Developmental Neurobiology

# Synapse Function and Formation in the CNS

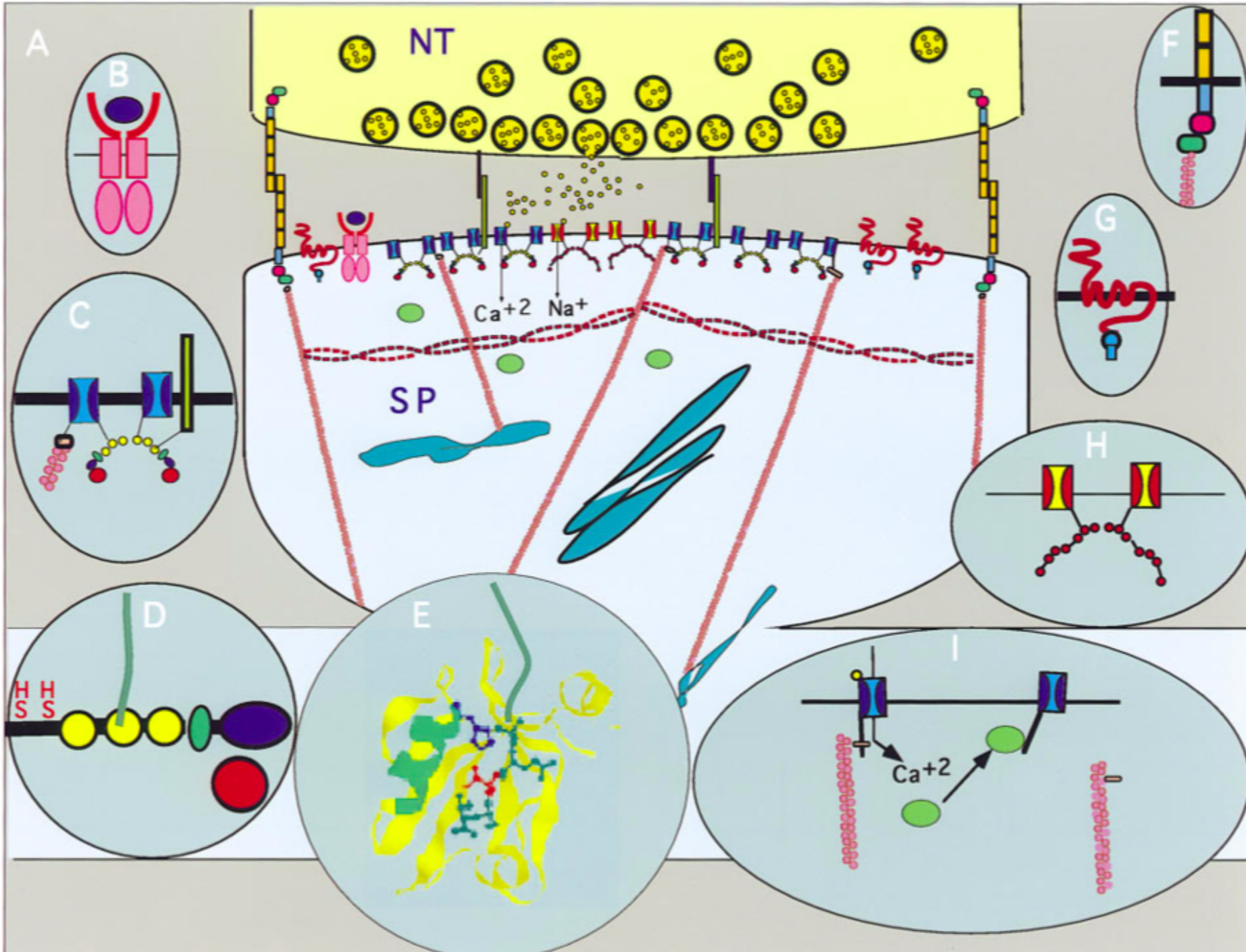
## Presynaptic Function

## Genesis of Excitatory and Inhibitory Synapses

March 23, 2016

Ed Ziff

# Overview of Pre- and Postsynaptic Organization

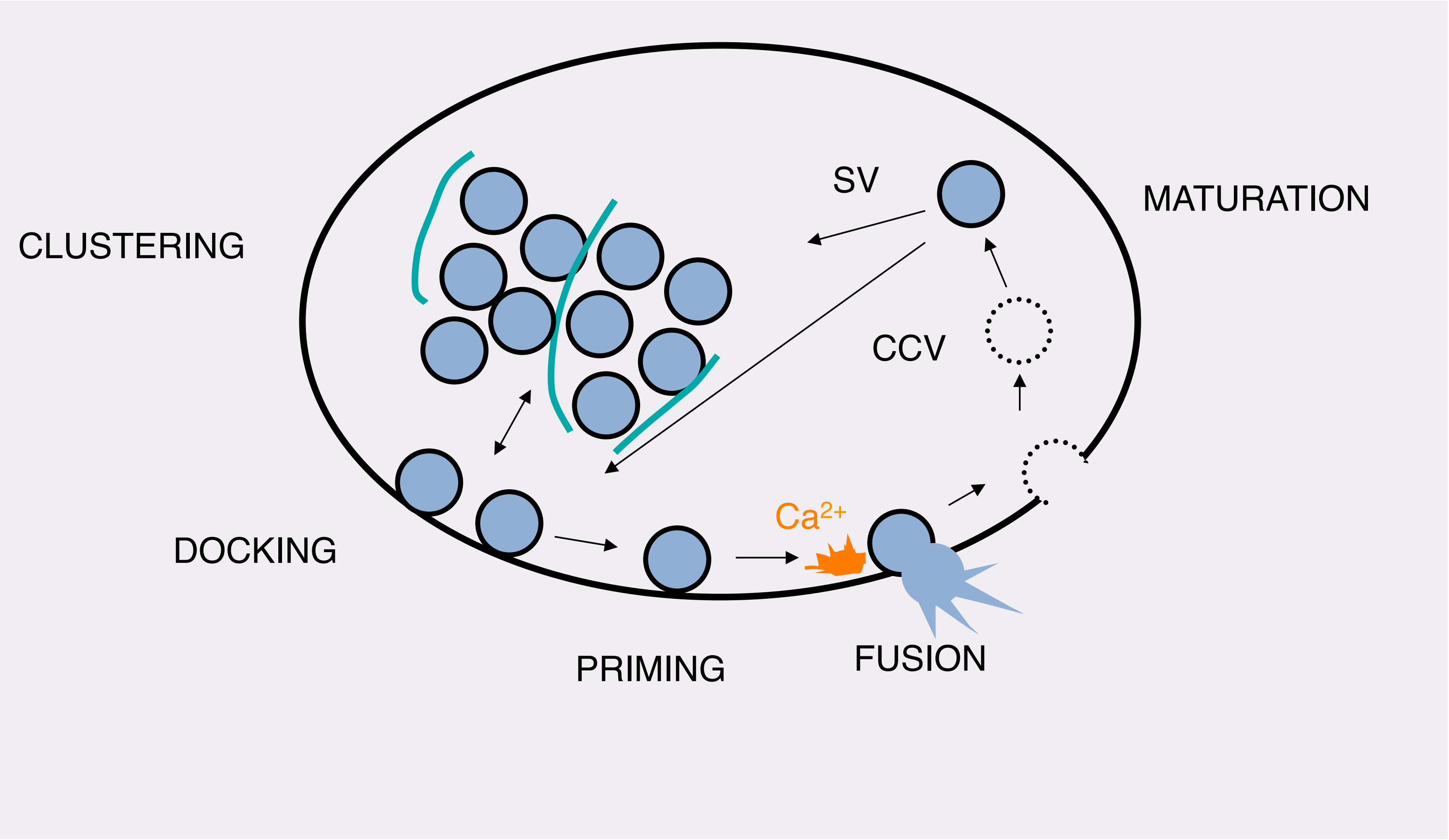



Ziff, 1997

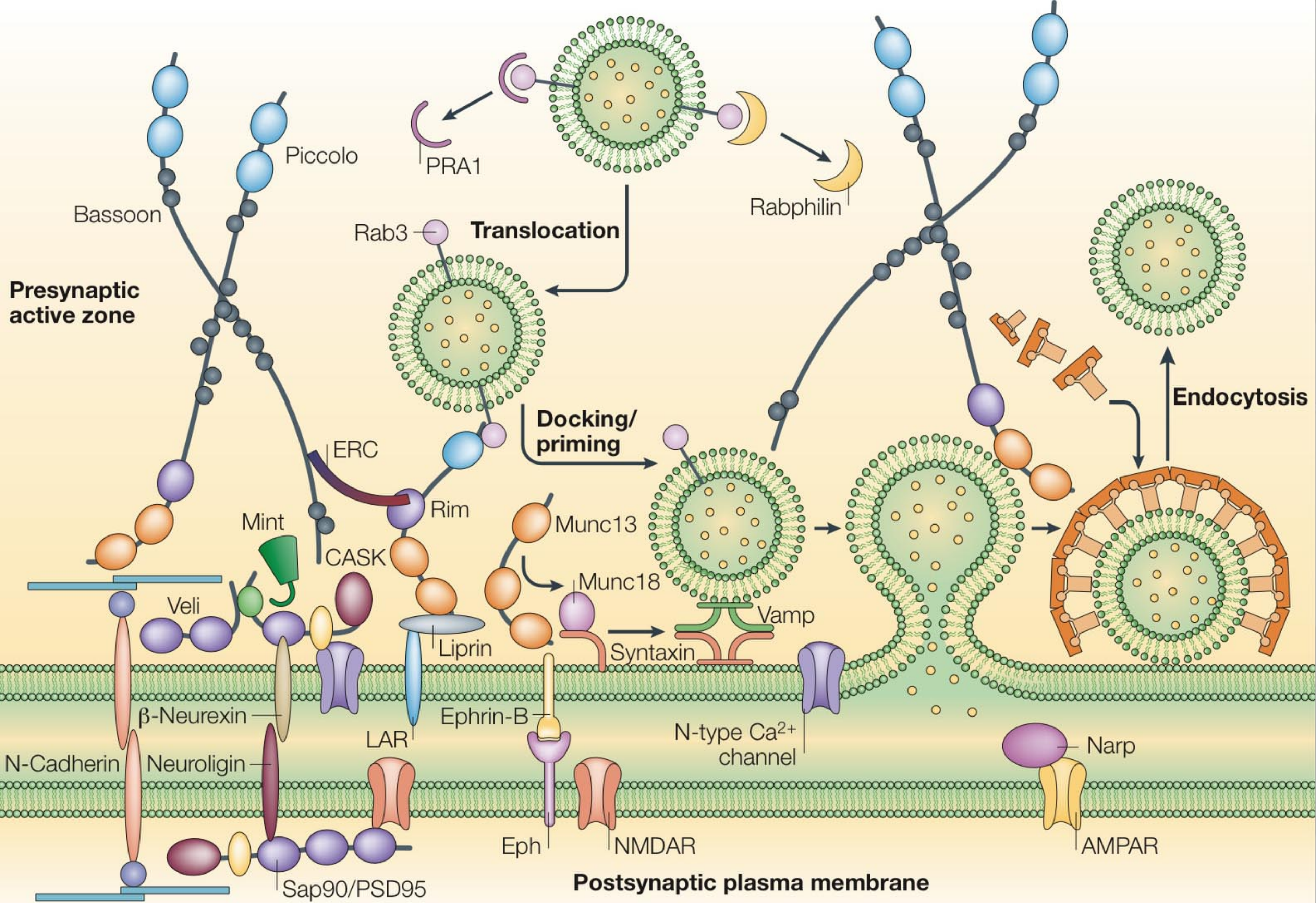
# Presynaptic Terminal

- The major feature of the presynaptic terminal is the active zone
- This is the site of synaptic vesicle fusion and neurotransmitter release
- A major functional specialization of the active zone is the restriction of sites of vesicle fusion and endocytic recycling.

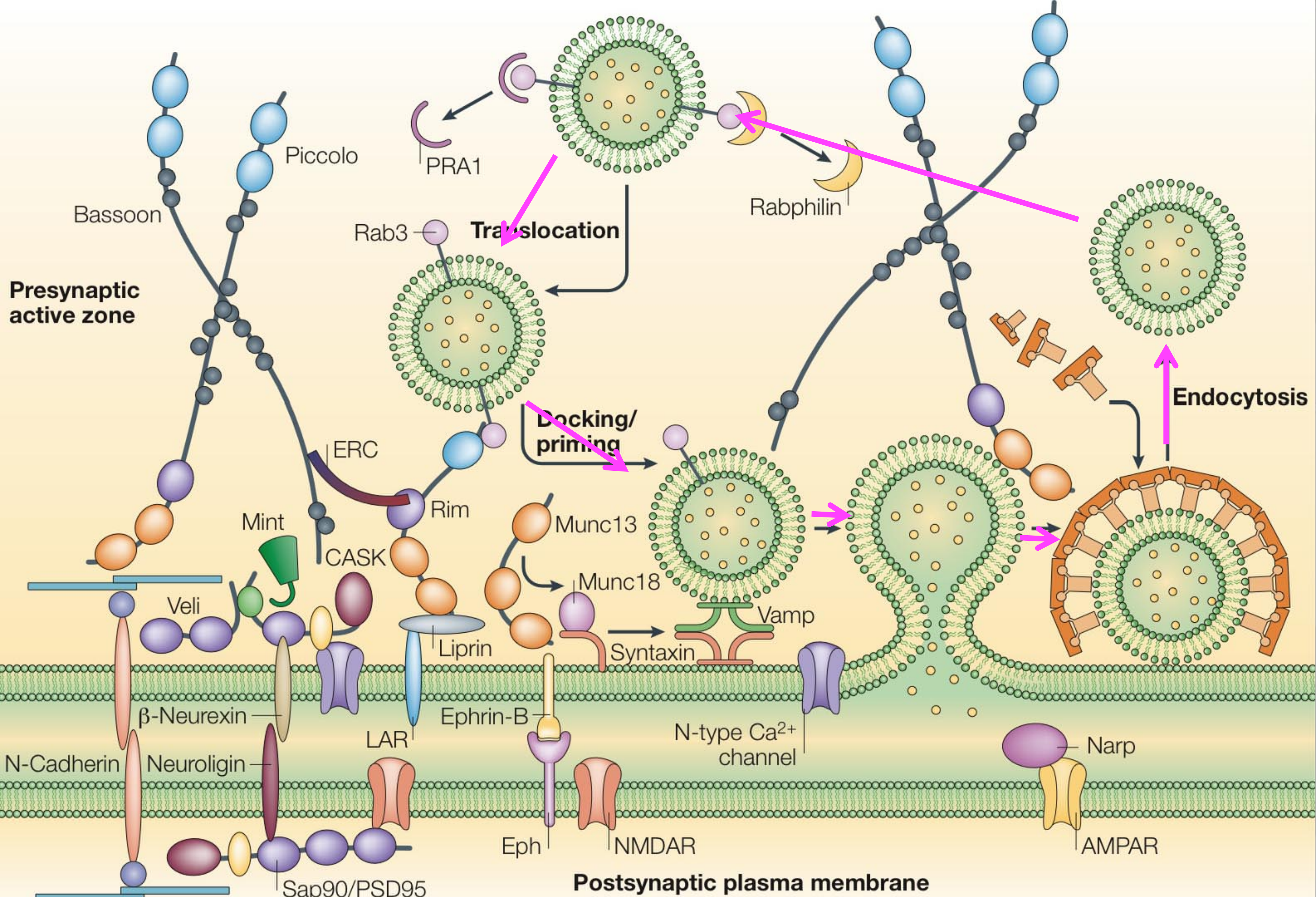
# Synaptic Vesicle Cycle




Adapted from Hilfiker et al., (1999)



Adapted from Ziv and Garner (2004)

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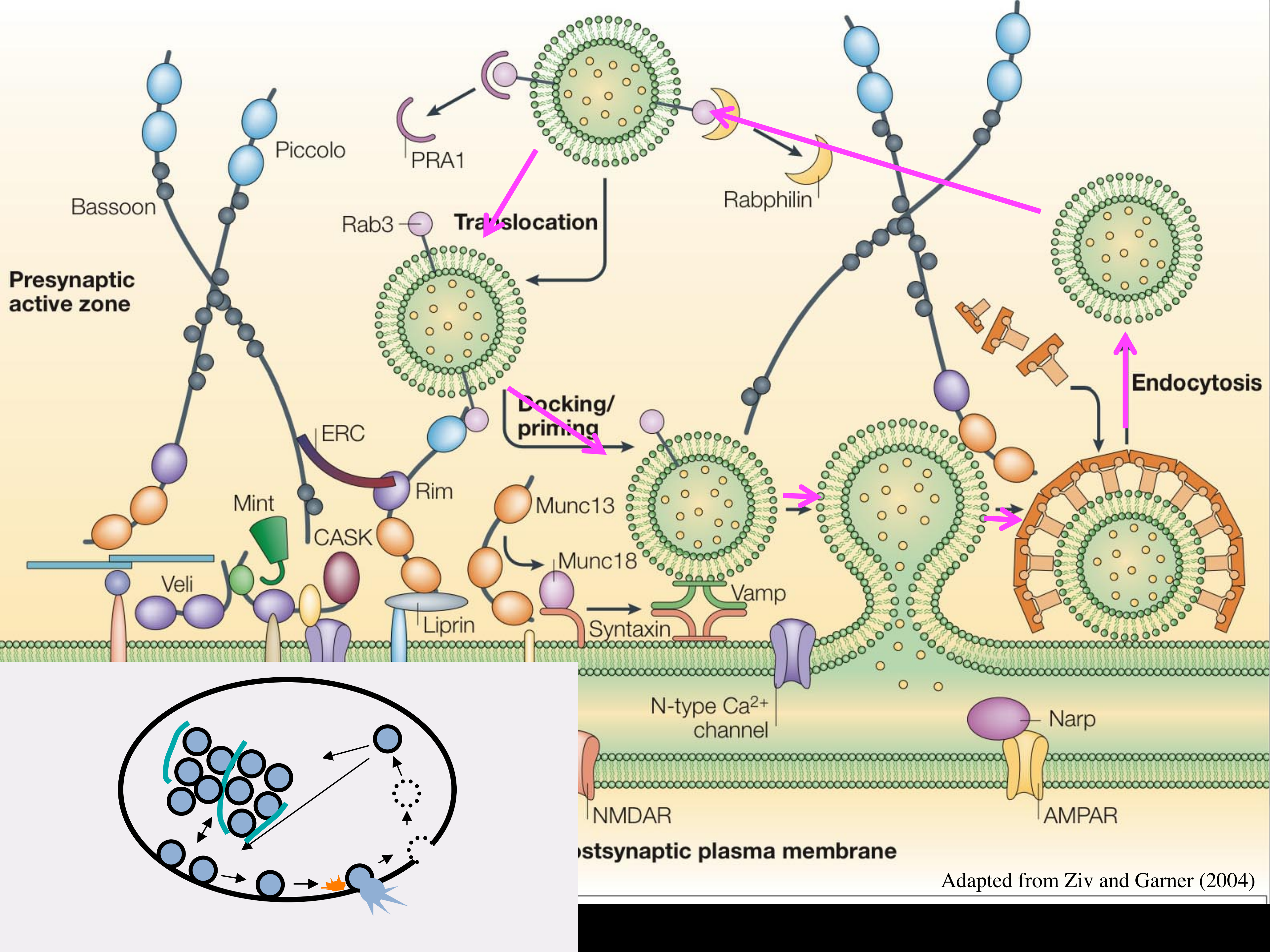
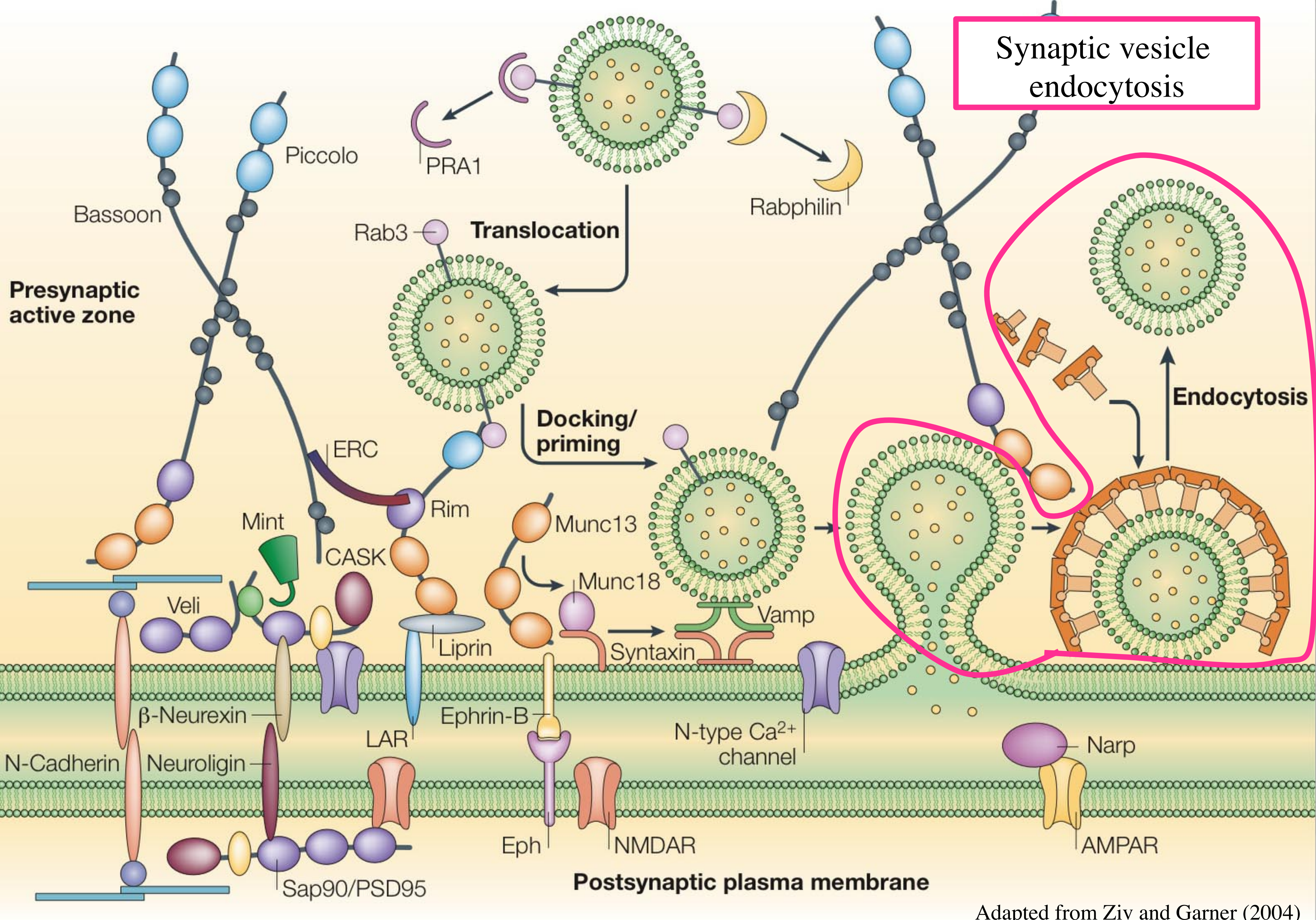


