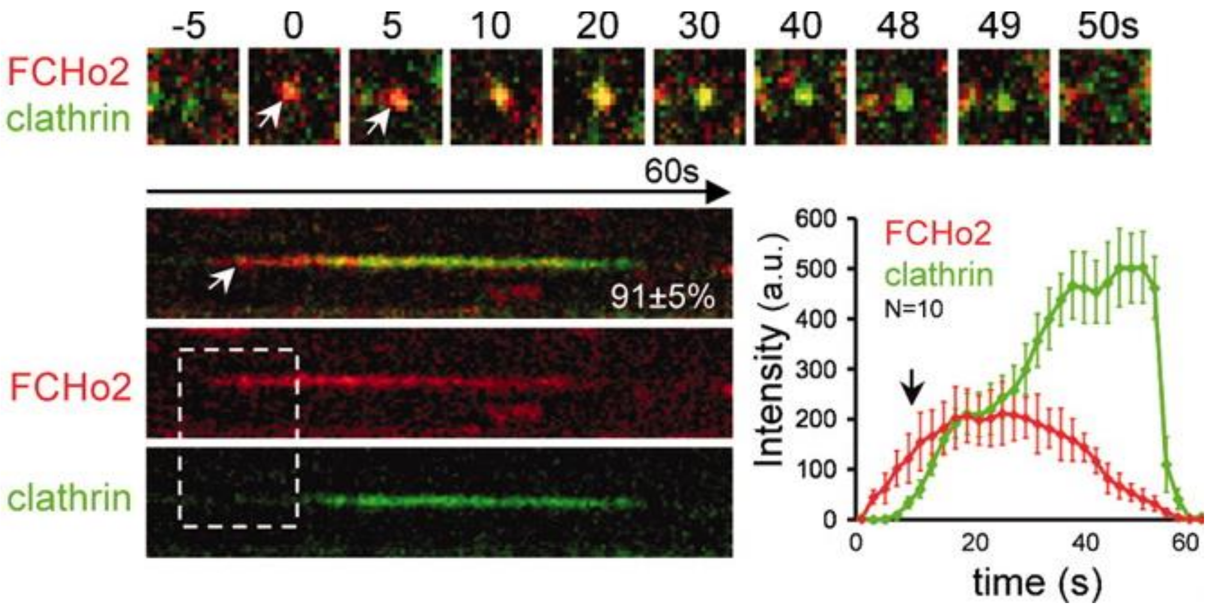



(Frost et al., 2008)

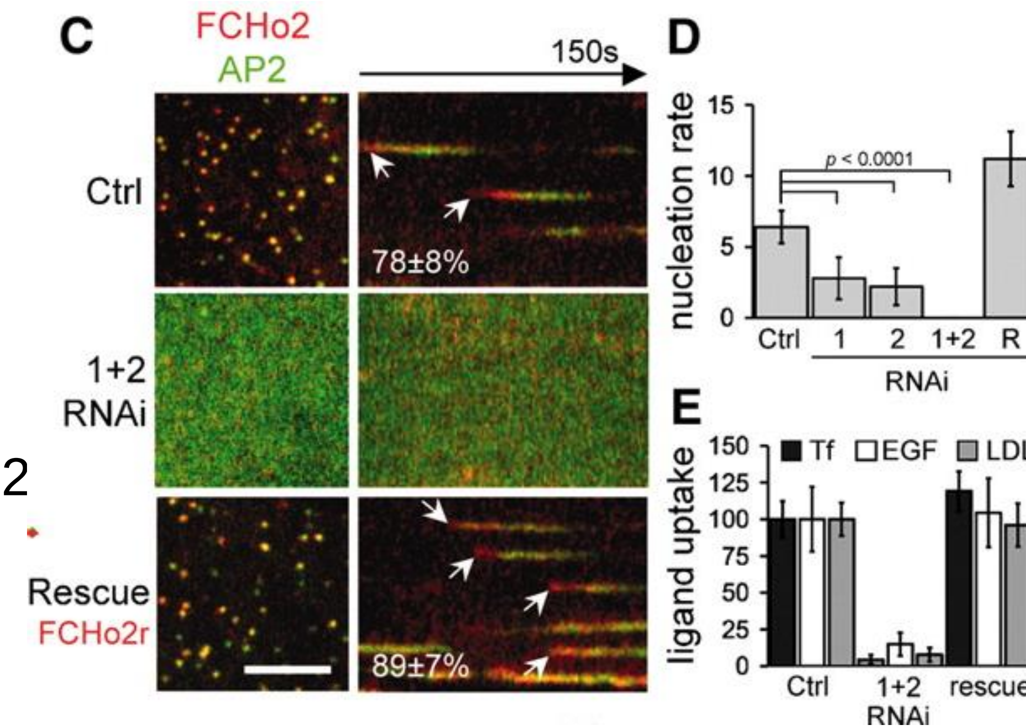
in cells as well as *in vitro*

## FCHo proteins

*precede* AP2, clathrin




(Henne et al., 2010)



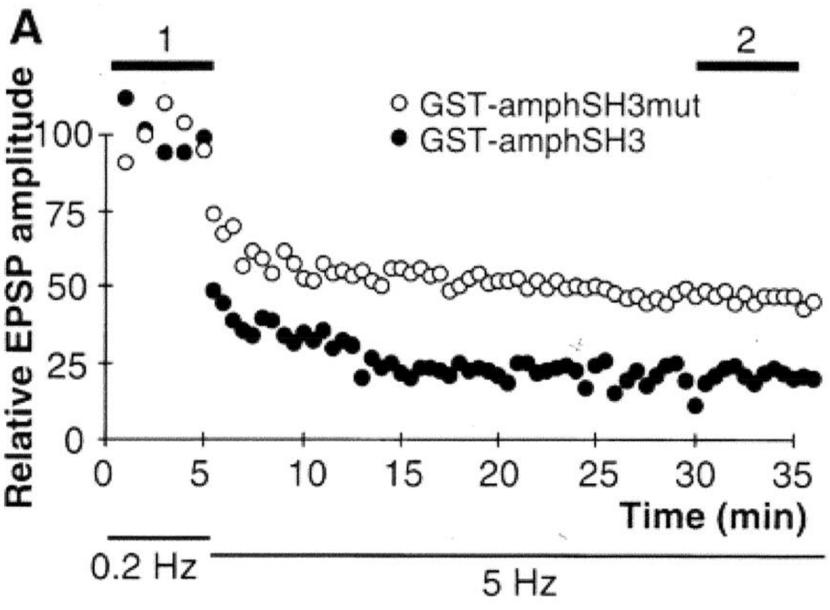

required for clathrin assembly

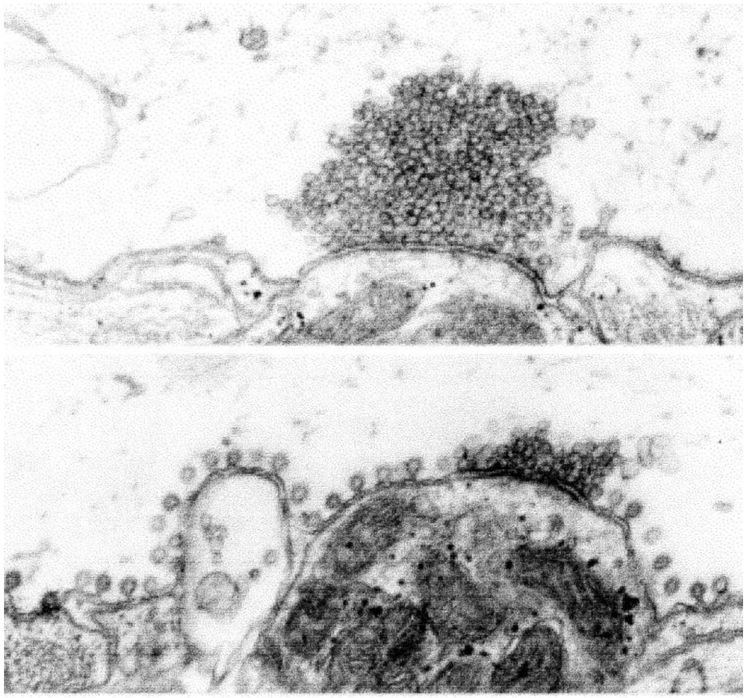
AP-2 accumulates at surface

FCHo allosterically activates AP-2

# BAR domain protein:  amphiphysin

lamprey reticulospinal synapse
(microinject nerve terminal)

