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
assembly of clathrin heavy chain triskelia into lattice produces invagination that may drive endocytosis
cargo recognition: adaptors

motifs
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
heterotetramer
\(\alpha, \beta\) bind to clathrin
\(\sigma/\alpha\) recognize dileucine motifs
\(\mu\) 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 \textit{C. elegans} suggests limited or no role for clathrin
BAR (Bin/Amphiphysin/Rvs) domain proteins

endophilin

| BAR | CC | SH3 |

sense/promote membrane curvature

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

(Weissenhorn, 2004)
BAR domain proteins produce tubules of different sizes in cells as well as \textit{in vitro} (Frost et al., 2008)
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

dynamin forms collars around neck of vesicle
GTP hydrolysis triggers scission

(Takei et al, 1998)
regulation: why are SVs not coated with clathrin?

AP-2

Open conformation

Locked conformation

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:

recycling slowed
defect in endocytosis

(diPaolo et al, 2004)
uncoating

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

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

(Kim et al, 2002)
compensatory endocytosis
(how does endo = exo): calcium?

lamprey reticulospinal synapse stimulated at high frequency incubated in 0 Ca\(^{++}\) for 90 min then Ca\(^{++}\) added back

Ca\(^{++}\) required but as low as 11 \(\mu\)M suffices

(Gad et al, 1998)
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
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
dual roles in function/recruitment
dynamin: scission / amphiphysin-endophilin
endophilin: invagination / synaptojanin
synaptojanin: ?fission/uncoating
kiss-and-run

clastrin-mediated endocytosis (CME)

CME

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
kiss-and-run

clathrin-mediated endocytosis (CME)

CME

bulk endocytosis
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)

(Heuser and Reese)
direct visualization--freeze-slammer

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

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

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

FM dye photoconversion

(Rizzoli and Betz, 2005)

(Harata et al., 2001)
spontaneous release of spontaneously loaded SVs 
evoked release of SVs loaded by stimulation
--distinct pools retain their identity after recycling

(Sara et al., 2005)
**Drosophila** neuromuscular junction postsynaptic Ca$^{++}$ imaging spontaneous and evoked release at different sites -- not correlated maturation state vs. diff pools?

(Melom et al., 2013)
perhaps different endocytic pathways make different pools
VAMP7 (not synaptophysin) depends on AP-3 for SV localization

(Scheuber et al, 2006)
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

References