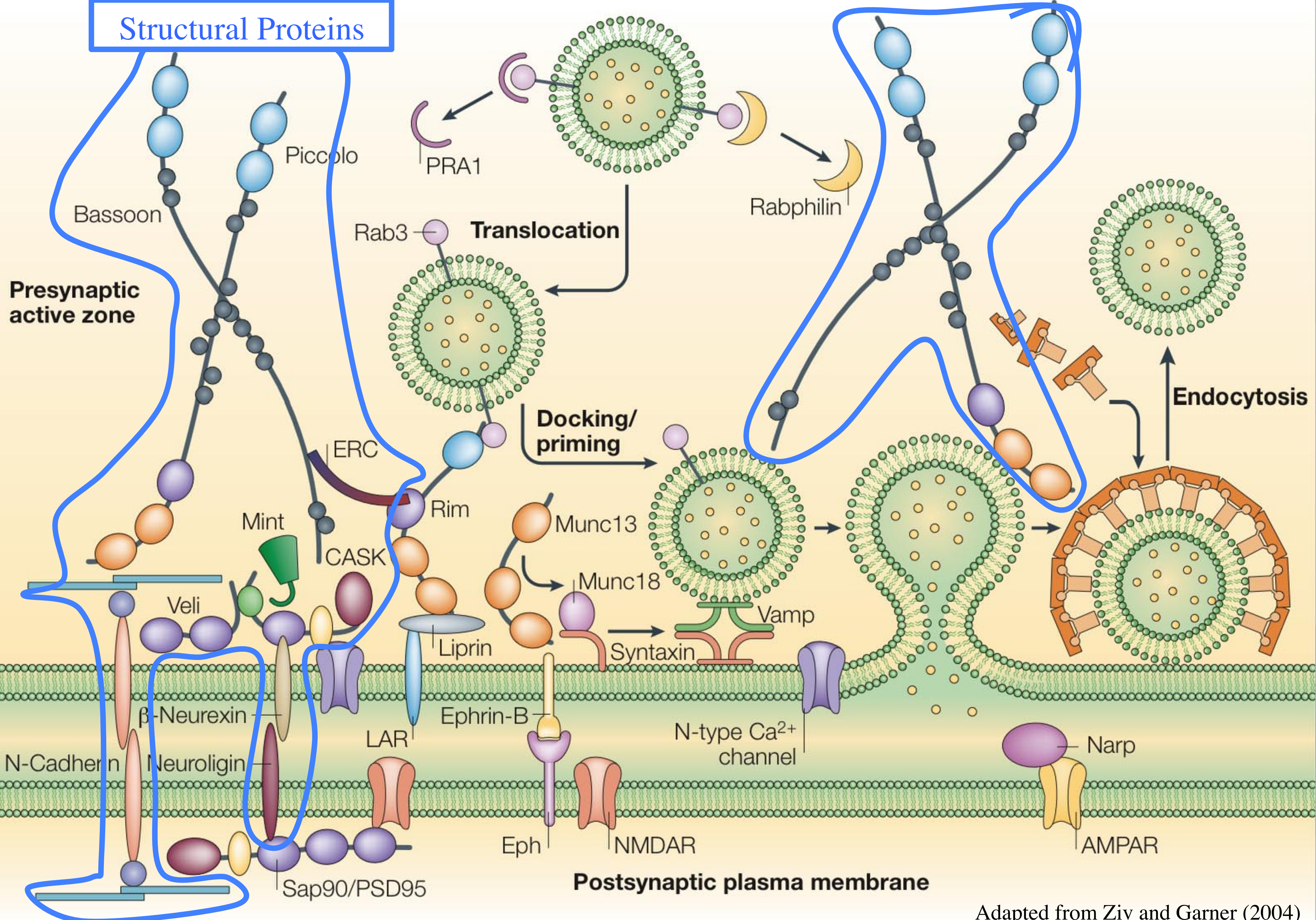

Adapted from Ziv and Garner (2004)

# Synaptic vesicle endocytosis



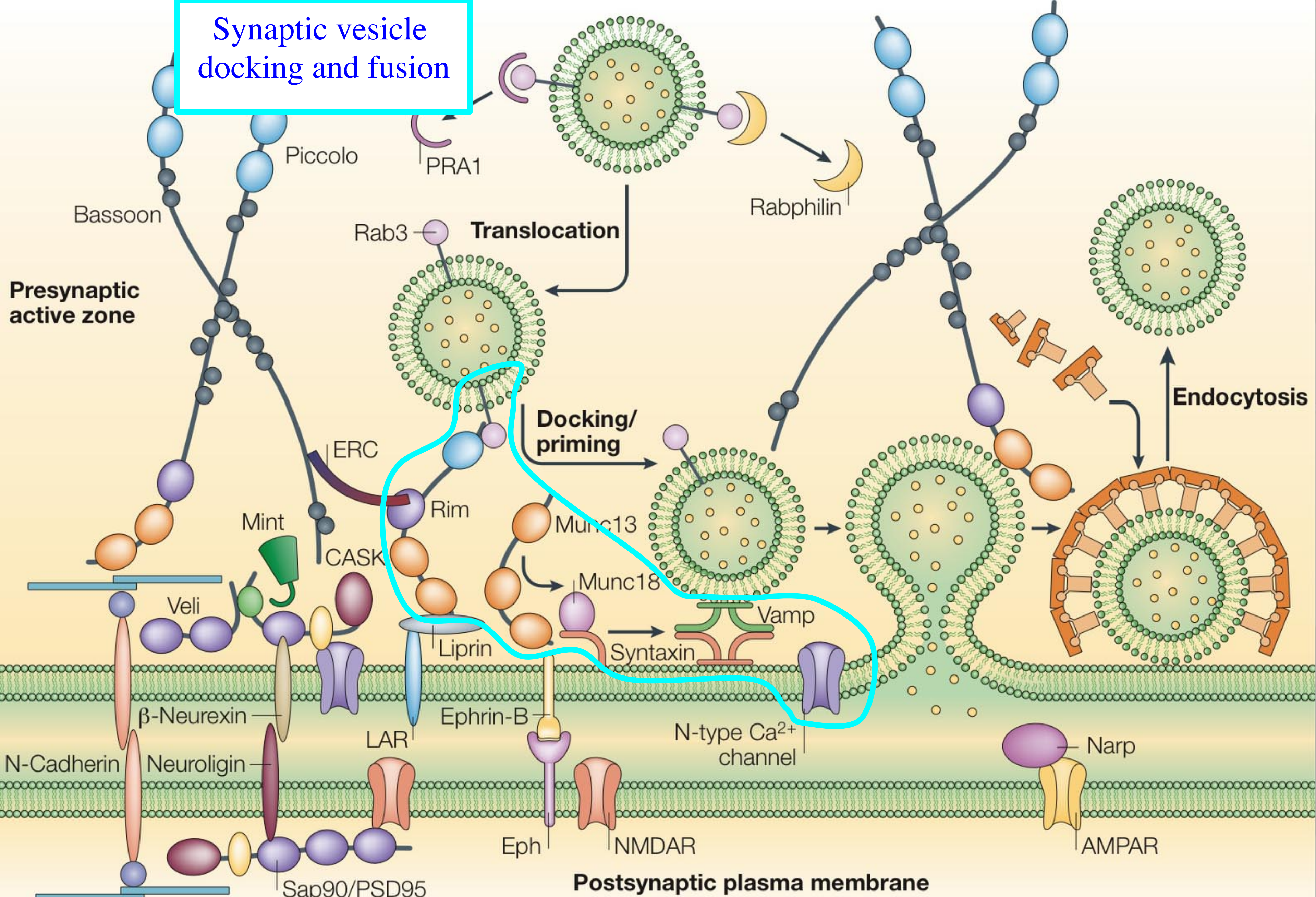

Adapted from Ziv and Garner (2004)

Structural Proteins

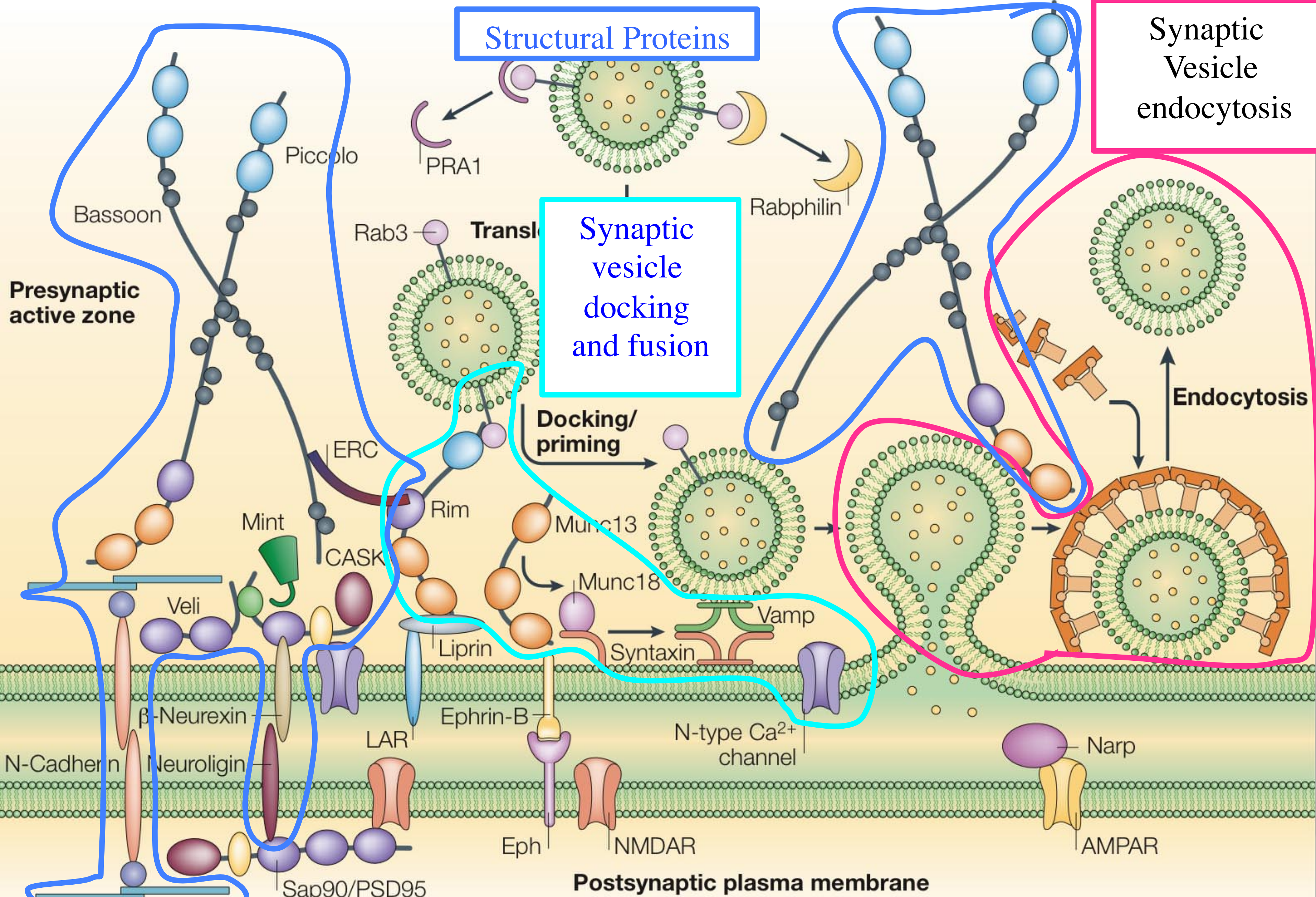



Adapted from Ziv and Garner (2004)

# Synaptic vesicle docking and fusion



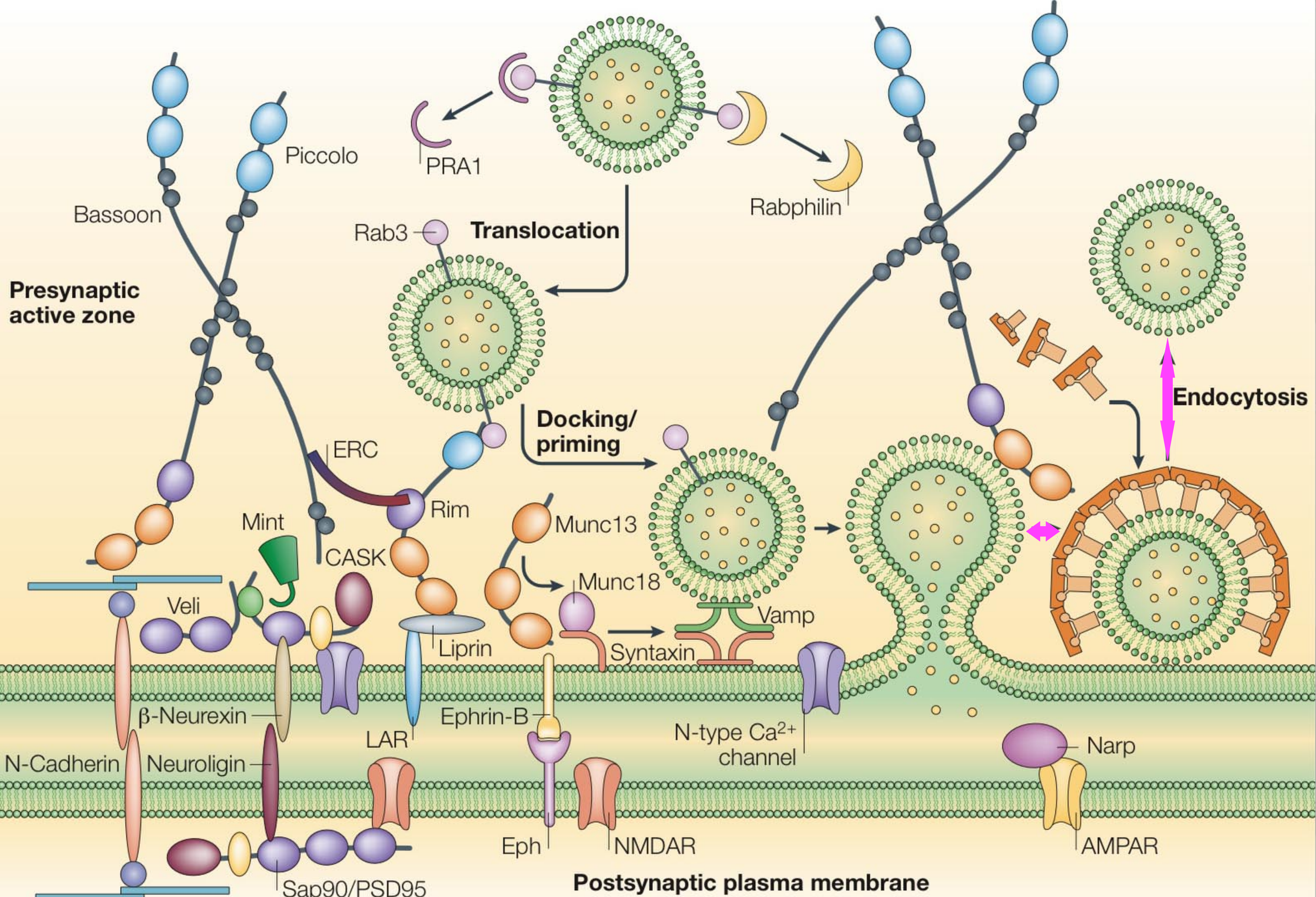

Adapted from Ziv and Garner (2004)




Adapted from Ziv and Garner (2004)

# Endocytosis

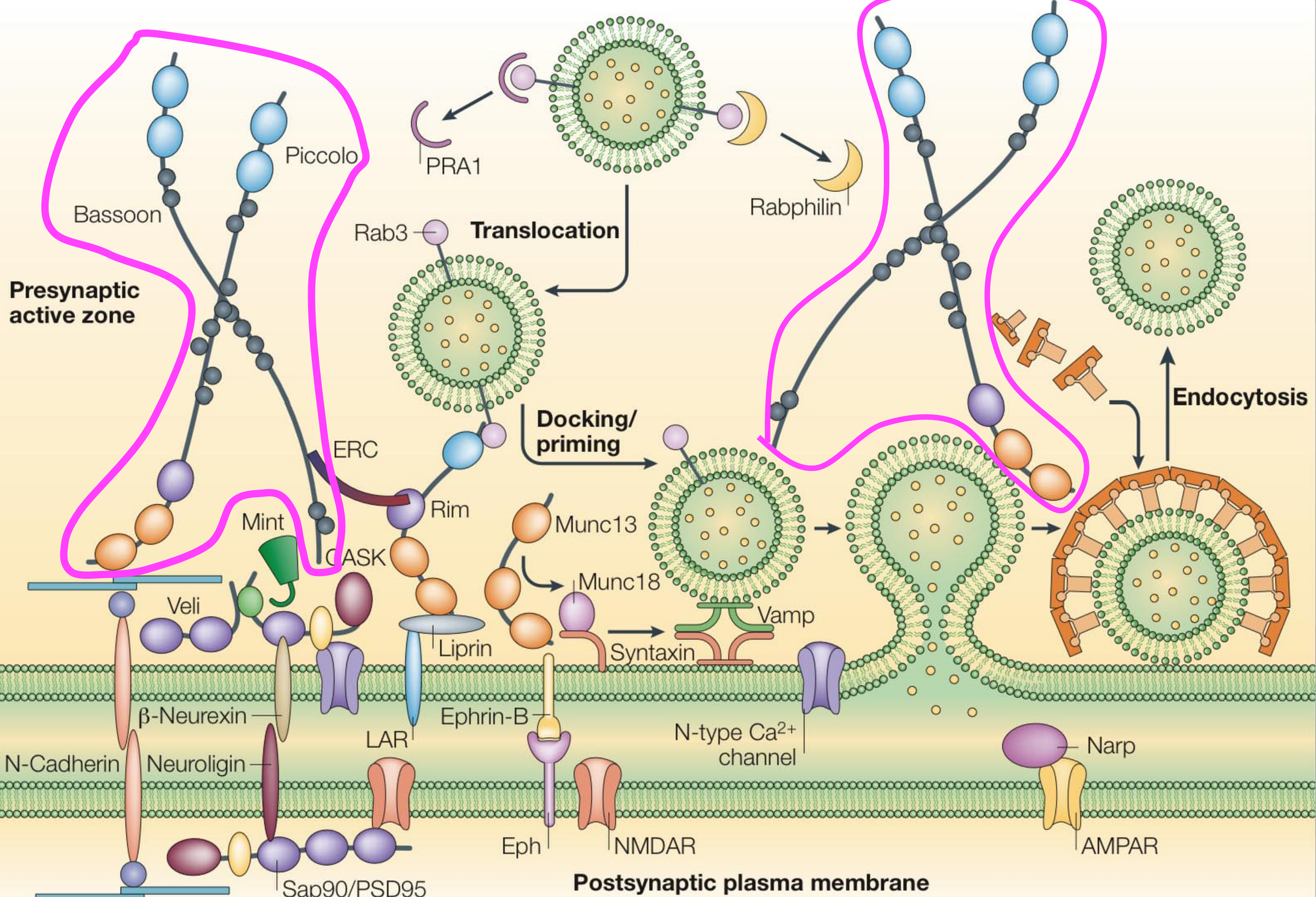
- After neurotransmitter release, a clathrin coated pit is generated, from which the vesicle reforms.



