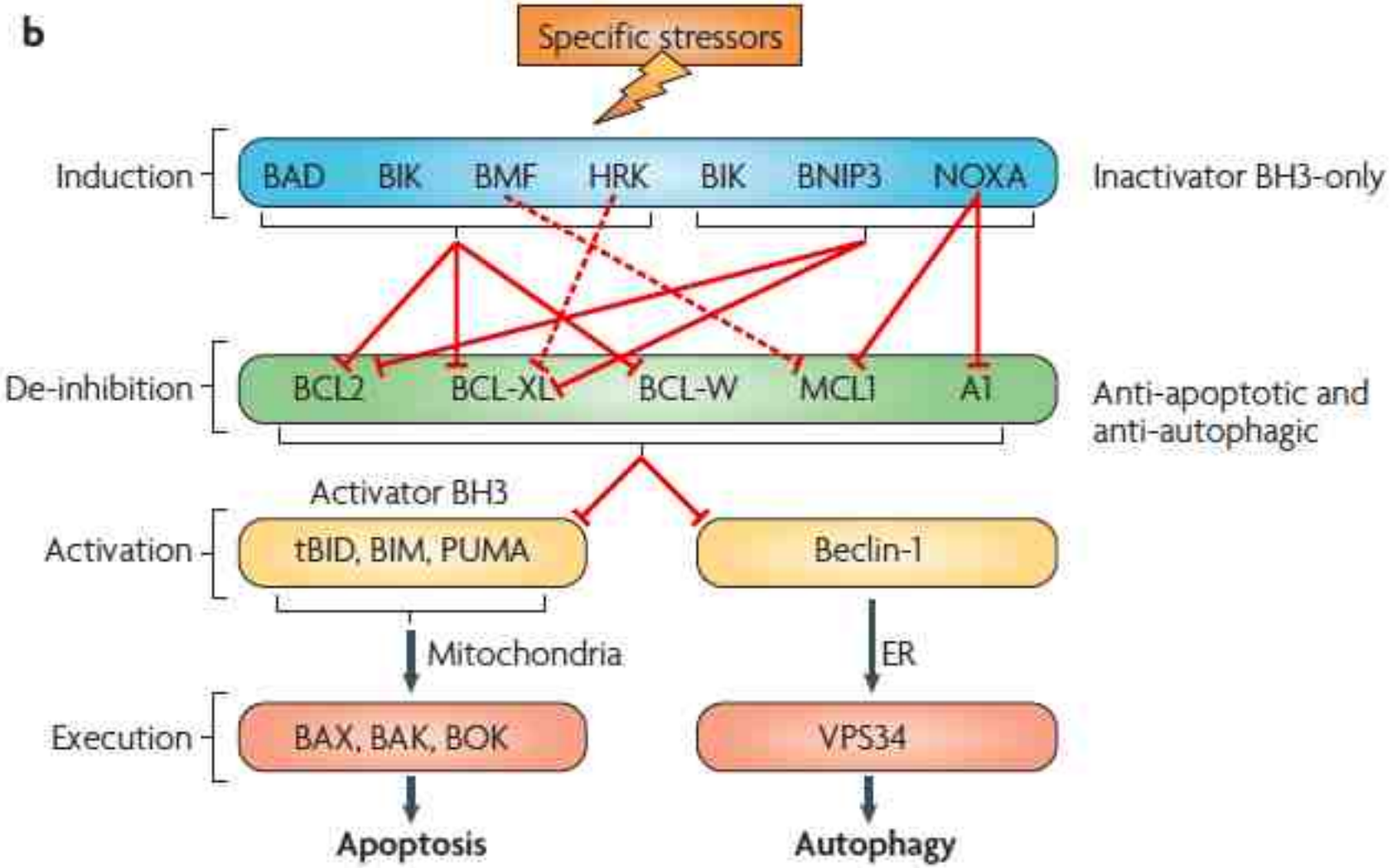
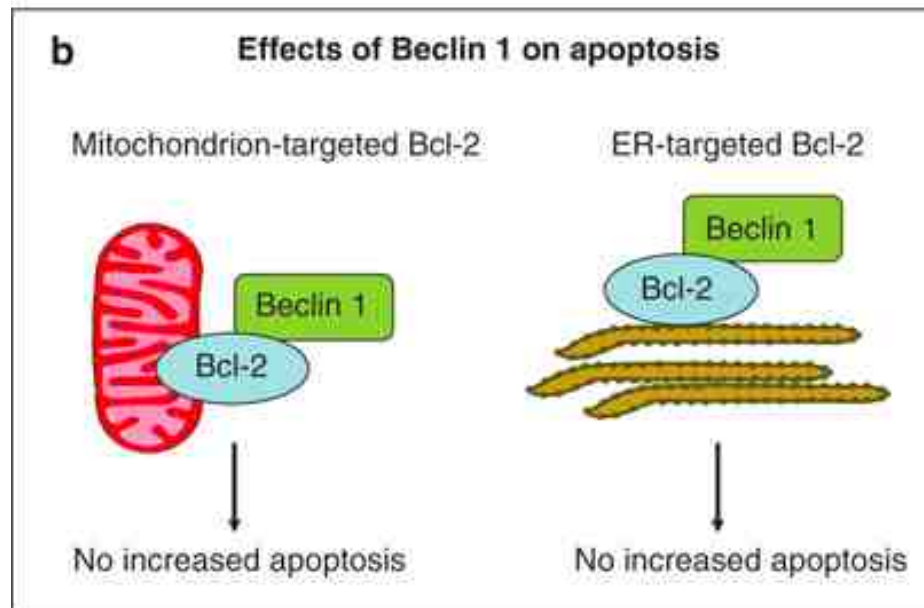
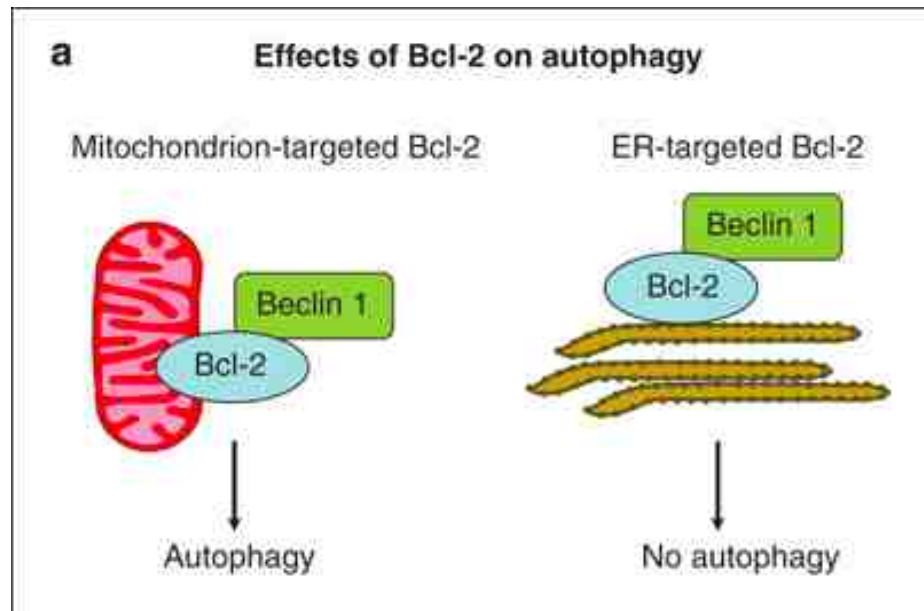


