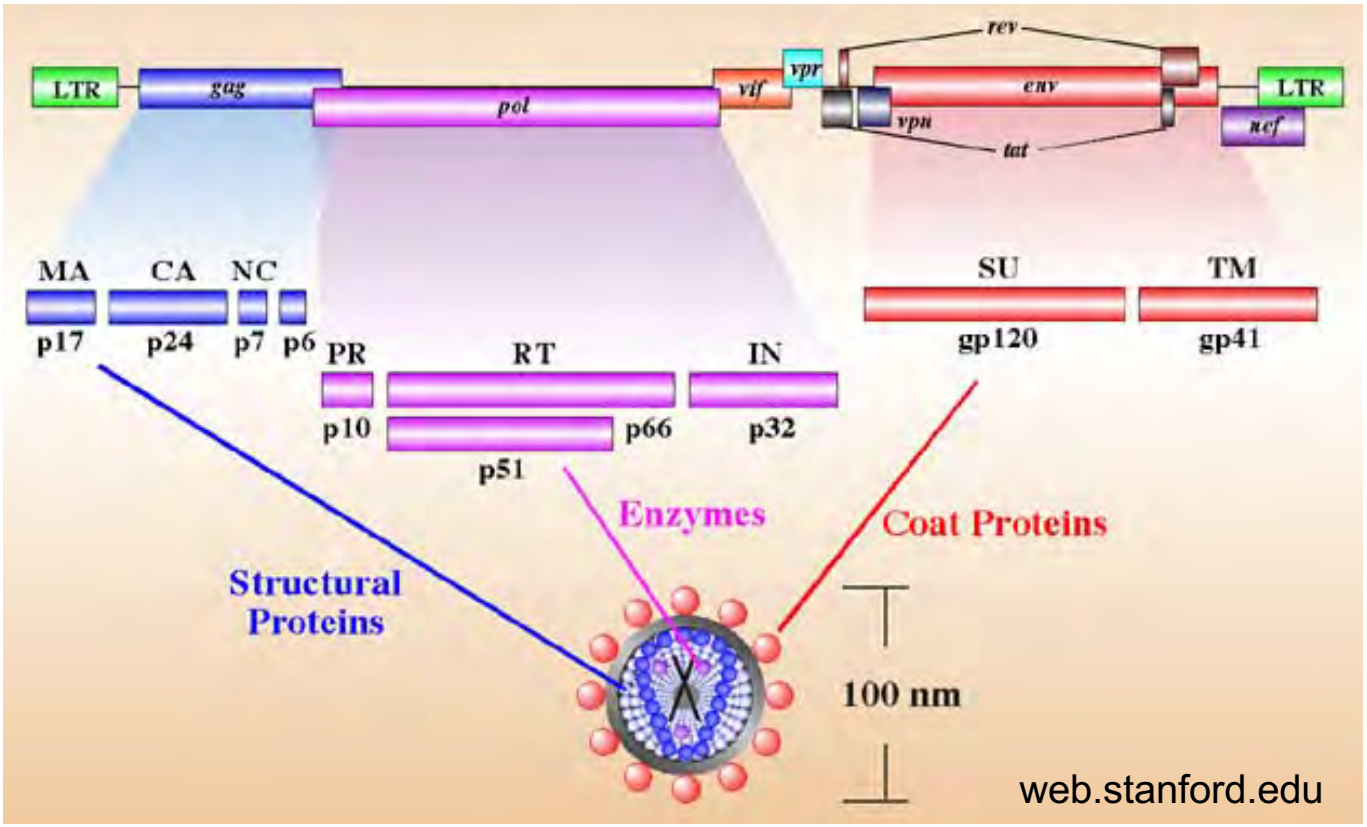
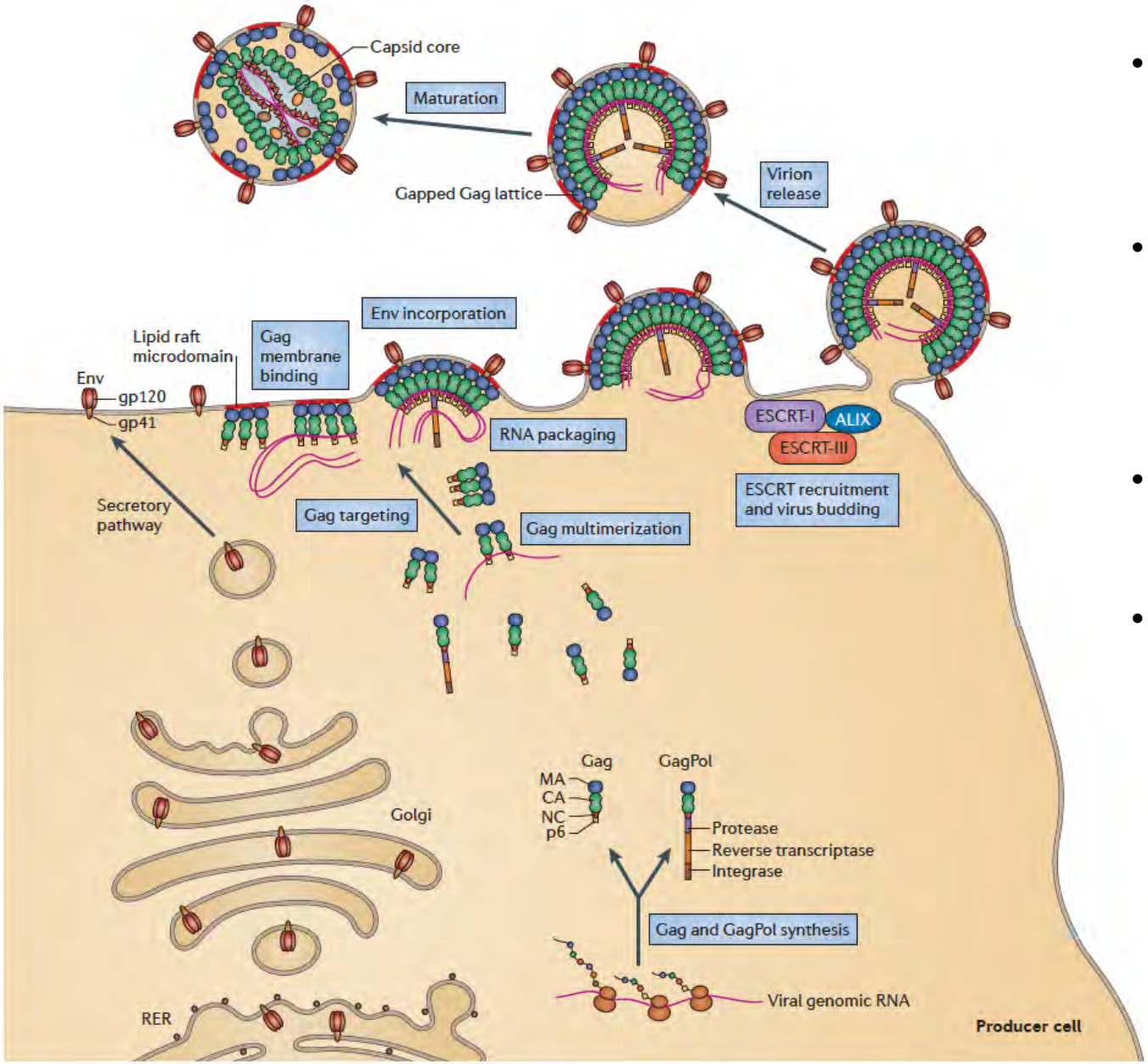
HIV-1 genome organization



- Gag encodes for a polyprotein that is cleaved into 4 proteins by HIV-1 protease.
- Gag is present in 2 transcripts: Gag polyprotein precursor and GagPol polyprotein precursor (5%).
- Cleavage occurs during virus "maturation," after viral budding.

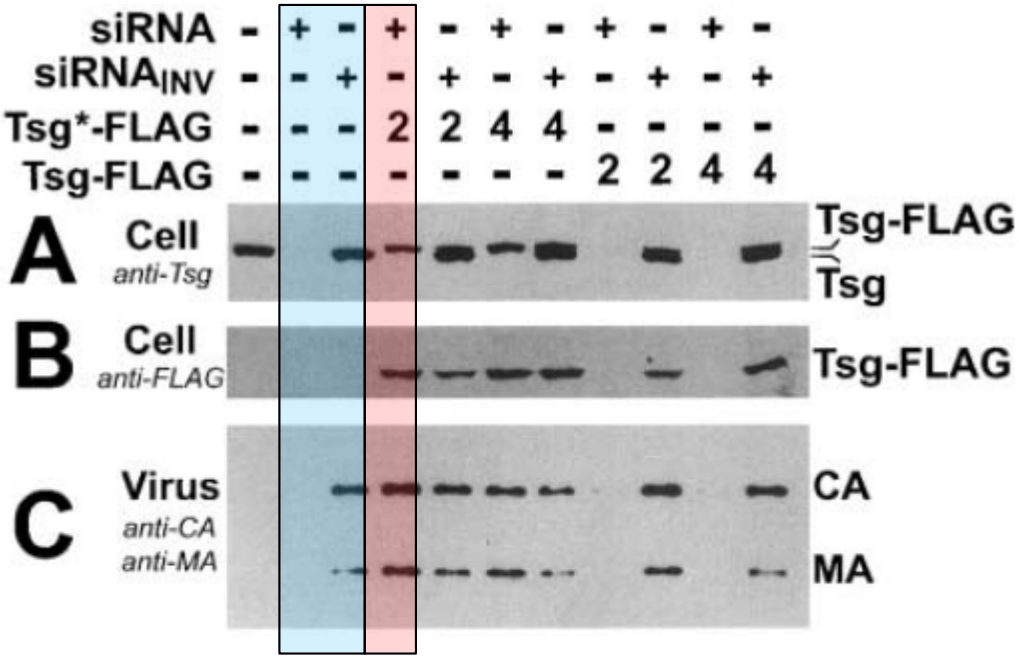
Late stage of HIV-1 replication

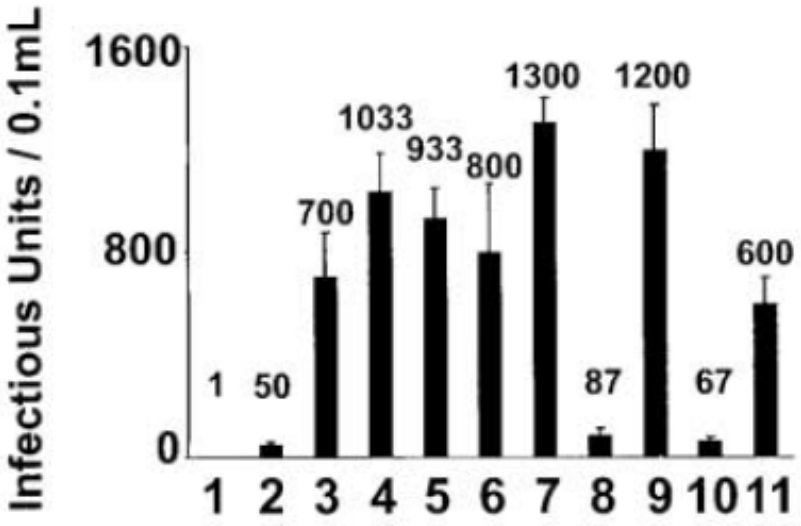


- Gag recruits viral RNA and is targeted to inner leaflet of plasma membrane.
- Virus budding involves assembly of Gag into lattice, evagination of bud, and scission of bud.
- **P6 of Gag is necessary for budding.**
- After virion is released, it undergoes maturation associated with cleavage of Gag and morphological change (radially symmetric to containing a conical capsid core).