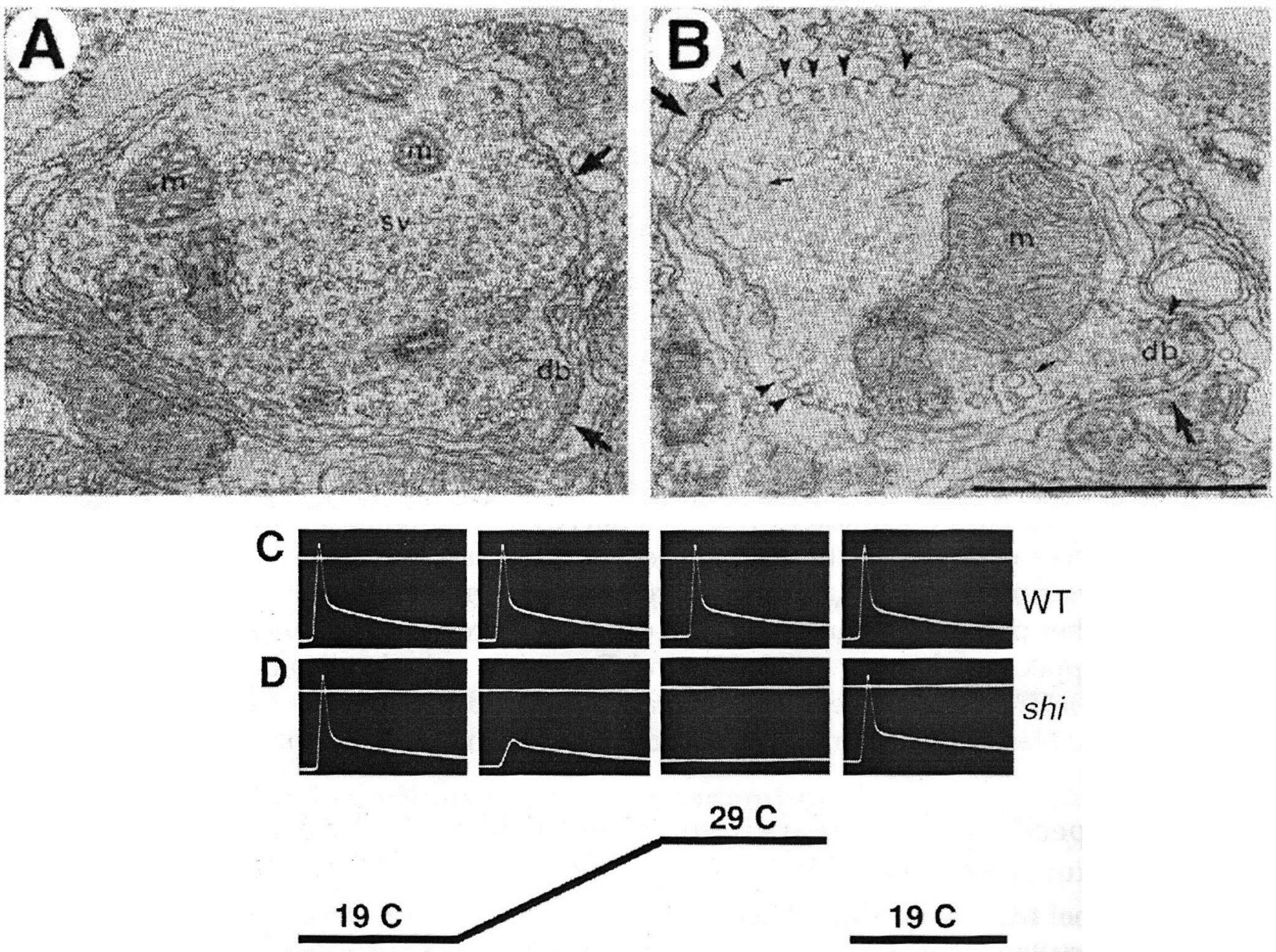


(Shupliakov et al, 1997)

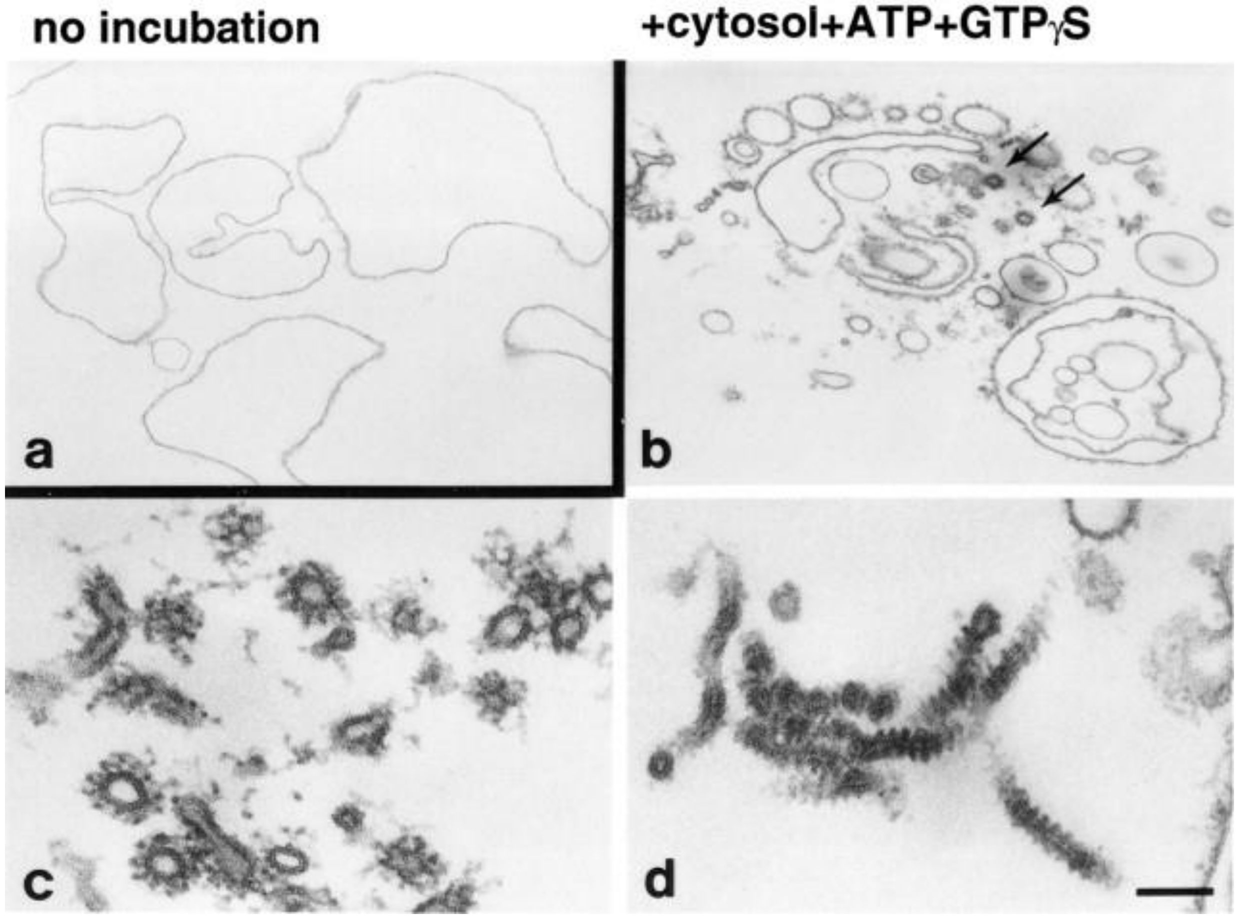
dominant negative amphiphysin blocks endocytosis
at a relatively late stage (~scission)

# **scission:** *shibire* (*Drosophila*)




ts dynamin (*shibire*) shows paralysis at non-permissive temp
failure of neurotransmission and depletion of SVs (late stage accumulates)

inside-out red blood cell membranes




clathrin

clathrin

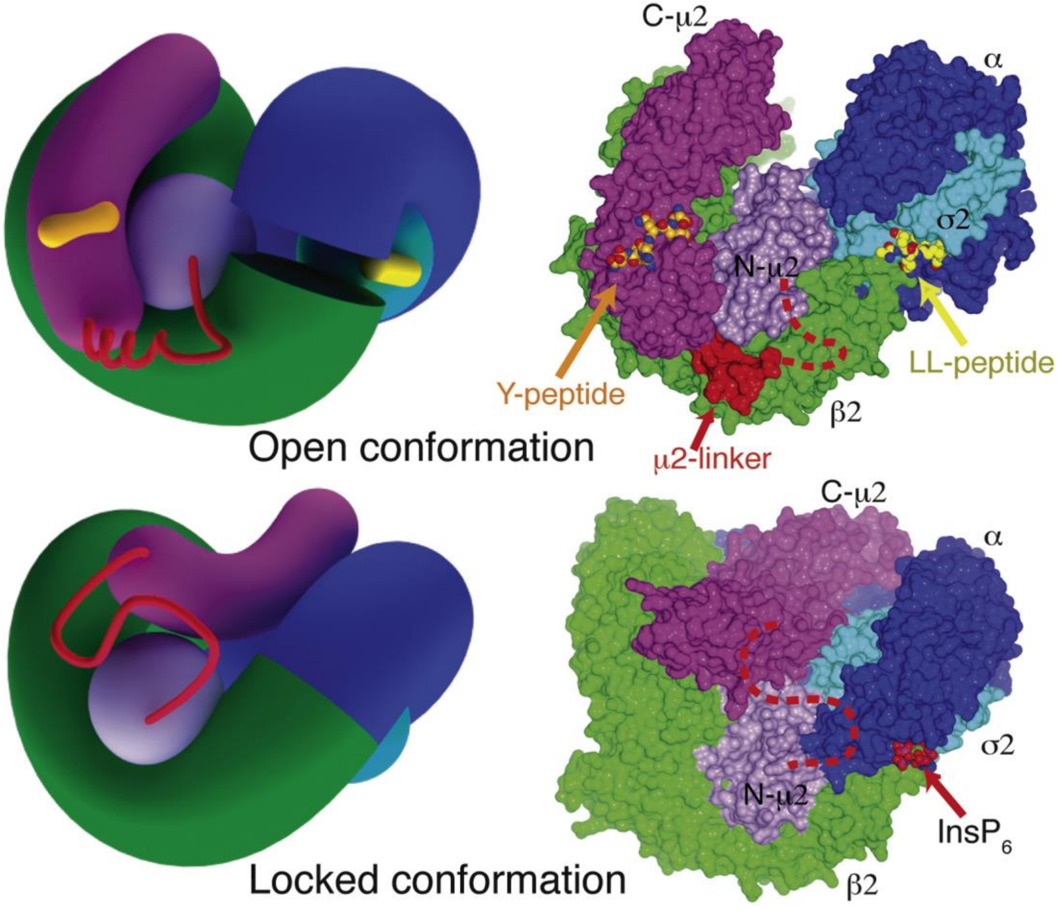
dynamin

(Takei et al, 1998)

dynamin forms collars around neck of vesicle
GTP hydrolysis triggers scission

# regulation:  why are SVs not coated with clathrin?

**AP-2**

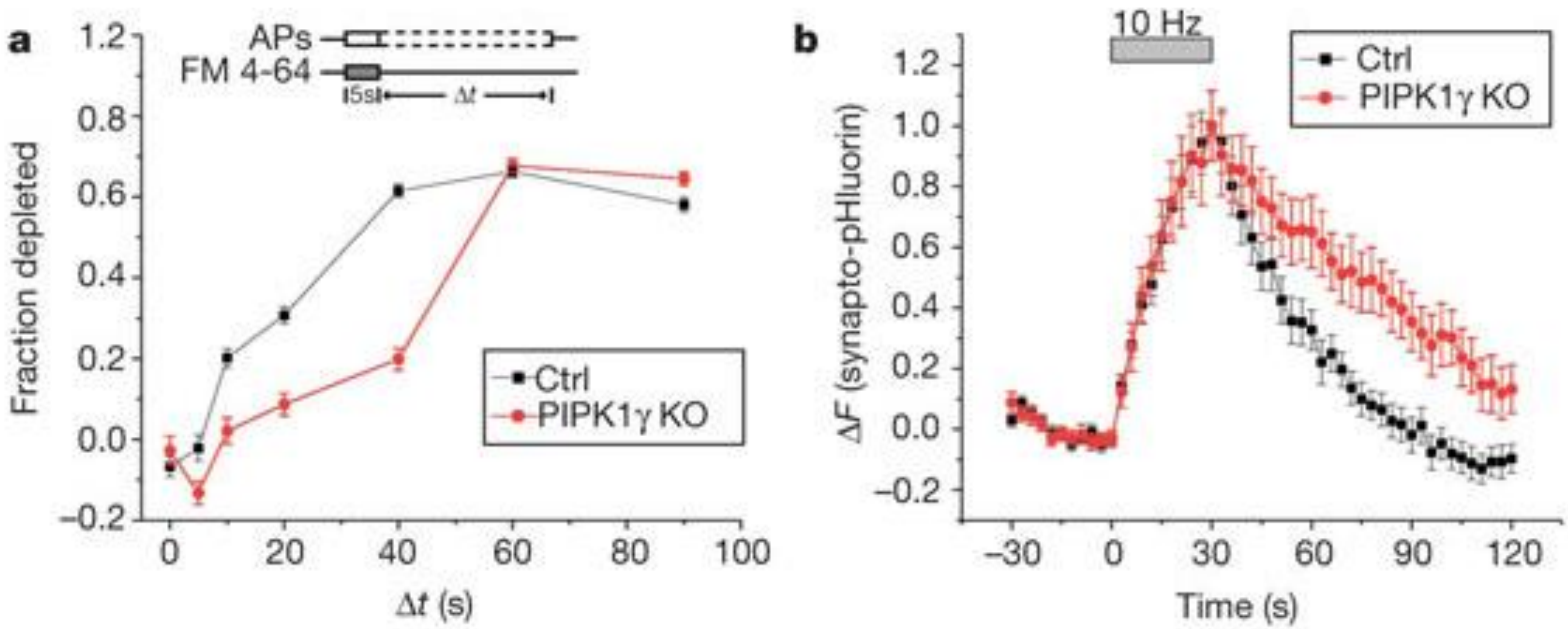



PIP2 binding unlocks cargo recognition sites

and PIP2 only in the plasma membrane (not SVs)