Molecular switches between apoptosis and autophagy

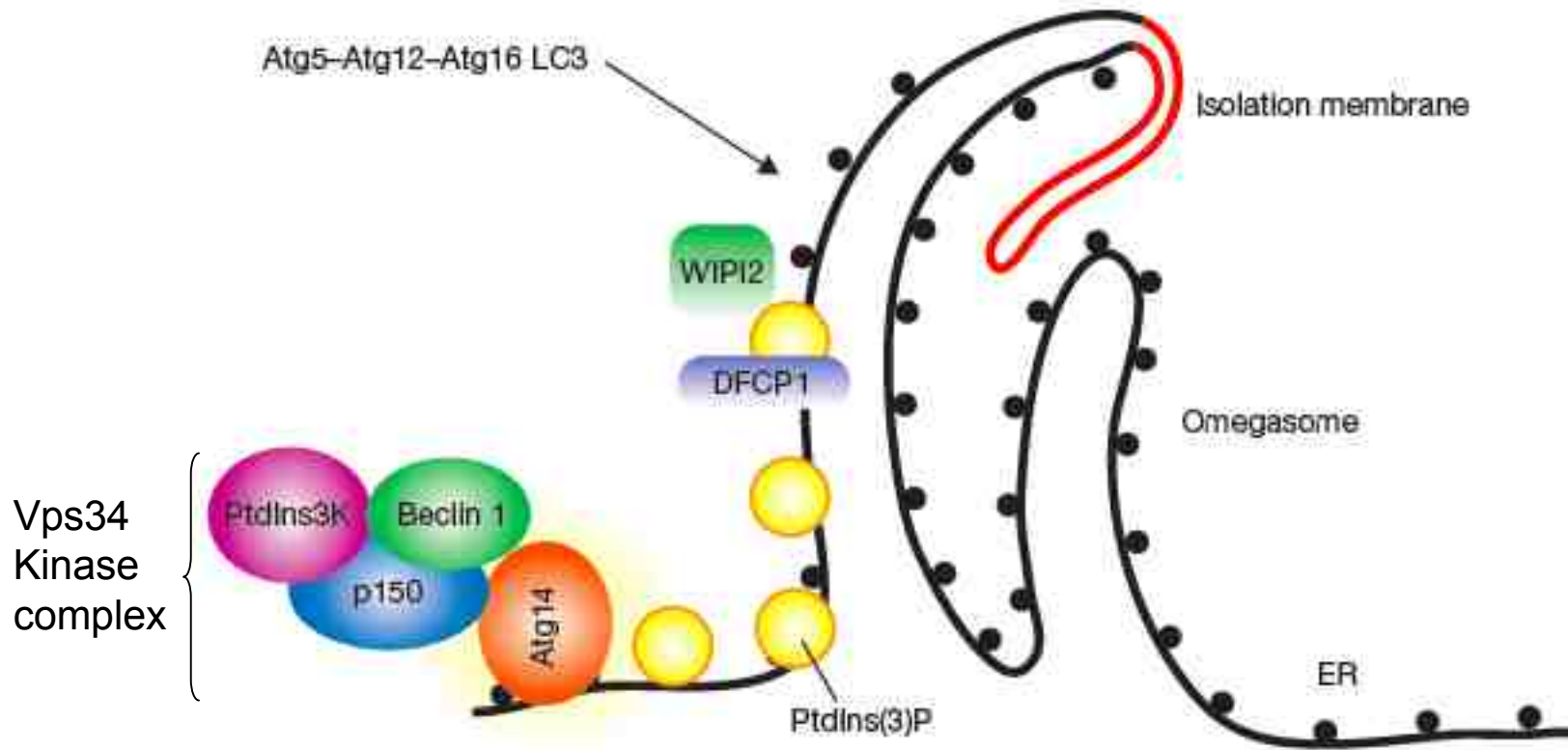


Beclin 1: a BH3-only protein that fails to induce apoptosis



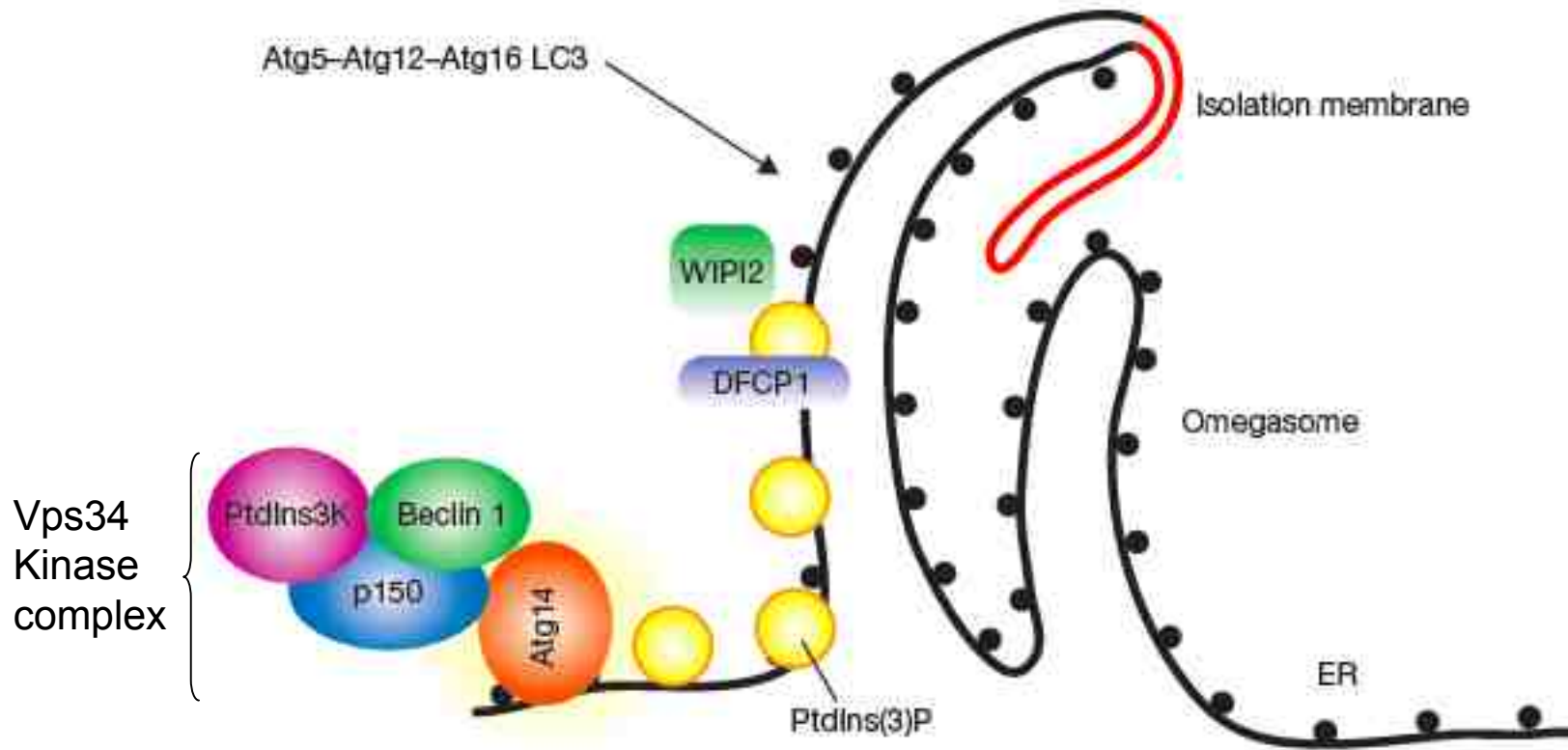
Model for recruitment of Atg proteins and DFCP1 to the ER-derived membrane and formation of the phagophore.