Freed (2015) Nat Rev Microbiology

Tsg101 is essential for HIV-1 virion release



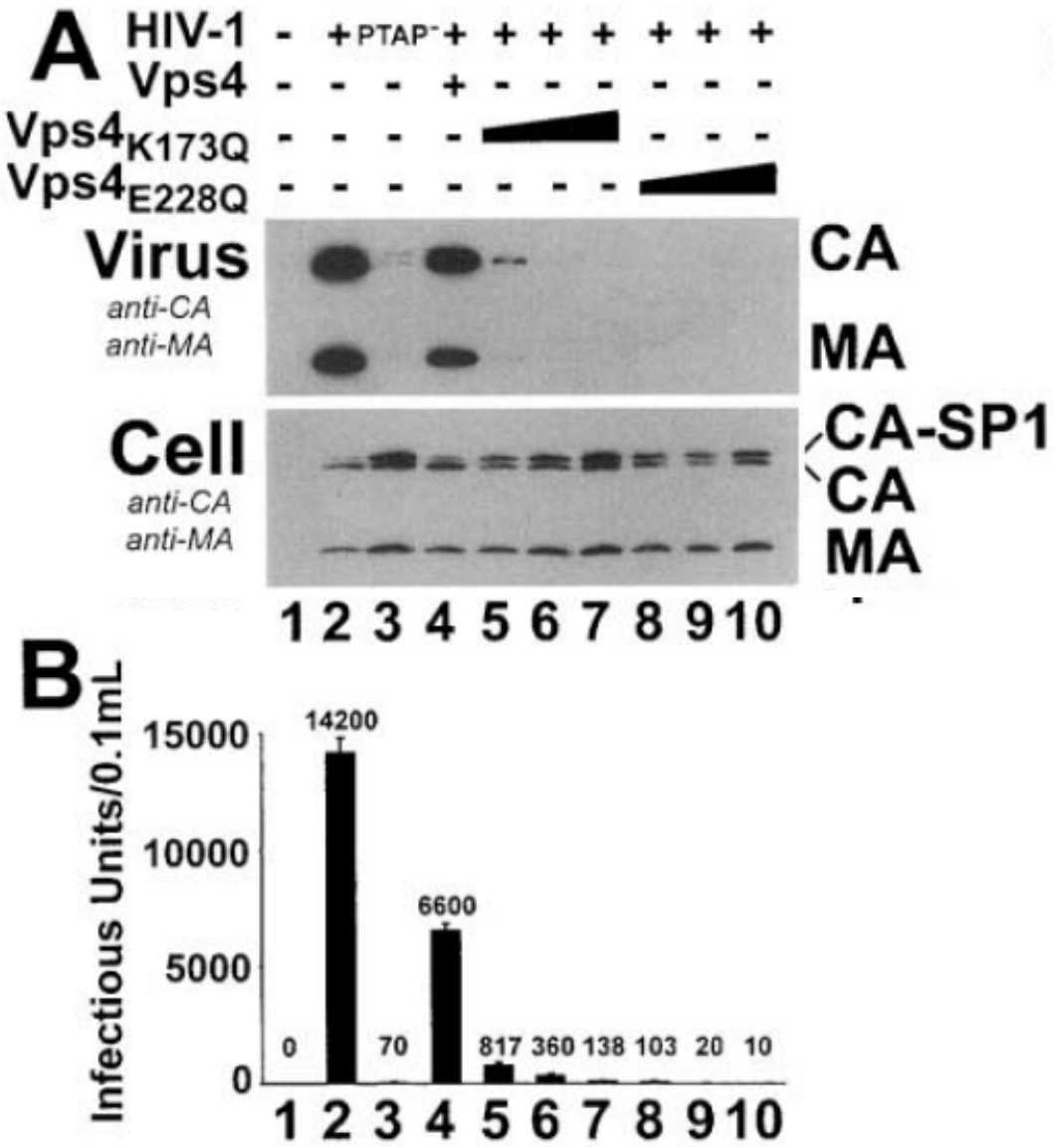


siRNA: against Tsg101
siRNA$_{INV}$: inverted siRNA (control)
Tsg*: siRNA resistant Tsg101

- Knockdown of Tsg101 prevents release of virions into supernatant of 293T cells.
- This effect can be rescued with siRNA-resistant Tsg101 construct.
- In other experiments, knockdown of Tsg101 did not affect MLV virion production.

Garrus et al (2001) Cell

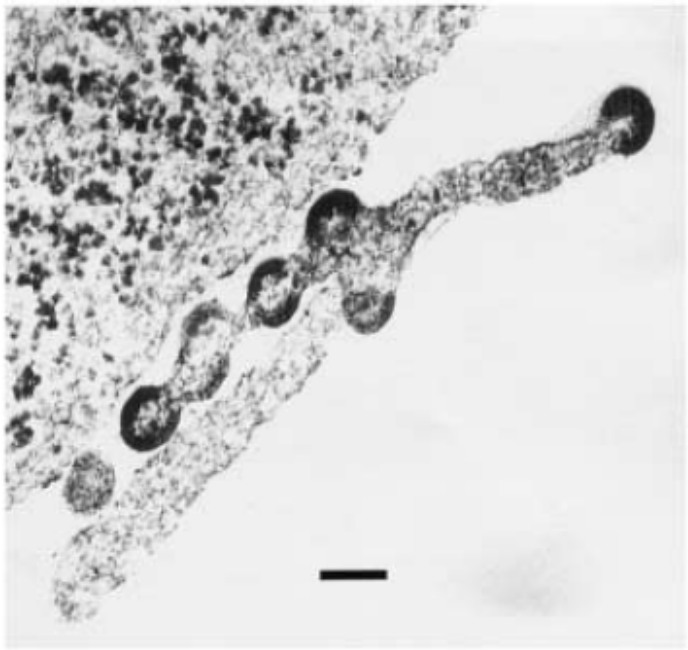
Inhibition of Vps4 blocks HIV-1 virion release



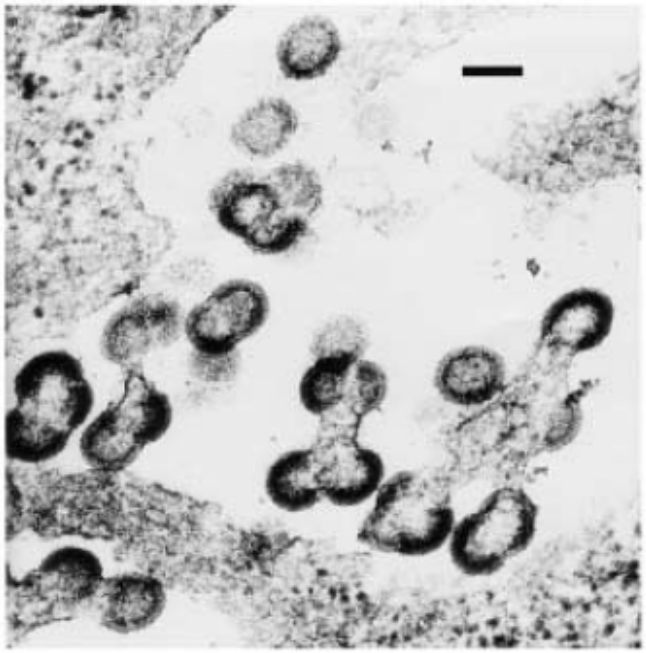
- K173Q (blocks ATP binding) and E228Q (blocks ATP hydrolysis) are dominant negative mutants.
- PTAP- is a mutant Gag (p6) that cannot be interact with Tsg101; used as control for defective virion production.
- Expression of WT Vsp4 reduced virion production several fold.

Garrus et al (2001) Cell

Arrested budding intermediates

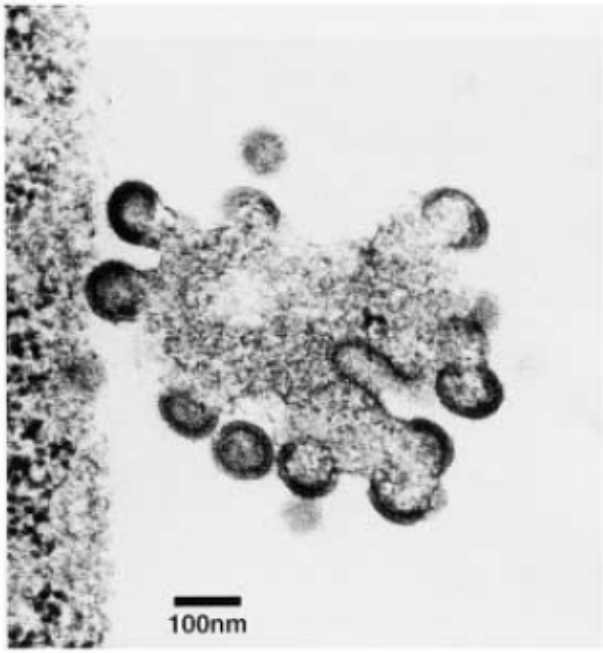


HIV-1 PTAP$^-$



HIV-1 + siRNA

(Tsg101 siRNA)

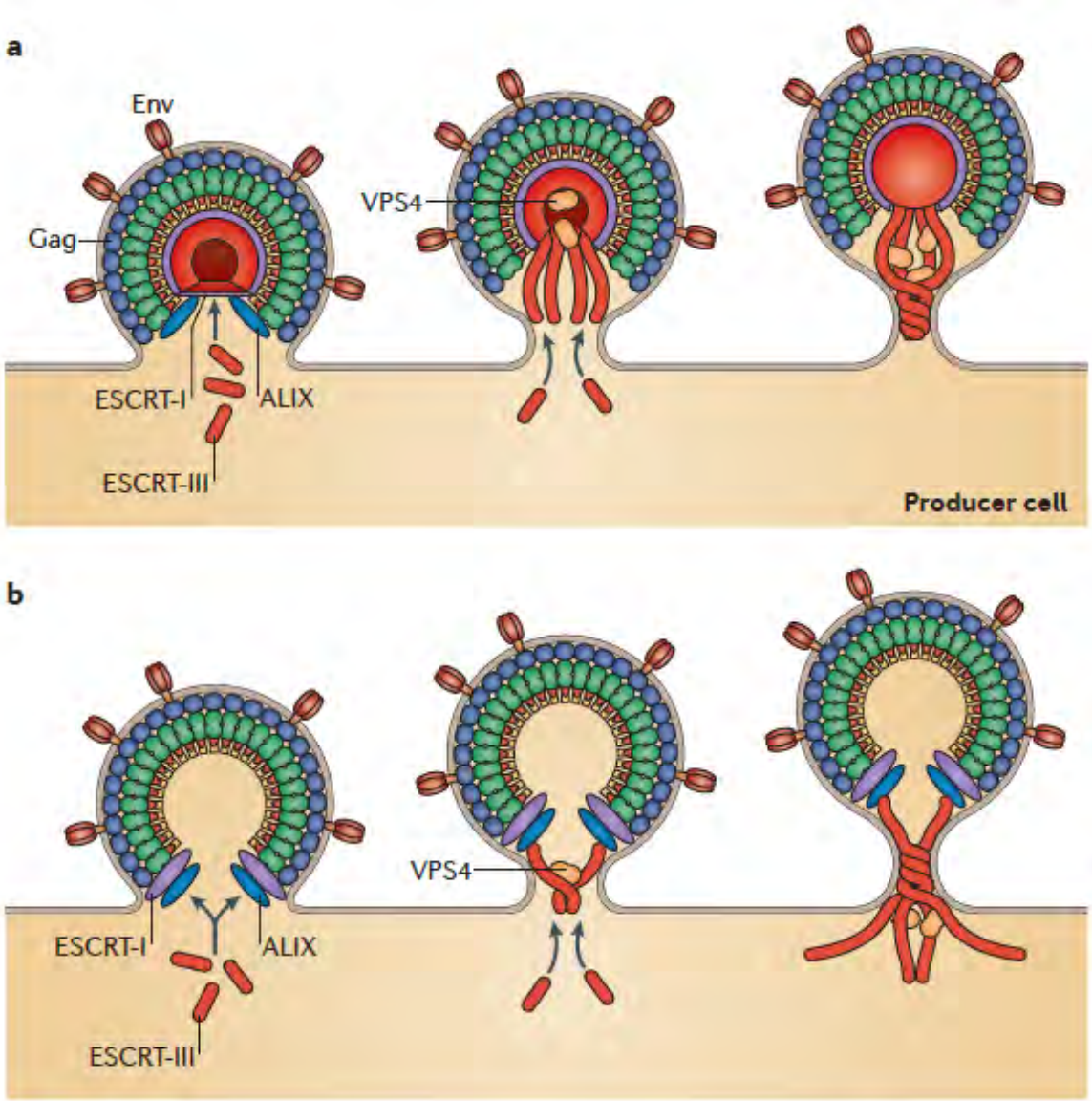


HIV-1 + $Vps4_{E228Q}$

- In cells lacking Tsg101 or Vsp4, late budding intermediates were found by EM. Immature viral particles connected to plasma membrane by membrane stalks.
- Similar to defect found in PTAP- mutant.

Garrus et al (2001) Cell

Model of ESCRT-III in HIV-1 budding and virion release



- The p6 protein of Gag interacts with Tsg101, a component of ESCRT-I.
- Gag also interacts with ALIX, which interacts with Tsg101 and ESCRT-III.
- This leads to recruitment of ESCRT-III/Vps4.
- ESCRT-III polymerization at the bud neck causes scission.