# PIP(4,5)2

synthesis activated by stimulation
PI4Kgamma KO:

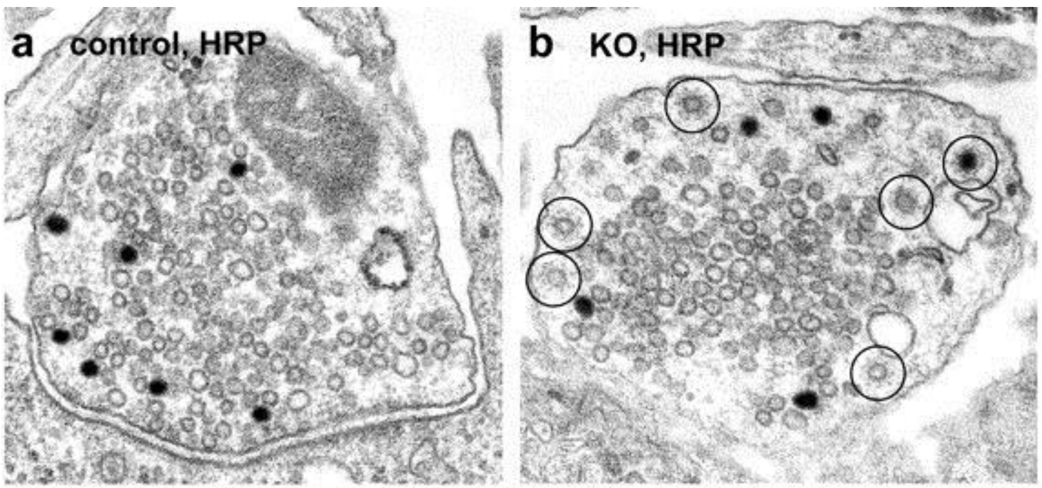


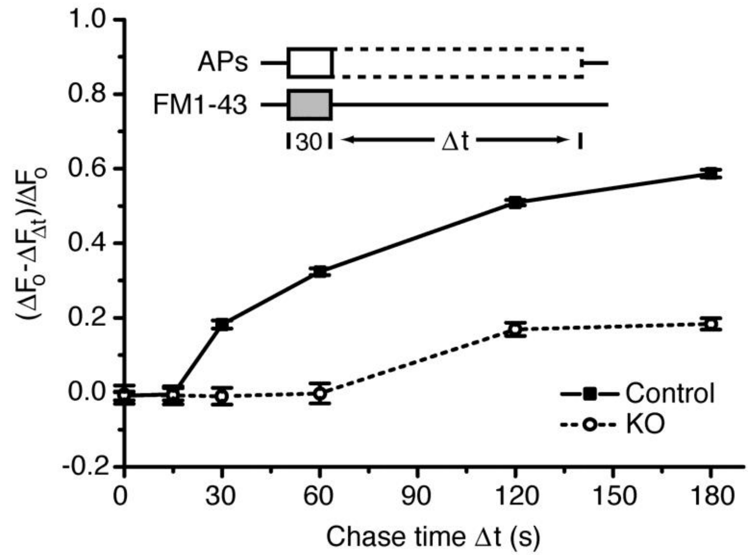

(diPaolo et al, 2004)

recycling slowed
defect in endocytosis

# uncoating

dephosphorylation of PIP2 releases AP2, clathrin
synaptojanin is a lipid phosphatase




(Kim et al, 2002)

synaptojanin KO accumulates coated vesicles
impaired SV recycling
specific mutation in Sac1 domain causes Parkinson’s

# compensatory endocytosis

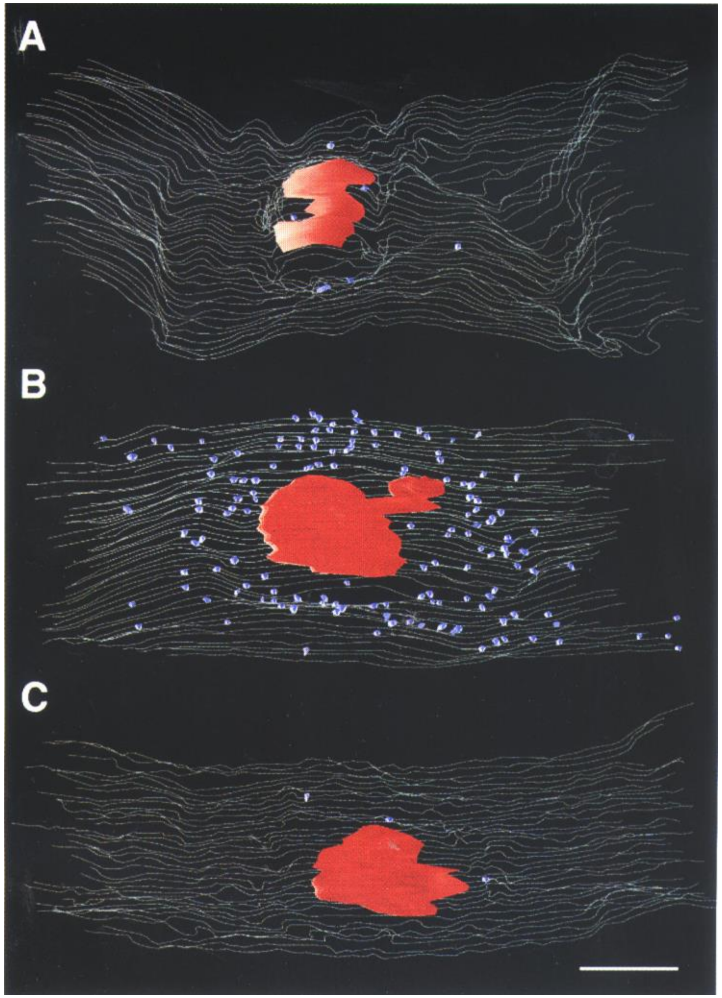
(how does endo = exo): calcium?

active zone
coated pits

0 min

40 sec

15 min




(Gad et al, 1998)

<u>lamprey reticulospinal synapse</u>
stimulated at high frequency
incubated in 0 $Ca^{++}$ for 90 min
then $Ca^{++}$ added back

$Ca^{++}$ required
but as low as 11 µM suffices

**mechanisms for compensatory endocytosis**

$Ca^{++}$ regulates rate of endocytosis but not extent

endocytic proteins dephosphorylated by increased $Ca^{++}$

presence of SV proteins at plasma membrane?

but many in substantial amounts there already

--VAMP2 (readily retrievable pool)

recognized as a complex?  STED suggests yes

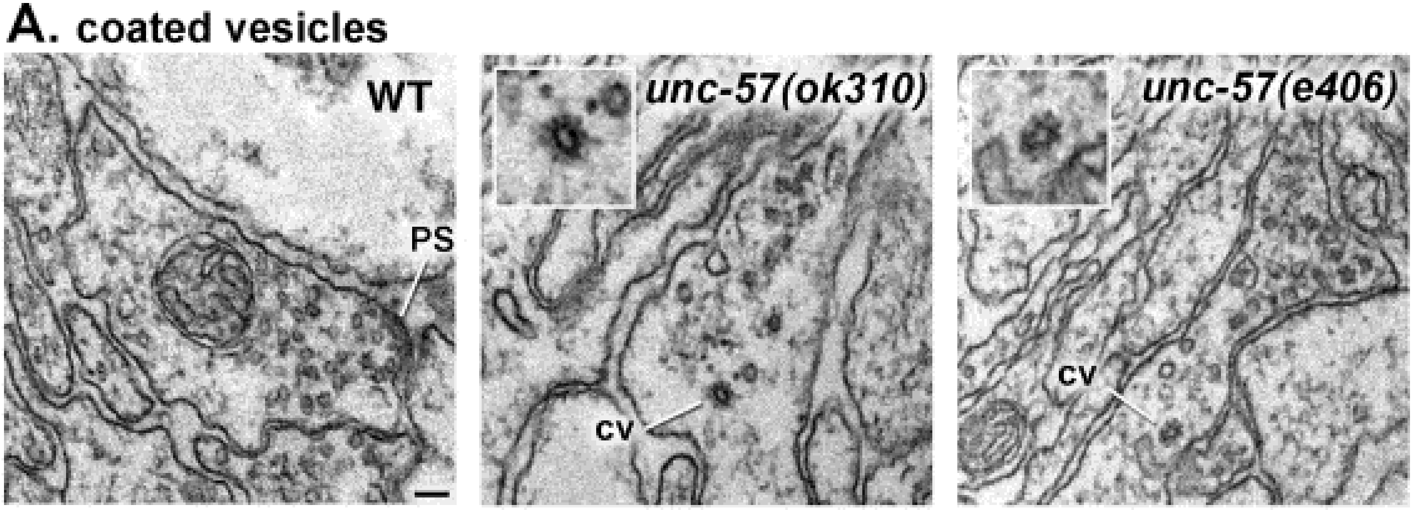
synaptotagmin thought to be receptor for AP2

*flower*:  $Ca^{++}$ channel on SVs?