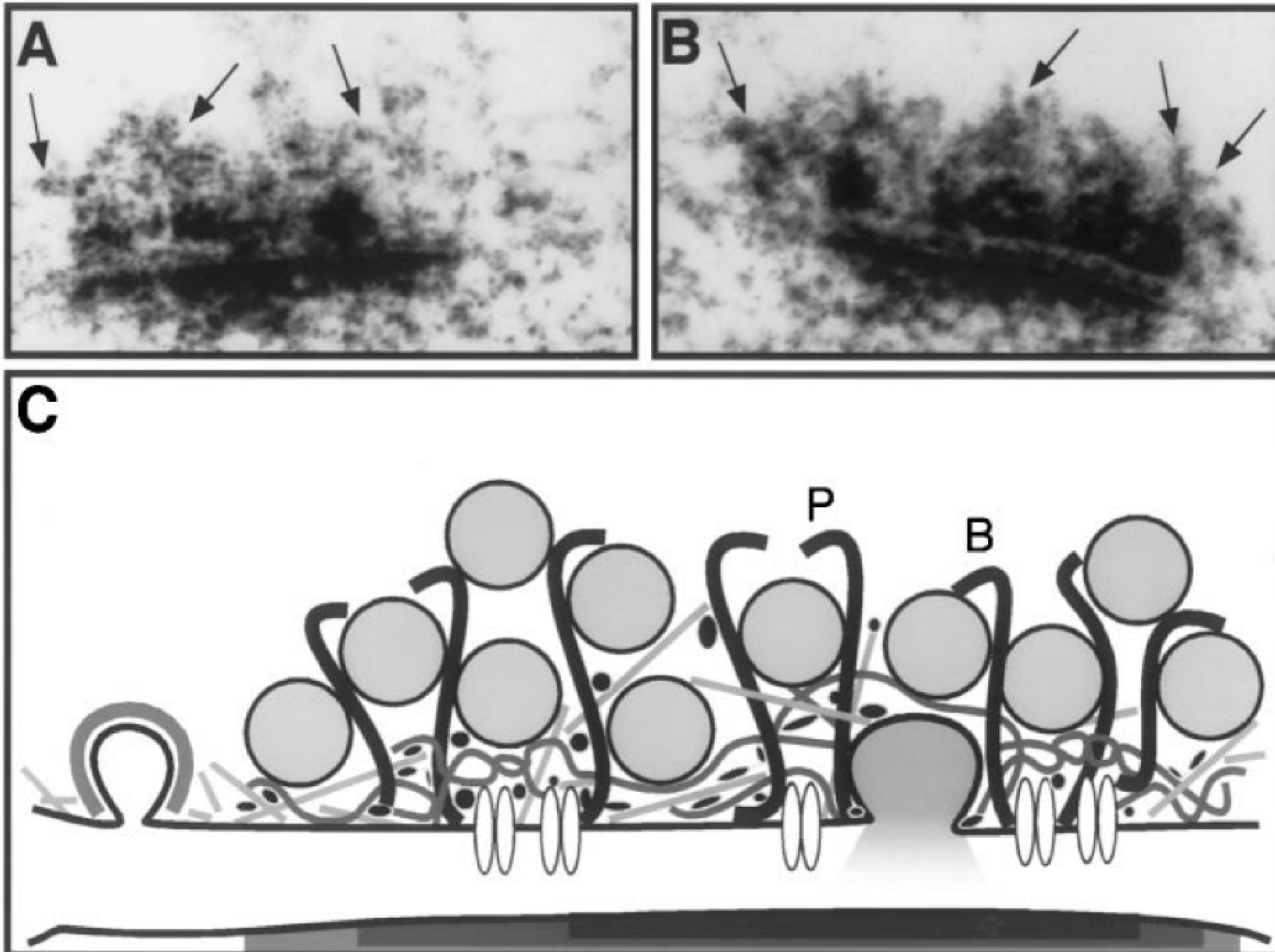
Adapted from Ziv and Garner (2004)

# Presynaptic scaffold proteins

- Piccolo and bassoon: high molecular weight multi-domain proteins that are structural components of the presynaptic CAZ (cytoskeletal matrix assembled at active zones).



Adapted from Ziv and Garner (2004)




Adapted from Dresbach et al., 2001

# Presynaptic scaffold proteins

- Piccolo and bassoon make a series of interactions with cytoskeletal proteins, plasma membrane proteins and vesicle associated proteins.
- These interactions may assemble the readily releasable pool of vesicles at the plasma membrane.

# Three Sets of Interactions for Piccolo and Bassoon

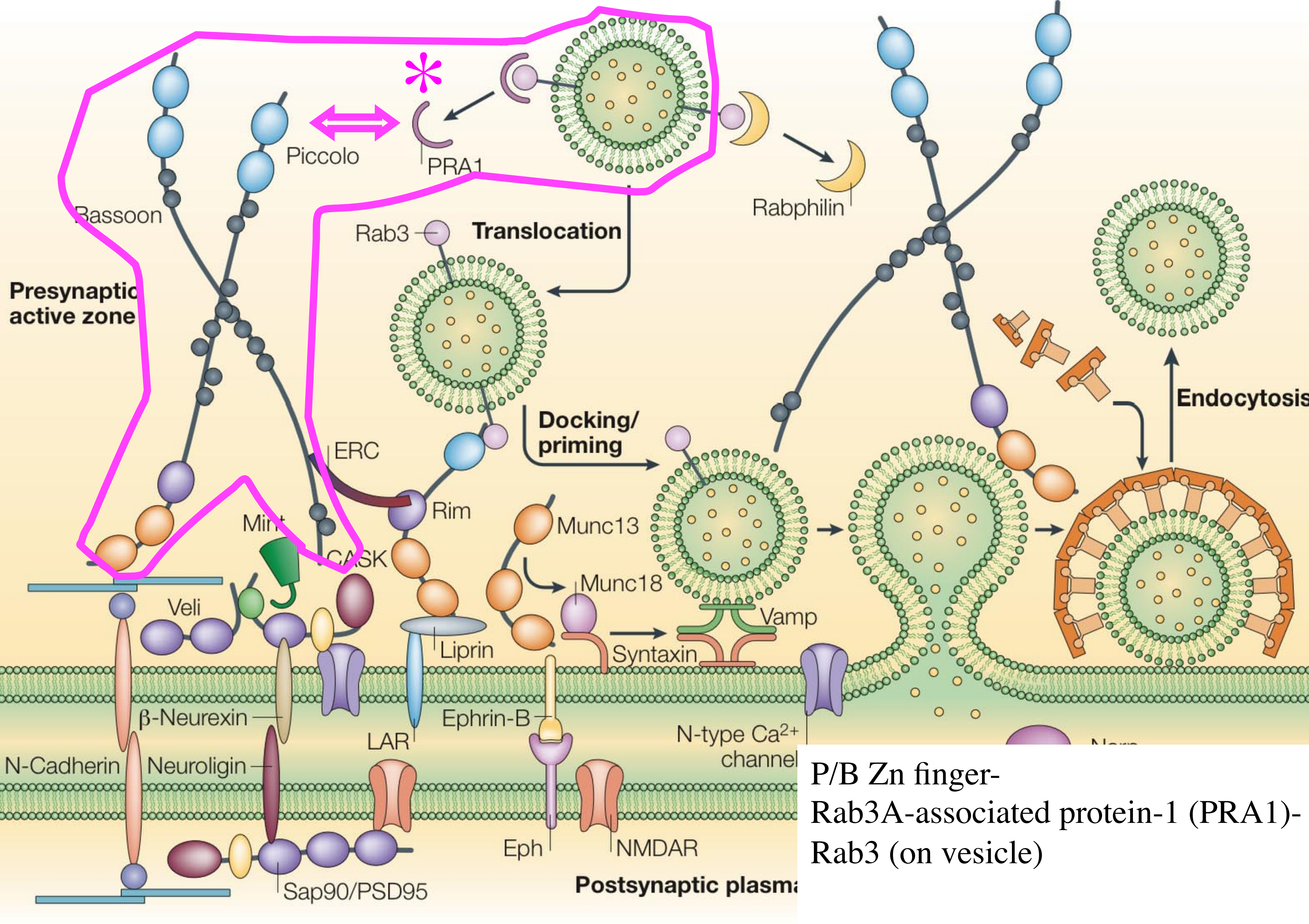
- P/B Zn finger-Rab3A-associated protein-1 (PRA1)-Rab3 (on vesicle)

- P/B-MINT, Veli, CASK-plasma membrane proteins (including neurexins)

- P/B-ERC-Rim-Rab3 (on vesicle)

# Bassoon and Piccolo

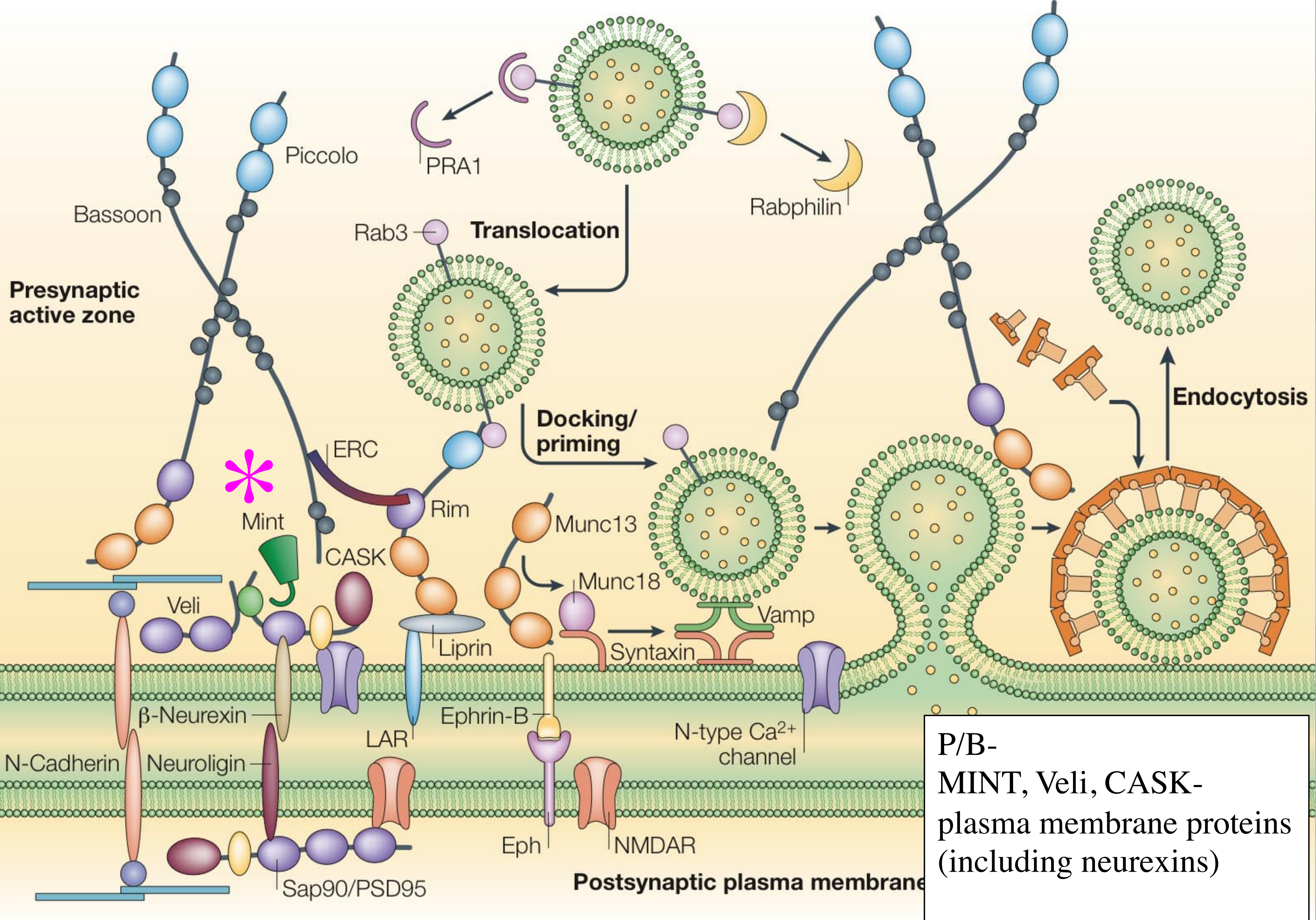
- Zn finger domains of Bassoon and Piccolo bind to Rab3A-associated protein-1 (PRA1).
- Rab3A is associated with synaptic vesicles.



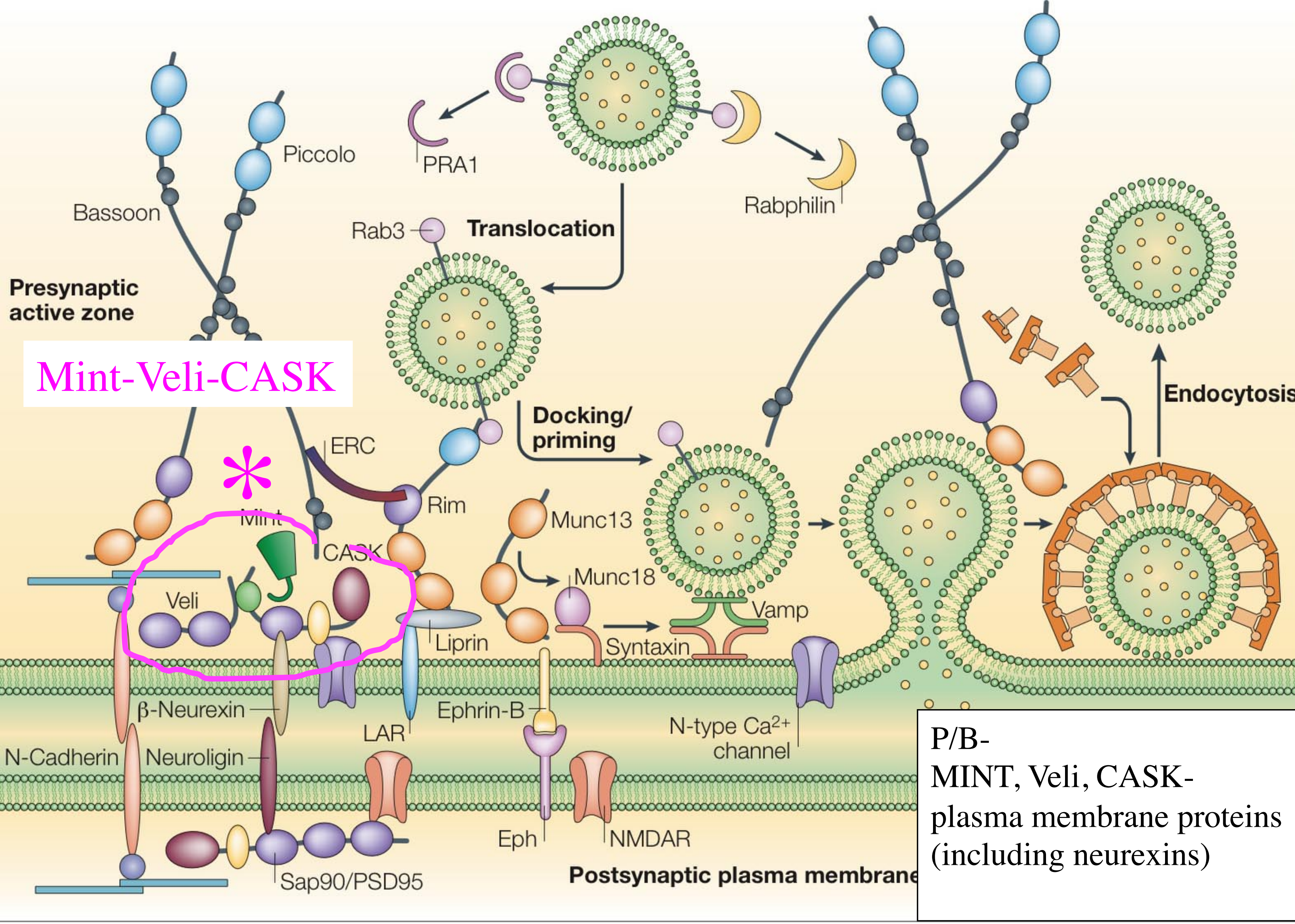

P/B Zn finger-
Rab3A-associated protein-1 (PRA1)-
Rab3 (on vesicle)

# Bassoon and Piccolo

- Piccolo and Bassoon are tethered to the membrane via MINT, a PDZ protein that is in a multiprotein complex (MINT, Veli, CASK).



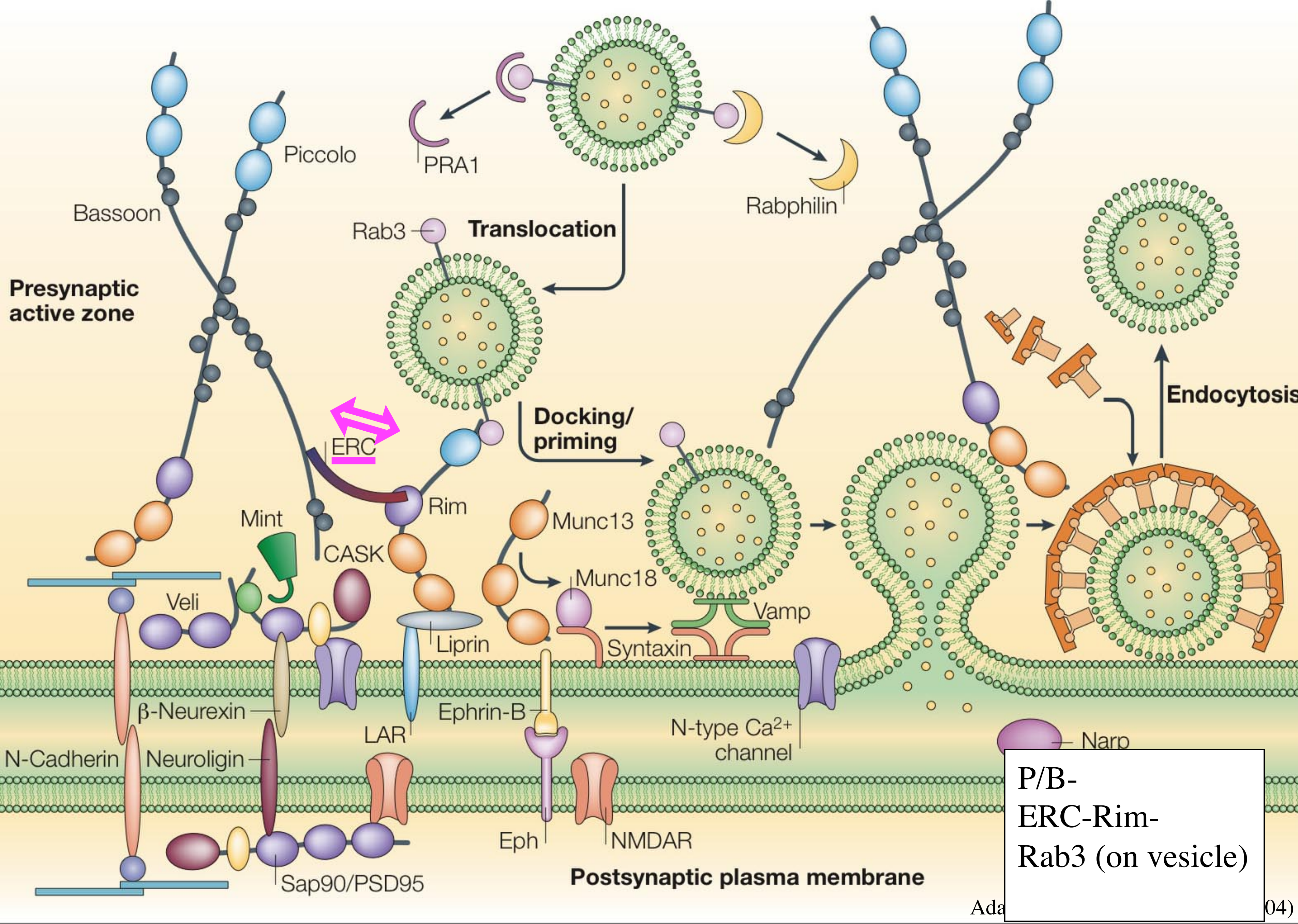

P/B-
MINT, Veli, CASK-
plasma membrane proteins
(including neurexins)




P/B-
MINT, Veli, CASK-
plasma membrane proteins
(including neurexins)

# Piccolo and Bassoon

- Piccolo and Bassoon are coupled by ERC to RIM (Rab interacting molecule).
- This may initiate docking of the associated vesicles through a translocation of the vesicles to the docking machinery.




P/B-
ERC-Rim-
Rab3 (on vesicle)

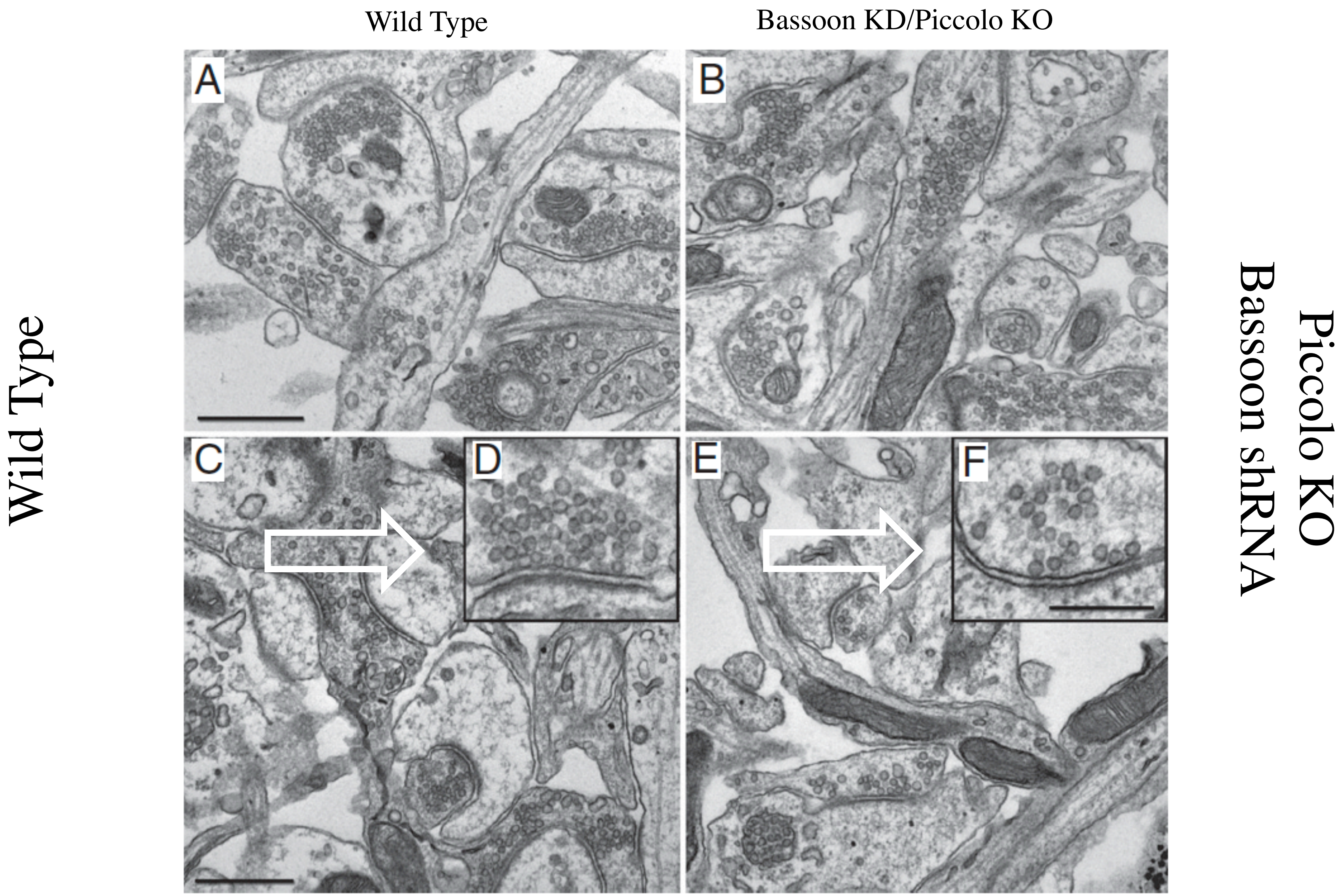
Ada… 04)

# Bassoon and Piccolo

- These interactions support a function for Piccolo and Bassoon in vesicle clustering.