PtdIns(3)P is generated by the Vps34 kinase complex which is recruited to this site by Atg14. PtdIns(3)P is then recognized by the effectors DFCP1 and WIPI2. The phagophore shown in red extends from this PtdIns(3)P-rich structure and elongates, cupped between two ER cisternae.



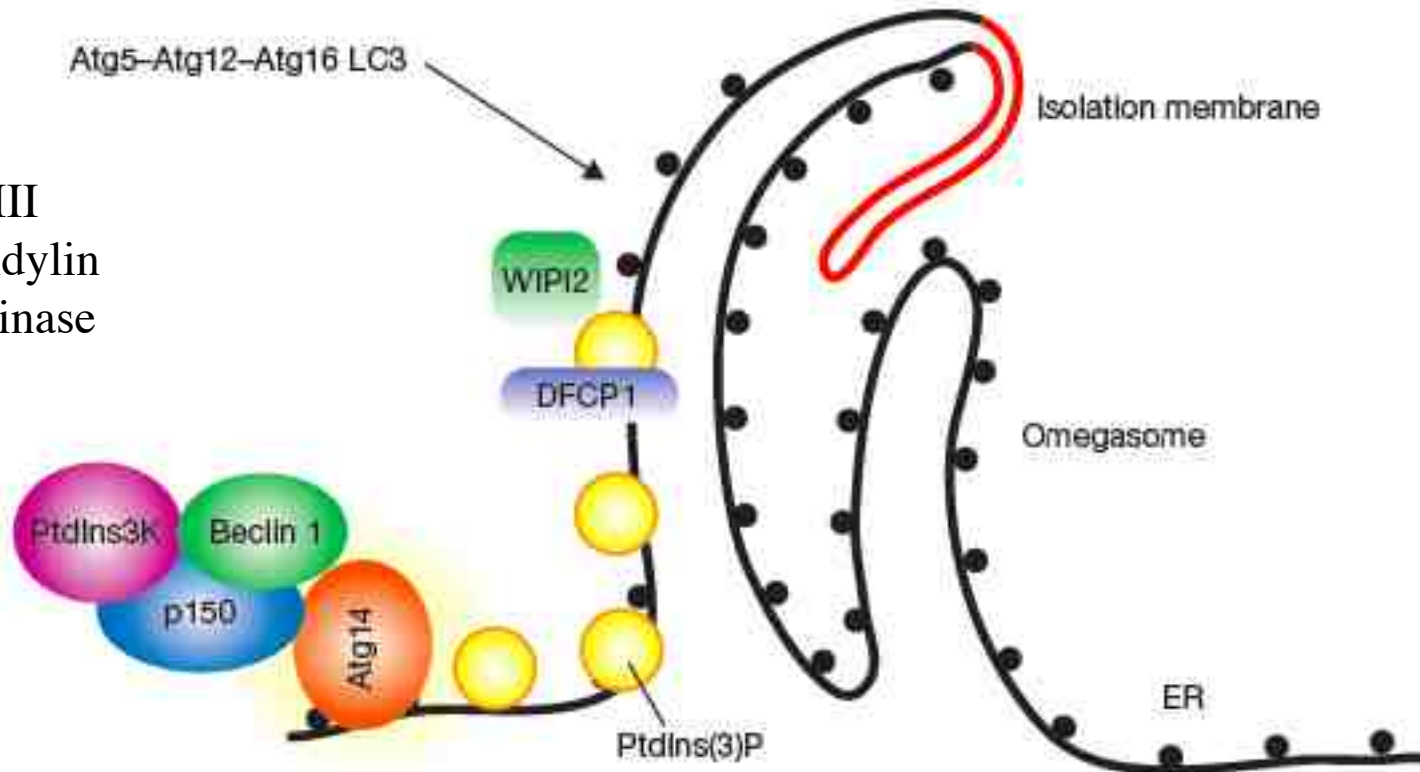
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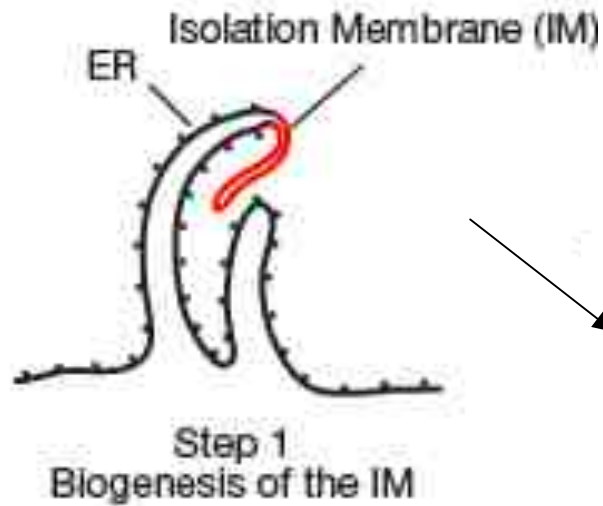
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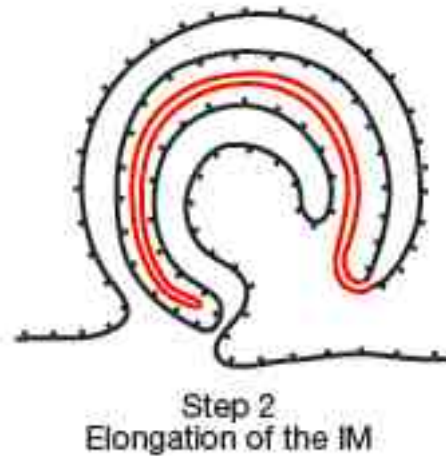
In mammalian cells, the key components seem to be the kinase-containing ULK1 complex (Atg1 in yeast), the class III phosphatidylinositol 3-kinase (PtdIns3K) complex Vps34, **the ubiquitin-like conjugation systems producing Atg5–Atg12–Atg16 and LC3-II** (the phosphatidylethanolamine-containing LC3 conjugate), the sole multi-spanning membrane protein mammalian Atg9 (mAtg9, also known as Atg9a) and the phosphatidylinositol 3 phosphate (PtdIns(3)P)-binding protein WIPI2 (Atg18 in yeast).