delivery of endocytic proteins (endophilin)

membrane tension

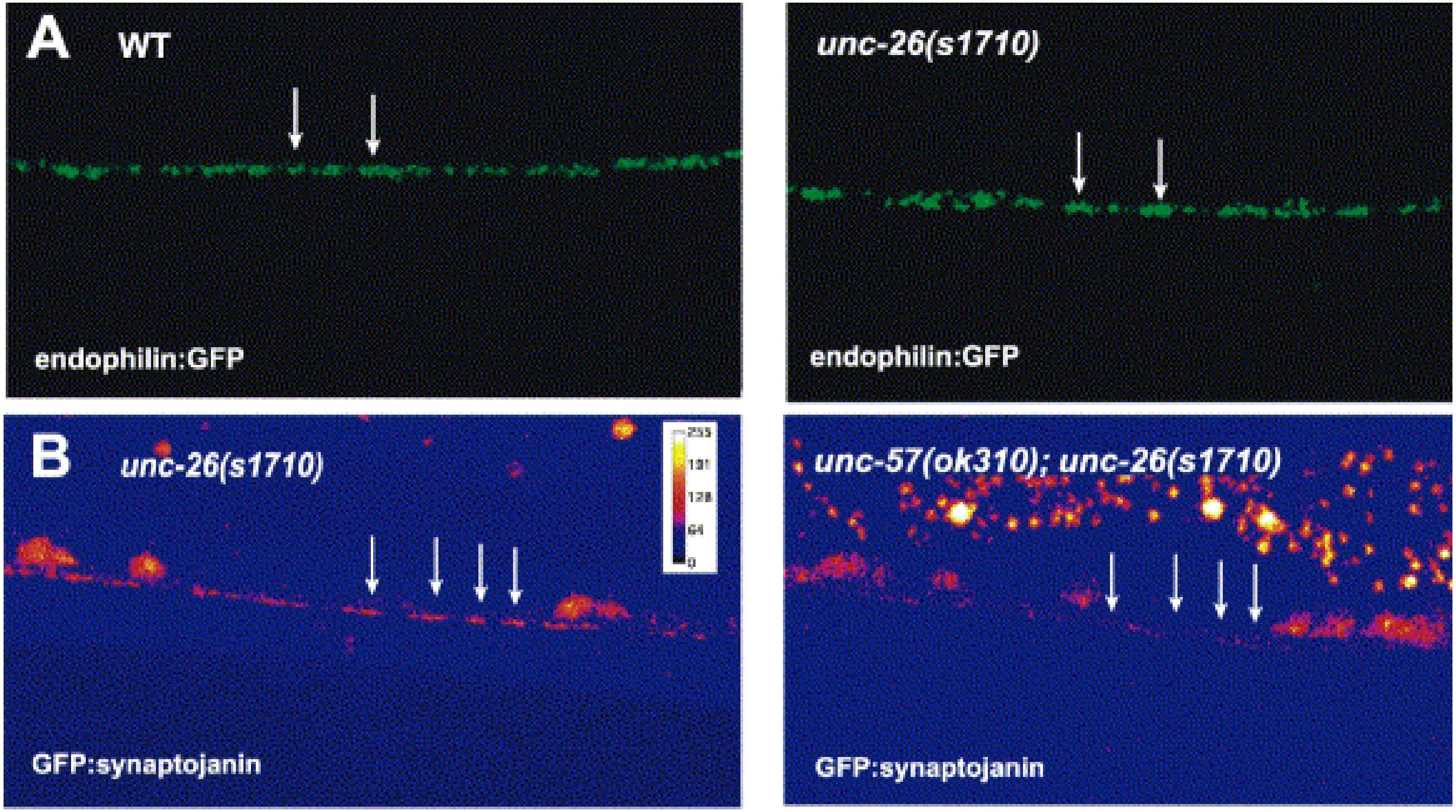
# coordination



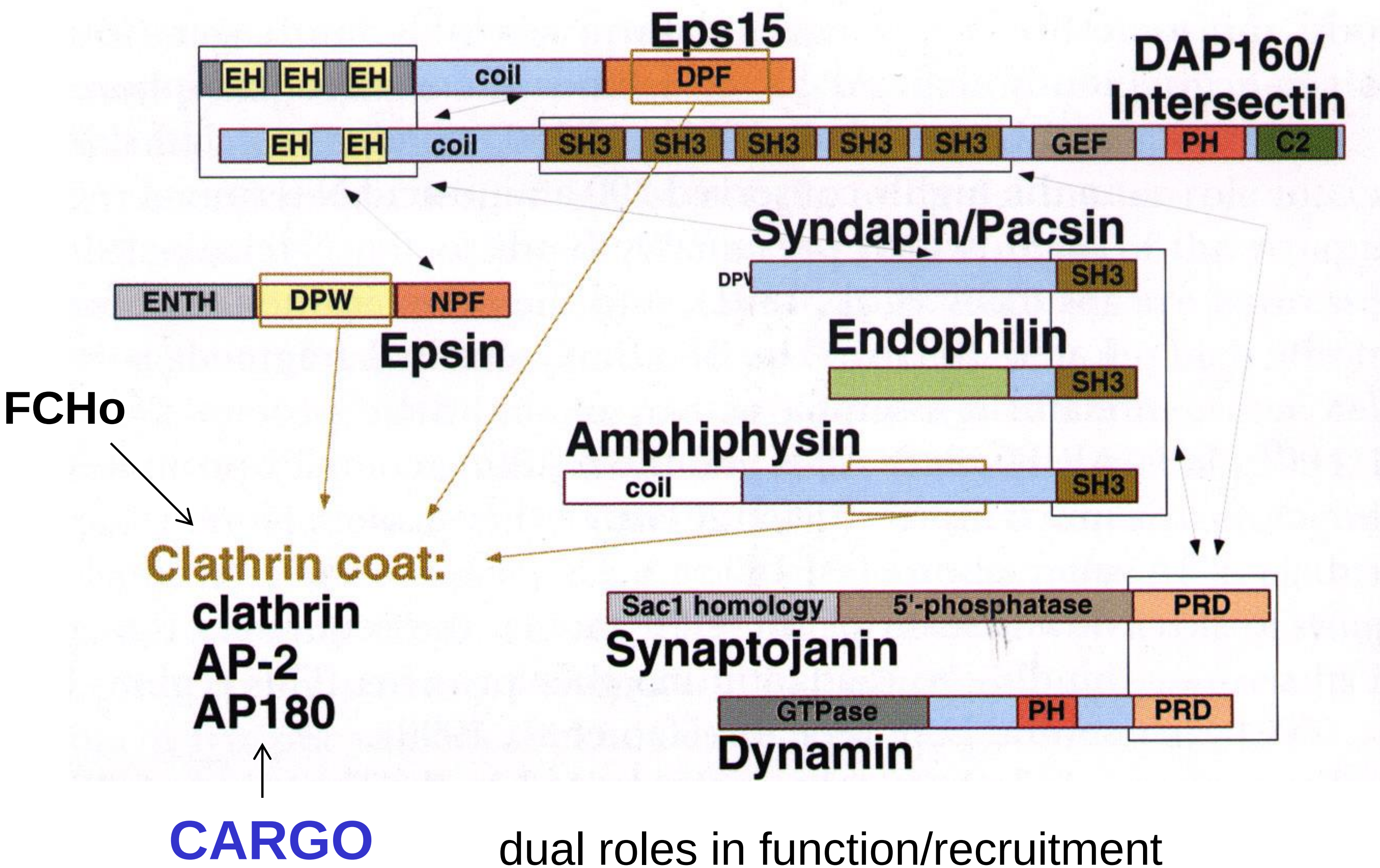

(Schuske et al, 2003)

endophilin mutant (*unc-57*) shows defect in clathrin uncoating very similar to synaptojanin mutant (*unc-26*) (*C. elegans*)

unc-57 = unc-26 and over-expression of other does not rescue --both required




endophilin required for synaptojanin localization
more recent data supports a role for BAR domain as well