# Synaptic Vesicle Clustering by Piccolo and Bassoon

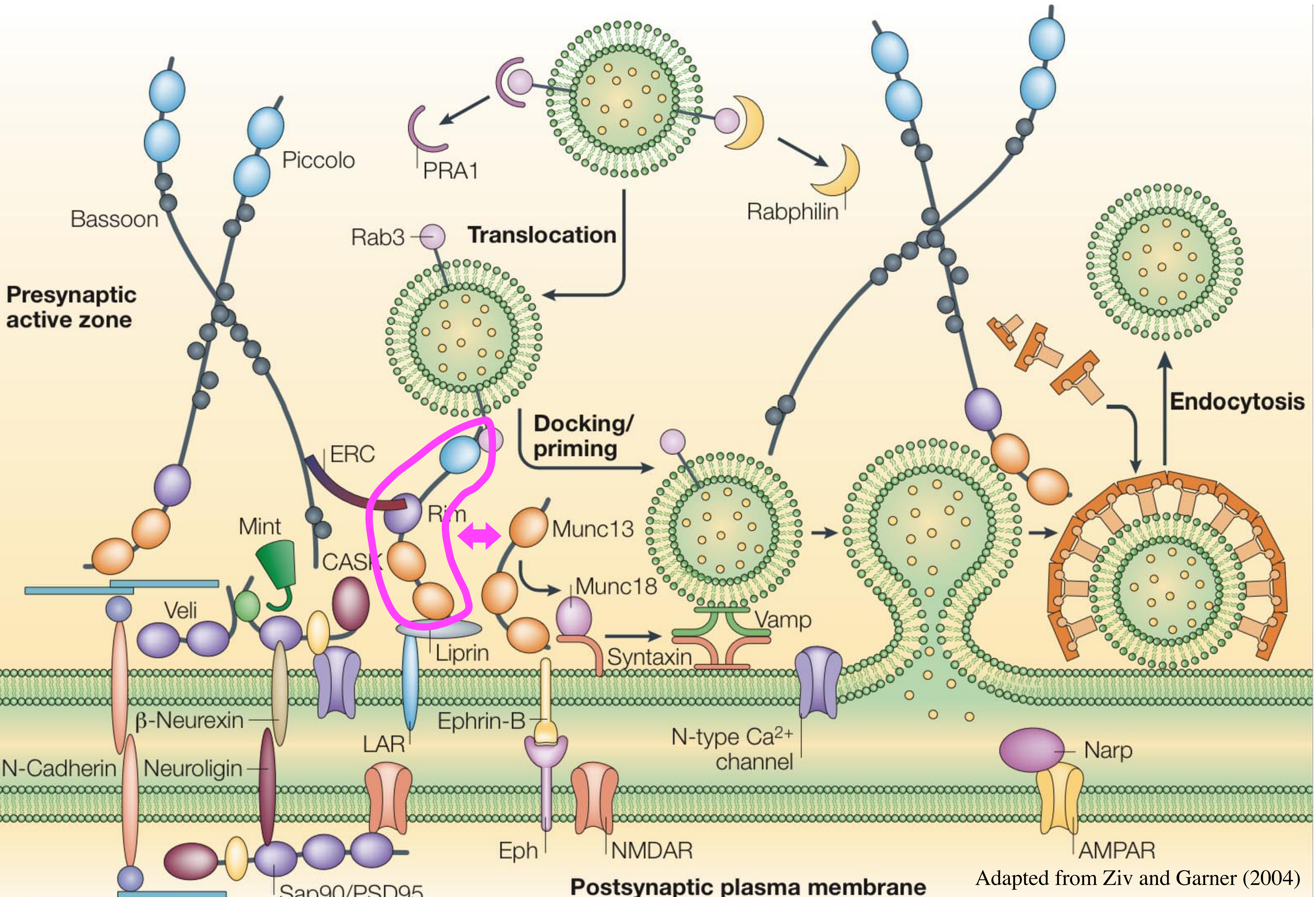
Bassoon KD/Piccolo KO reduces number of vesicles in nerve terminal by 40% and overall density by 33%



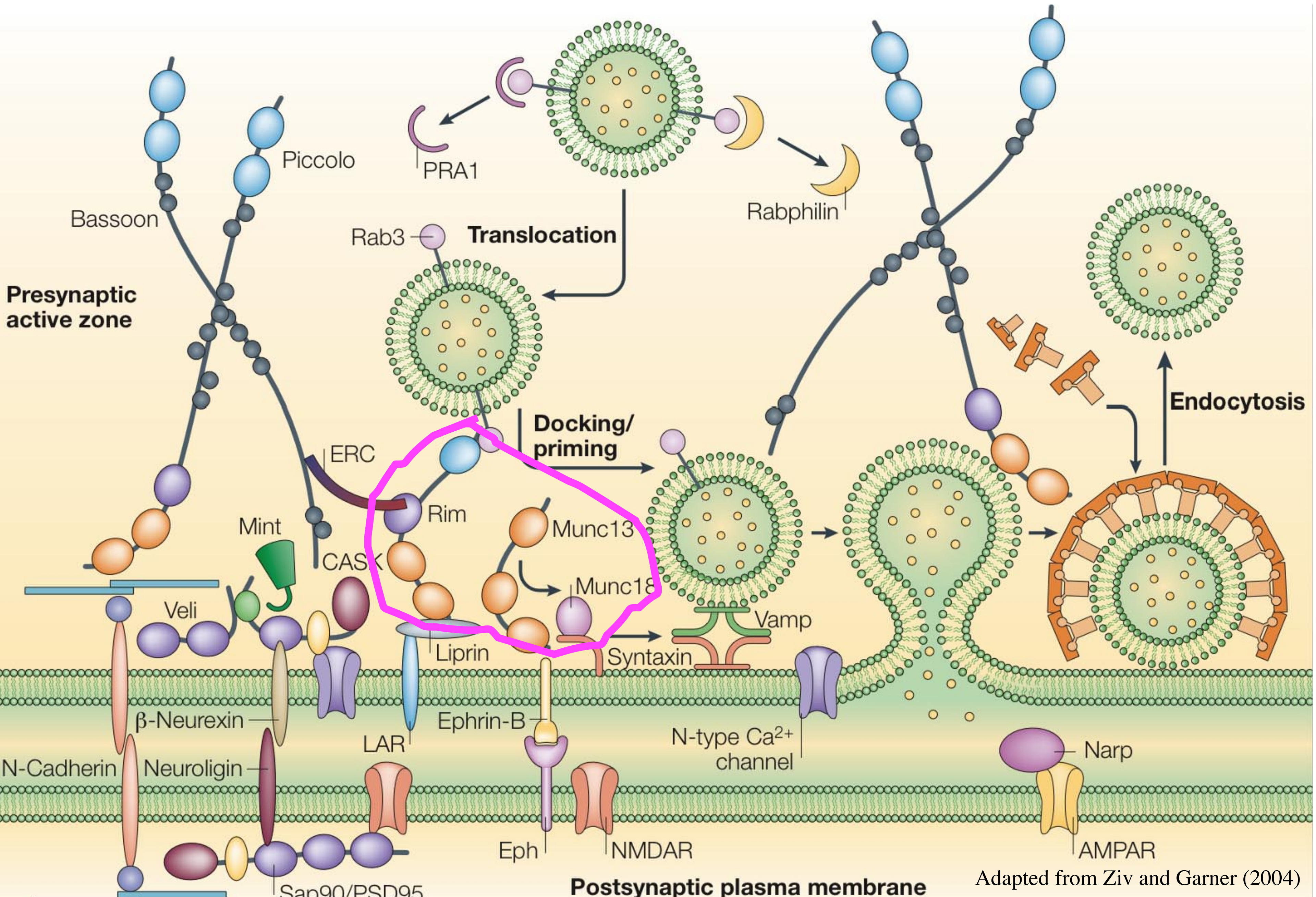

Muckherjee et al., 2010

# Presynaptic scaffold proteins

- RIM1 and -2 (Rab3 interacting molecules): Involved in synaptic vesicle priming; interact with Munc13, RimBPs and synaptotagmin.



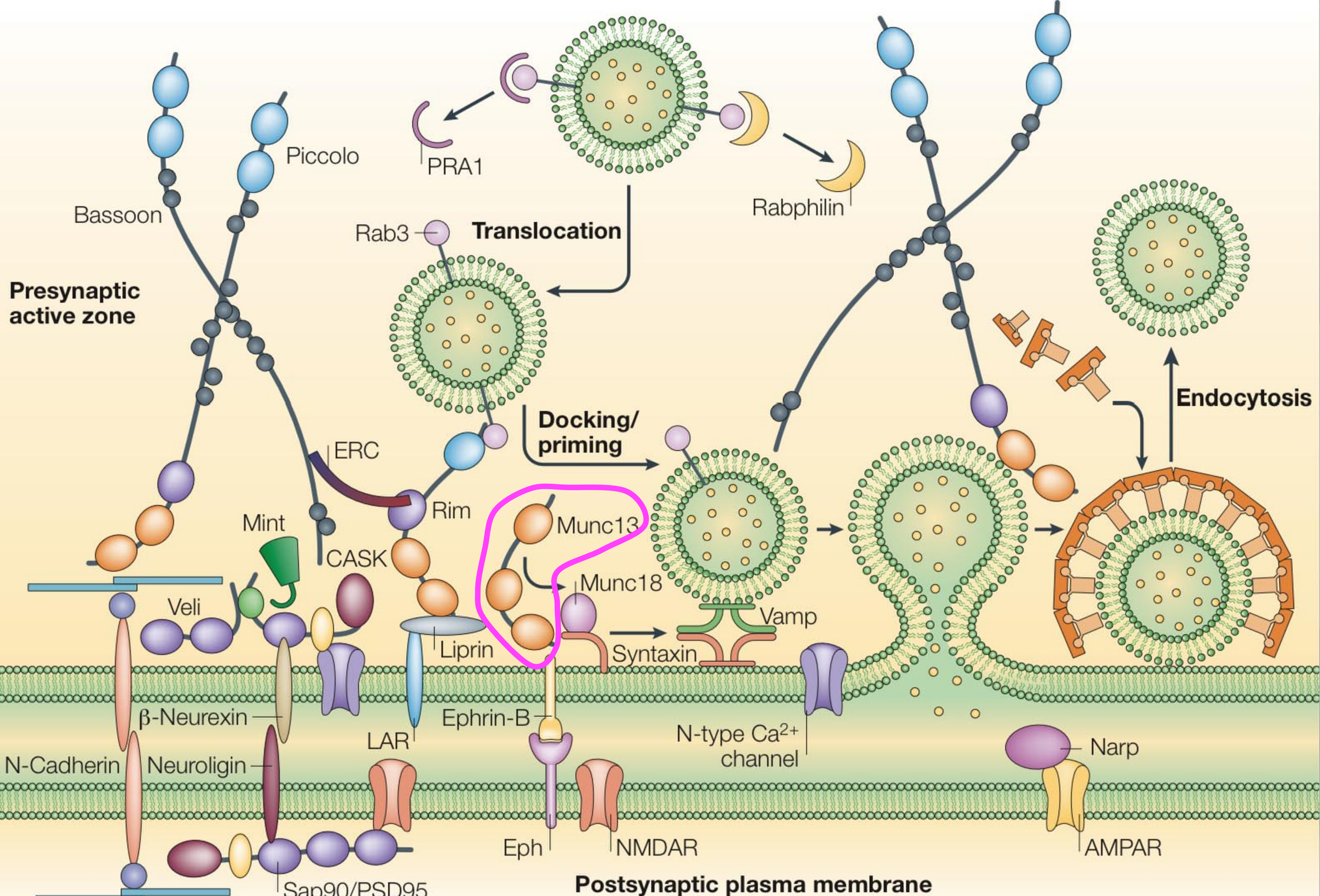

Adapted from Ziv and Garner (2004)



Adapted from Ziv and Garner (2004)

# Presynaptic scaffold proteins

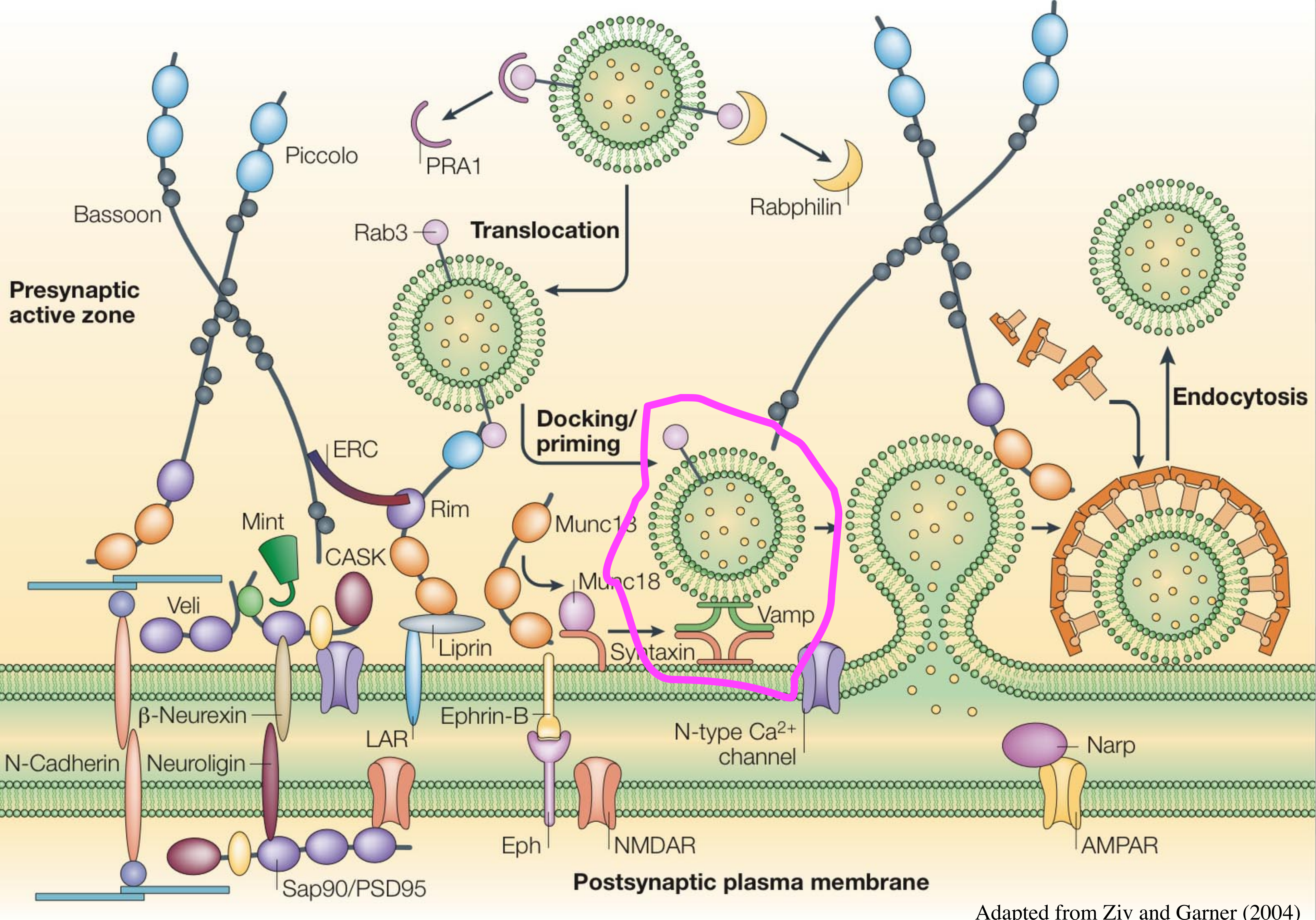
- Piccolo and bassoon
- RIM1 and -2 (Rab3 interacting molecules)
- MUNC 13: Involved in synaptic vesicle priming; interacts with Rim and displaces MUNC18.



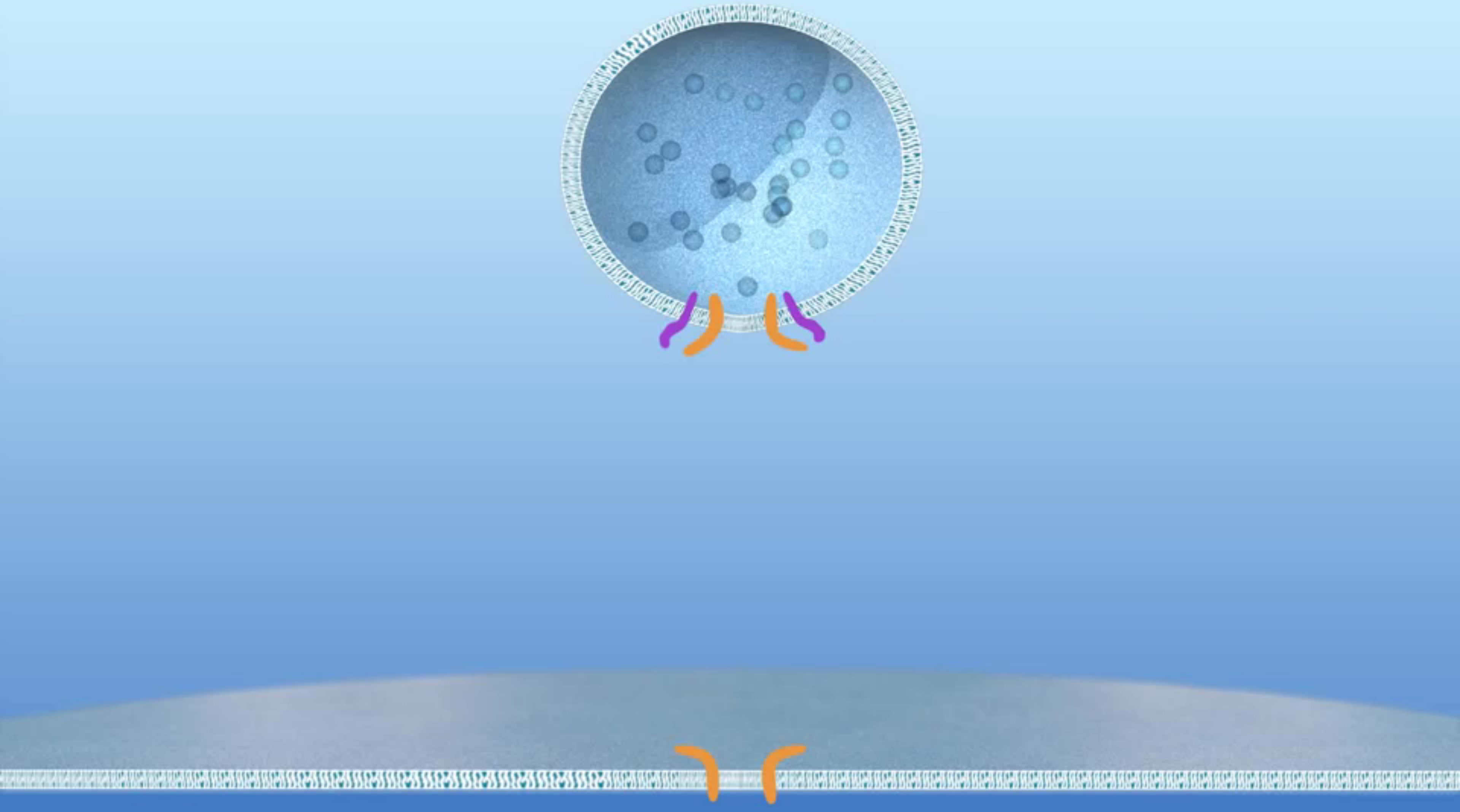

Adapted from Ziv and Garner (2004)

# Docked Vesicle

- The vesicle docks at the active zone through interaction with the SNARE complex and thus joins the “readily releasable pool” of vesicles.