Freed (2015) Nat Rev Microbiology

Epidermal growth factor receptor (EGFR)

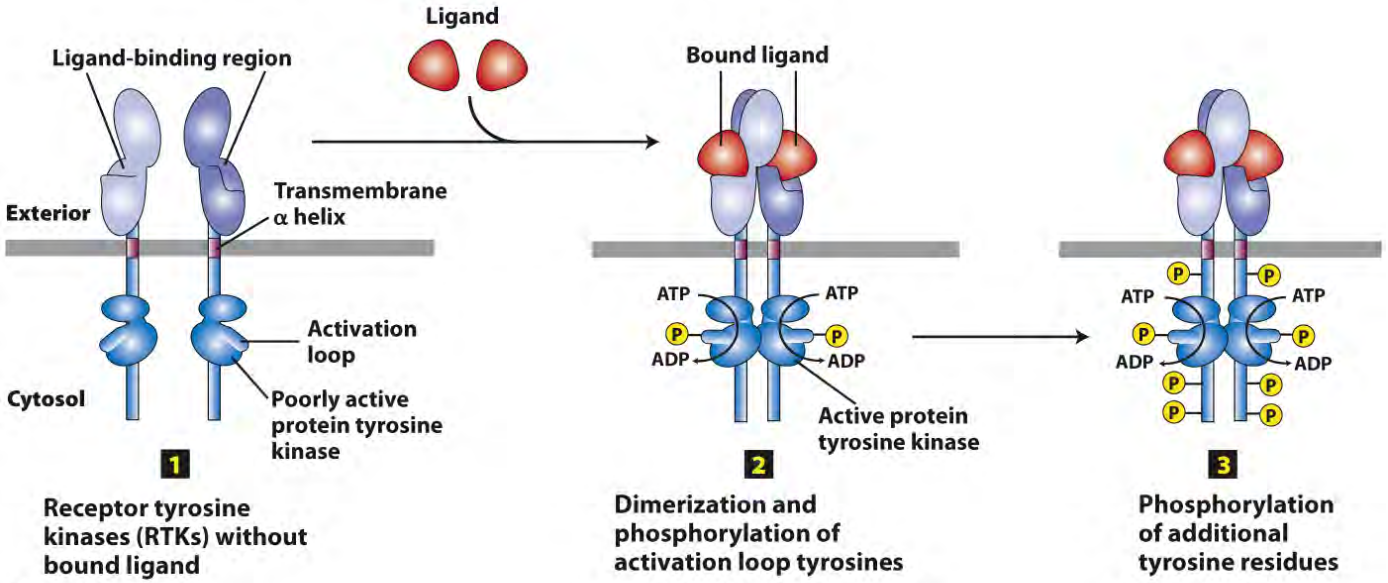
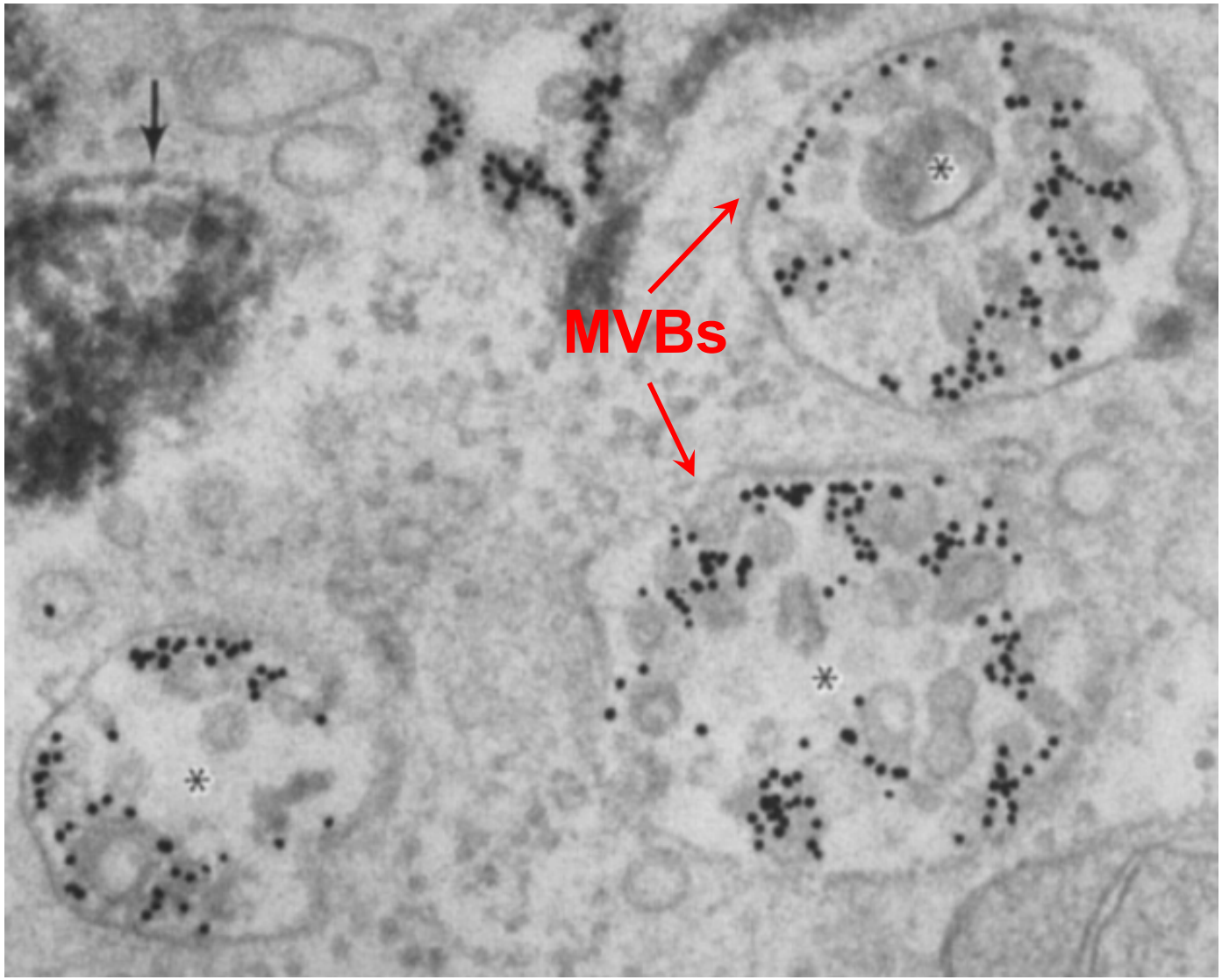


Figure 16-14
Molecular Cell Biology, Eighth Edition

Lodish et al.

- EGFR is a receptor tyrosine kinase. Binding of EGF activates EGFR kinase activity.
- This leads to MAPK signal transduction cascade for cell growth regulation.
- **Receptor down regulation:** Activation of EGFR causes reduction in EGFR numbers; the EGF/EGFR complexes are endocytosed and delivered to the lysosome.
- Failure to downregulate can result in dysregulated growth.

EGFR is delivered to multivesicular bodies



- EGFR was labeled with anti-EGFPR conjugated with gold particles.
- EGF was added.
- Chase 1 hour.

Futter et al (1996) JCB

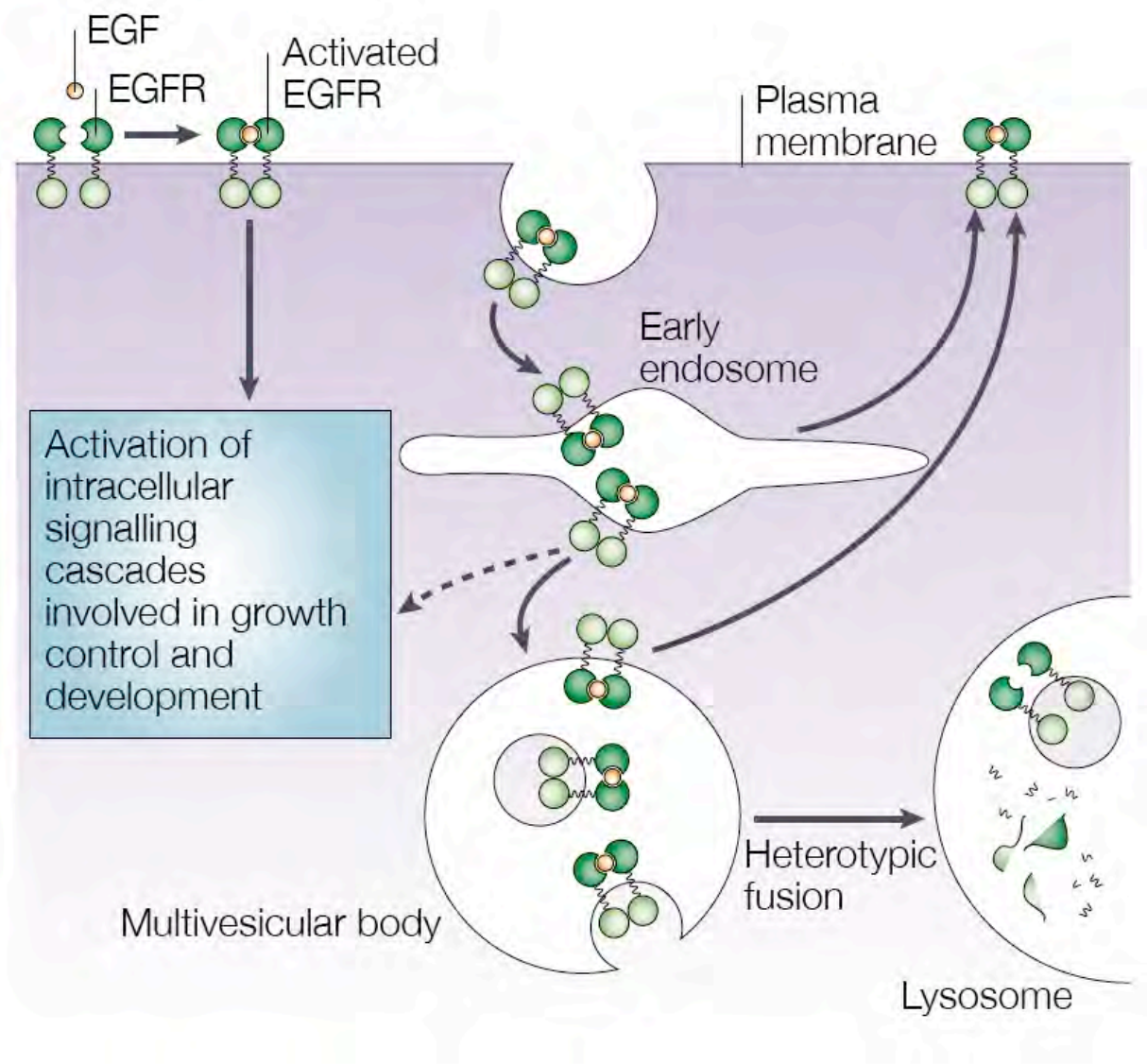
Sorting of cargo into MVBs is selective

- Some surface receptors, like LDL receptor and transferrin receptor, go through many rounds of internalization and recycling; they stay in the limiting membrane of the lysosome and get resorted to the plasma membrane.

- Proteins destined for the lysosomal membrane do not get sorted into lumenal vesicles.

- Sorting of EGFR:
  - Cytosolic tail
  - Kinase activity
  - Ubiquitin added to cytosolic tail

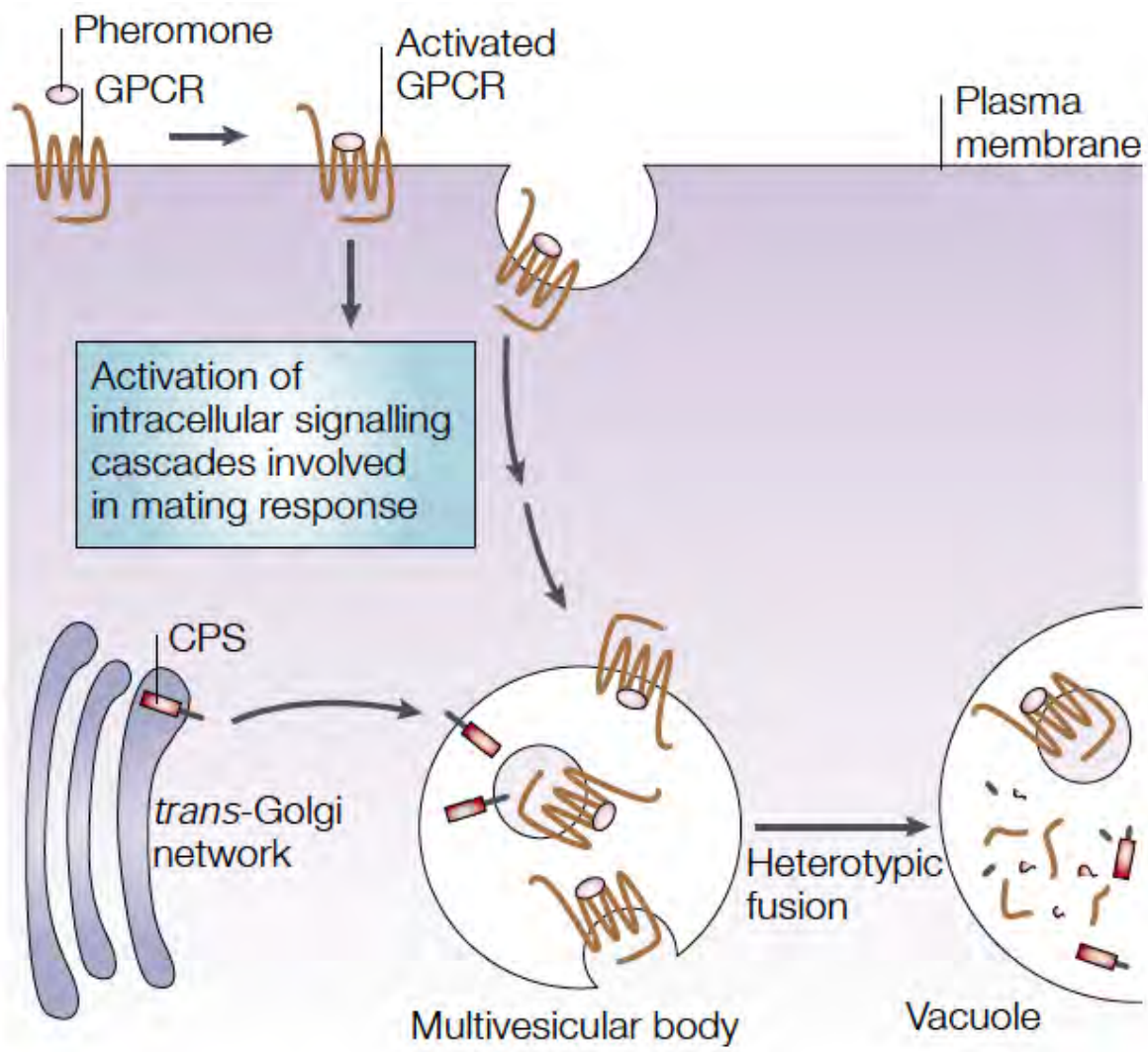
Receptor downregulation by the multivesicular body



- EGFR is normally distributed uniformly on cell surface.
- Addition of EGF causes clustering of EGFR at clathrin-coated pits.
- Within 1-2 min, the EGF-EGFR complexes are internalized.
- Some are recycled to the cell surface.
- Some go to the MVB and are degraded in the lysosome—this is a major mechanism for regulating cell surface EGFR levels. Requires ubiquitination by Cbl.