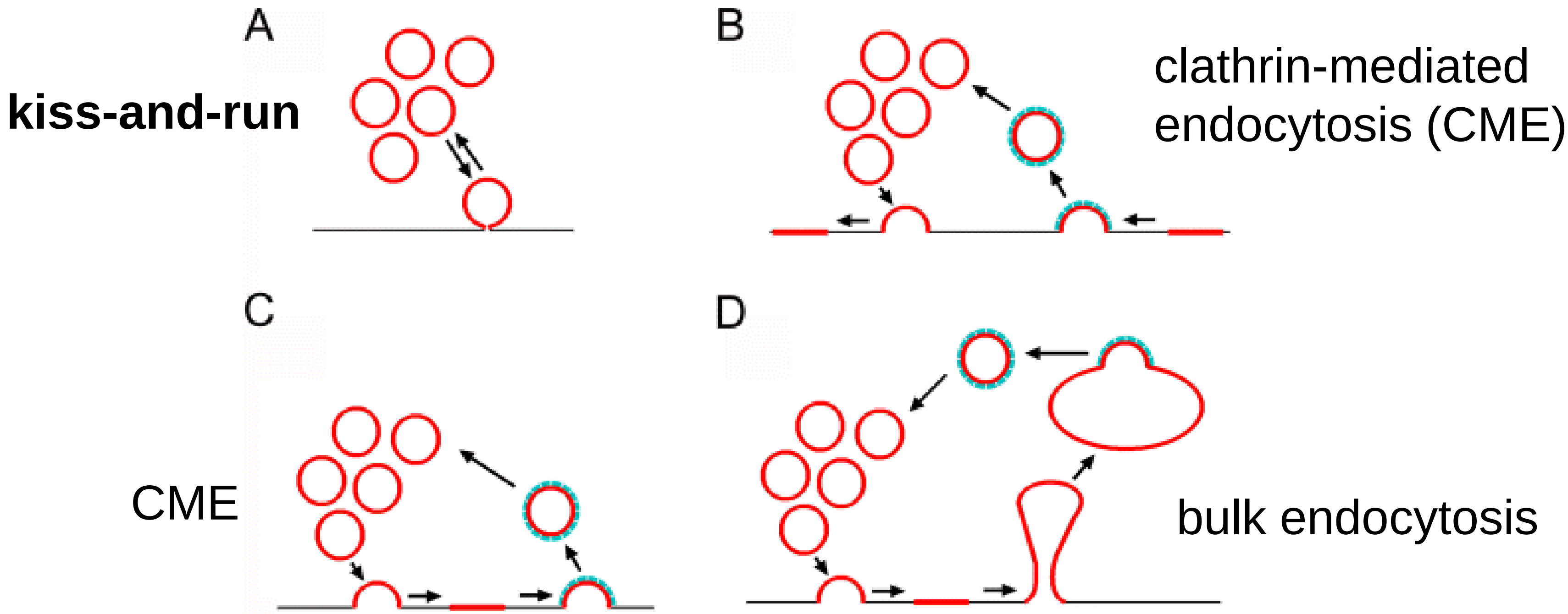



<u>dual roles in function/recruitment</u>

dynamin:  scission / amphiphysin-endophilin

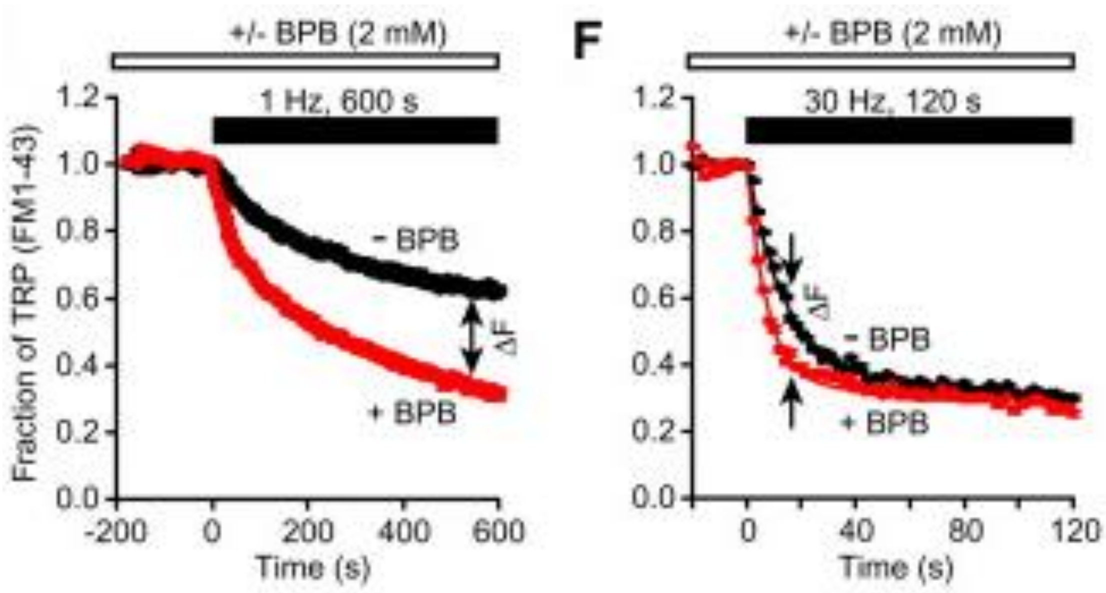
endophilin:  invagination / synaptojanin

synaptojanin:  ?fission/uncoating


A
kiss-and-run
B
clathrin-mediated
endocytosis (CME)
C
CME
D
bulk endocytosis

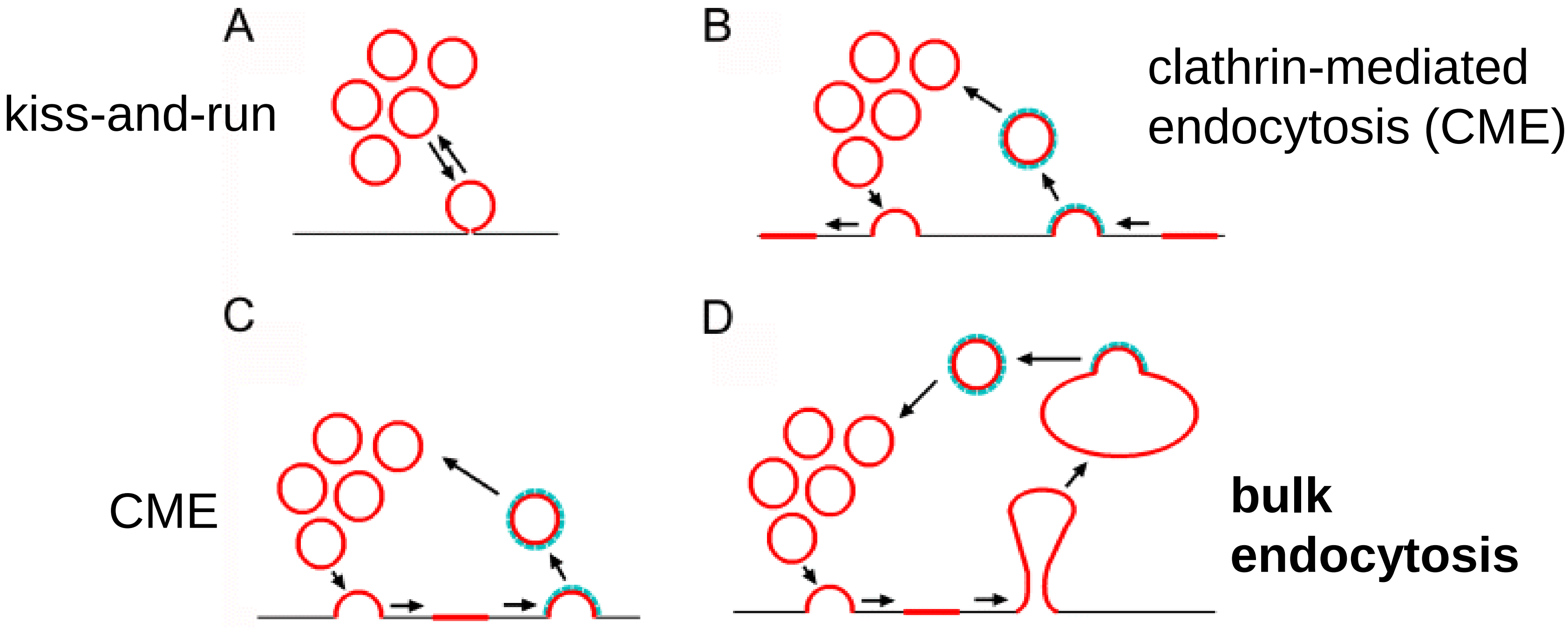
## synaptic vesicles

do SVs need more k-and-r than LDCVs?
differential unloading of FM dyes:
bigger difference at low frequency?!

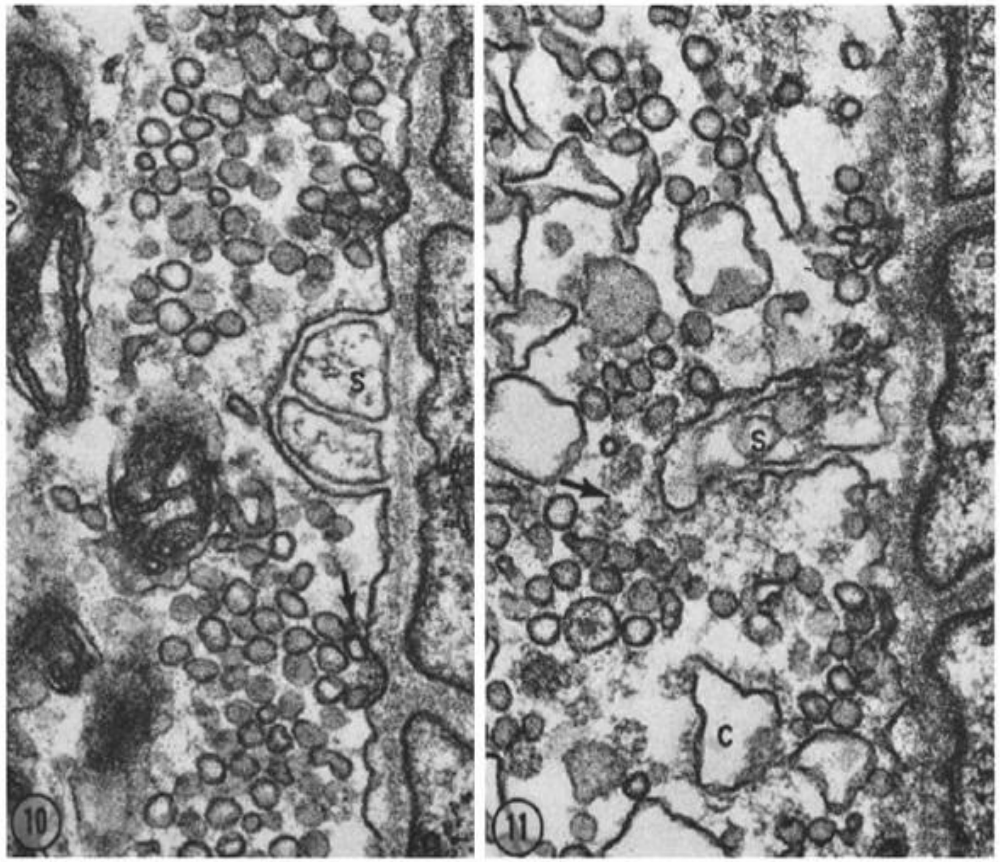



(Harata et al., 2006)

BPB accelerates loss of FM fluorescence


A
kiss-and-run
B
clathrin-mediated
endocytosis (CME)
C
CME
D
**bulk**
**endocytosis**

unstimulated stimulated (10 Hz x 15 min)