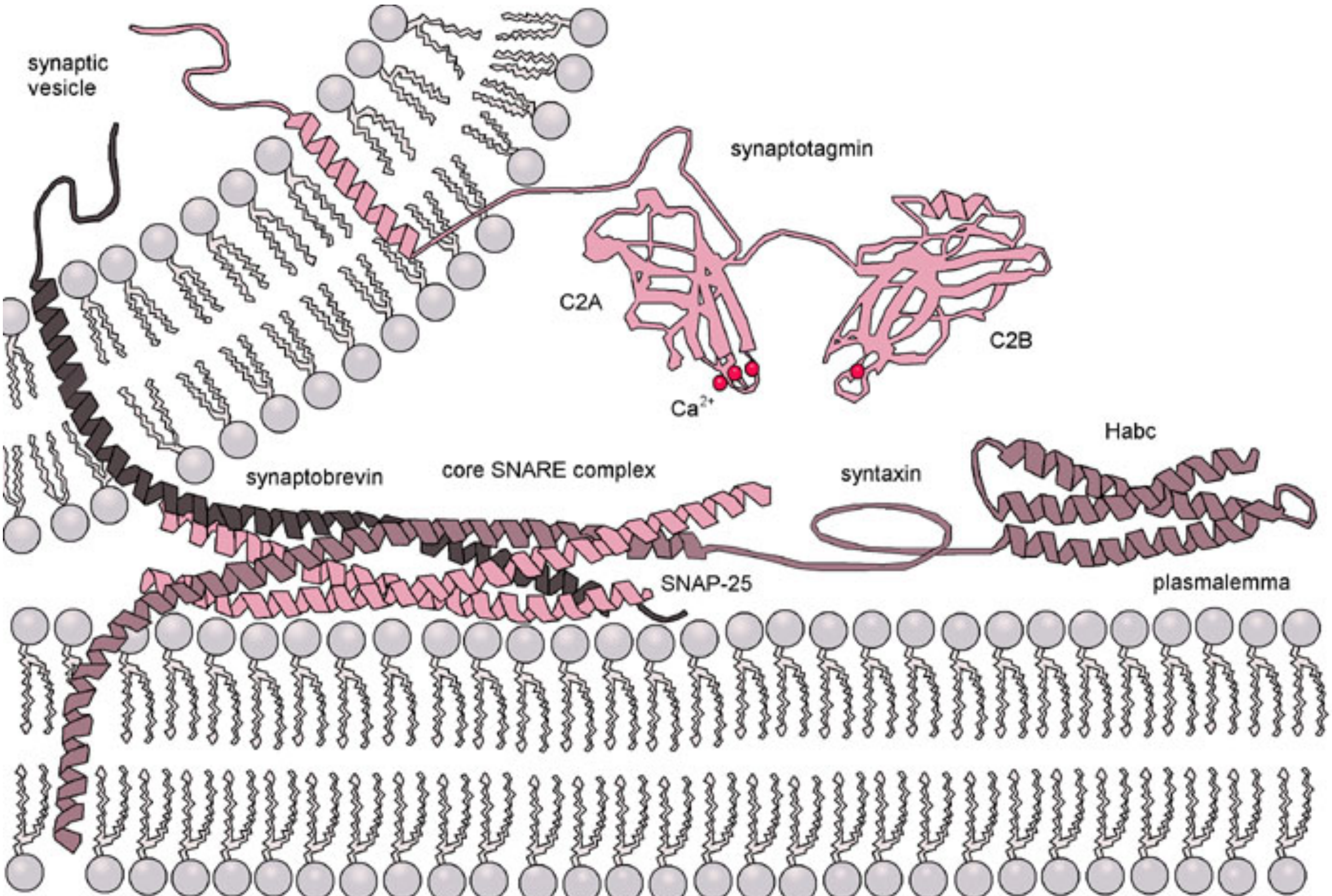



Adapted from Ziv and Garner (2004)





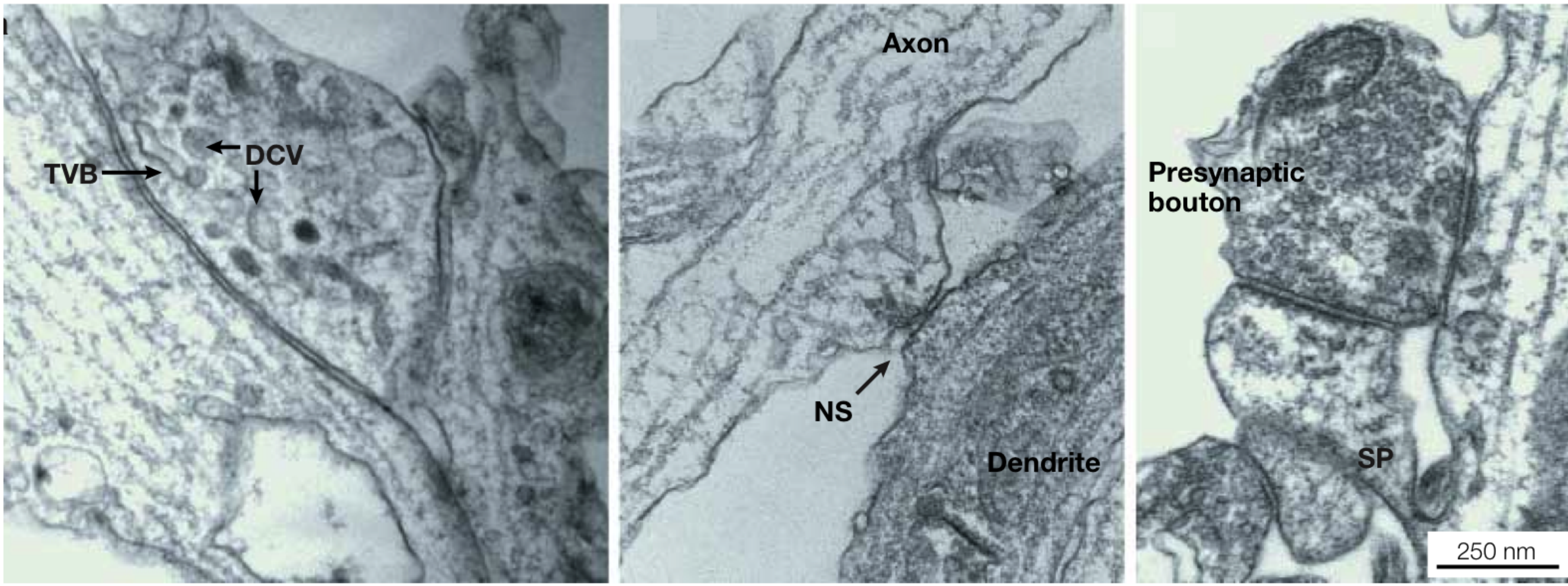
Animation: Mattias Karlén



synaptic vesicle
synaptotagmin
C2A
C2B
$Ca^{2+}$
Habc
synaptobrevin
core SNARE complex
syntaxin
SNAP-25
plasmalemma

# Mechanism of Synaptogenesis: Pathway

# Ultrastructure of axons and synapses at various stages of development



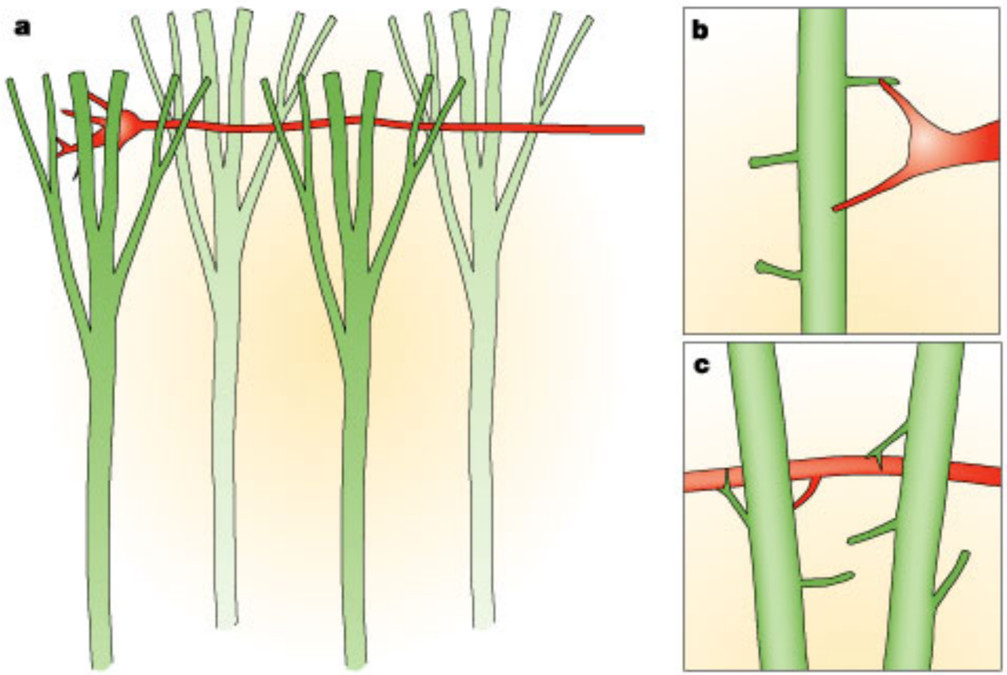

Axonal varicosity:
(TVB) tubulovesicles
(DCV) dense core vesicles

Nascent synapse (NS)

Mature synapse:
Spine (SP)

Adapted from Ziv and Garner (2004)

# Early stage of synaptogensis:
# Axon growth cone passes dendritic arbor




Nature Reviews | Neuroscience

Adapted from Ziv and Garner (2004)

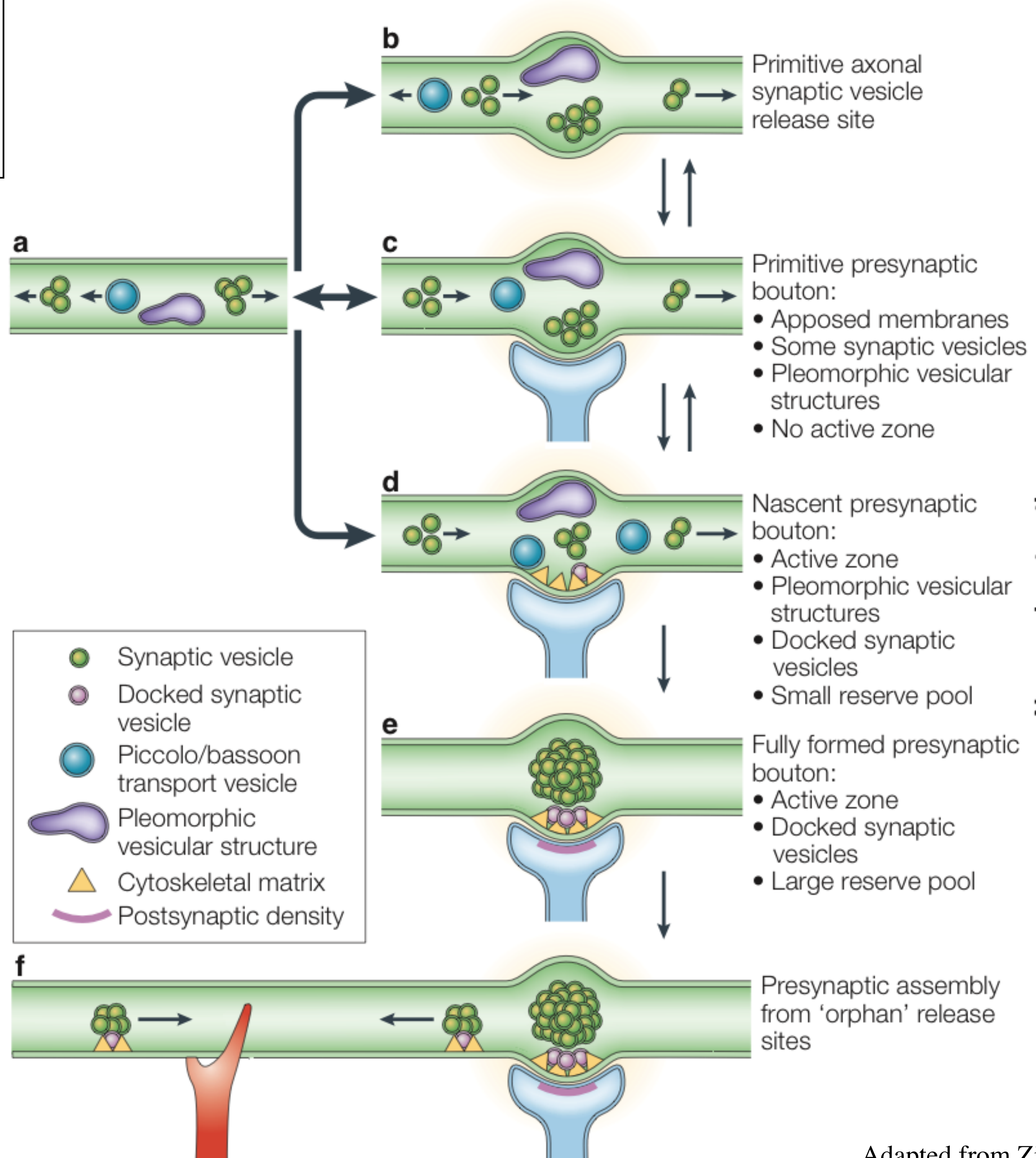
# Unitary Assembly Pathway

- "Presynaptic compartments may be assembled from macromolecular complexes transported along axons and recruited to nascent presynaptic membranes as preformed precursors". (Shapria et al., 2003)

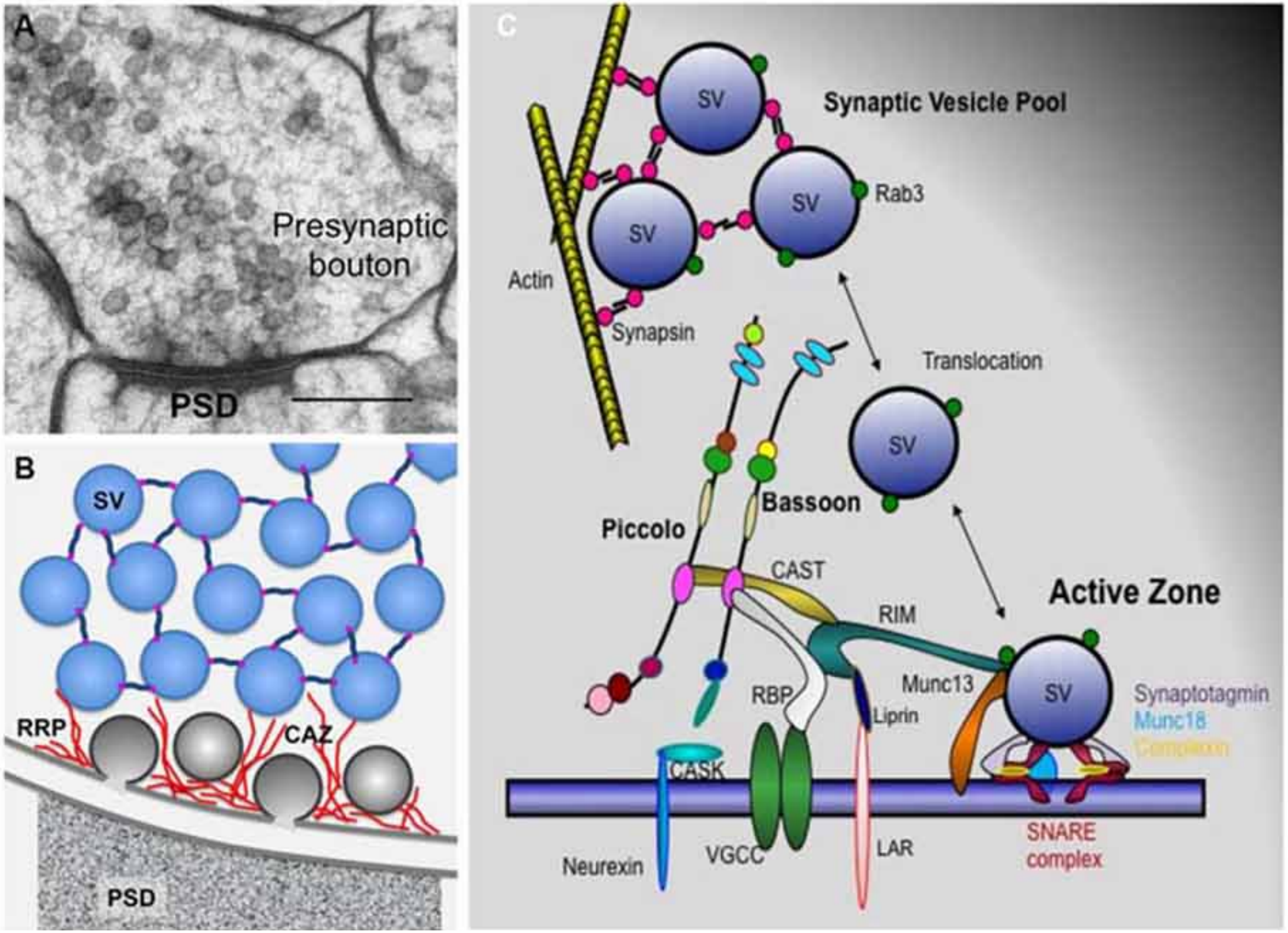
# Unitary Assembly Pathway

- Active zones may be assembled from preassembled AZ (active zone) precursor vesicles.
- Piccolo-Bassoon transport vesicles (PTV) have about 1/2 the Piccolo, Bassoon and RIM synaptic bouton content .
- AZs may thus be assembled from unitary (integral) quantities (2 or 3) of PTVs.
- Synaptic vesicle proteins may be transported separately.

# A model for presynaptic assembly

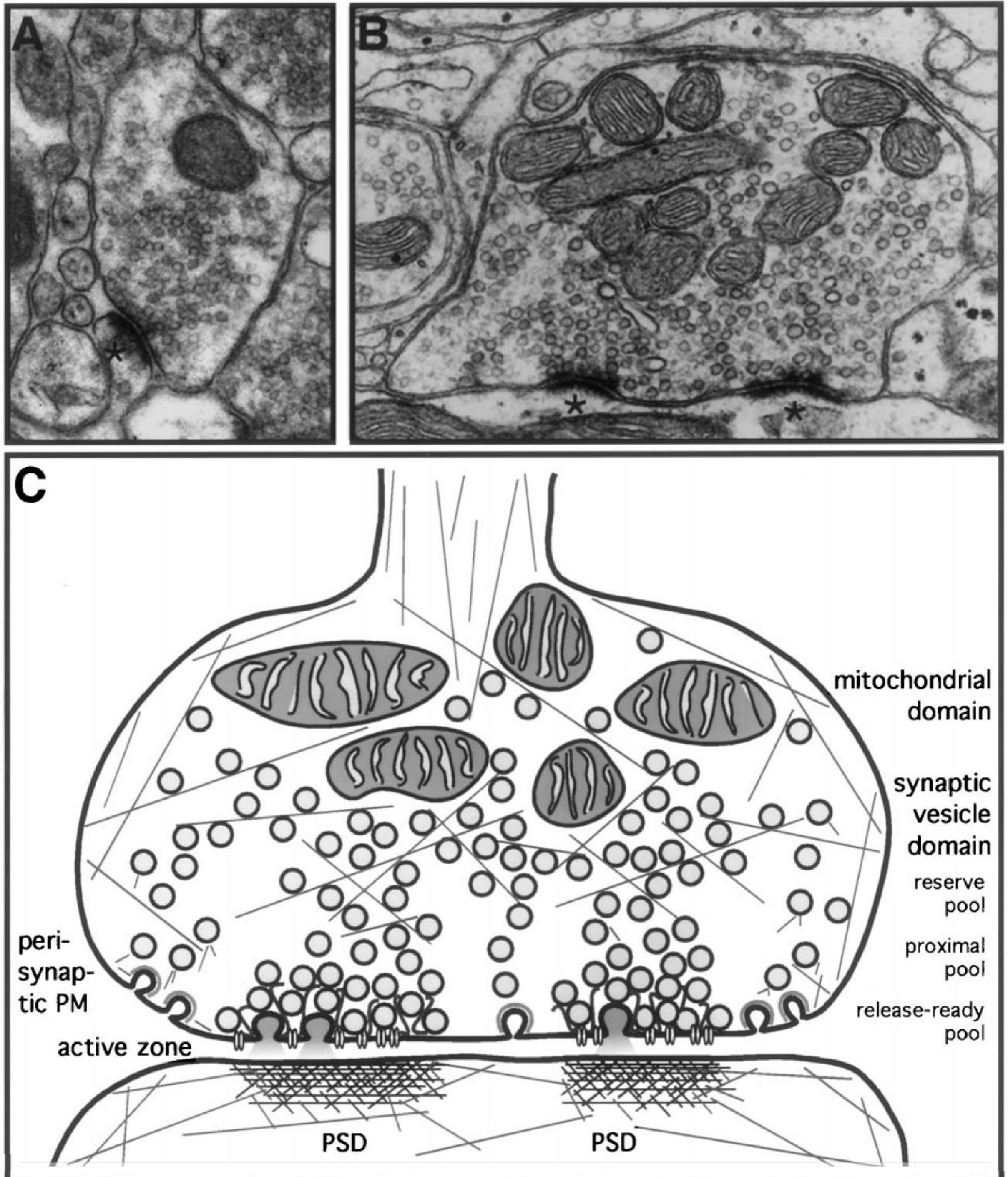



Adapted from Ziv and Garner (2004)