the class III
phosphatidylin
ositol 3-kinase

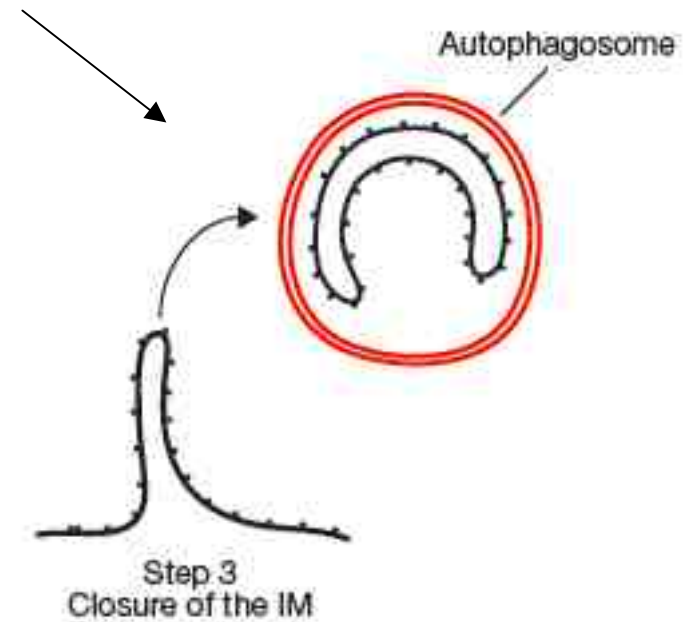




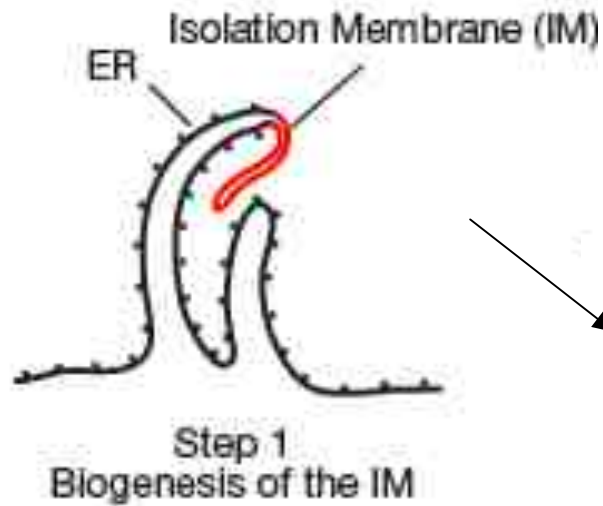
The phagophore shown in red extends from this PtdIns(3)P-rich structure and elongates, cupped between two ER cisternae.



Resident ER proteins are prevented from entering the phagophore, perhaps through a diffusion barrier.

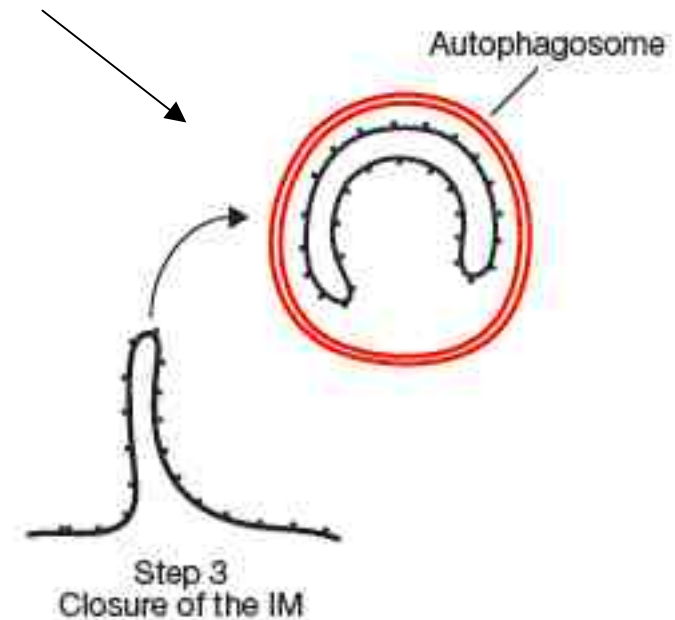
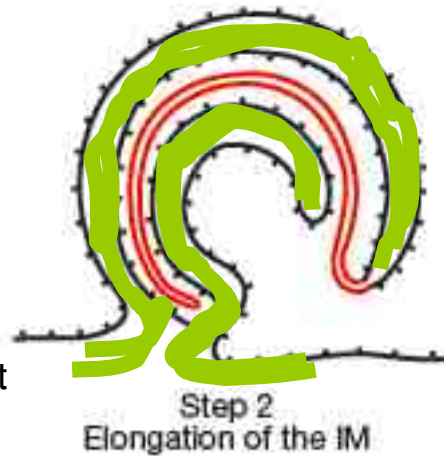


The final stages of elongation and closure of the phagophore and detachment from the ER is illustrated. To allow such a dramatic re-organization of the ER and detachment of the autophagosome other unidentified machinery must be involved.