Katzmann et al., 2002

Downregulation of Ste2 in yeast by MVB sorting



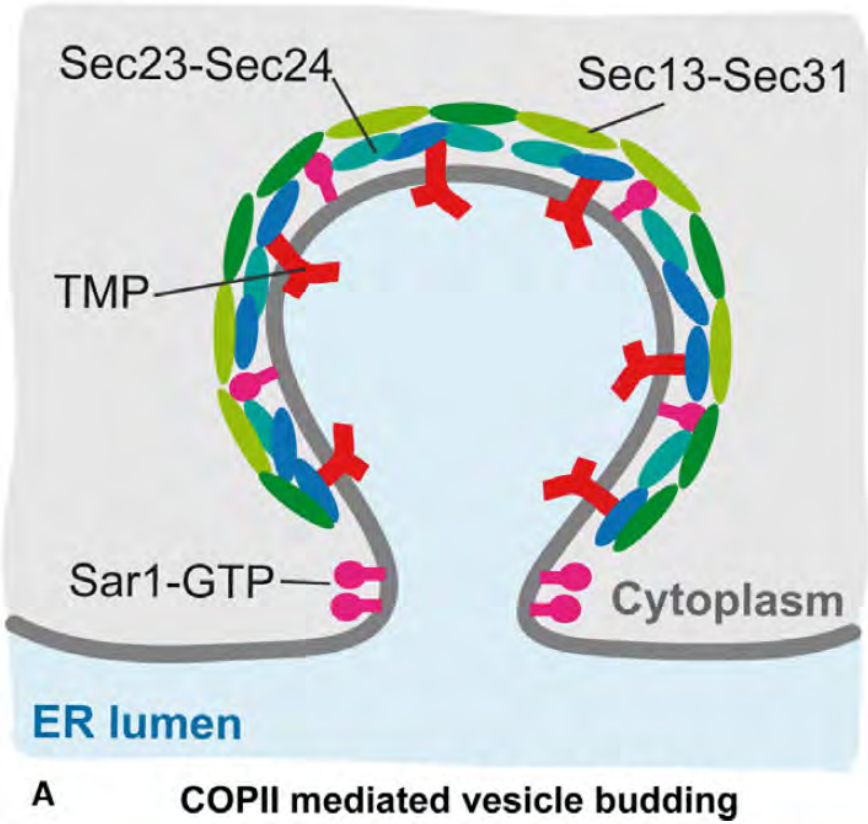
Katzmann et al., 2002

- Ste2 is a receptor (expressed in "*a*" cells) that binds to α-factor (peptide mating hormone) expressed by α cells.
- Ste2 activates MAPK cascade.
- Ste2 is downregulated by endocytosis and sorting to MVBs.
- Mutation of lysines from Ste2 impairs downregulation, showing importance of ubiquitylation.
- Mono-ubiquitination

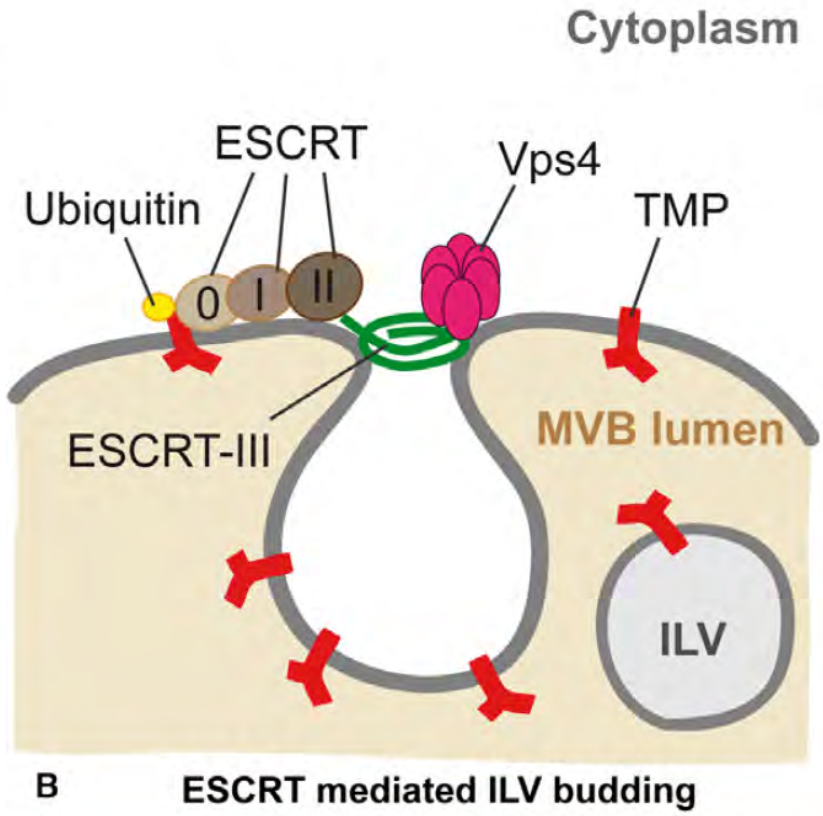
The multivesicular body delivers cargo to the lysosome

- MVBs deliver cargo to lysosome lumen for degradation.
- Endosomes form invaginating buds that pinch off and form internal vesicles within the endosome.
- The MVB are progressively acidified and fuse with lysosomes.
- Mono-ubiquitin targets certain cell surface receptors for downregulation via MVB formation and fusion with lysosome.
- ESCRT (endosomal sorting complex required for transport) proteins recognize ubiquitinated cargo and sort them into MVBs for degradation by the lysosome.
- ESCRT-III and Vps4 mediate the scission of lumenal vesicles.

ESCRT-III processes have topology opposite traditional budding events



A COPII mediated vesicle budding

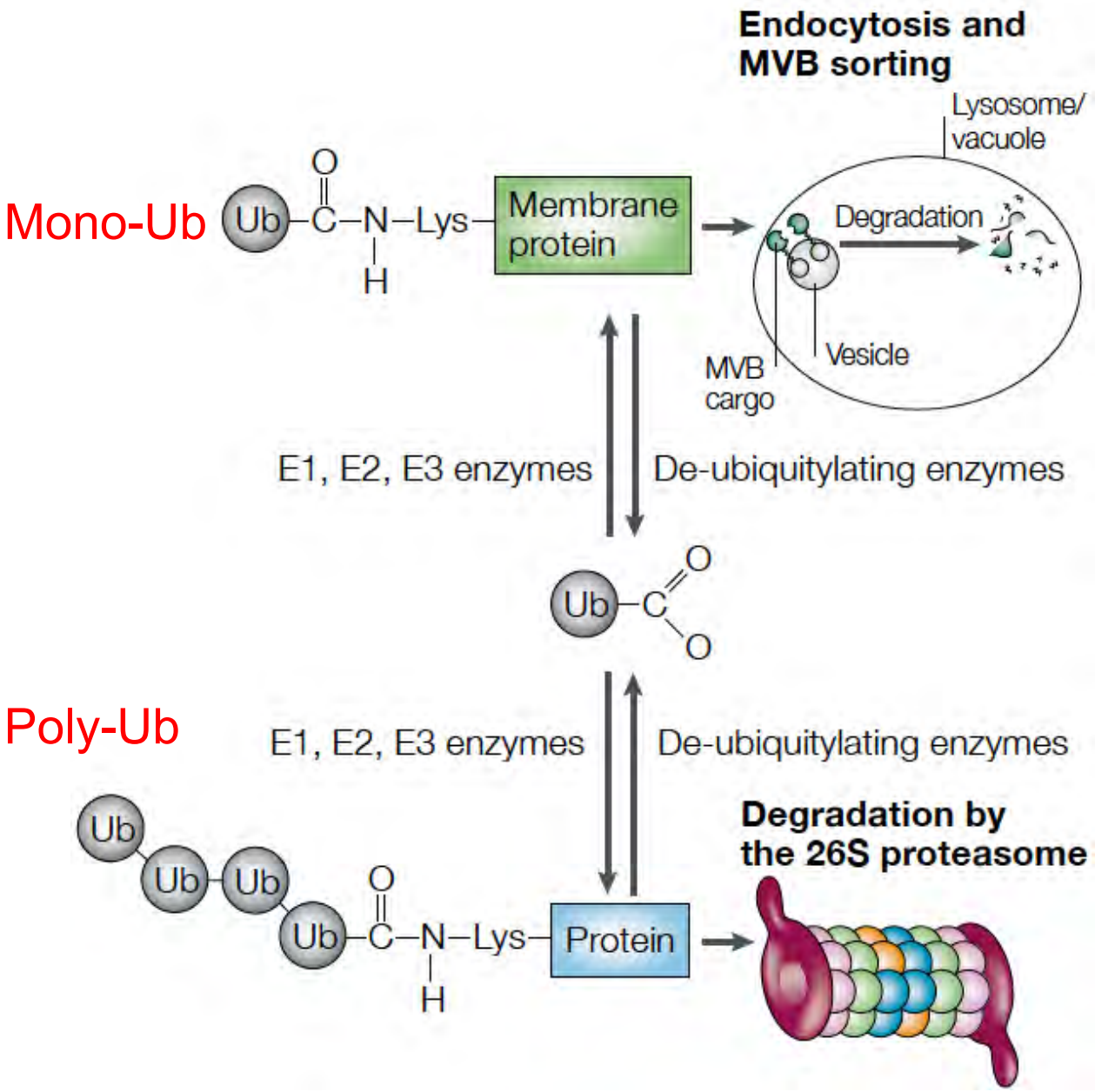


B ESCRT mediated ILV budding

- ESCRT-III processes bud *away* from cytosol.
- ESCRT-III protein assemble *inside* vesicle.