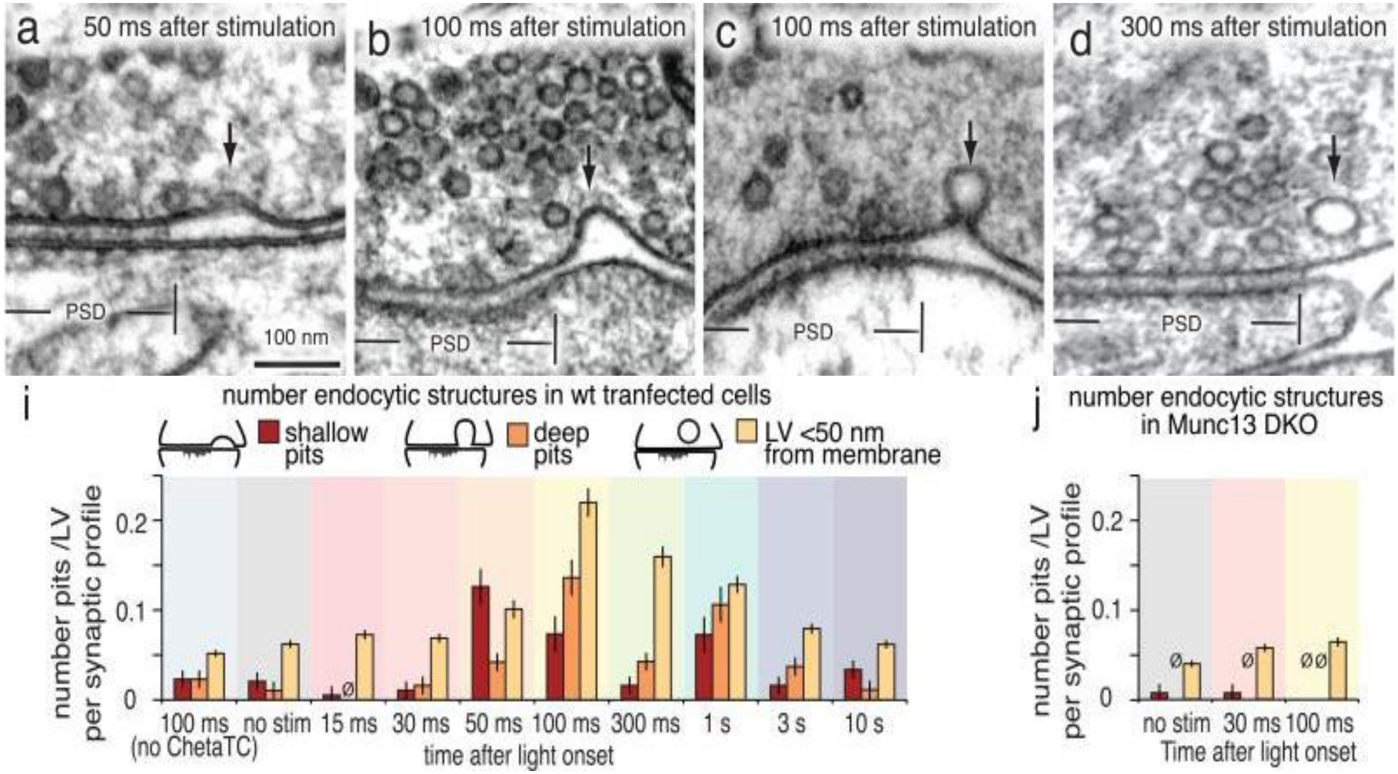



(Heuser and Reese)

follows prolonged stimulation or in absence of clathrin
SVs regenerated from cisternae (?AP3): slow
bulk endocytosis--at many synapses
requires actin, PI3 kinase (not required for clathrin)

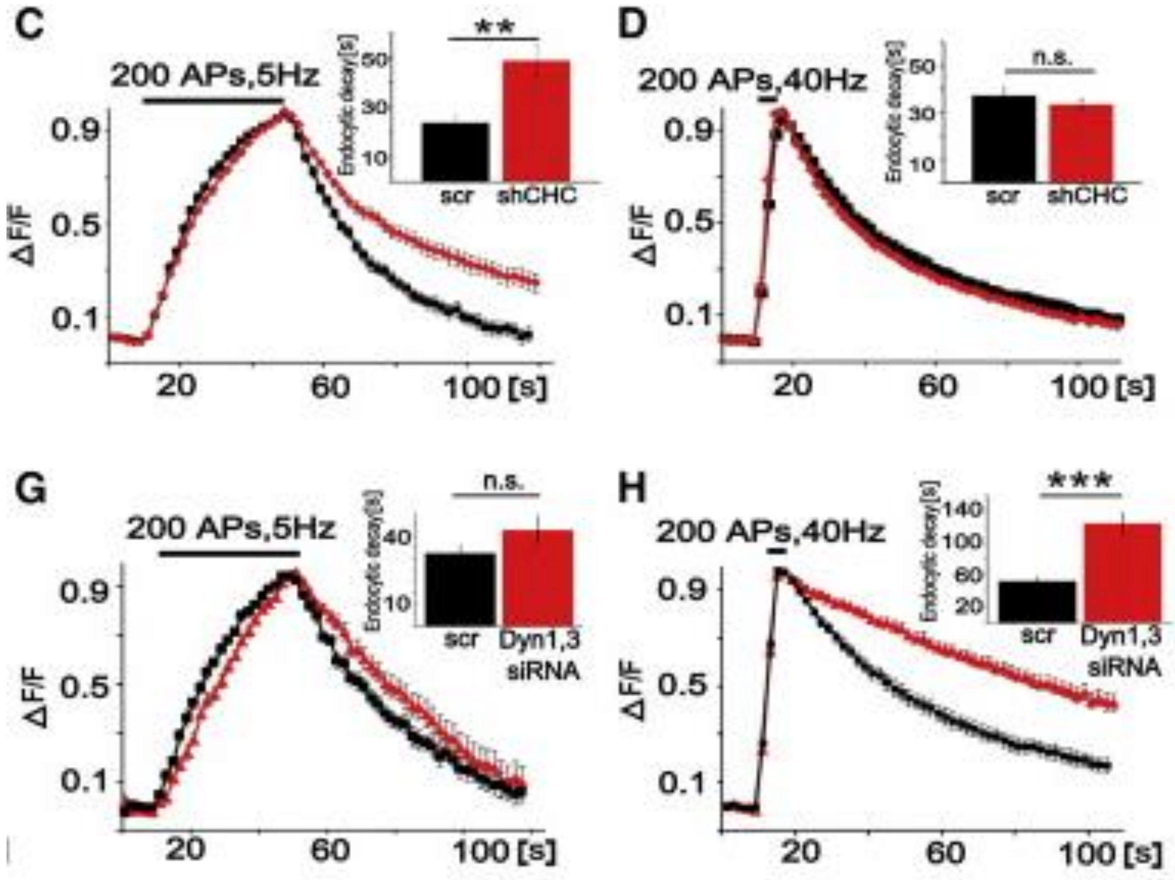
# direct visualization--freeze-slammer

ChR2 in neurons: blue light, then freeze rapidly and EM:




(Watanabe et al., 2014)

no kiss-and-run
no clathrin coated pits
larger endocytic vesicles (?bulk endocytosis?)
requires actin, dynamin, ?endophilin (not clathrin)
only at physiological temperature?!

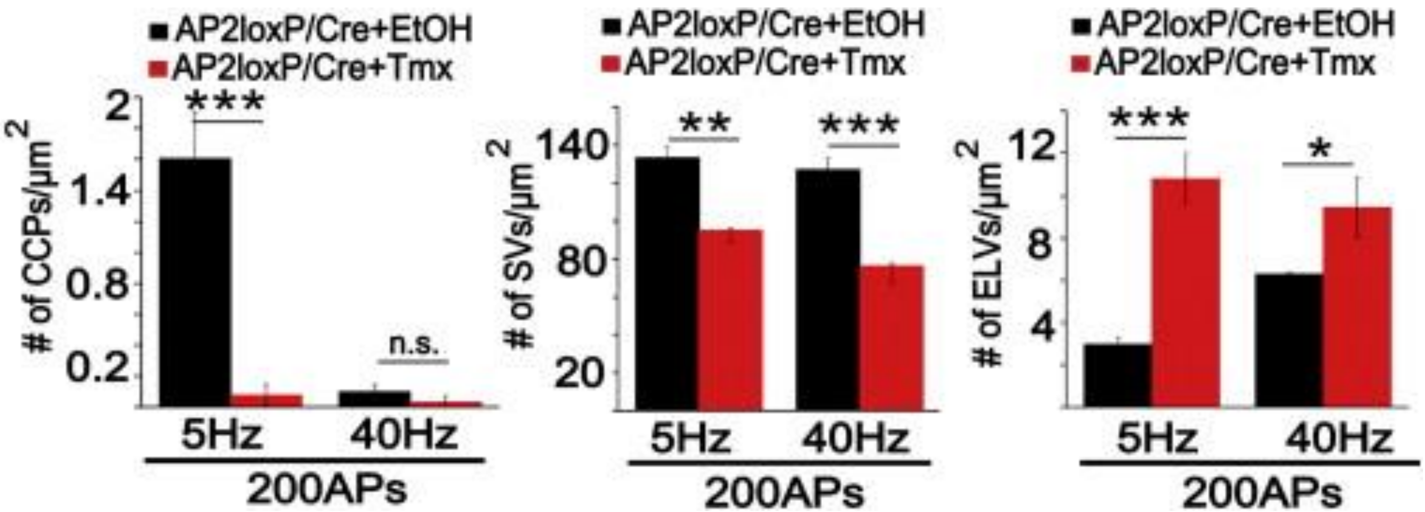


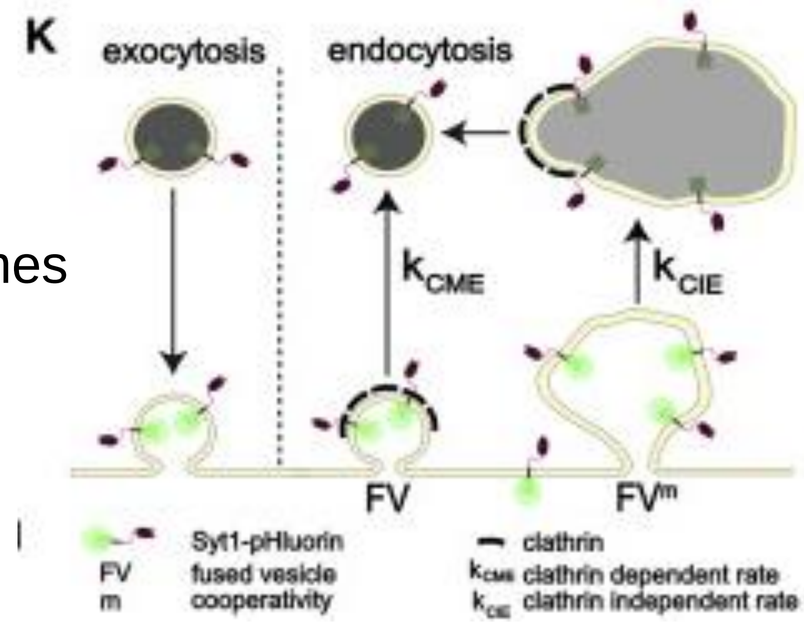

clathrin RNAi

dynamin RNAi

(Kononenko et al., 2014)