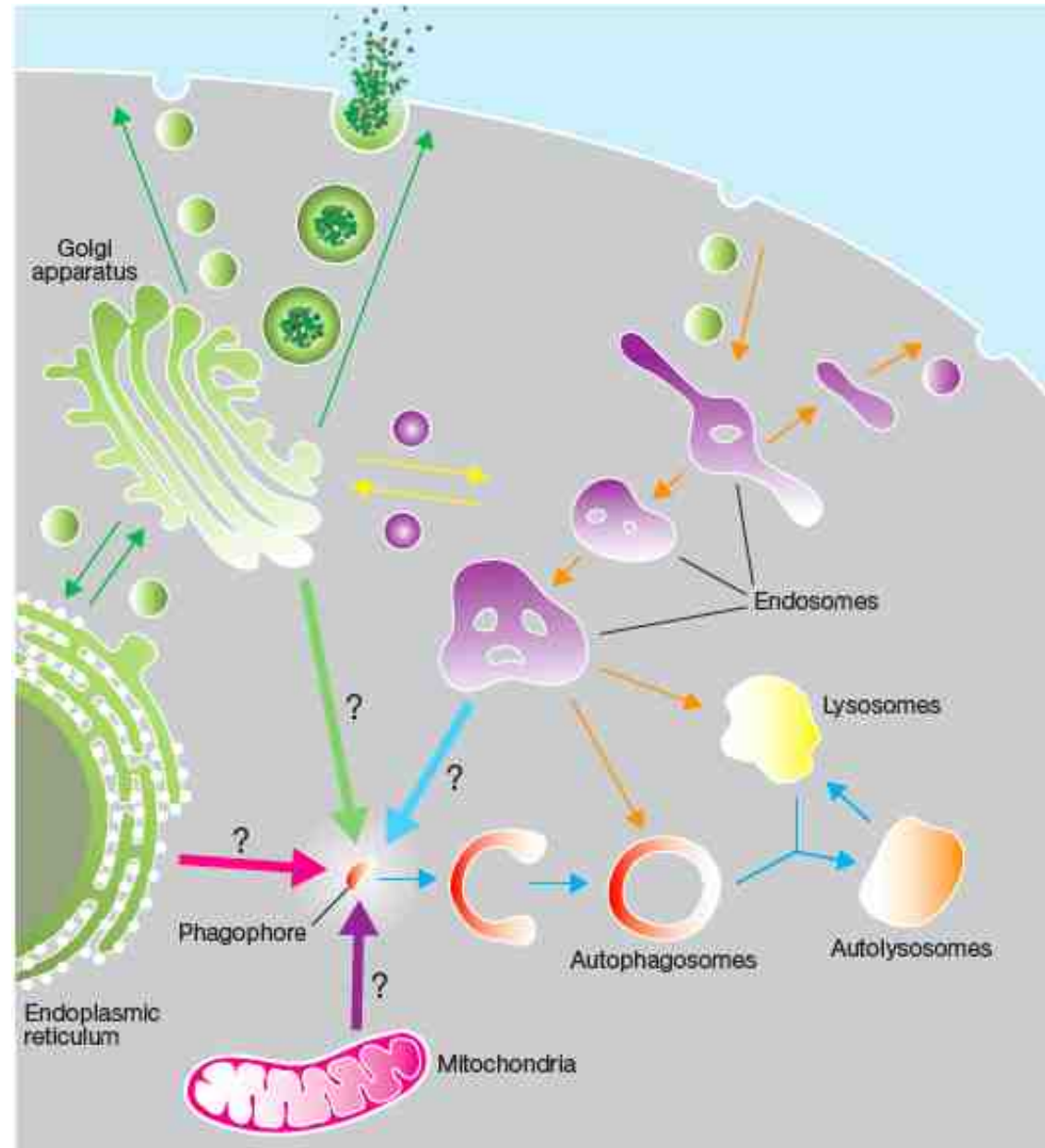
The phagophore shown in red extends from this PtdIns(3)P-rich structure and elongates, cupped between two ER cisternae.

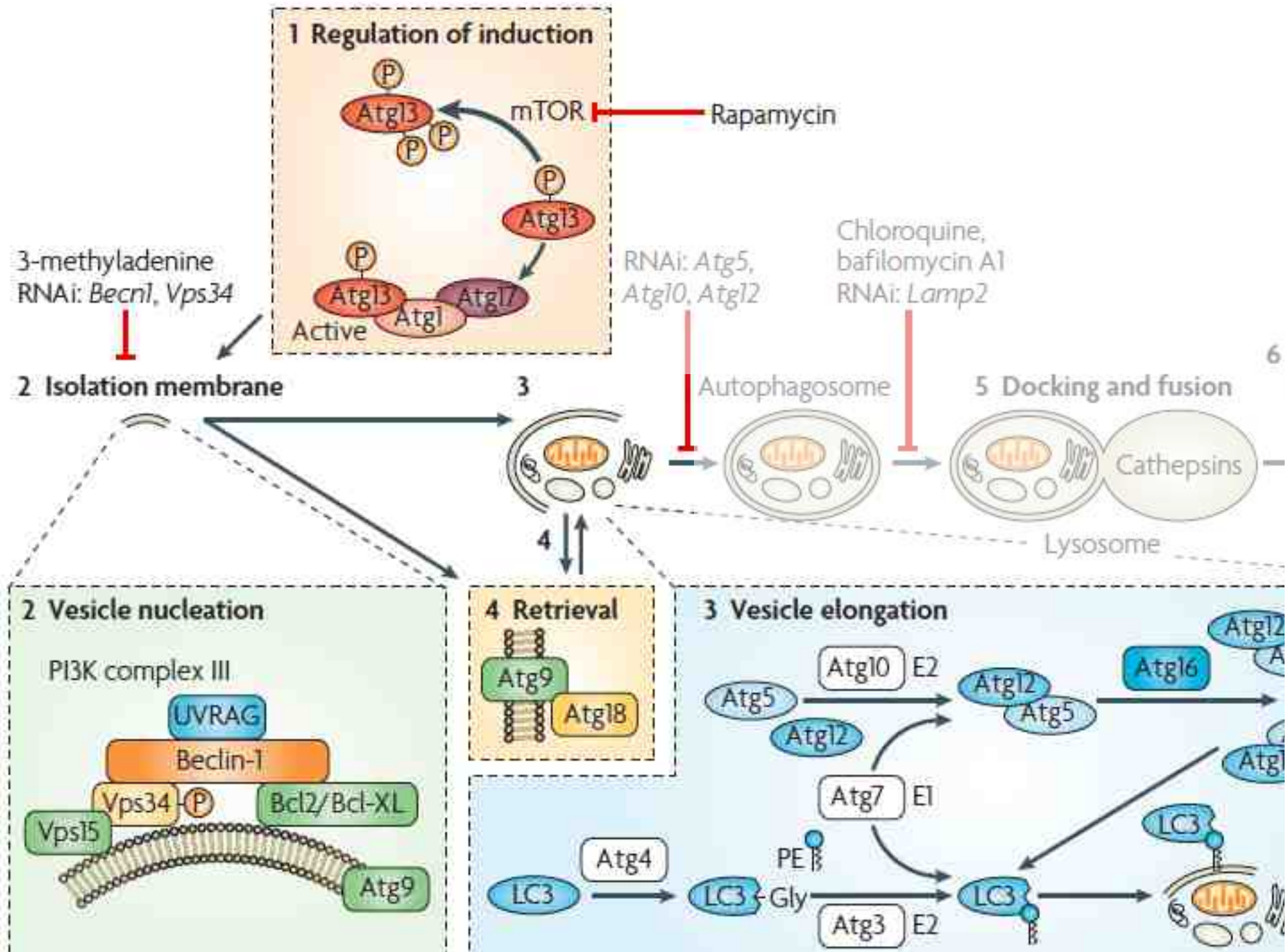
Resident ER proteins are prevented from entering the phagophore, perhaps through a diffusion barrier. It is not known if the PtdIns(3)P produced is localized to the ER or the phagophore, and if localized to the ER whether it is also prevented from entering the phagophore by a diffusion barrier.