Adell et al (2016) The FEBS Journal

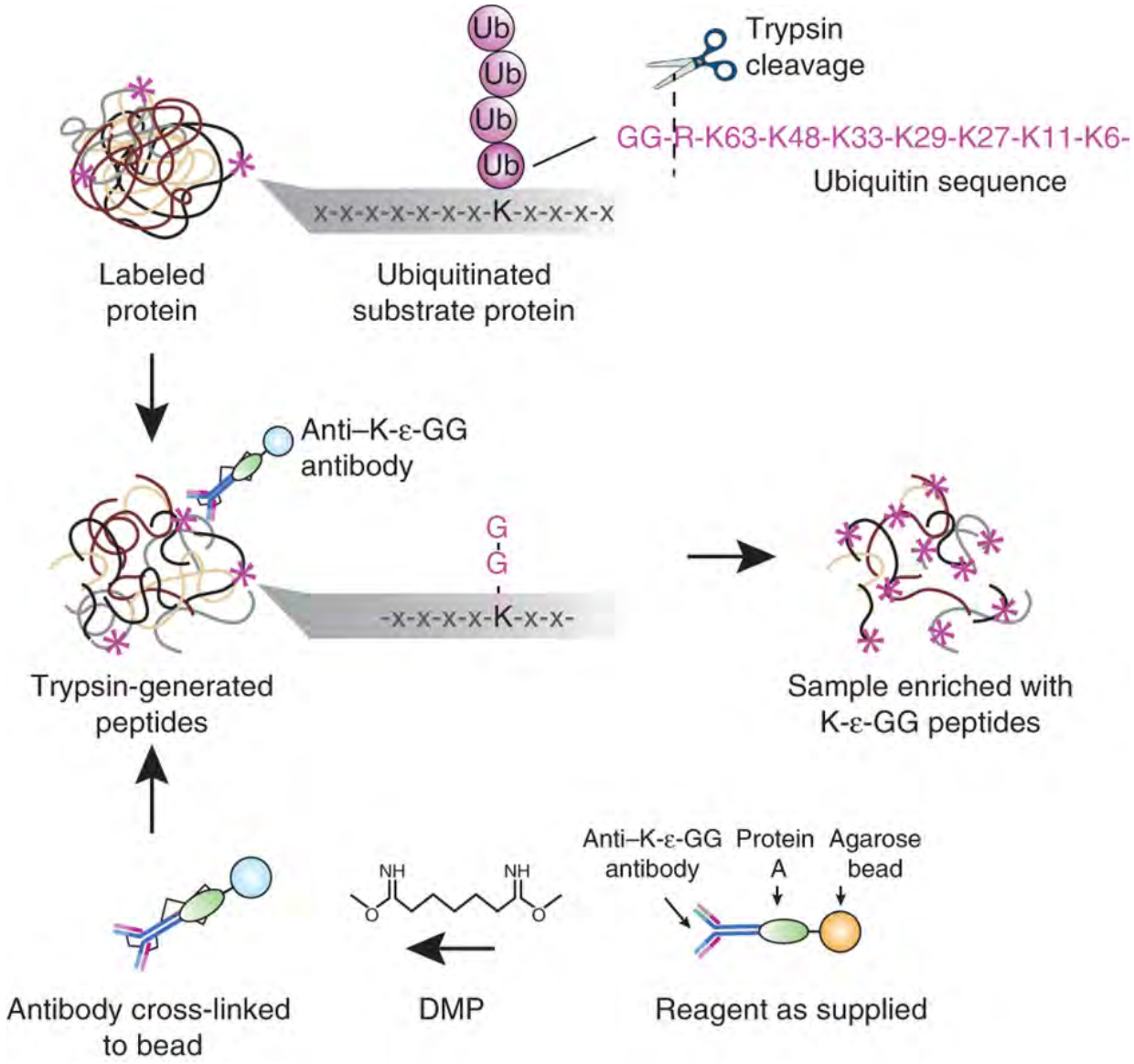
Two types of ubiquitin signals



Katzmann et al., 2002

- Ub is a 76 aa polypeptide
- Attached to lysine residues by isopeptide bond between C-term of Ub and ε-amino group ($NH3^+$) of lysine on substrate.
- Requires
  - E1 (ubiquitin-activating enzyme)
  - E2 (ubiquitin-conjugating enzyme)
  - E3 (ubiquitin ligase)
- Ubiquitylation can reversed by de-ubiquitylating enzymes.
- Ub has 7 lysines; K48 polyubiquitylation is classic degradation signal for 26S proteasome.

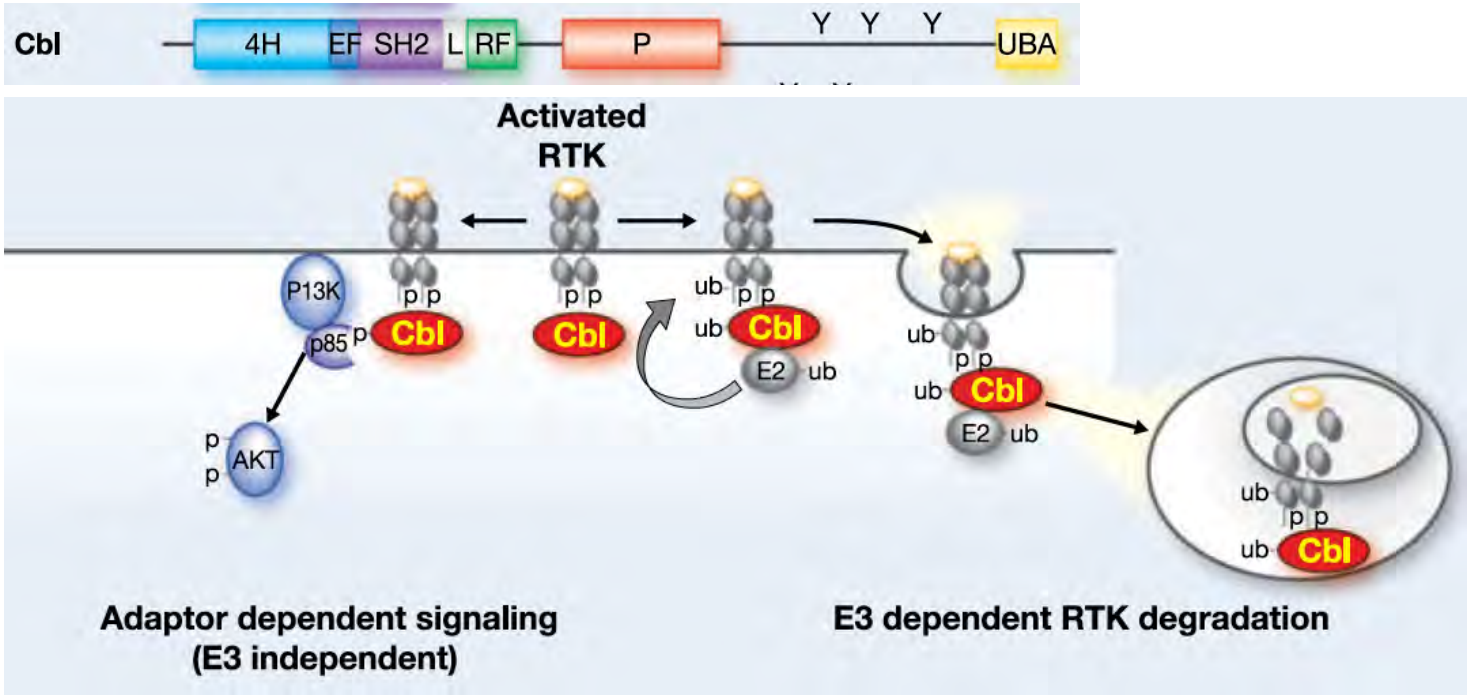
Identification of ubiquitylation sites by mass spectrometry



- Trypsin is an endopeptidase that cleaves after R and K.
- Trypsin digestion of a protein creates an array of tryptic peptides that can be identified by mass spectrometry.
- In the case of a ubiquitylated protein, some of these tryptic peptides will have gly-gly signatures. The C-terminal sequence of Ub is: RGG*

Udeshi et al (2013) Nature Protocols

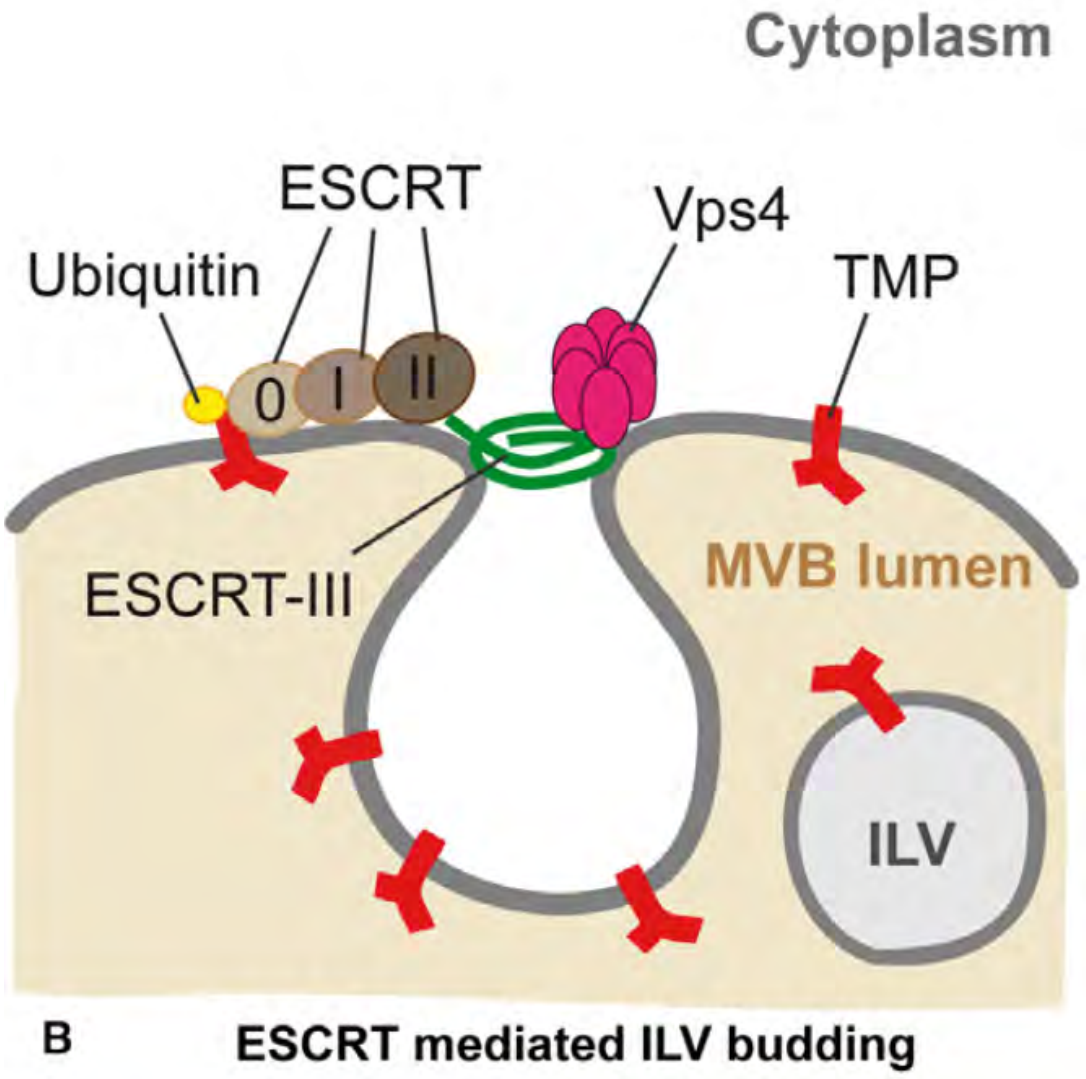
Inability to downregulate EGFR can lead to cancer



- Cbl is a Ring Finger E3 ubiquitin ligase (RF is the largest class of E3s)
- Cbl is recruited to activated EGFR and conjugates ubiquitin to it.
- Loss of Cbl E3 ligase activity is associated with myeloid cancers in humans and mouse.
- Note: Cbl has both positive and negative roles for EGFR activity.

Kales et al (2010) Cancer Research

ESCRT complexes mediate MVB sorting and scission

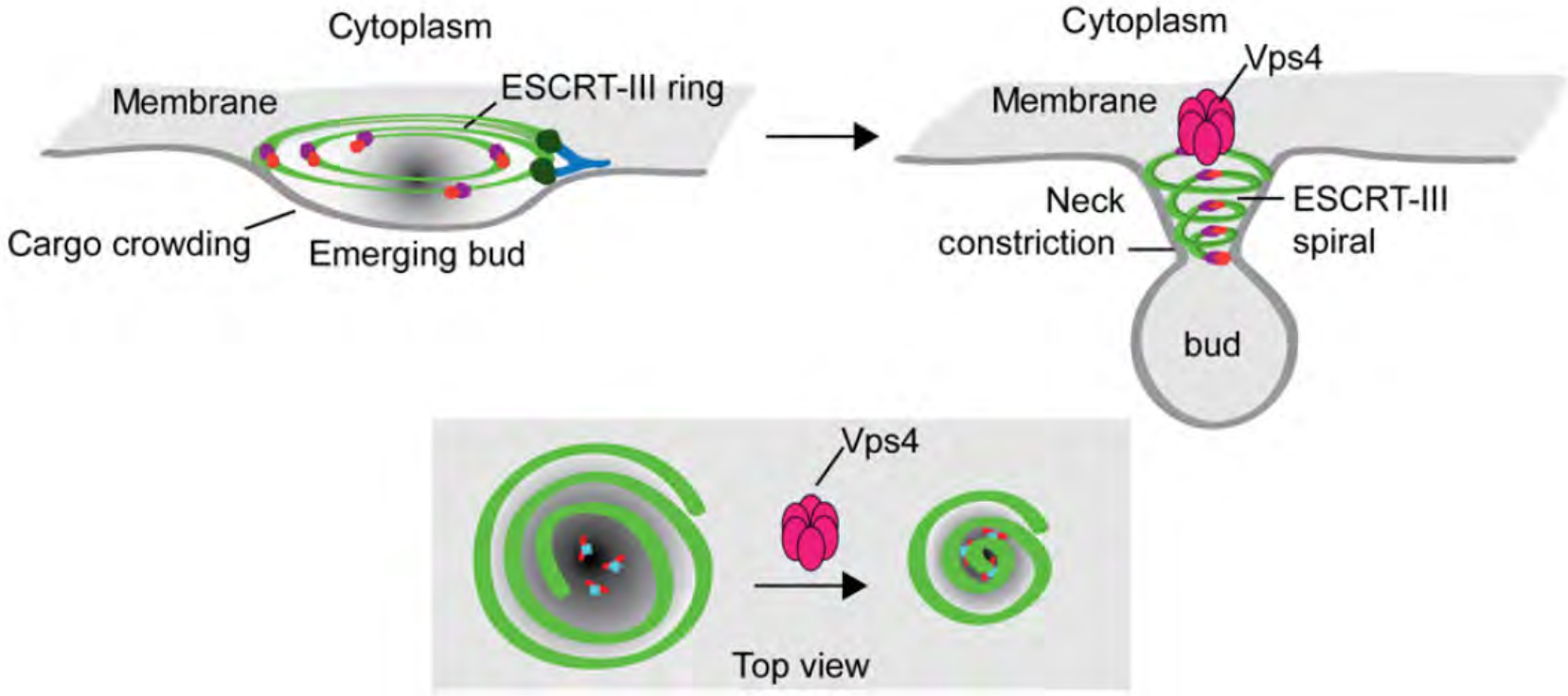


B ESCRT mediated ILV budding

Adell et al (2016) The FEBS Journal

- Mechanism of ESCRTs not fully understood.
- ESCRT-0, I, II act “early” to sort cargo and recruit ESCRT-III.
- ESCRTI plays a major role in cargo recognition: several components (Vps23/Tsg101, Vps27) bind Ub.
- ESCRTIII assembles into spiral filaments that likely mediate scission.
- A key component of ESCRT-III is Chmp4/Snf7, which constitute the bulk of the filament.
- Disassembly of ESCRT-III requires the AAA ATPase Vps4.