endocytosis after low, high frequency stimulation differ in mechanism

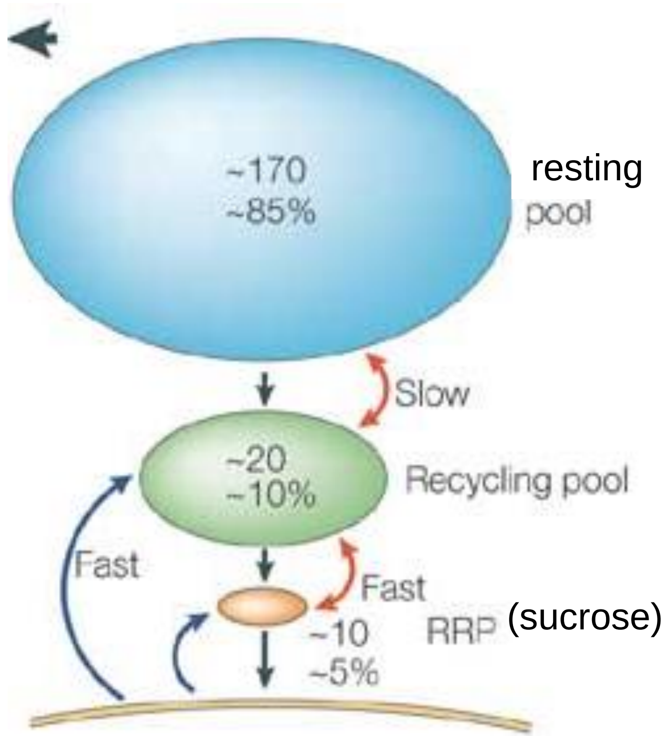



clathrin/AP2 are still important

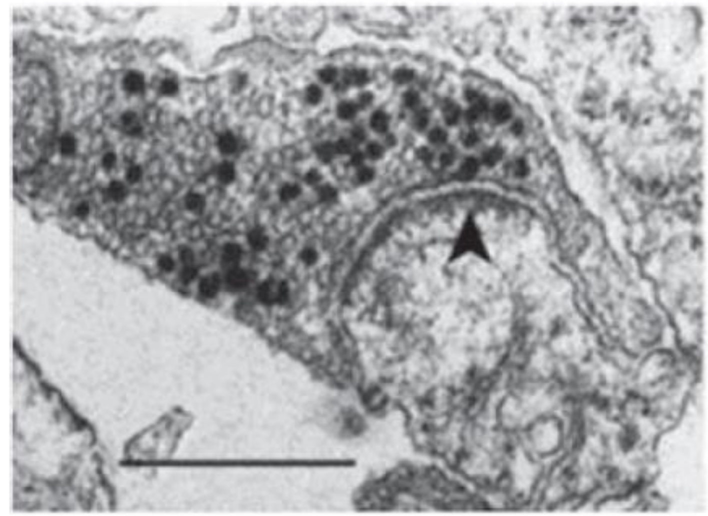
- --in SV formation from endosomes
- --not endocytosis

# SV Pools




(Rizzoli and Betz, 2005)

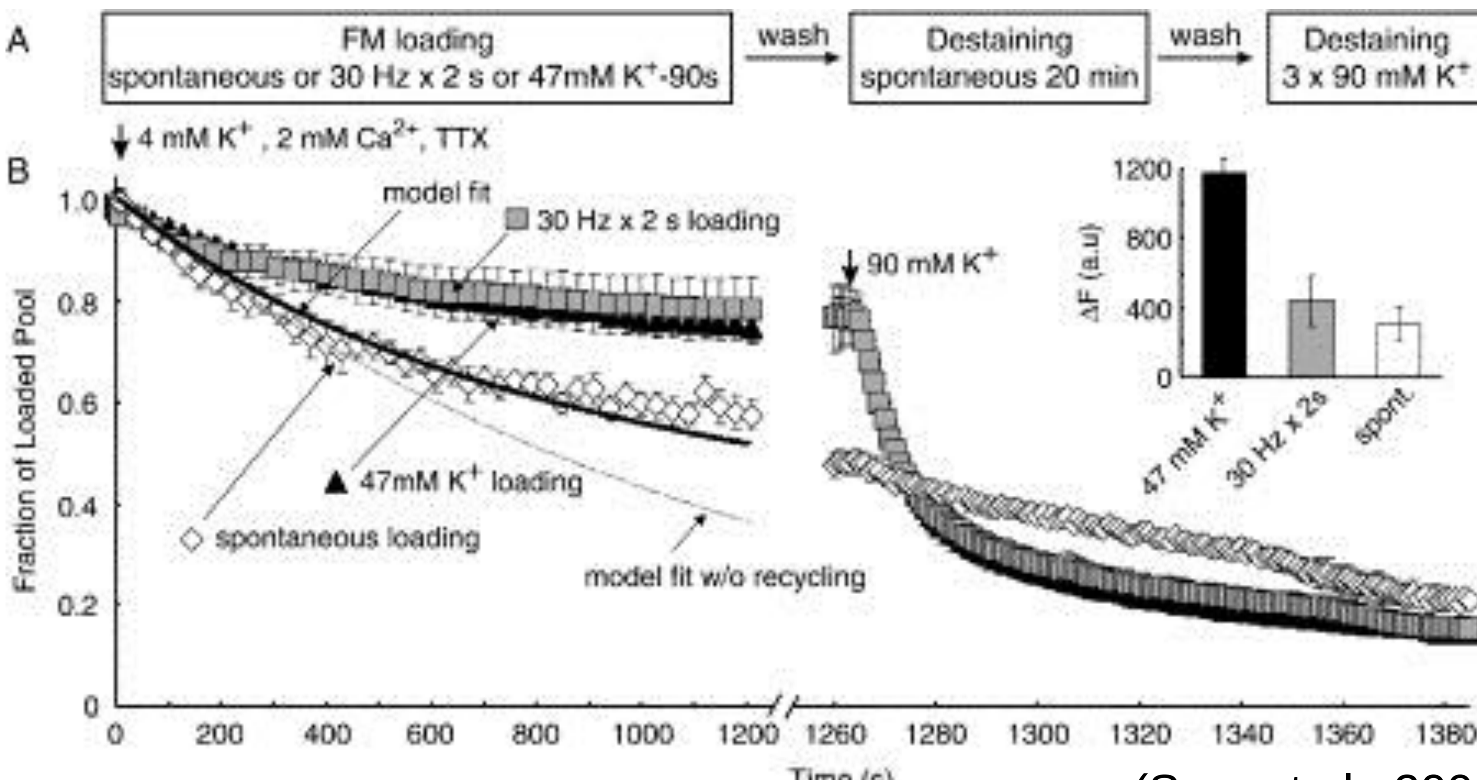
**FM dye photoconversion**



(Harata et al., 2001)

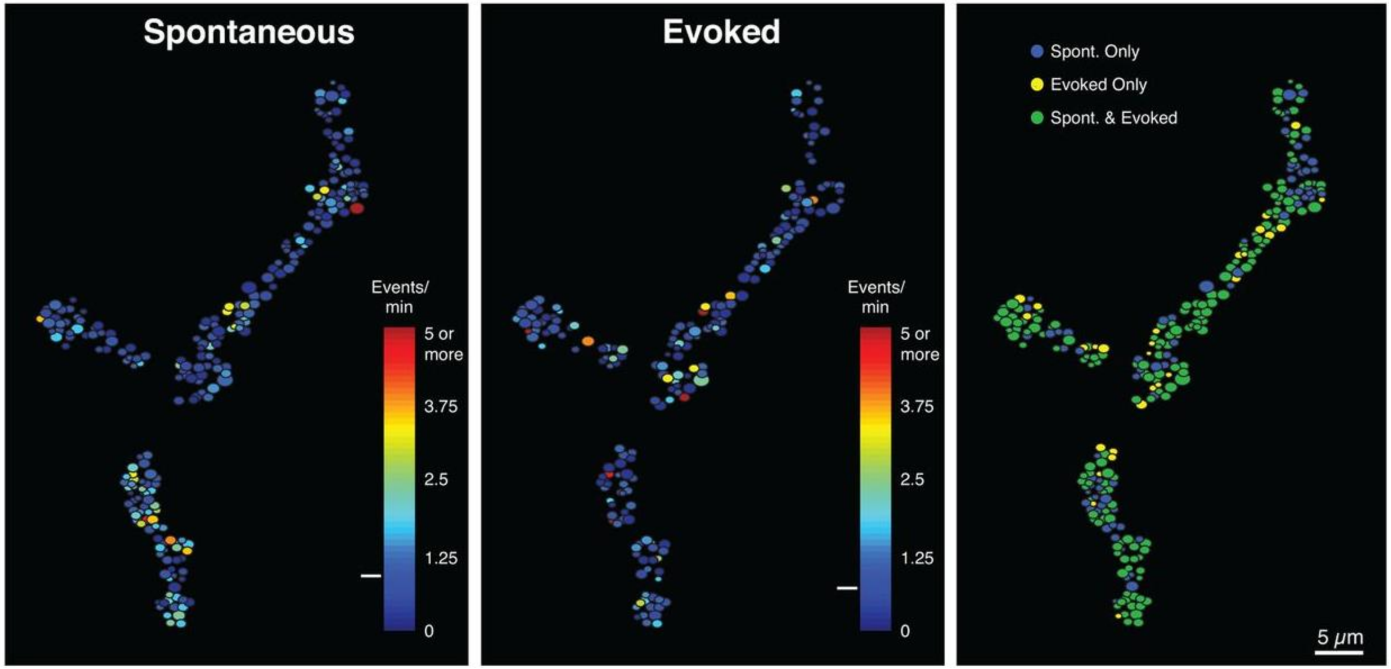
extreme functional heterogeneity
differ in history, association (e.g., cytoskeleton)?
--interconvertible at different rates?
or biochemically distinct?

do different endocytic pathways make different SVs?

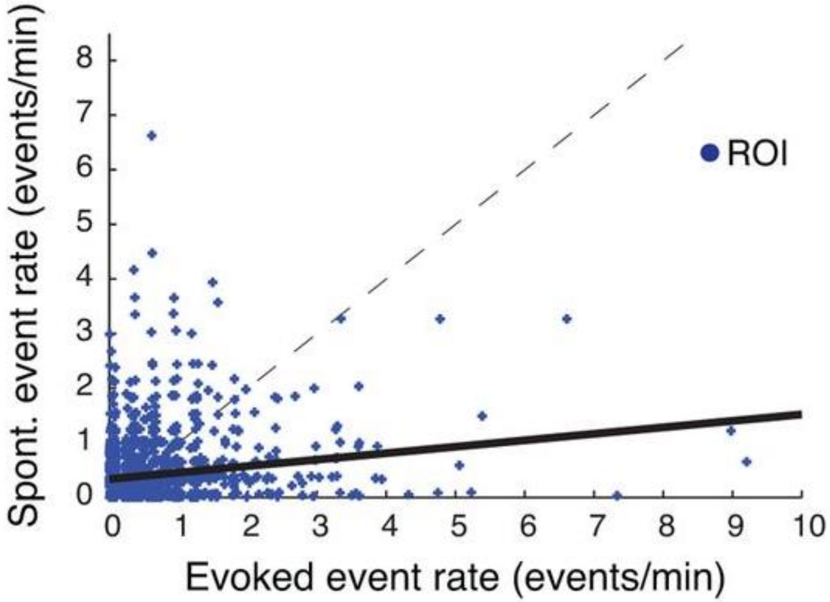



(Sara et al., 2005)

spontaneous release of spontaneously loaded SVs
evoked release of SVs loaded by stimulation
--distinct pools retain their identity after recycling