The final stages of elongation and closure of the phagophore and detachment from the ER is illustrated. To allow such a dramatic re-organization of the ER and detachment of the autophagosome other unidentified machinery must be involved^{36.15_bmca_2011}

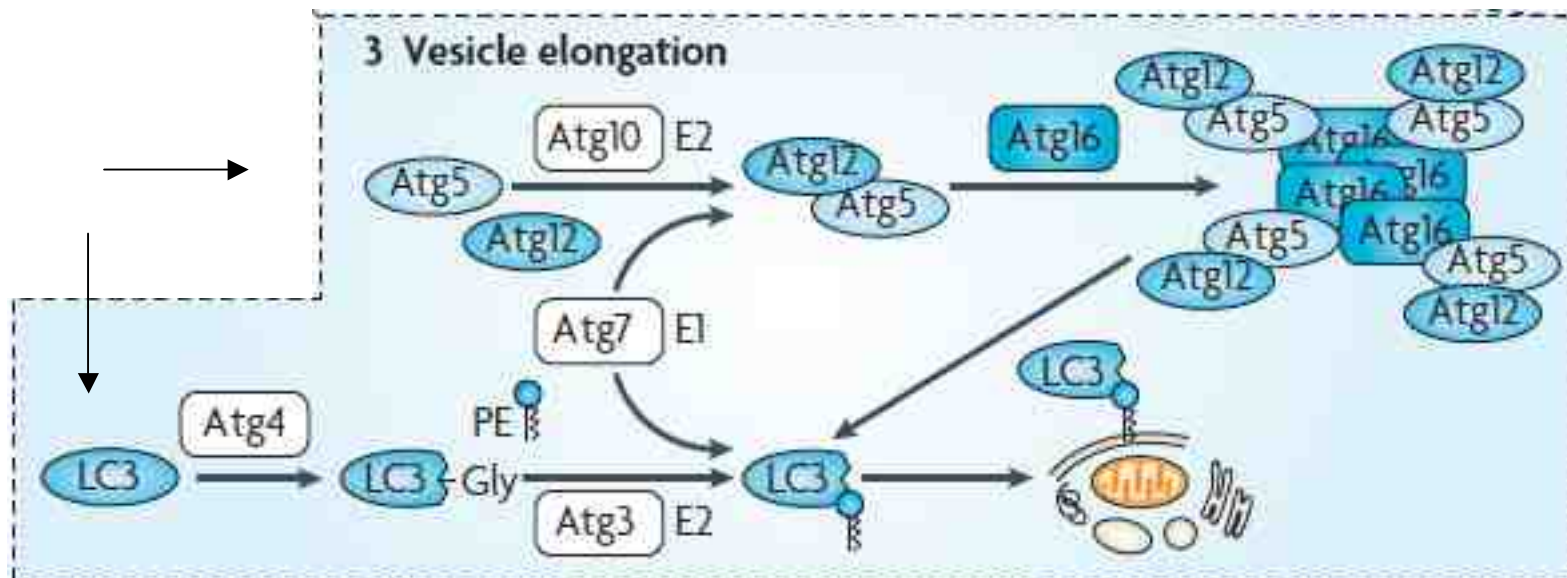
Potential sources for the phagophore include the Golgi complex, endosomes, ER and mitochondria.





step 3 Two ubiquitin-like conjugation systems are part of the vesicle elongation process.

- One pathway involves the covalent **conjugation of Atg12 to Atg5**, with the help of the E1-like enzyme Atg7 and the E2-like enzyme Atg10.
- The second pathway involves the **conjugation of phosphatidylethanolamine (PE) to LC3/Atg8** (LC3 is one of the mammalian homologues of Atg8) by the sequential action of the protease Atg4, the E1-like enzyme Atg7 and the E2-like enzyme Atg3. Lipid conjugation leads to the conversion of the soluble form of LC3 (named LC3-I) to the autophagic vesicle-associated form (LC3-II). LC3-II is used as a marker of autophagy because its lipidation and specific recruitment to autophagosomes provides a shift from diffuse to punctate staining of the protein and increases its electrophoretic mobility on gels compared with LC3-I. Moreover, green fluorescent protein–LC3 fusion proteins can be used to visualize autophagosomes by fluorescence videomicroscopy⁹¹



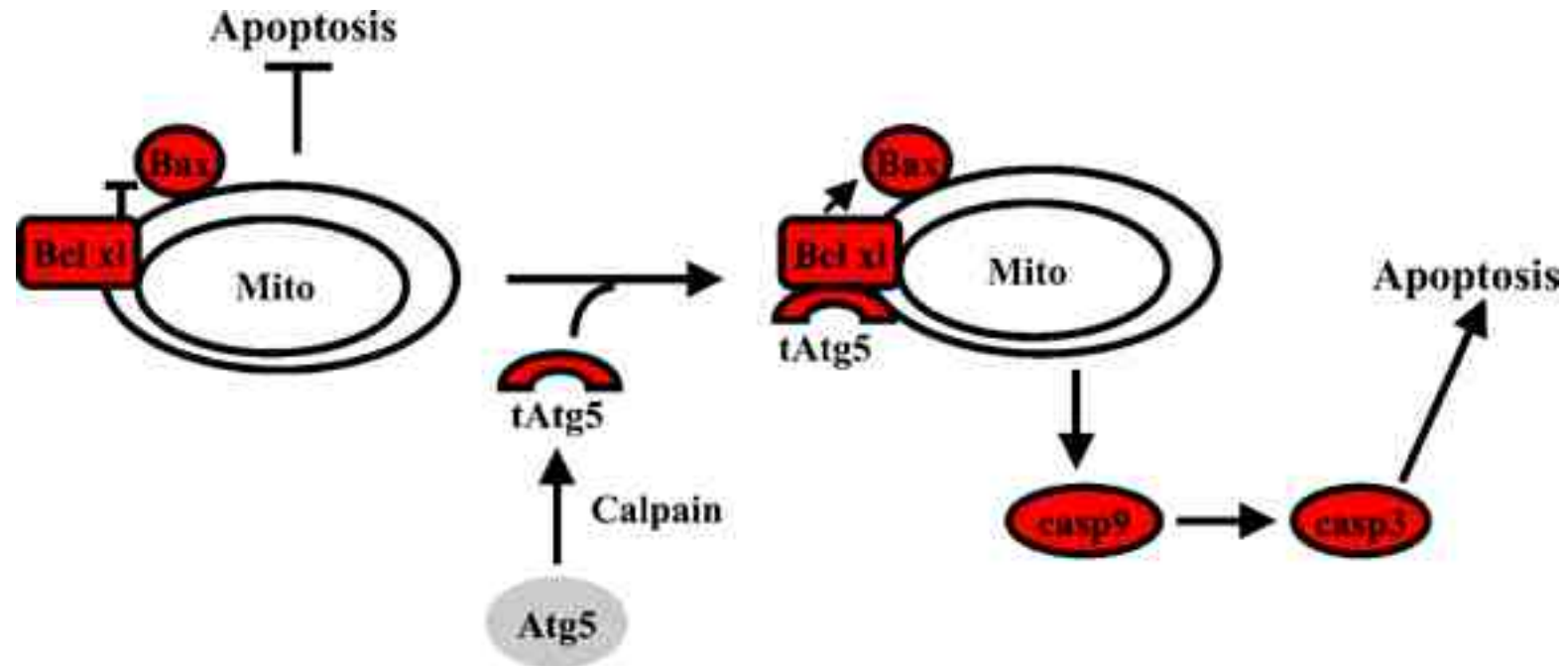
Cell Death and Differentiation (2007) **14**, 1247–1250; doi:10.1038/sj.cdd.4402149; published online 13 April 2007