Model of ESCRT-III scission

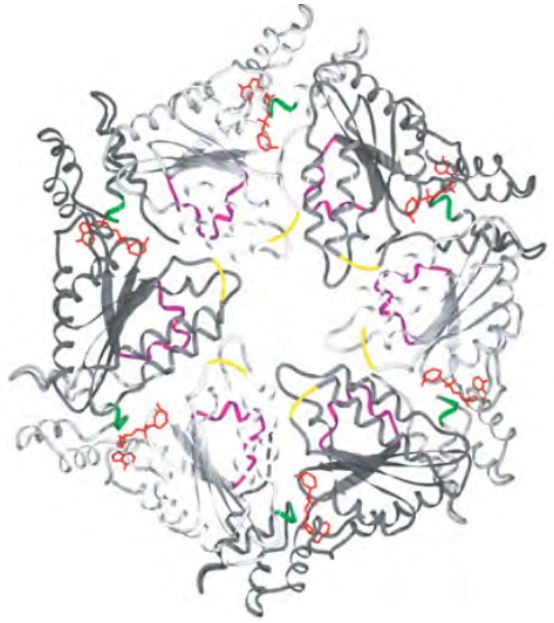


- ESCRT-III forms spiral filaments on lipid membranes.
- The diameters of these spirals can change; a small enough diameter may lead to membrane merger.
- Vps4 is usually thought to mediate disassembly of ESCRT-III after scission, so that the proteins can be used again; however, it may also contribute to the scission process.

Adell et al (2016) The FEBS Journal

AAA ATPases

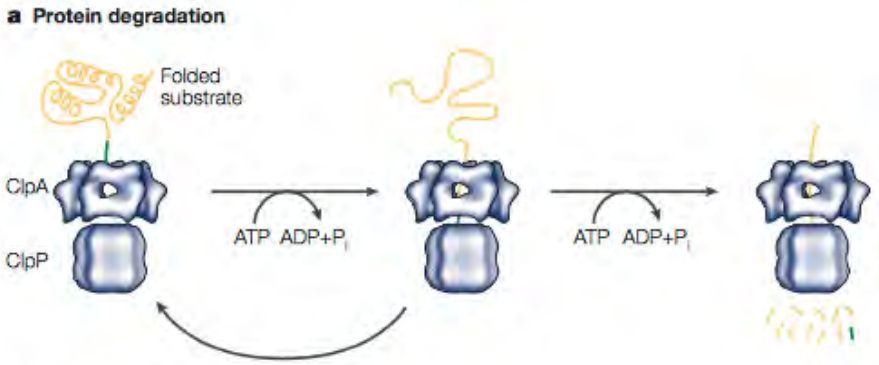
- AAA (**A**TPases **A**ssociated with various cellular **A**ctivities); also called AAA+ family.
- A large family of ATPases characterized by certain sequence motifs.
- Typically form oligomeric rings that use the power from ATP hydrolysis to undergo conformational changes and perform work on substrates.
- Examples:
  - Protein unfolding and degradation (ClpXP, ClpAP, Lon, 26S proteasome)
  - Protein disassembly (NSF, Vps4, VCP/p97)
  - Solubilization of protein aggregates (Hsp104)



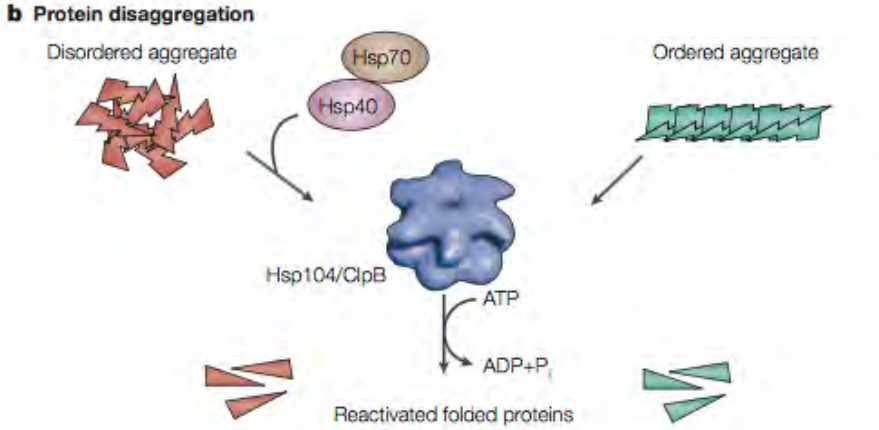
Hexameric AAA domain from NSF

Hanson & Whiteheart (2005) NRMCB

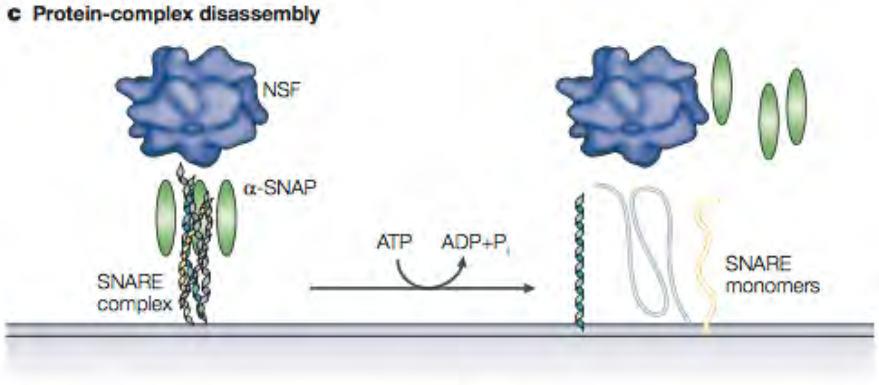
Examples of protein remodeling by AAA ATPases



AAA domain unfolds protein and delivers it to proteolytic chamber.



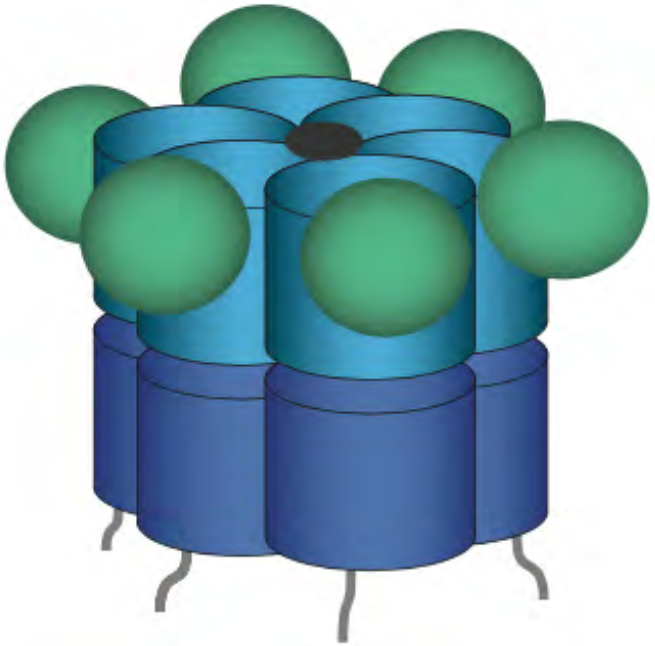
Protein aggregates are solubilized by 104 kD heat shock protein.

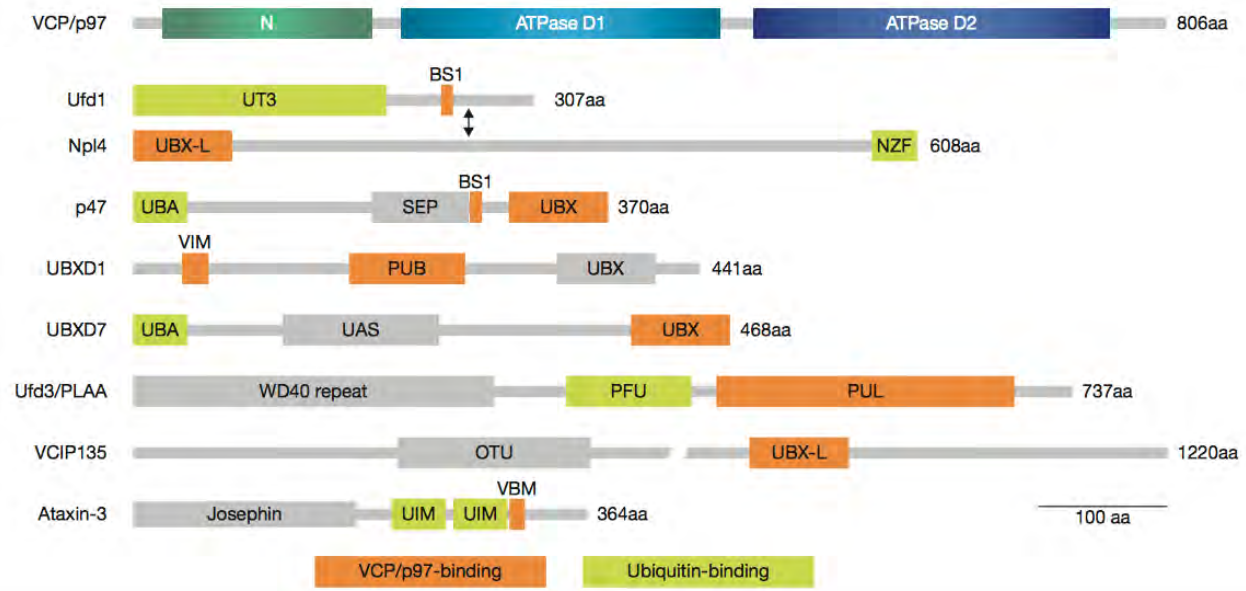


NSF, together with SNAP, disassemble 4-helix bundle SNARE complexes so that they can perform additional rounds of membrane fusion.