(Melom et al., 2013)




*Drosophila* neuromuscular junction

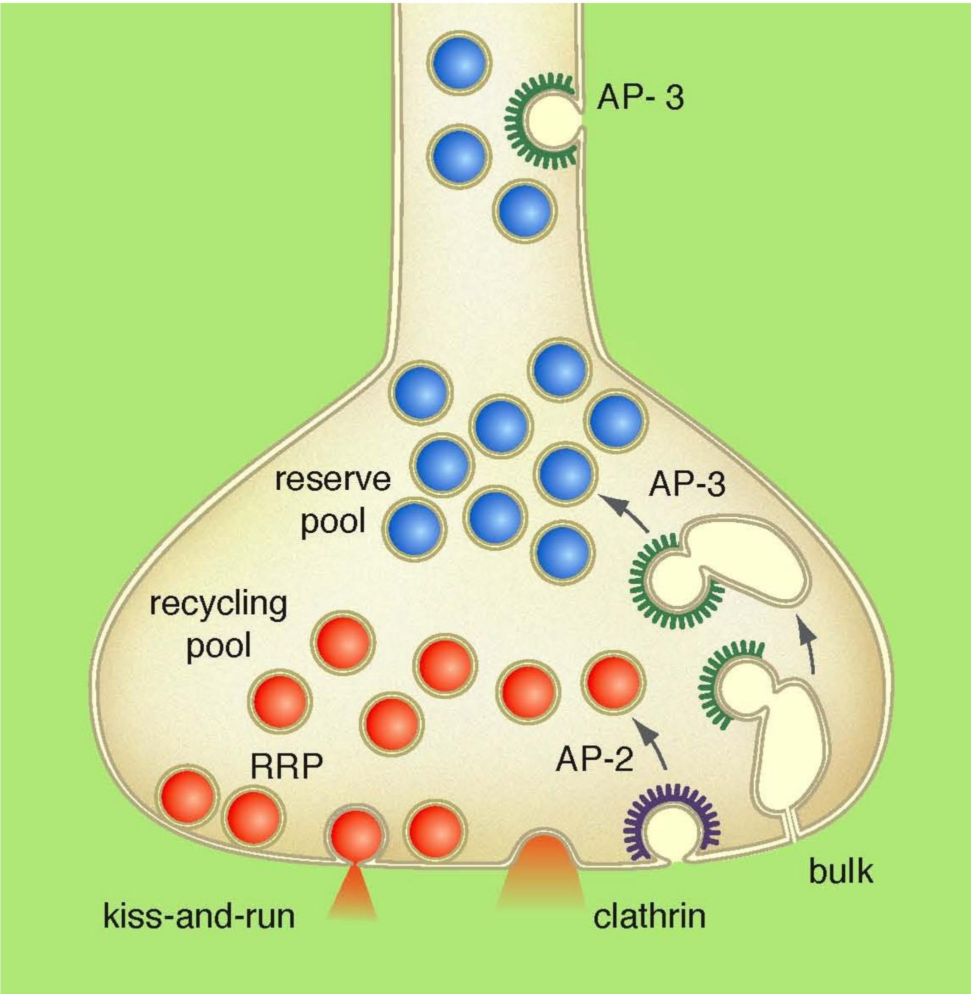
postsynaptic $Ca^{++}$ imaging

spontaneous and evoked

release at different sites

--not correlated

maturation state vs. diff pools?



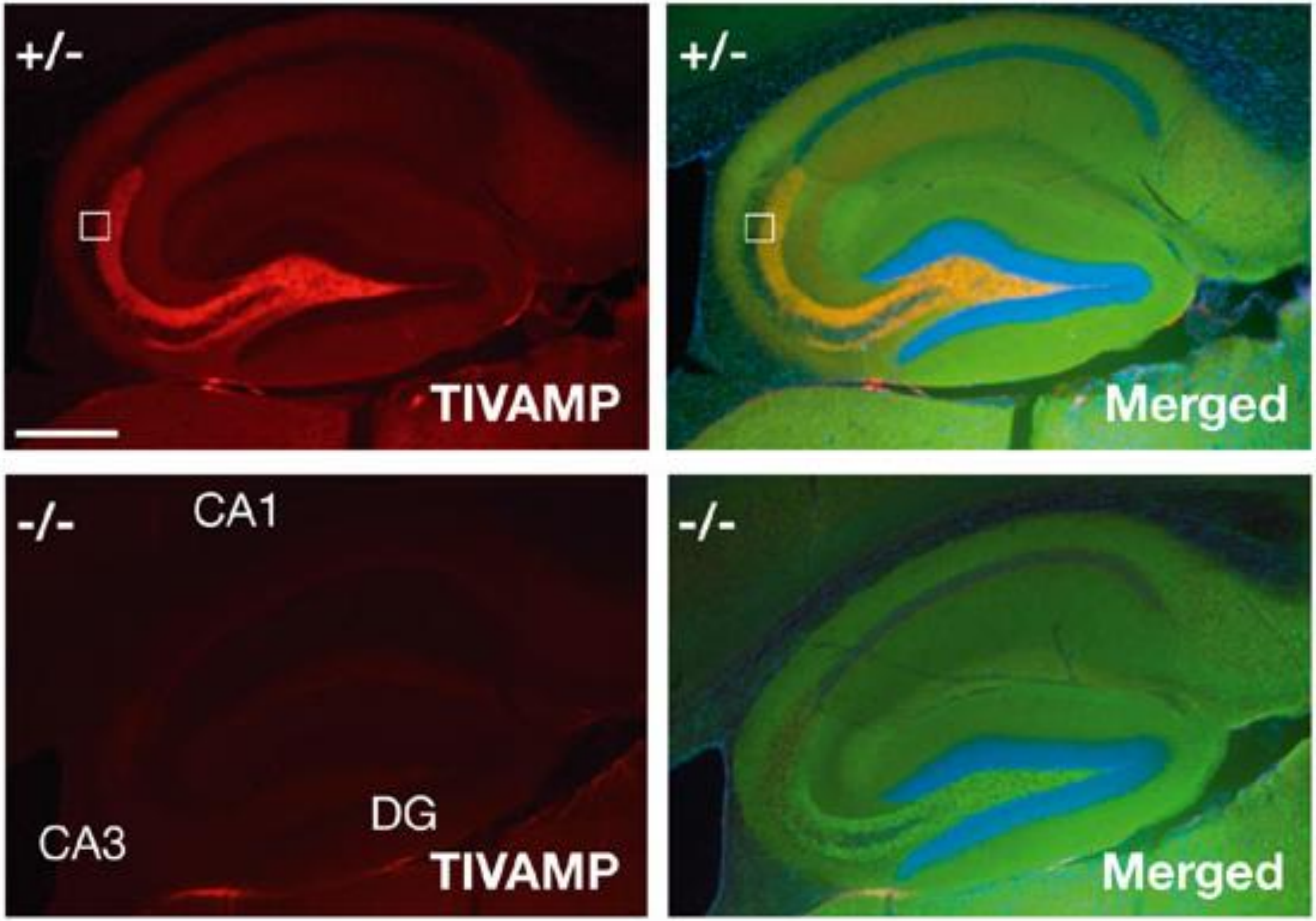

perhaps different endocytic pathways make different pools

TI-VAMP (VAMP7)/synaptophysin/DAPI

(Scheuber et al, 2006)

WT

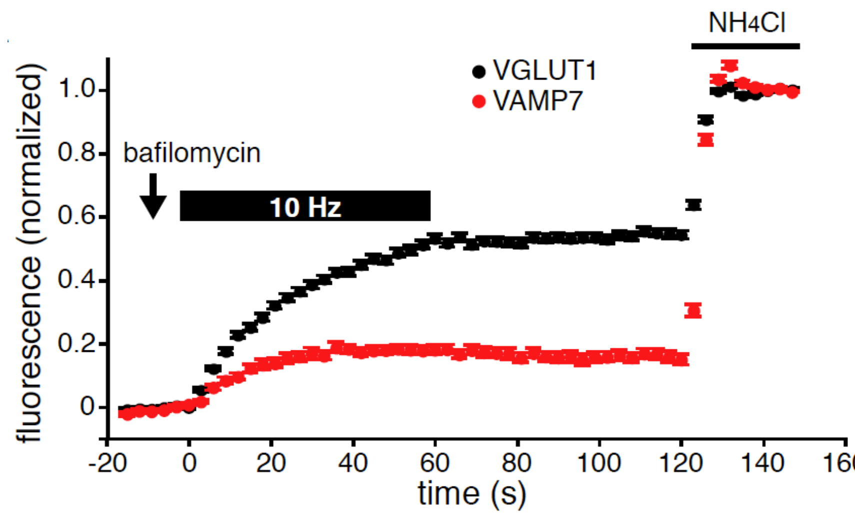
*mocha*
(no AP-3)



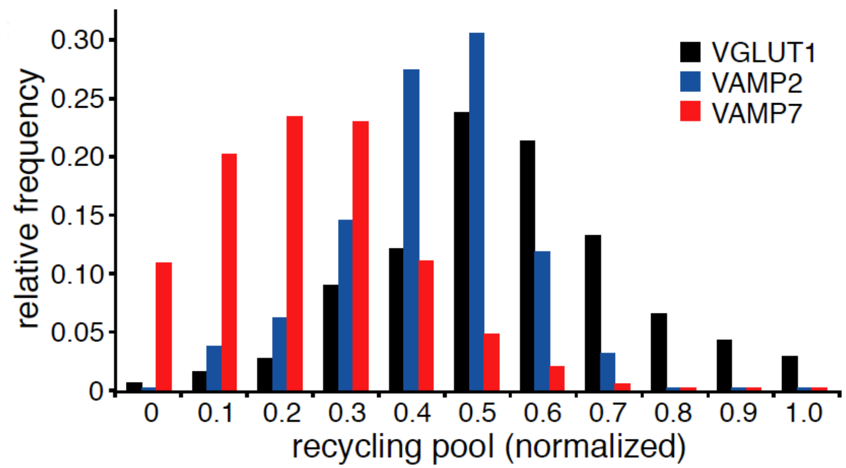

VAMP7 (not synaptophysin) depends on AP-3 for SV localization

does AP-3 make SVs with different properties?
--make pHluorin fusion to VAMP7

in bafilomycin,
SVs cannot reacidify
(works from lumen)
--reveals recycling pool




VAMP7 mostly in resting pool
releases spontaneously
--first evidence for difference
in composition of pools




**what is the role of spontaneous release?**
**subdivisions with recycling pool and RRP?**

(Hua et al, 2011)

**Reading: The Synapse, ed. Sheng, Sabatini, Sudhof, pp. 79-146**

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