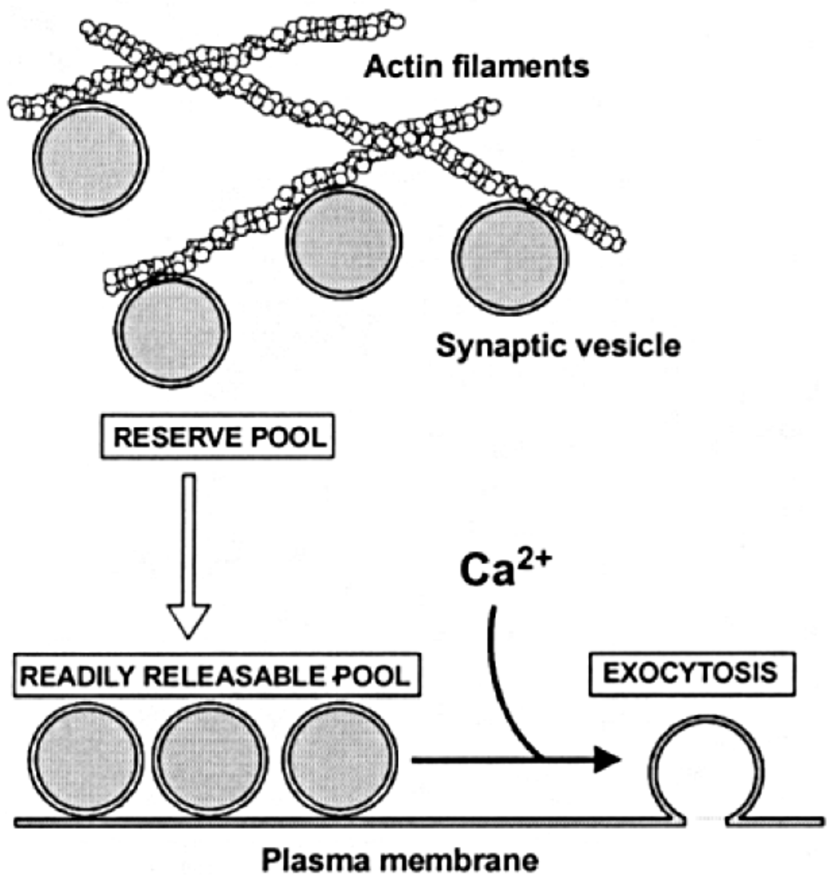

Gundelfinger et al., 2016

# Actin and Regulation of Synaptic Vesicles in the Readily Releasable Pool



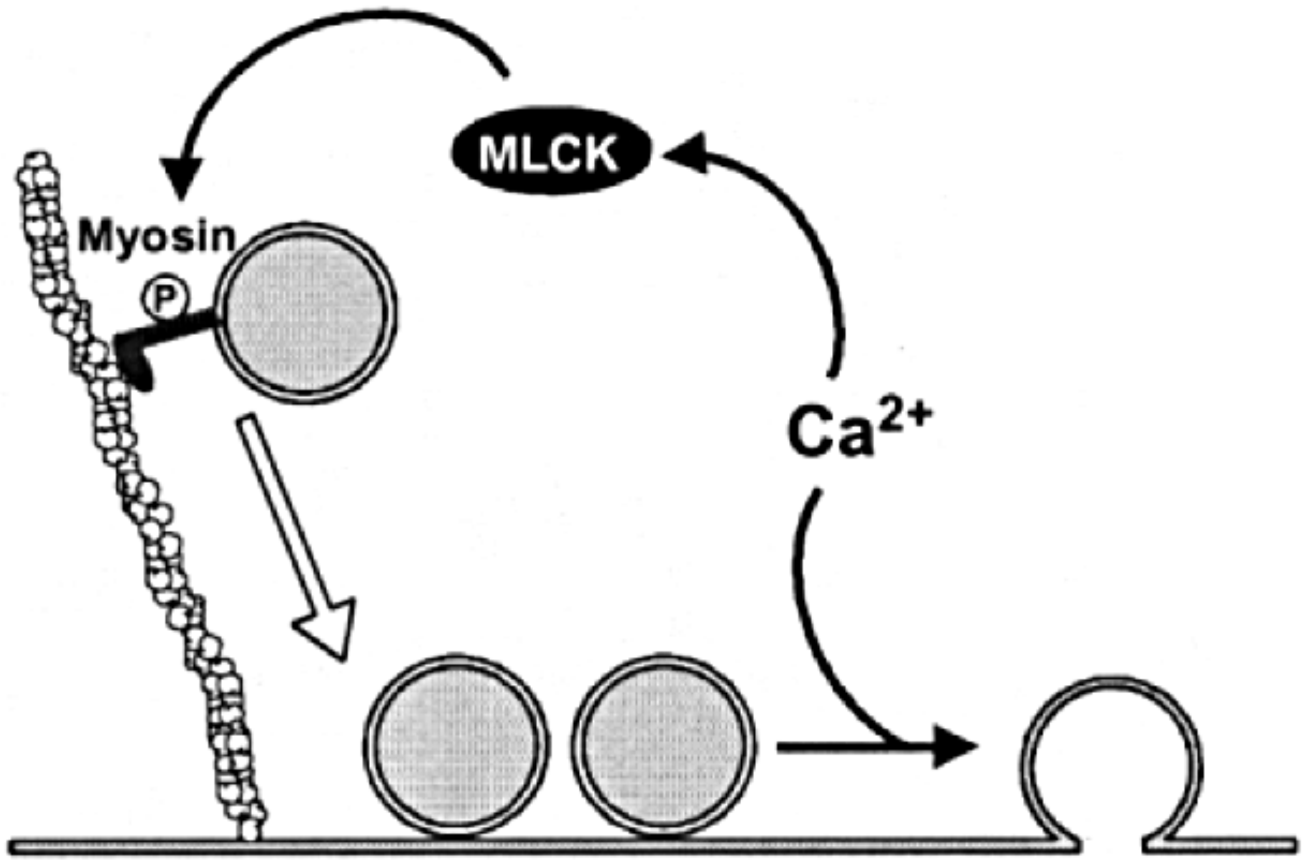

Dresbach et al., 2001

# Involvement of actin in translocation of SV from the reserve pool to the readily releasable pool



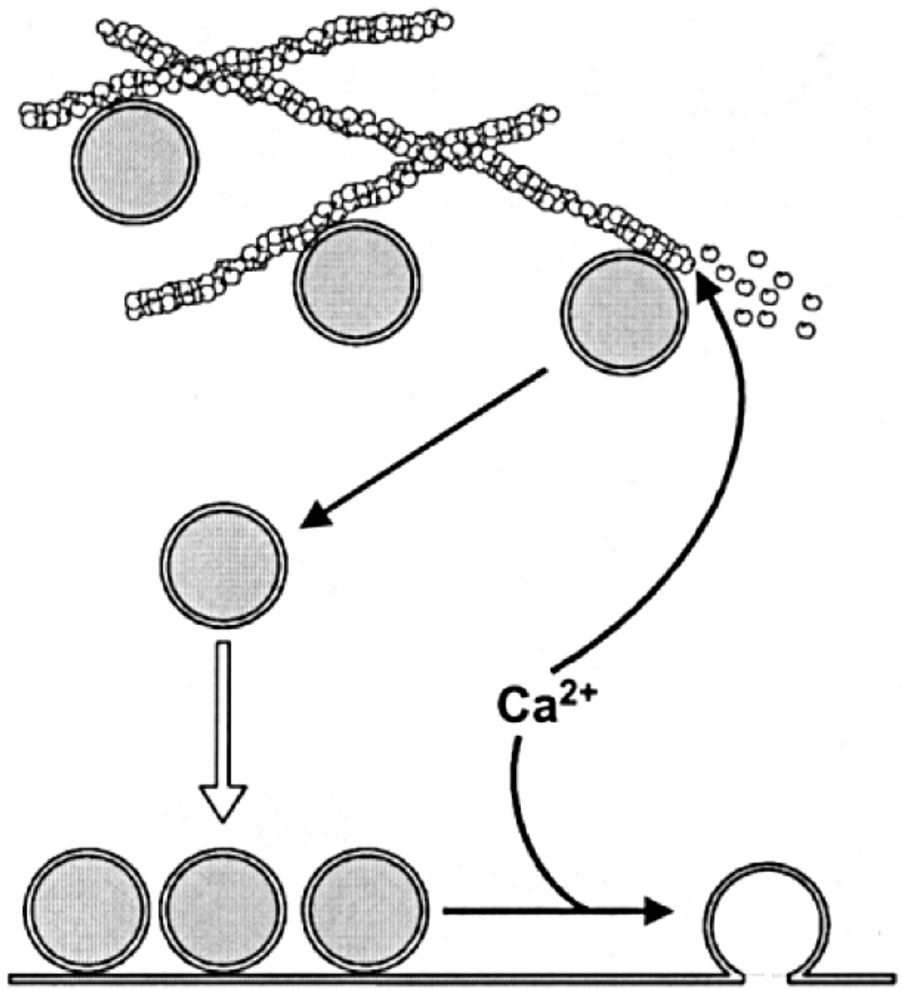

Doussau and Augustine, 2000

Actin may serve as a track for movement between the two pools



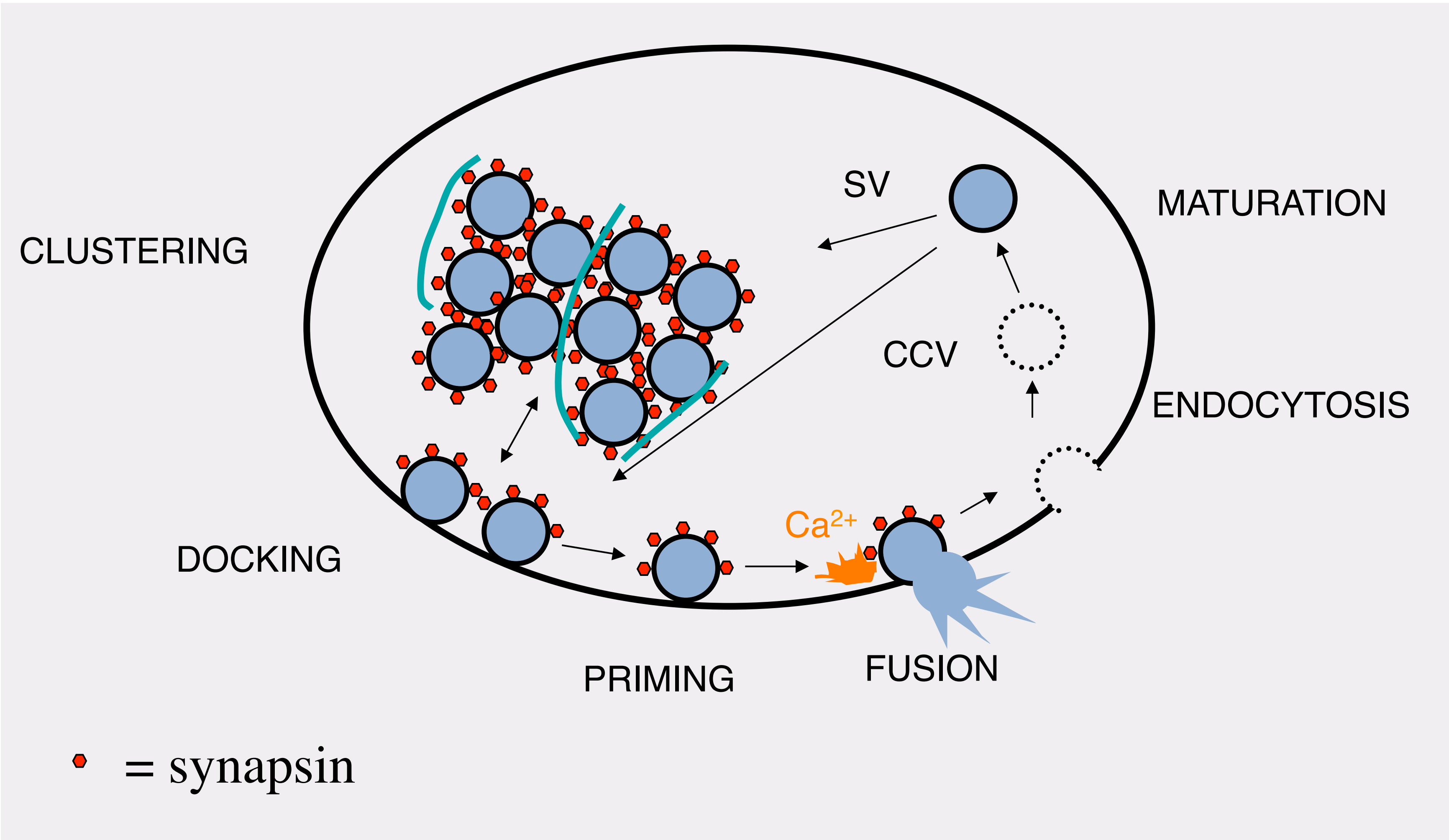

Doussau and Augustine, 2000

# Depolymerization of actin may enable vesicles to move between the two pools



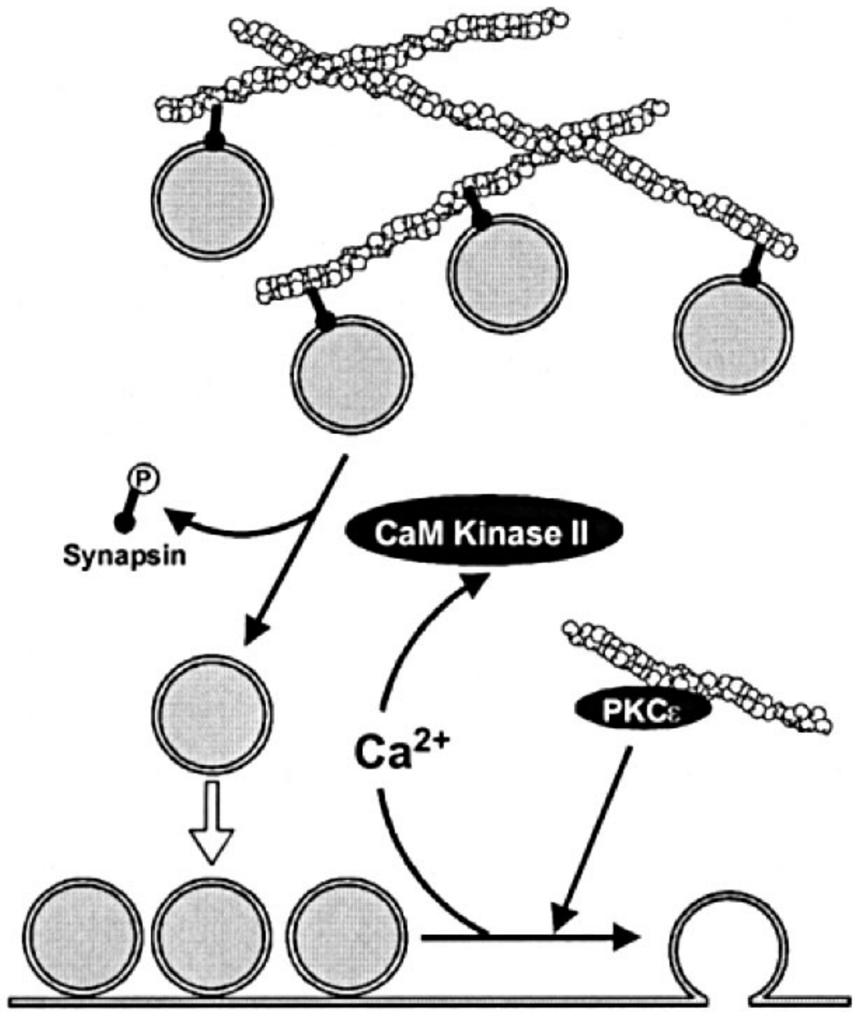

Doussau and Augustine, 2000

# Model for Synapsin Function

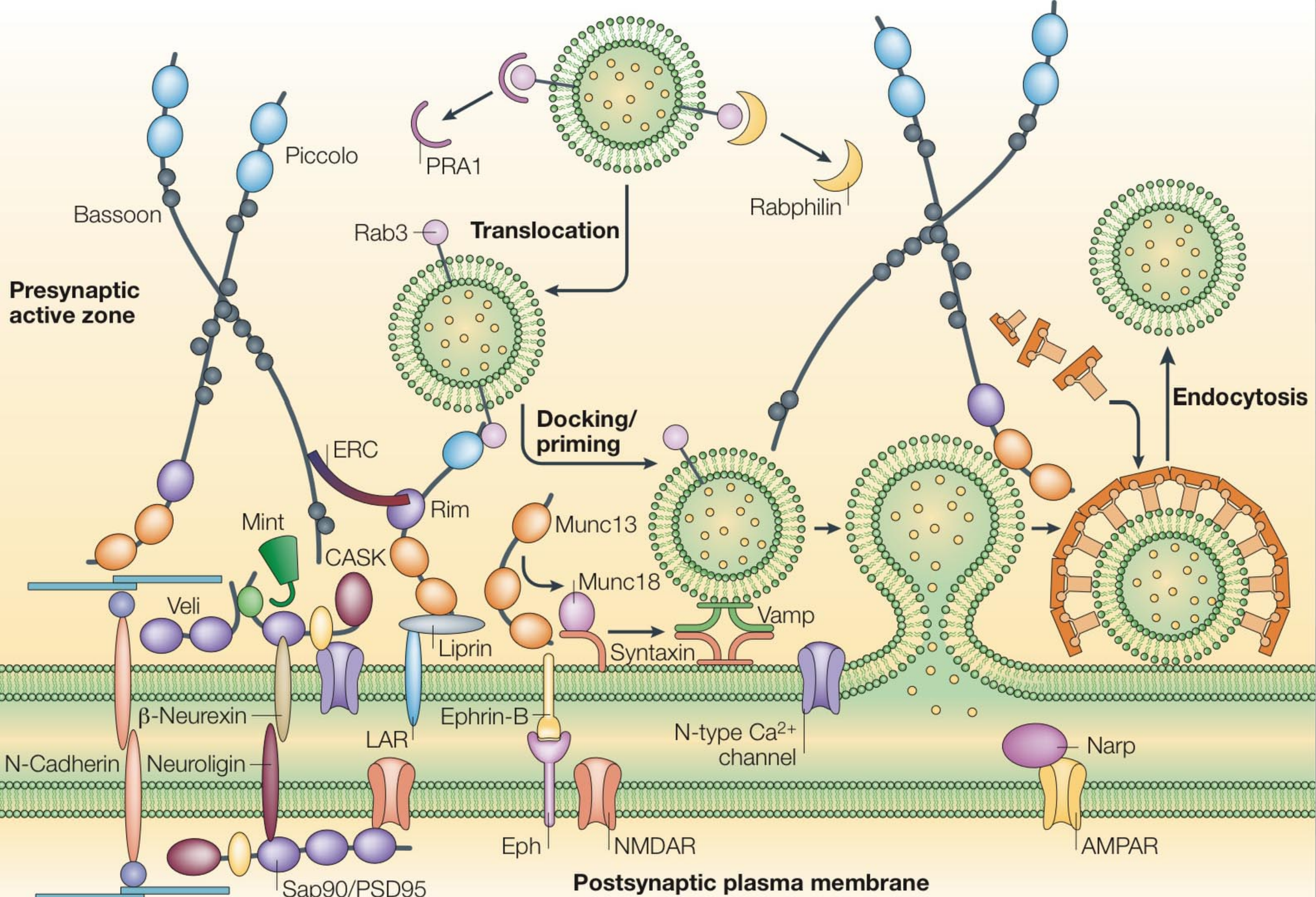



Adapted from Hilfiker et al., (1999)

# Phosphorylation of synapsin may release SV from actin




Doussau and Augustine, 2000

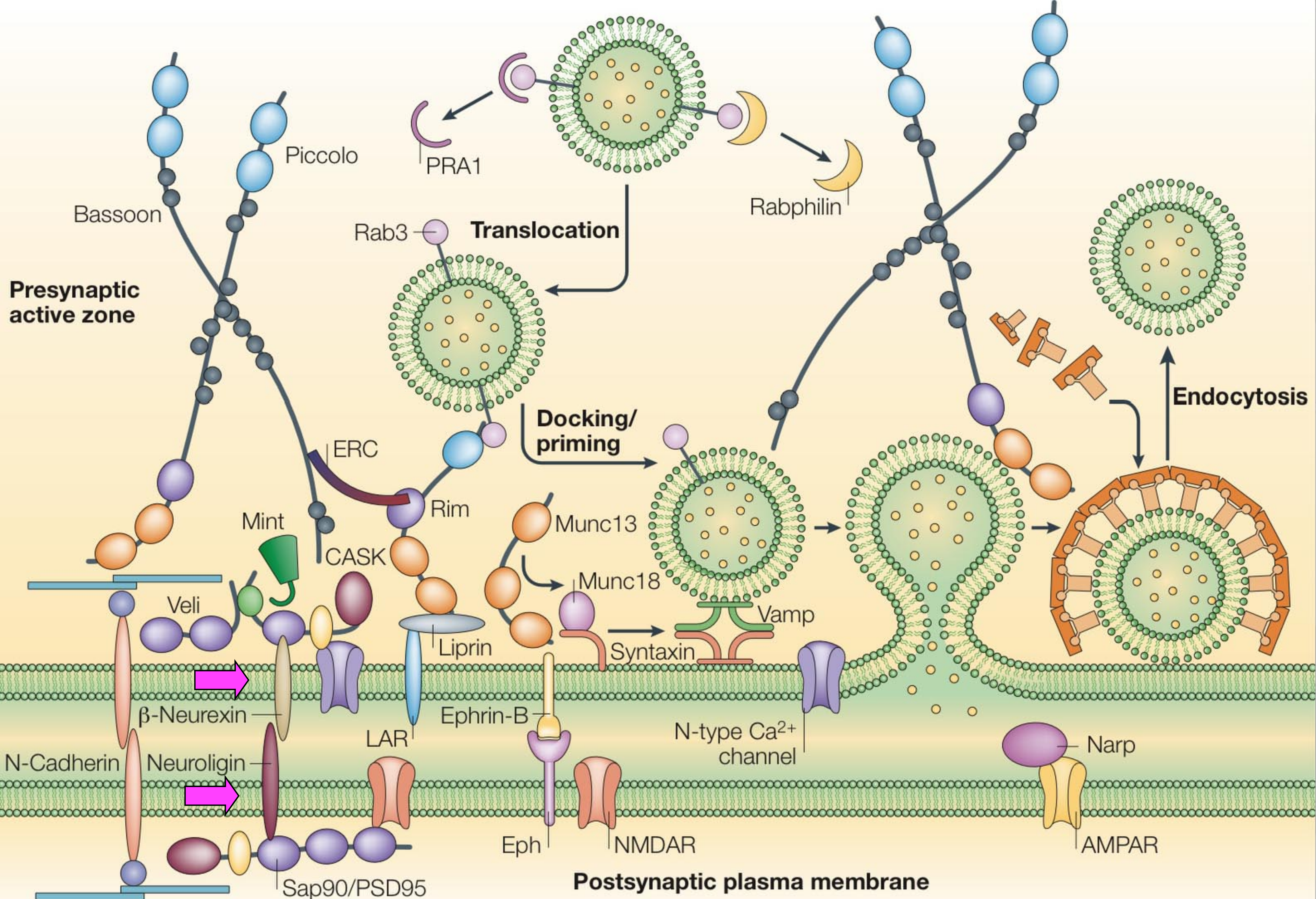


Adapted from Ziv and Garner (2004)