Hanson & Whiteheart (2005) NRMCB

p97/VCP is a AAA ATPase with many functions

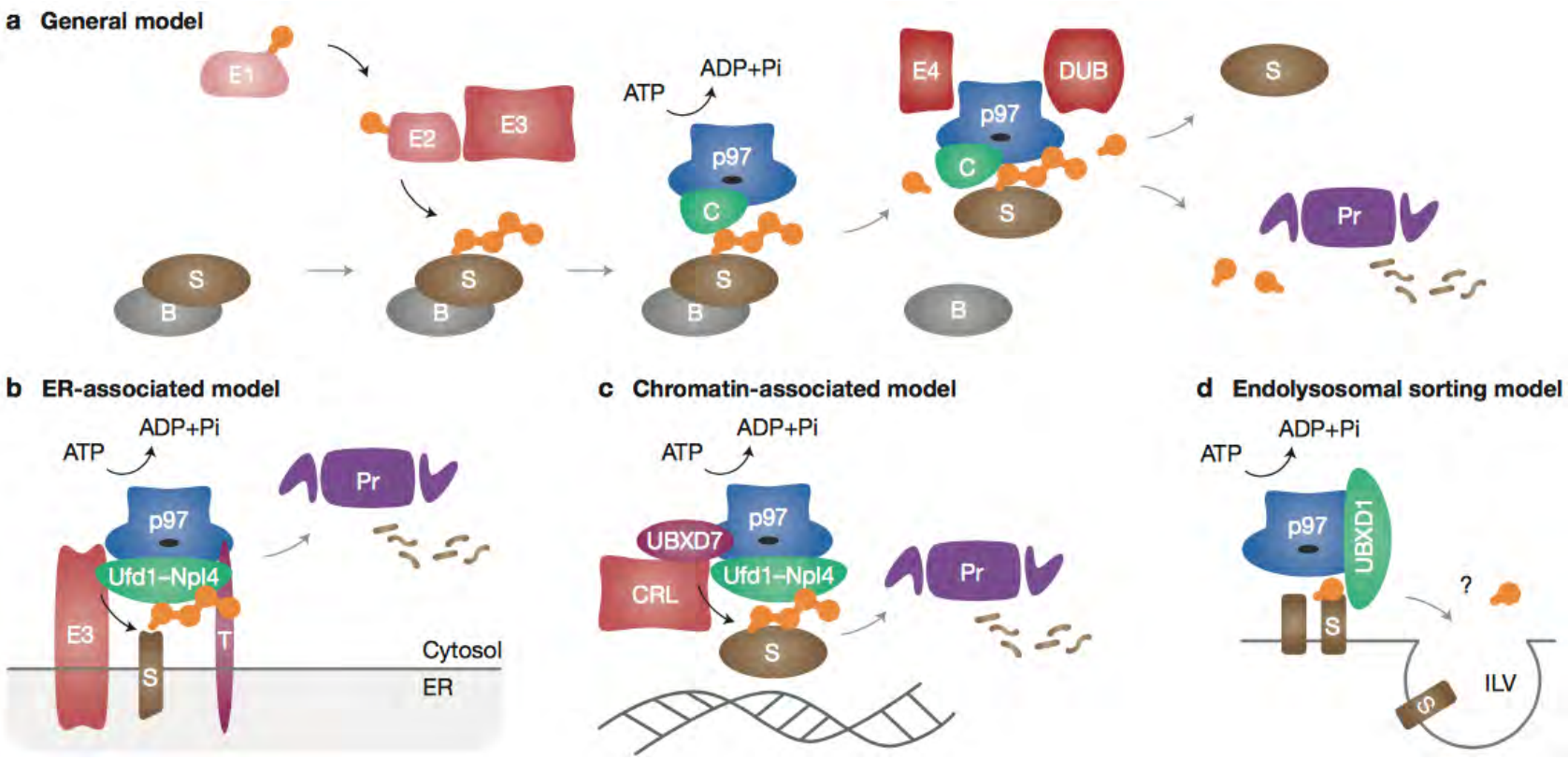




- p97/VCP (valosin-containing protein), Cdc48 in yeast
- Highly homologous to NSF.
- D2 has higher ATPase activity than D1.
- p97 operates with many adaptors/co-factors.
- Mutations in p97 cause IBMPFD (inclusion body myopathy associated with Paget disease of the bone and frontotemporal dementia)

Meyer et al (2012) Nat Cell Biol

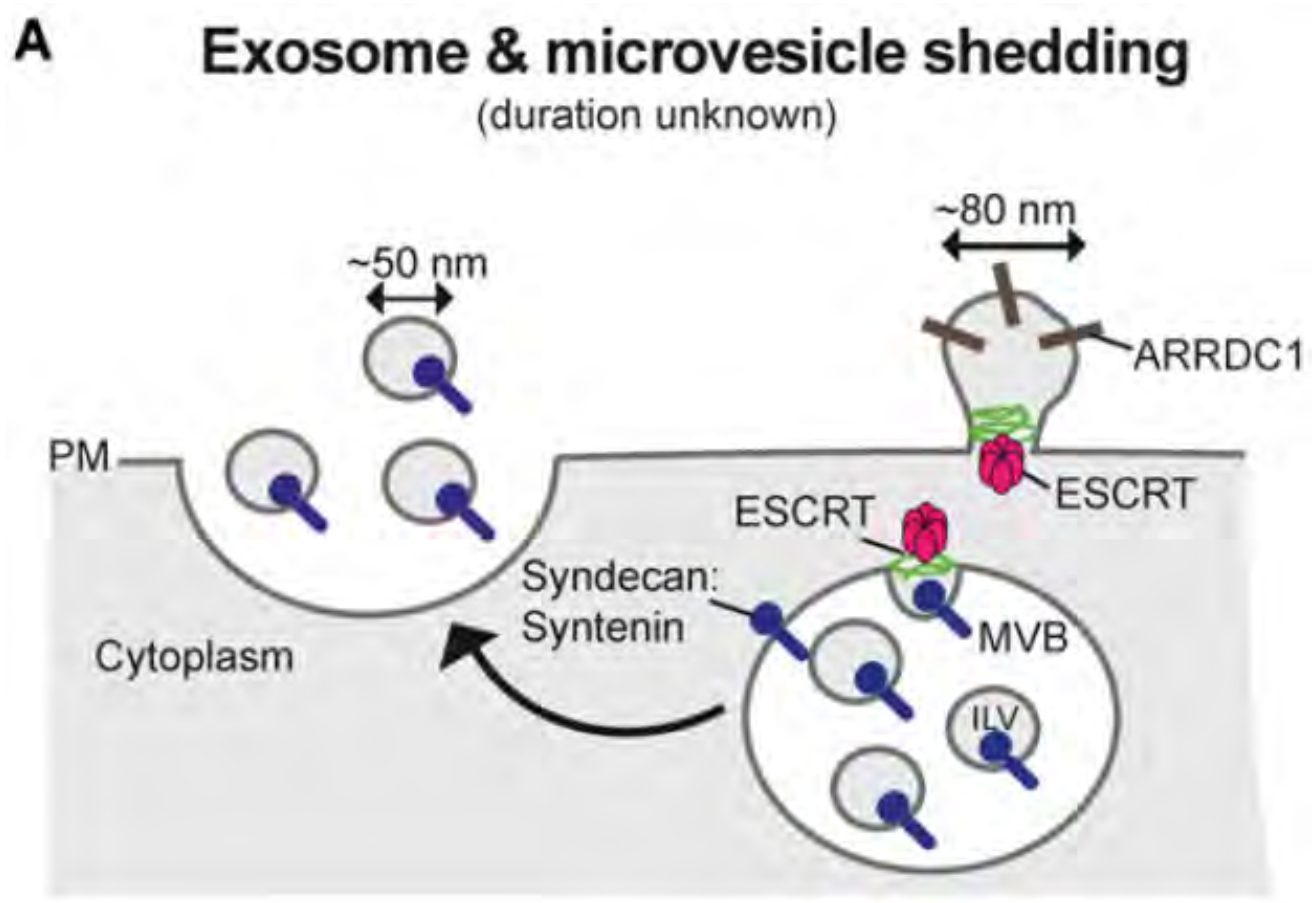
p97 often functions between ubiquitylation and the 26S proteasome



- p97 associates with ubiquitylated substrates that need remodeling before delivery to the 26S proteasome.
- Substrates might be in stable complexes or associated with surface.

Meyer et al (2012) Nat Cell Biol

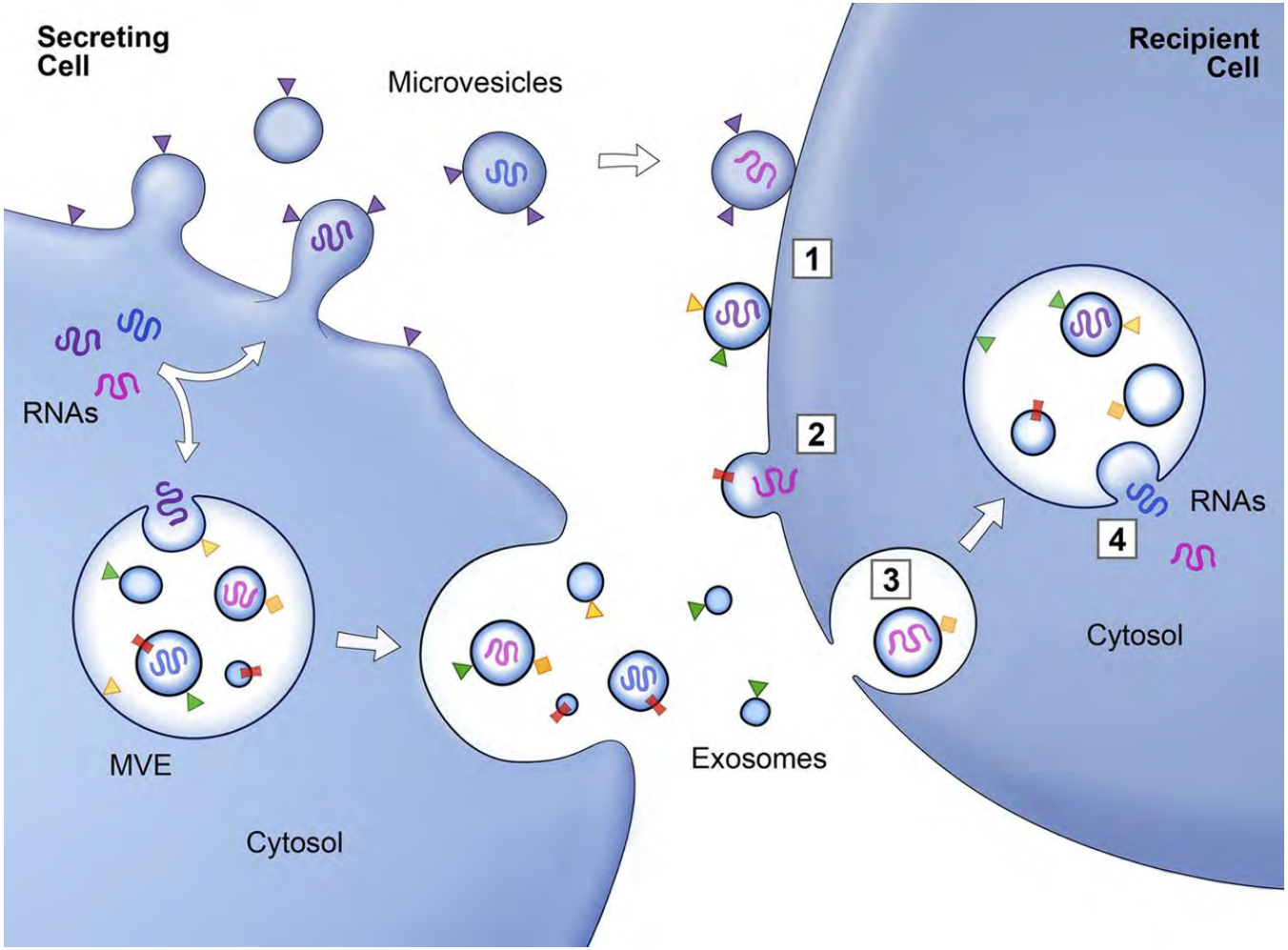
Additional functions of ESCRT-III pathway



- Exosomes: MVBs can fuse with plasma membrane, releasing vesicles to the extracellular space.
- 50-100 nm diameter.
- Ectosomes are microvesicles (100-1000 nm) that are shed directly from the plasma membrane.
- Tsg101 often used as a marker
- Some are independent of ESCRT.

Adell et al (2016) The FEBS Journal

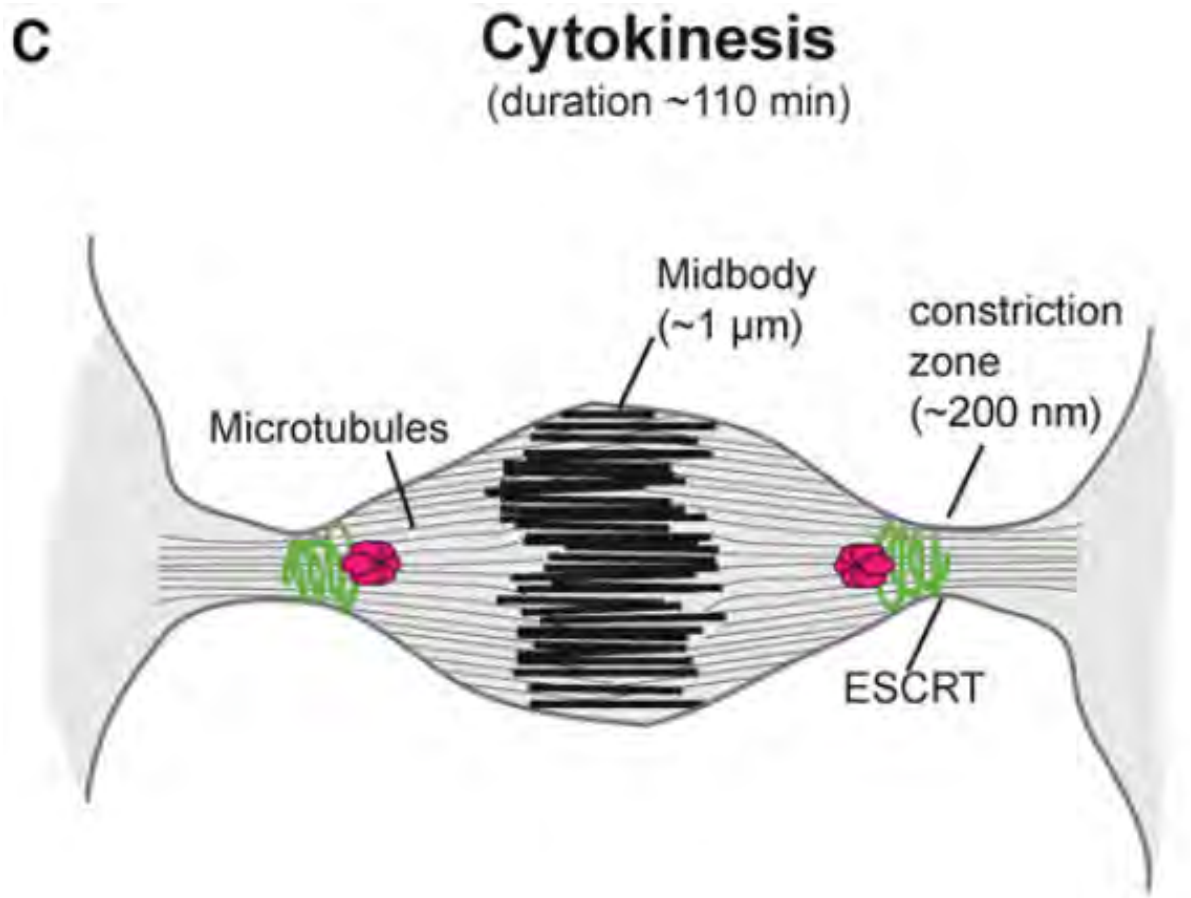
Exosomes may function in intercellular communication