Atg5 and Bcl-2 provide novel insights into the interplay between apoptosis and autophagy

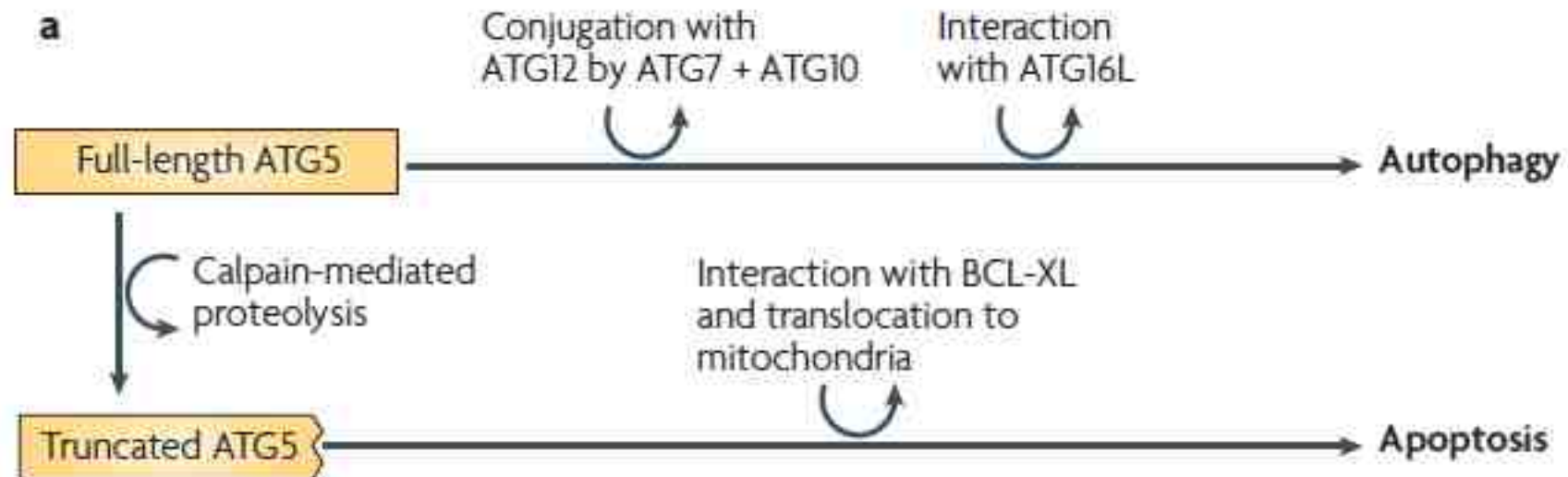
S Luo¹ and D C Rubinsztein¹

Atg5, previously considered to be an autophagy-specific gene involved in autophagosome precursor expansion and completion through an ubiquitin-like conjugation system, now appears to be an important mediator of apoptosis. Atg5 can be cleaved following death stimuli, and the cleavage product appears to promote mitochondria mediated apoptosis. Bcl-2, the well-characterised apoptosis guard, appears to be important in autophagy, as it binds to Beclin 1/Atg6 and inhibits Beclin 1-mediated autophagy and autophagic cell death. Thus, Bcl-2 and Atg5 are proteins that regulate both apoptosis and autophagy.

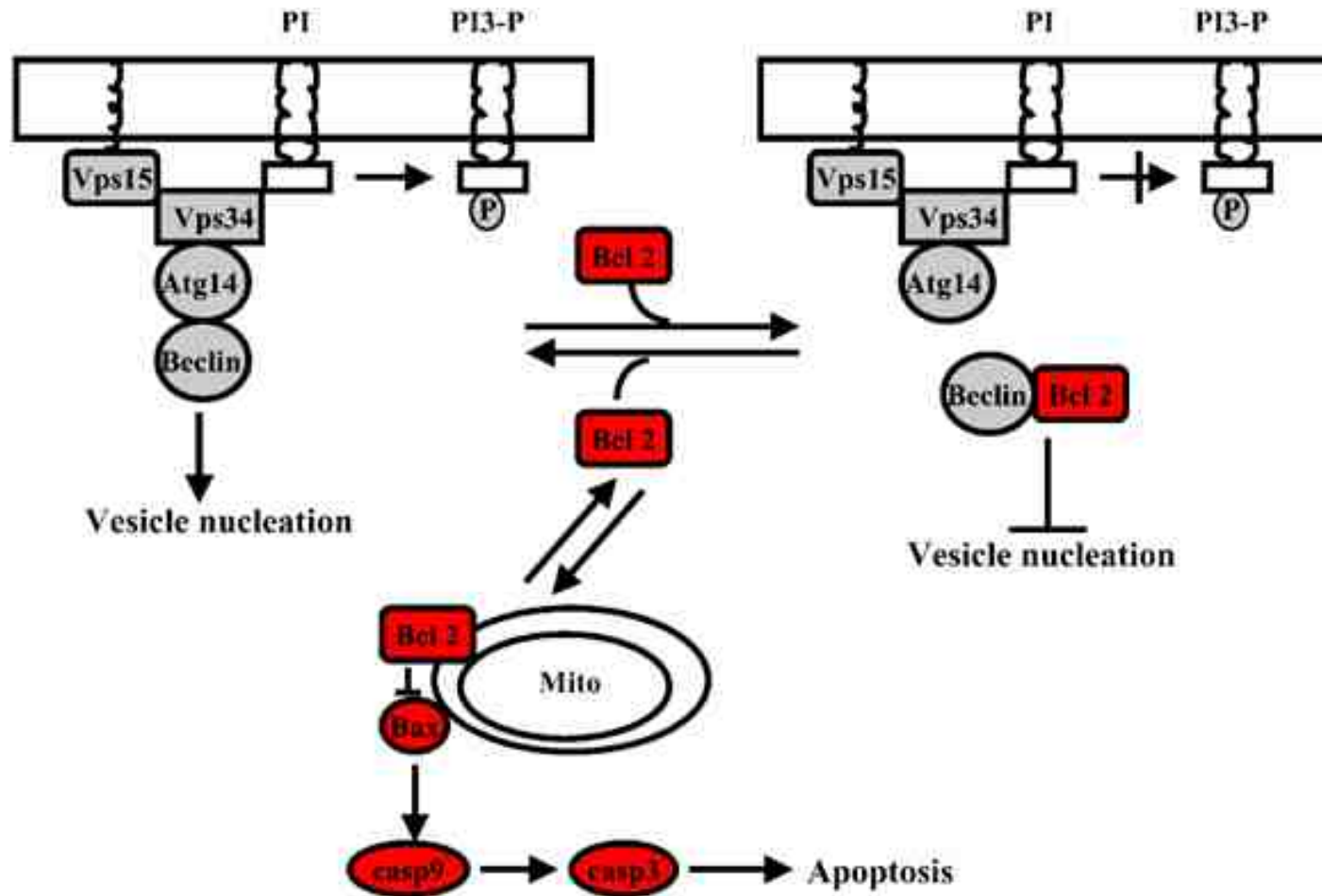
Atg5 is cleaved by calpain and induces apoptosis. Atg5, an autophagy protein is cleaved by calpain into truncated Atg5 (tAtg5), tAtg binds to Bcl-xl and leads to apoptosis