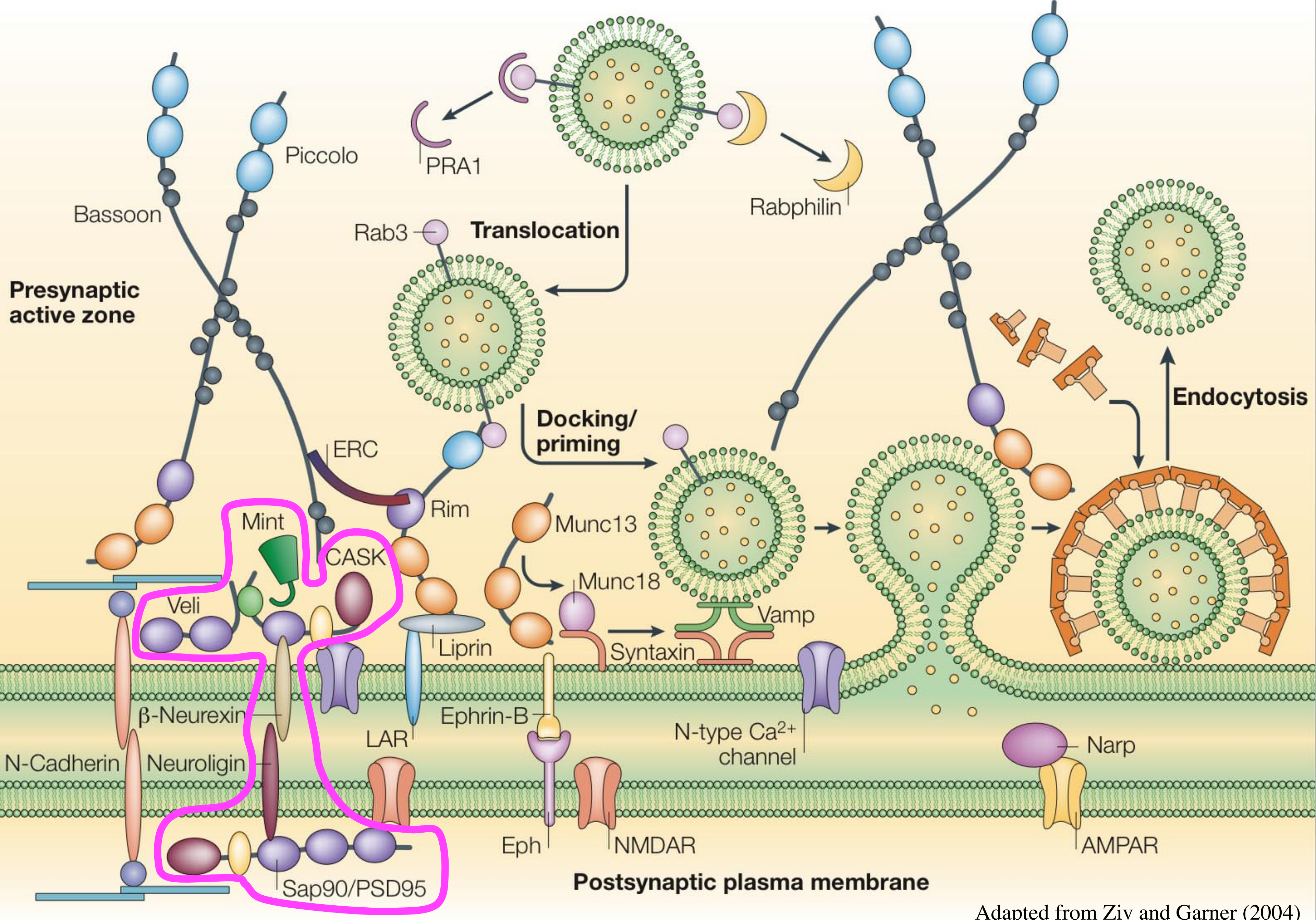
# Relationship of scaffold proteins to assembly

# Neurexin Synaptogenic Activity

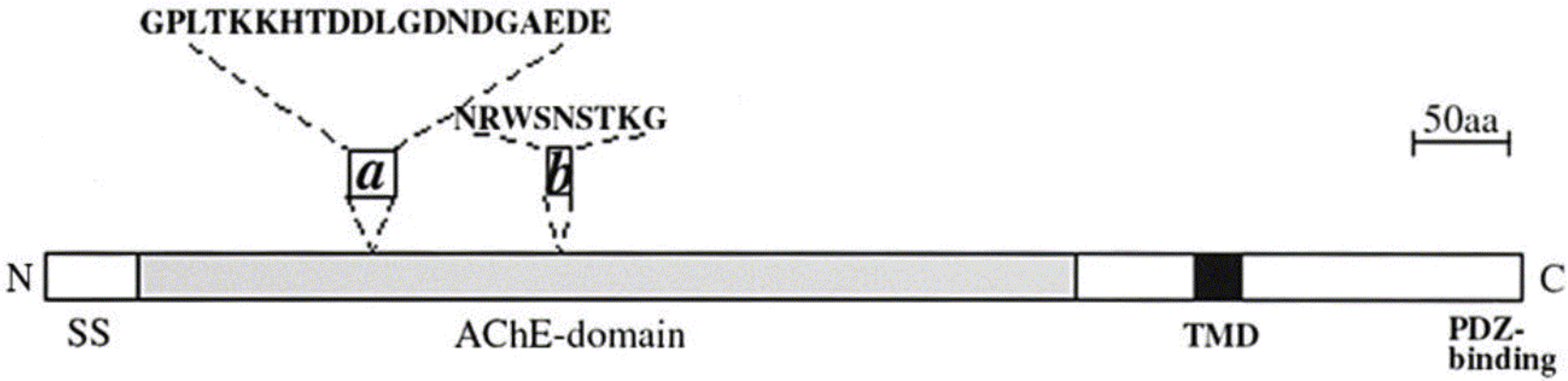
- The neuroligin-neurexin interaction can induce presynaptic assembly.



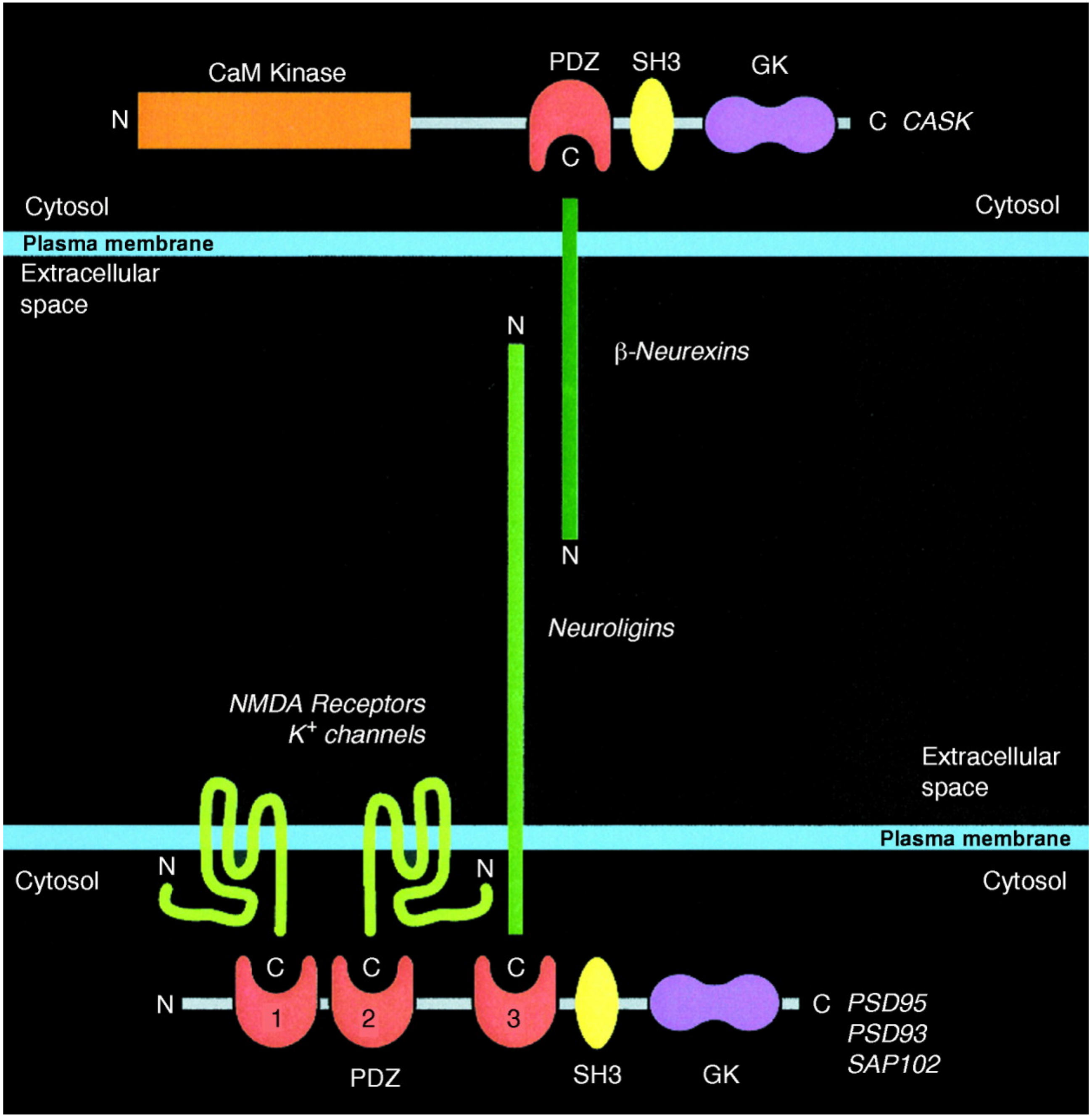

Adapted from Ziv and Garner (2004)




Adapted from Ziv and Garner (2004)

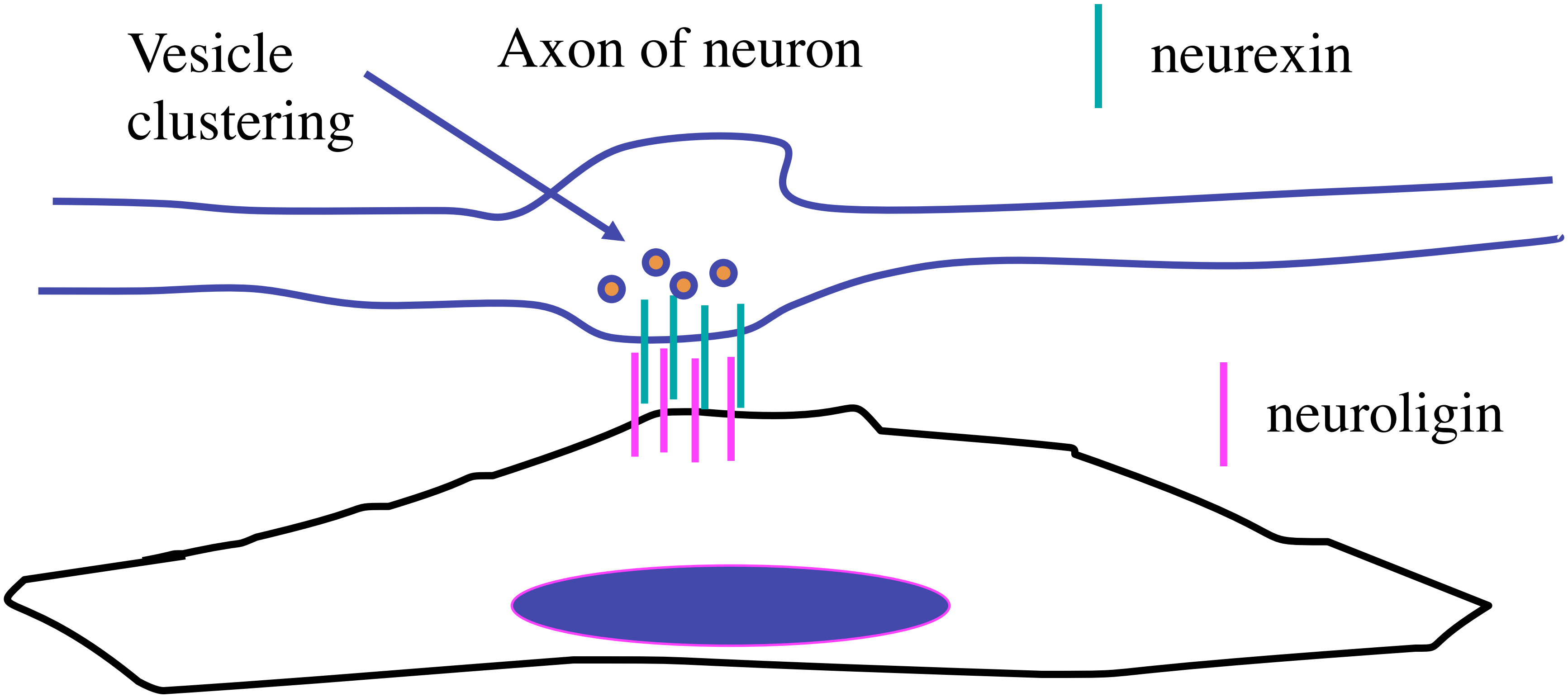



Neuroligin-1 and splice variants:
SS = signal peptide sequence
AChE-domain = homology with acetylcholinesterase
TMD = Trans membrane domain
PDZ binding site is shown



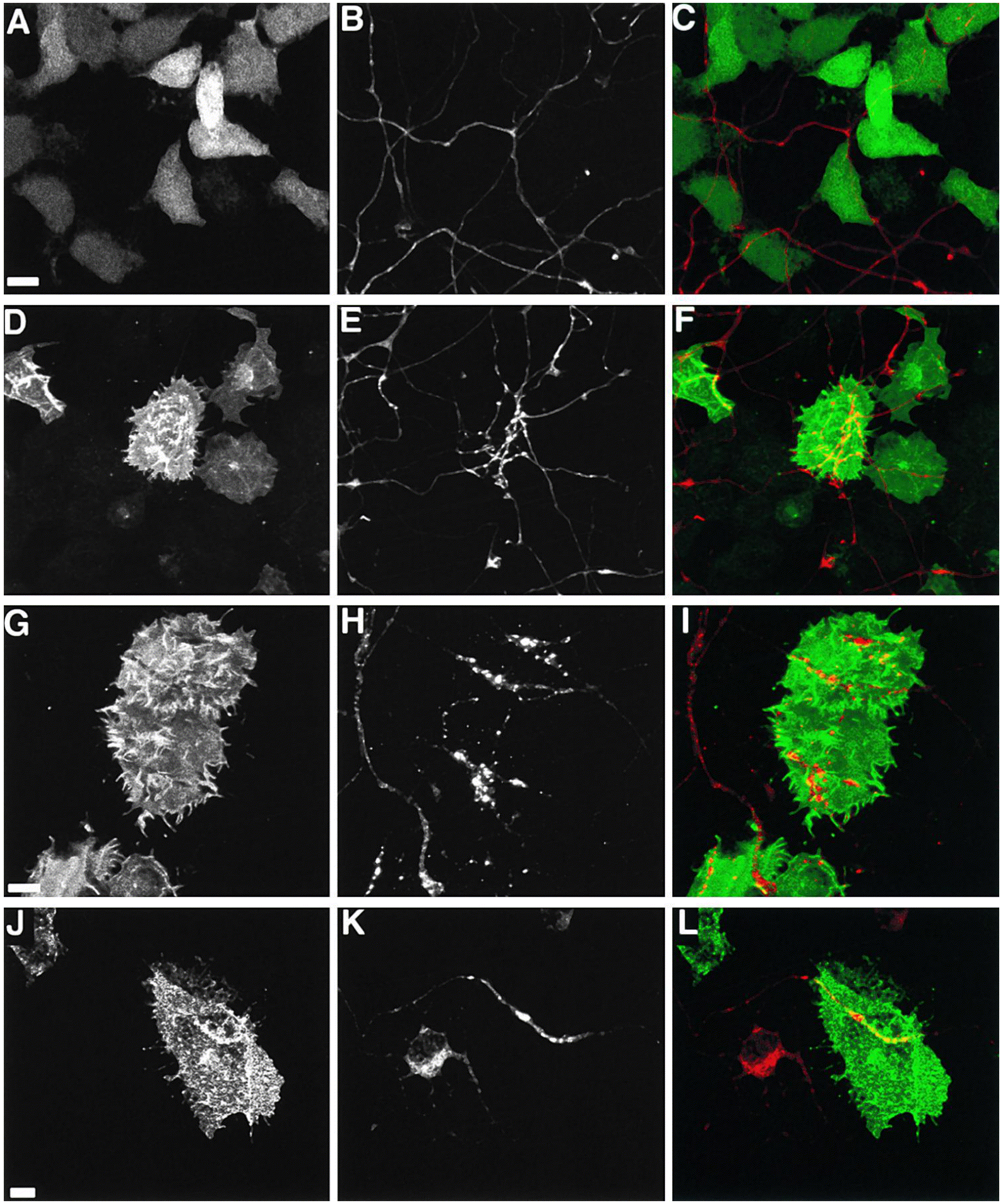

Irie et al., 1997

Presynaptic genesis by neuroligin in heterologus cell




Heterologous cell expressing exogenous neuroligins

# Clustering of axonal synapsin by neuroligins expressed in heterologous cells (293 cells)




<u>Transfection of 293 cells</u>

GFP

Neuroligin-1

Neuroligin-2

*

*Pontine explant coculture (synapsin)

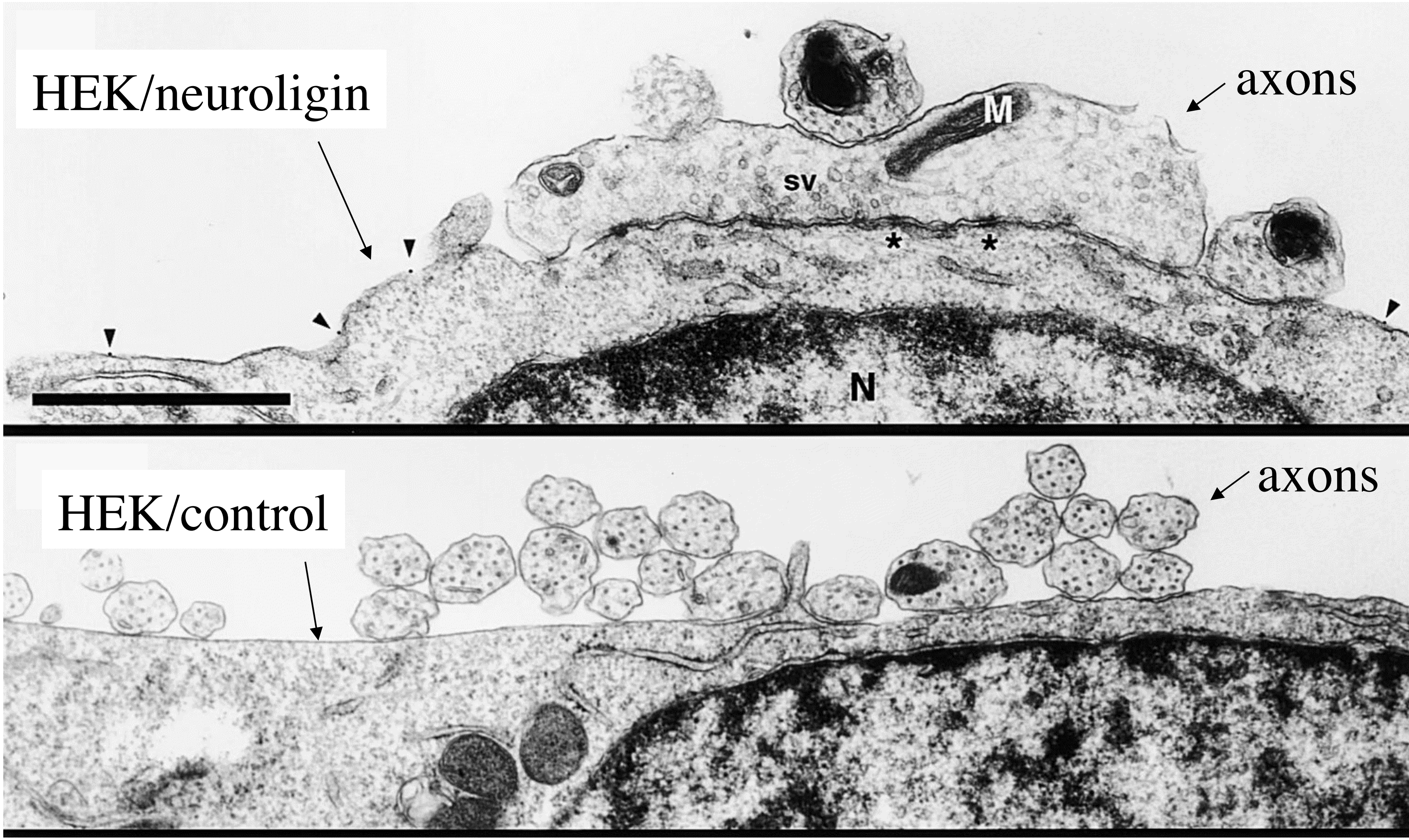
Neuroligin-1

- Cerebellar granule cell coculture (synapsin)