- Exosomes contain RNA, miRNA, and protein, and have been proposed to facilitate intercellular communication.
- Many proposed functions:
  - Waste removal
  - Antigen presentation
  - Tumor promotion
  - Signal transduction

Raposo & Stoorvogel (2013) JCB

Additional functions of ESCRT-III pathway

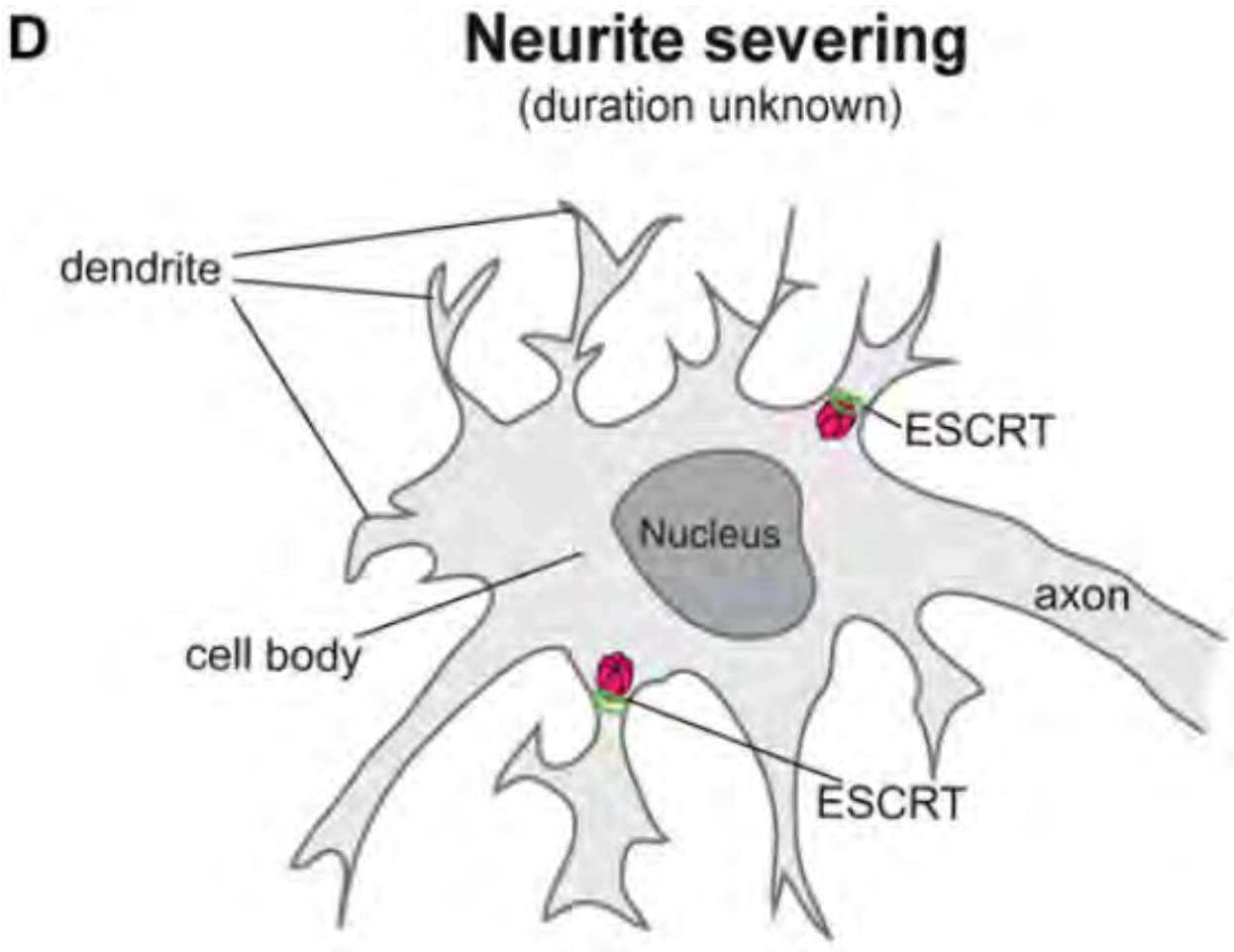


- Near the end of cytokinesis, the daughter cells are separated by the midbody.
- Midbody contains bundles of microtubules from the mitotic spindle.
- Cleavage at the midbody is termed abscission.

Adell et al (2016) The FEBS Journal

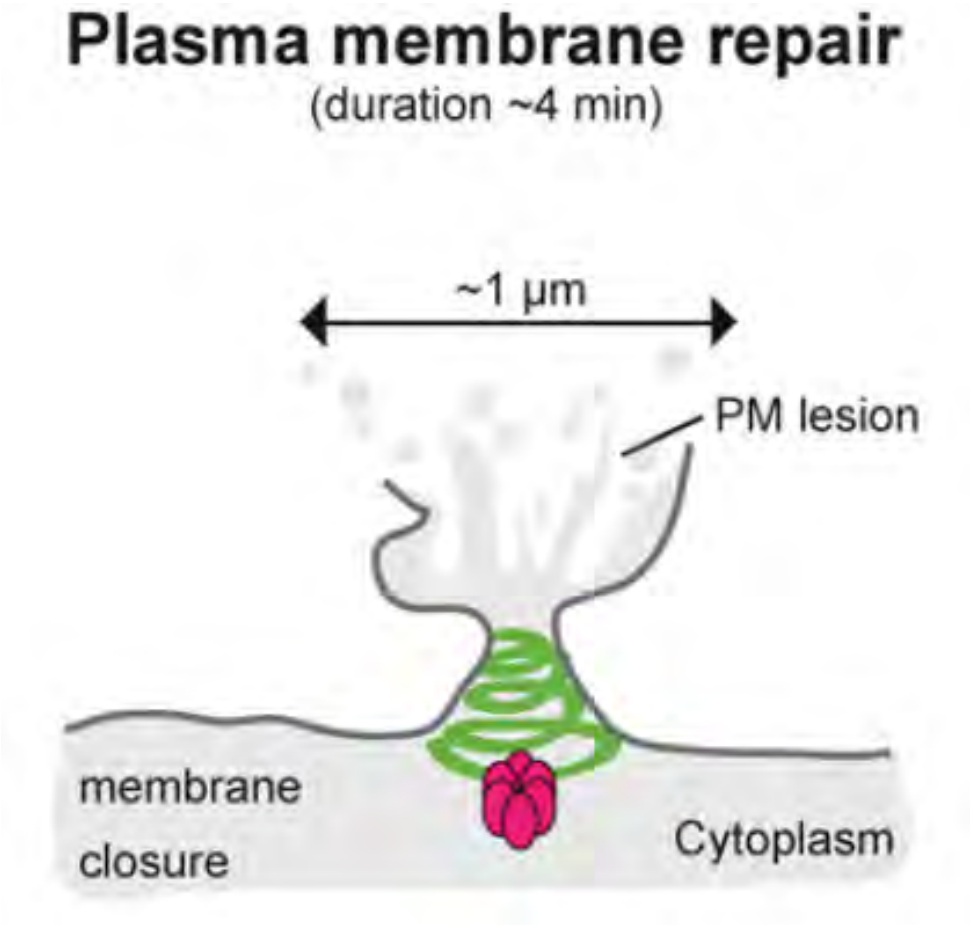
Additional functions of ESCRT-III pathway

- Neuronal processes no longer needed can be pruned to remodel neurons.



Adell et al (2016) The FEBS Journal

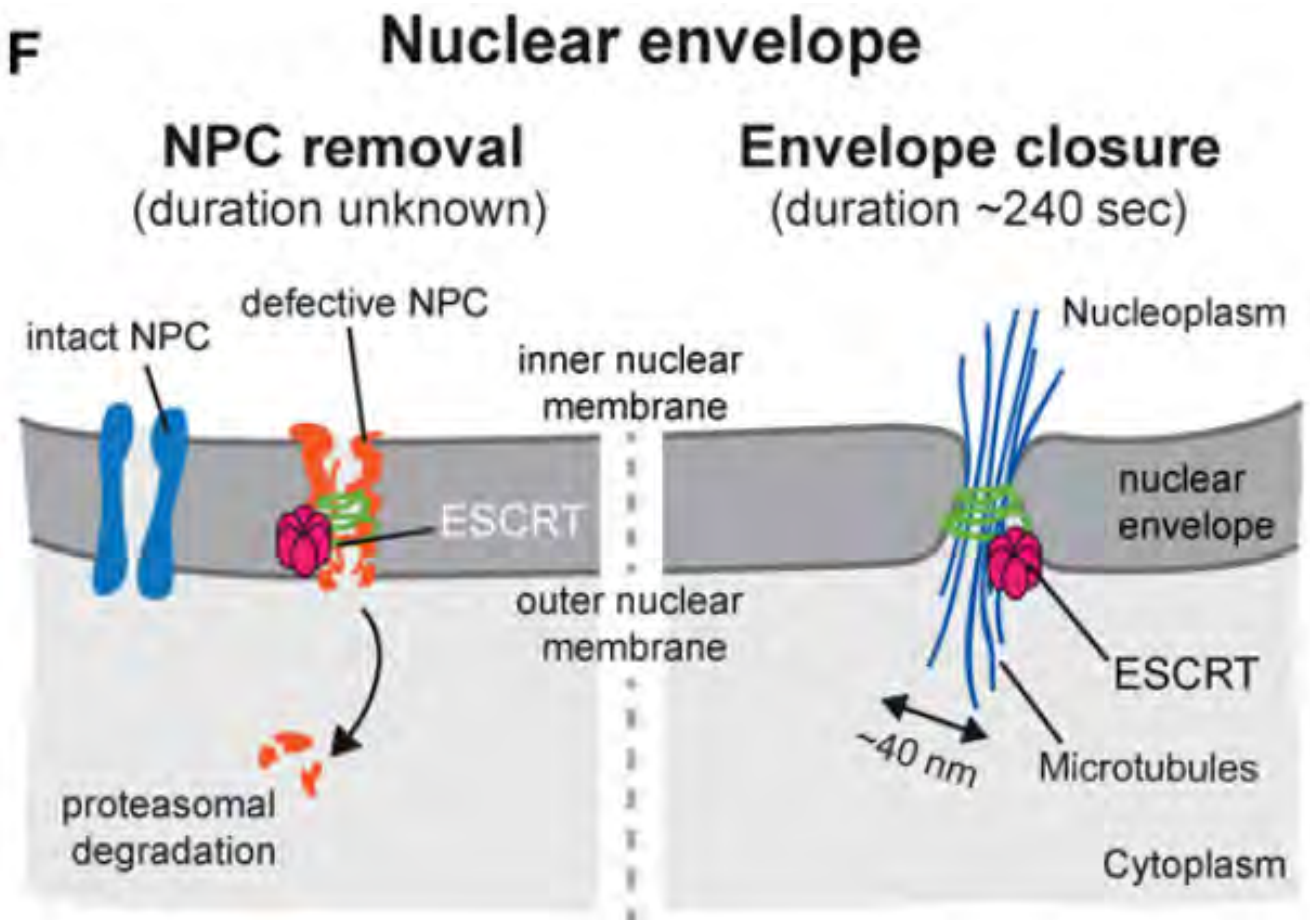
Additional functions of ESCRT-III pathway



- Holes in the plasma membrane can be caused by physical trauma or bacterial toxins.
- Such damage leads to recruitment of ESCRT-III proteins to injury site.
- By budding part of the plasma membrane, the damage can be repaired.

Adell et al (2016) The FEBS Journal

Additional functions of ESCRT-III pathway



- During late anaphase, the nuclear envelope must be re-formed; ESCRT-III is important for this process.
- Repair of defective nuclear pore complexes.

Adell et al (2016) The FEBS Journal