*

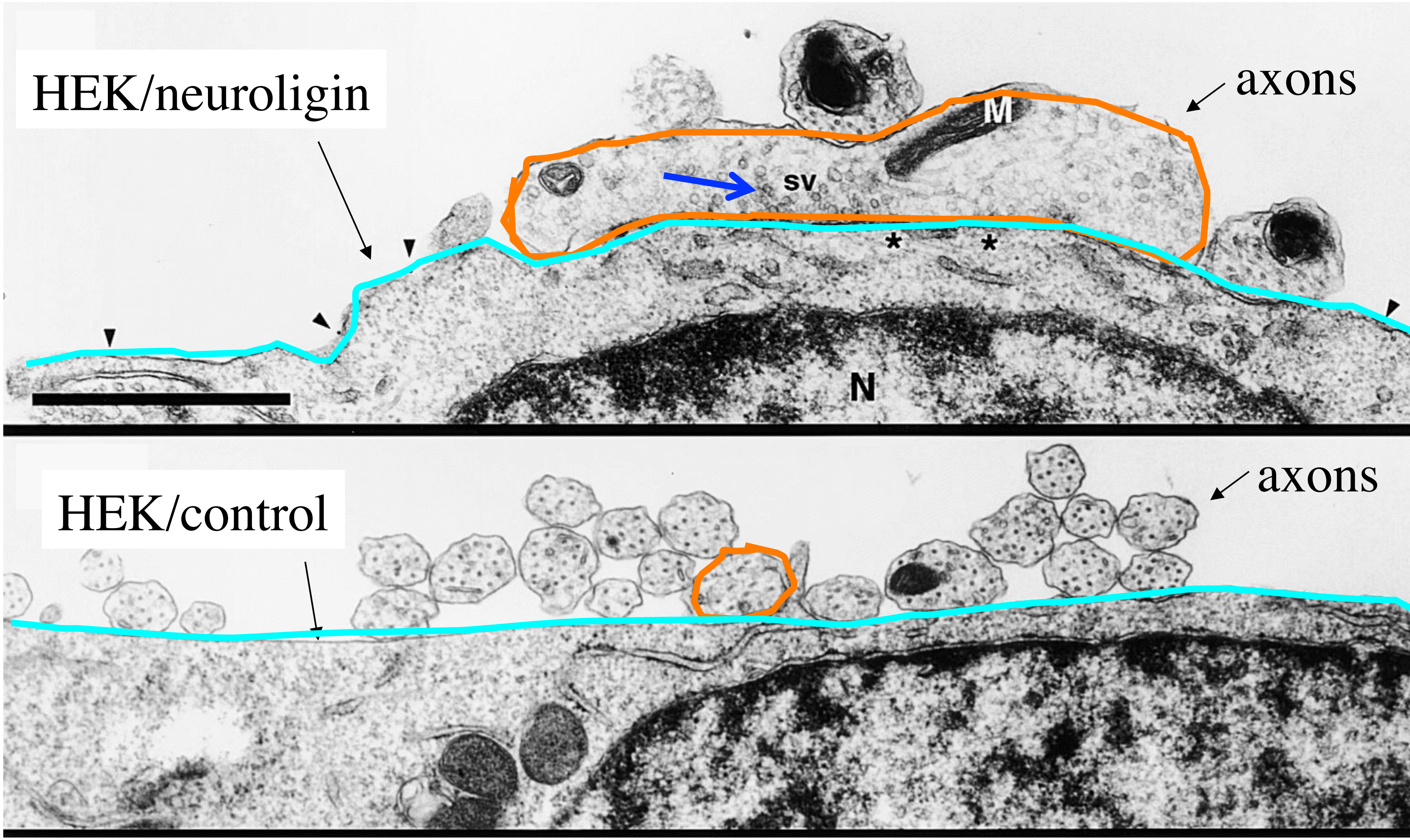
Scheiffele et al., (2000)

# Neuroligin in HEK cell induces synaptic vesicle clustering




Scheiffel et al., (2000)

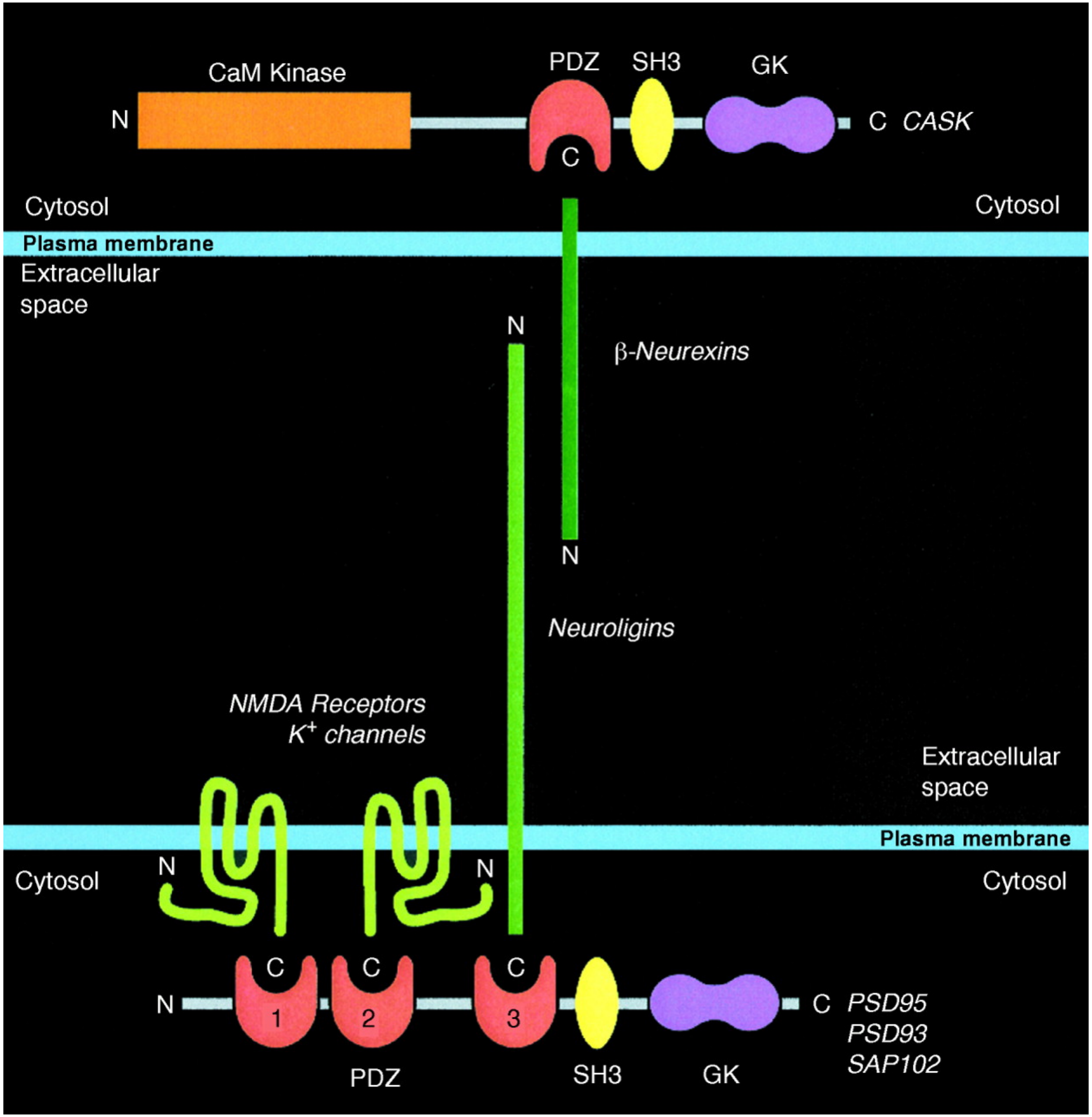
# Neuroligin in HEK cell induces synaptic vesicle clustering



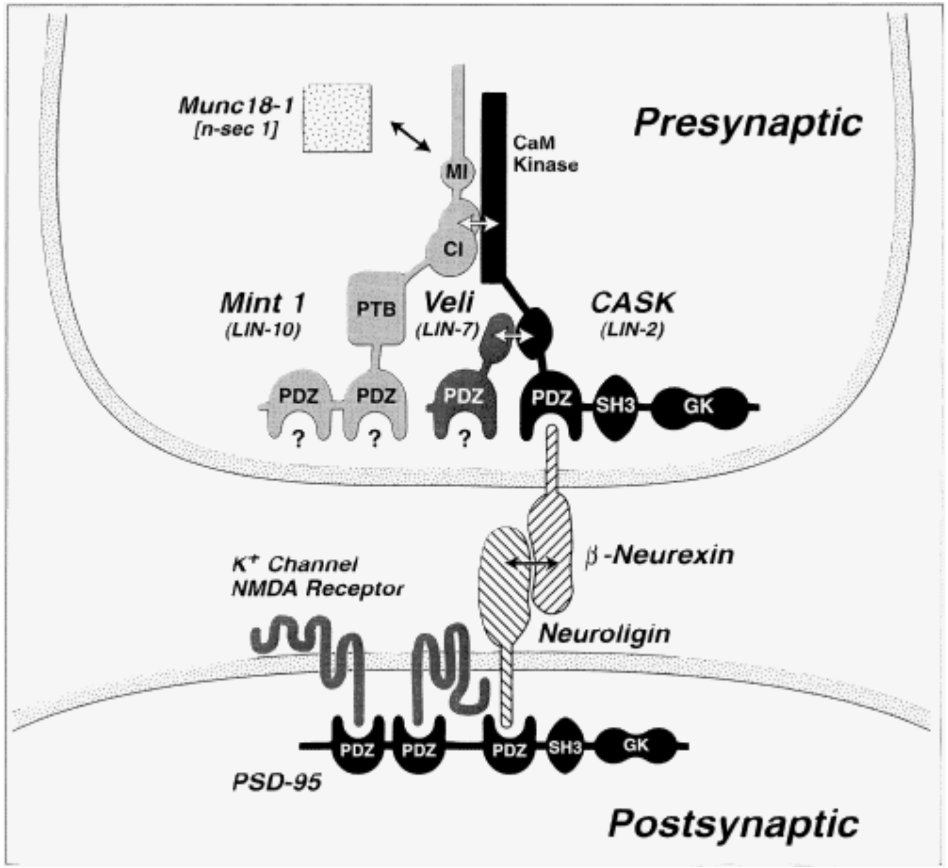

Scheiffel et al., (2000)

## Synaptic vesicle clustering by neuroligin:

- Depends upon neuroligin acetylcholinesterase domain
- Depends upon neuroligin interaction with neurexin (since loss of neurexin binding upon neuroligin mutagenesis parallels loss of SV clustering)
- Is blocked by soluble neurexin




Irie et al., 1997




Model of the Tripartite Complex of CASK, Mint1, and Velis: Implications for Neurexin/Neuroligin–Dependent Cell Adhesion and Synapse Function. Butz et al., 1998.

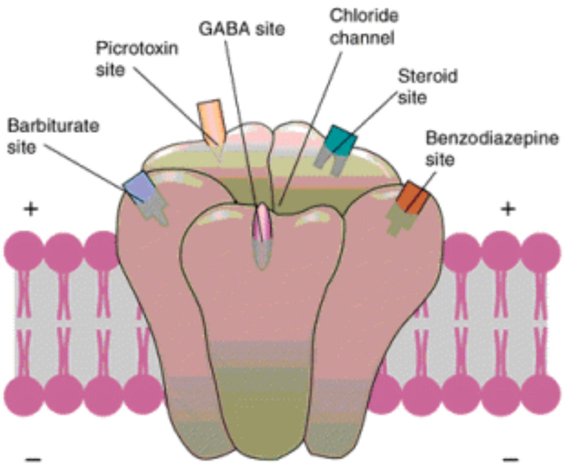
# $GABA_A R$ Function in Development

- Maturation of inhibitory circuits can be protracted and extend well into postnatal life.
- Chloride efflux through $GABA_A R$ early in development depolarizes.
- Later in development the KCC2 Chloride transporter lowers intracellular chloride concentration, leading to chloride influx through the $GABA_A R$.
- Chloride influx later in development inhibits (polarizes the cell).

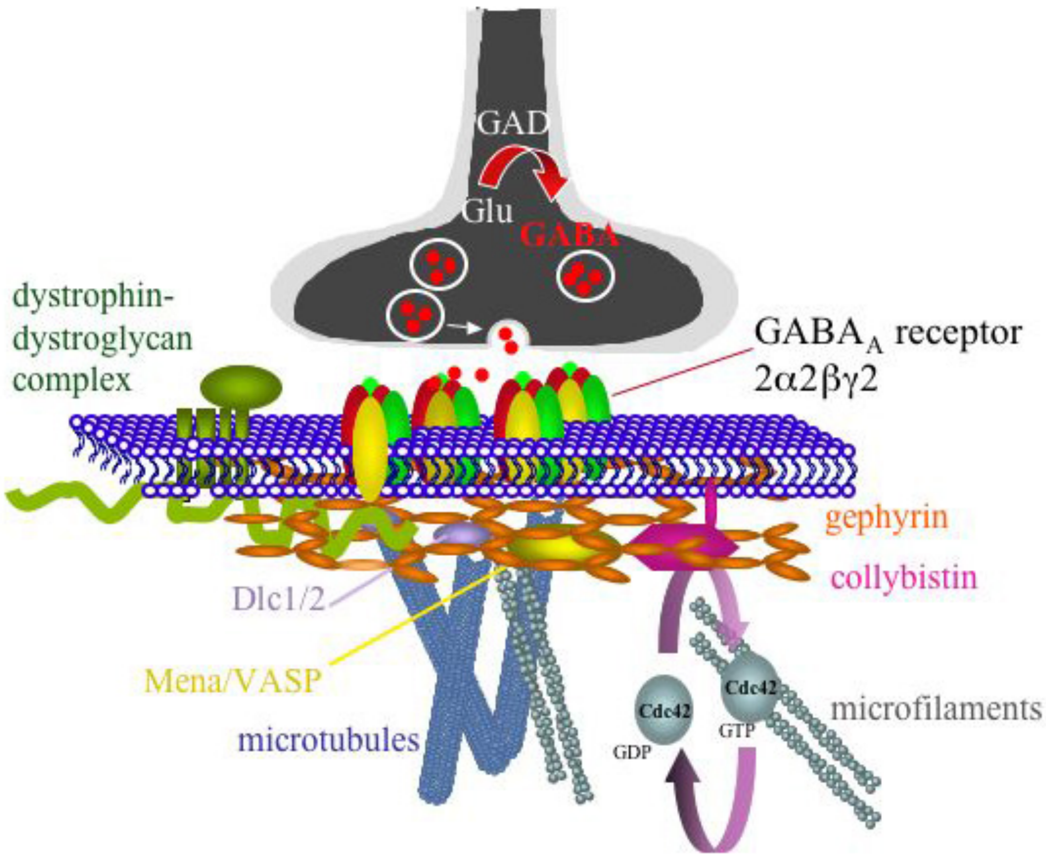
# GABA$_A$Rs

- GABA$_A$Rs are pentameric: α, β γ2 in 2:2:1 stoichiometry.

► Schematic Illustration of a GABA$_A$ Receptor, with Its Binding Sites

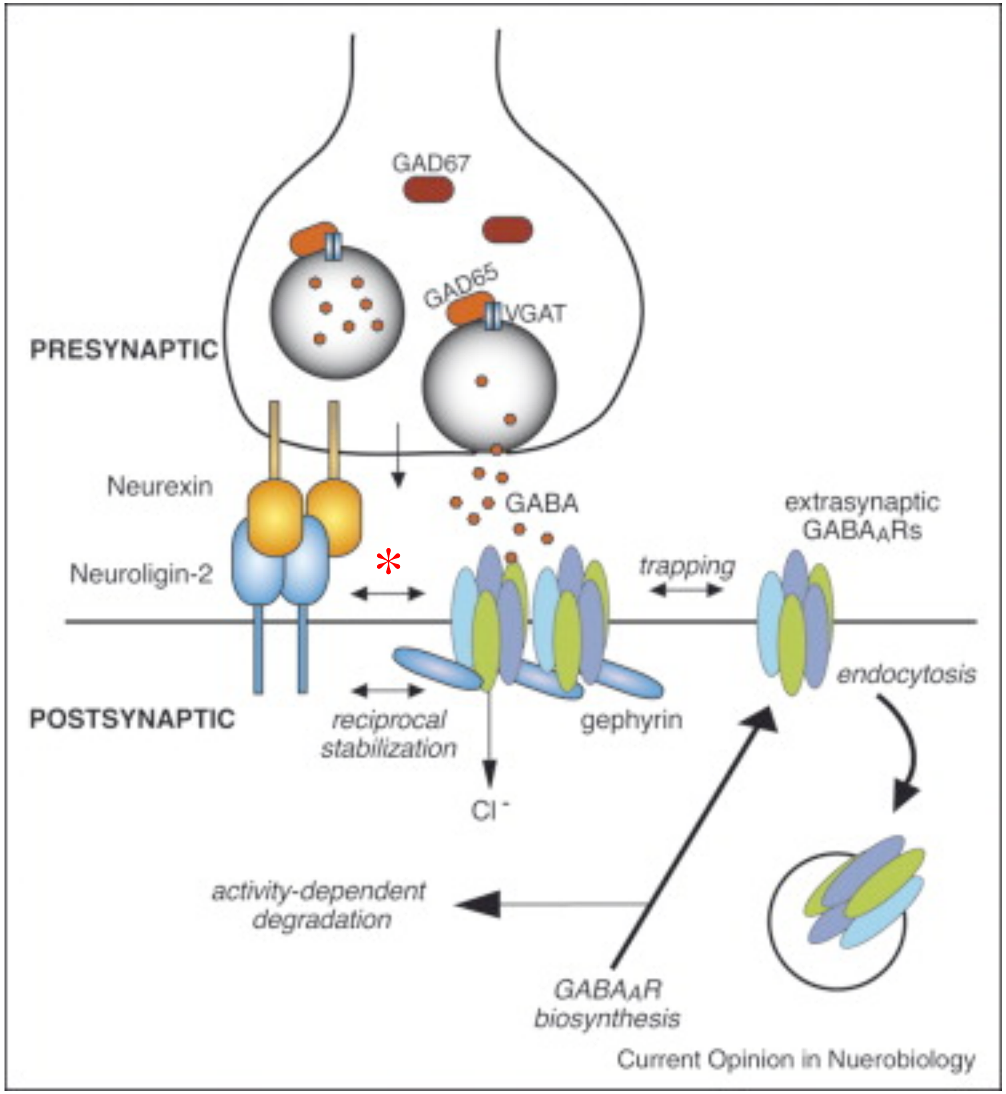


# GABA$_A$Rs Scaffolds



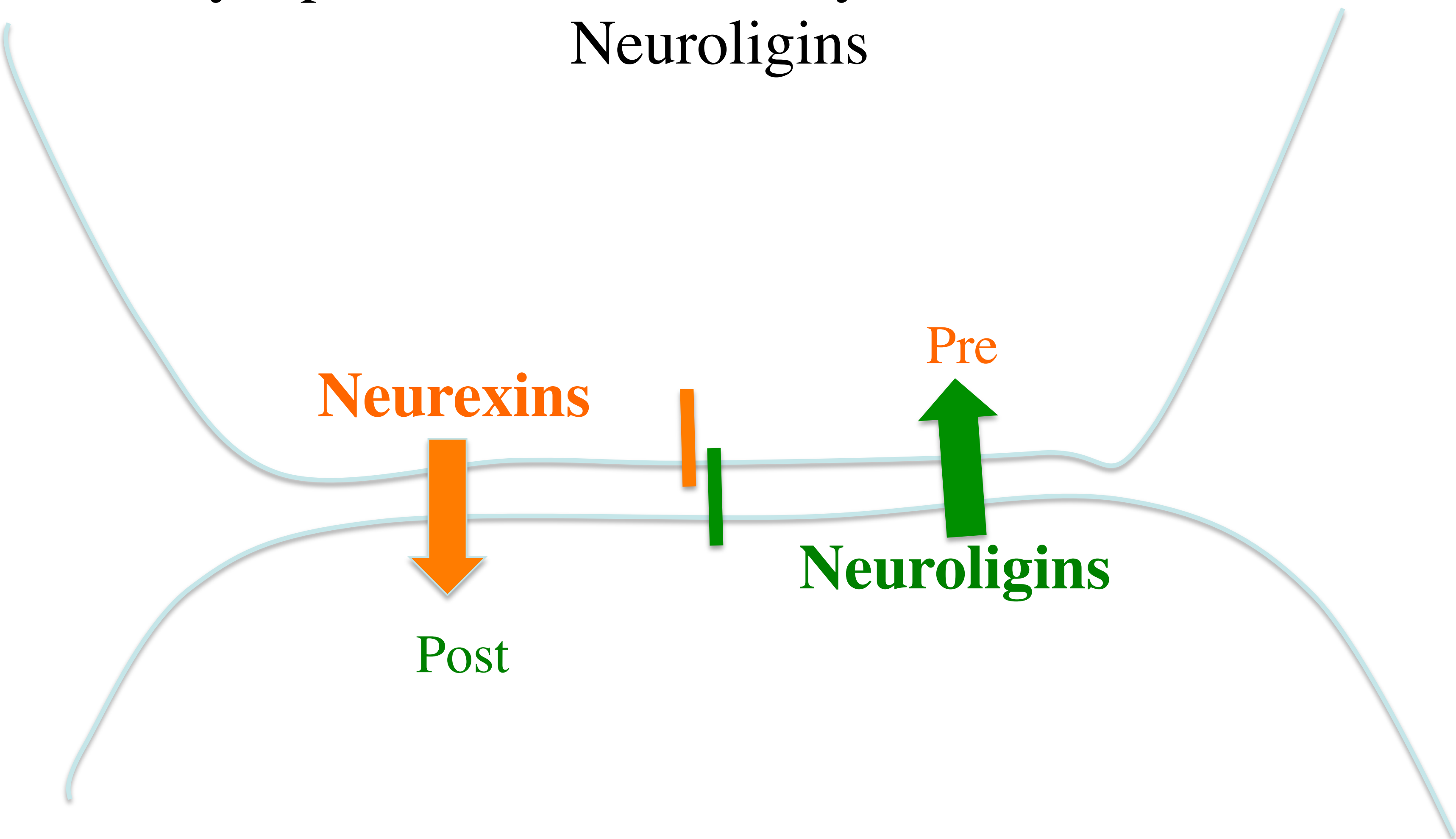
## Neurexins Induce Differentiation of GABA and Glutamate Postsynaptic Specializations via Neuroligins (Graf et al., 2004)

- Neurexin alone is sufficient to induce glutamate postsynaptic differentiation in contacting dendrites.
- Surprisingly, neurexin also induces GABA postsynaptic differentiation.
- Neuroligins induce presynaptic differentiation in both glutamate and GABA axons.
- Whereas neuroligins-1, -3, and -4 localize to glutamate postsynaptic sites, neuroligin-2 localizes primarily to GABA synapses.
- Direct aggregation of neuroligins reveals a linkage of neuroligin-2 to GABA and glutamate postsynaptic proteins, but the other neuroligins only to glutamate postsynaptic proteins.




NL2 and synaptic $GABA_A$R stabilize each other, either through intracellular reciprocal interactions aided by scaffolding proteins such as gephyrin or through extracellular *cis* interaction.

# Synaptic Differentiation by Neurexins and Neuroligins



# Discussion Reading

- Selective Capability of SynCAM and Neuroligin for Functional Synapse Assembly
- Sara, Y et al (2005) J. Neuroscience 25:260-170.

# Questions?

Contact Ed Ziff
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