Dual function of autophagy protein-5 (ATG5) in autophagy. Full-length ATG5 can participate in the initial stages of autophagy with the help of a ubiquitin-like conjugation system. ATG12 (activated by ATG7 and then transferred to ATG10) is conjugated with ATG5 at a lysine- ϵ -amino group, allowing it to bind to ATG16. The resulting ATG12–ATG5–ATG16 complex imposes curvature on the crescent phagophore and recruits activated LC3 to the elongating membrane (LC3 is one of the mammalian homologues of Atg8, which is conjugated to phosphatidylethanolamine by the action of ATG4, ATG7 and ATG3). Proteolysis by a calpain results in a truncated ATG5, which can translocate to mitochondria and induce MOMP.



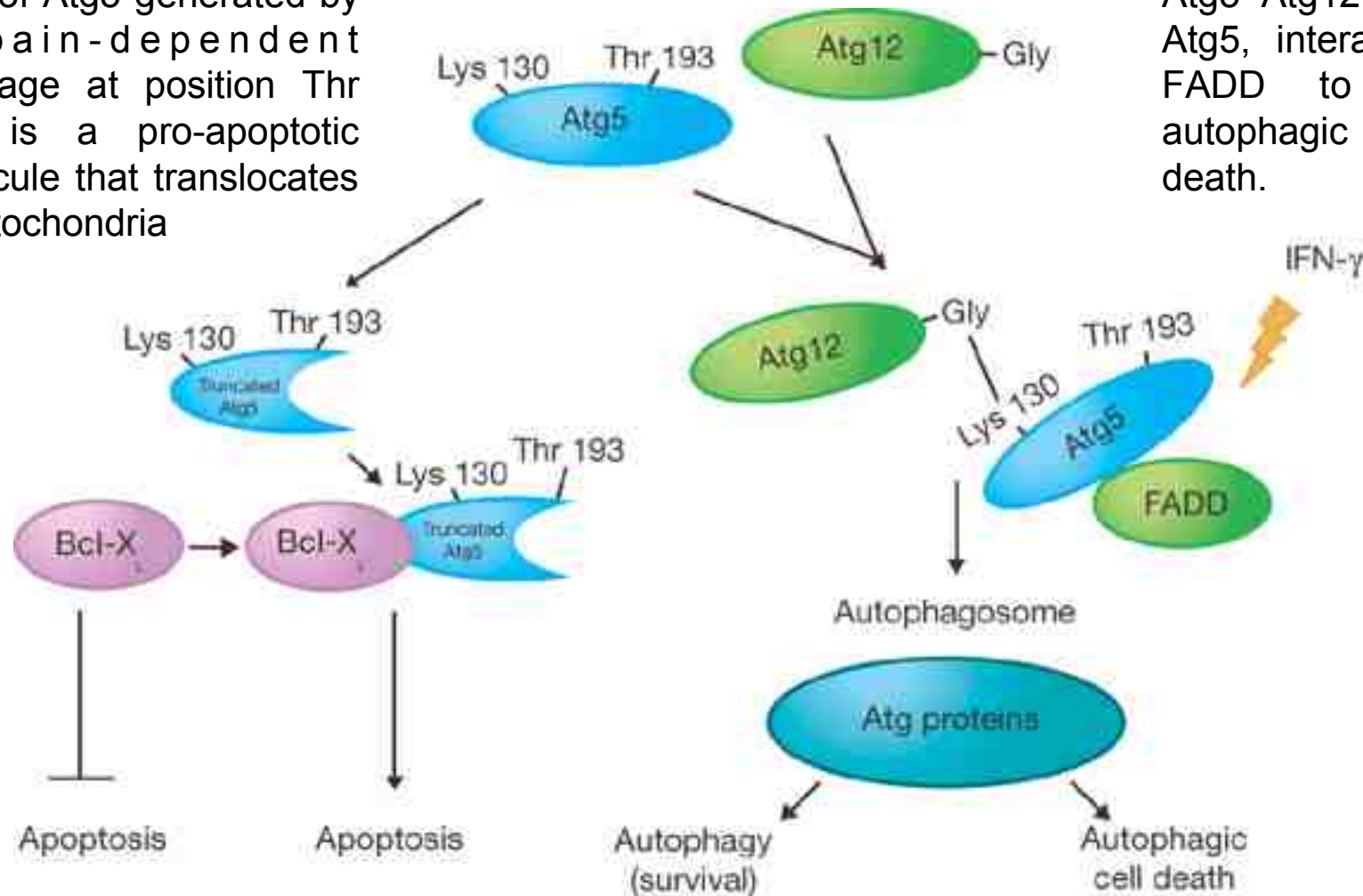
Bcl-2 regulates autophagy and apoptosis. Bcl-2/Bcl-xl binds to Beclin 1 (possibly affecting Beclin 1-Vps34 complex) and inhibits autophagy.



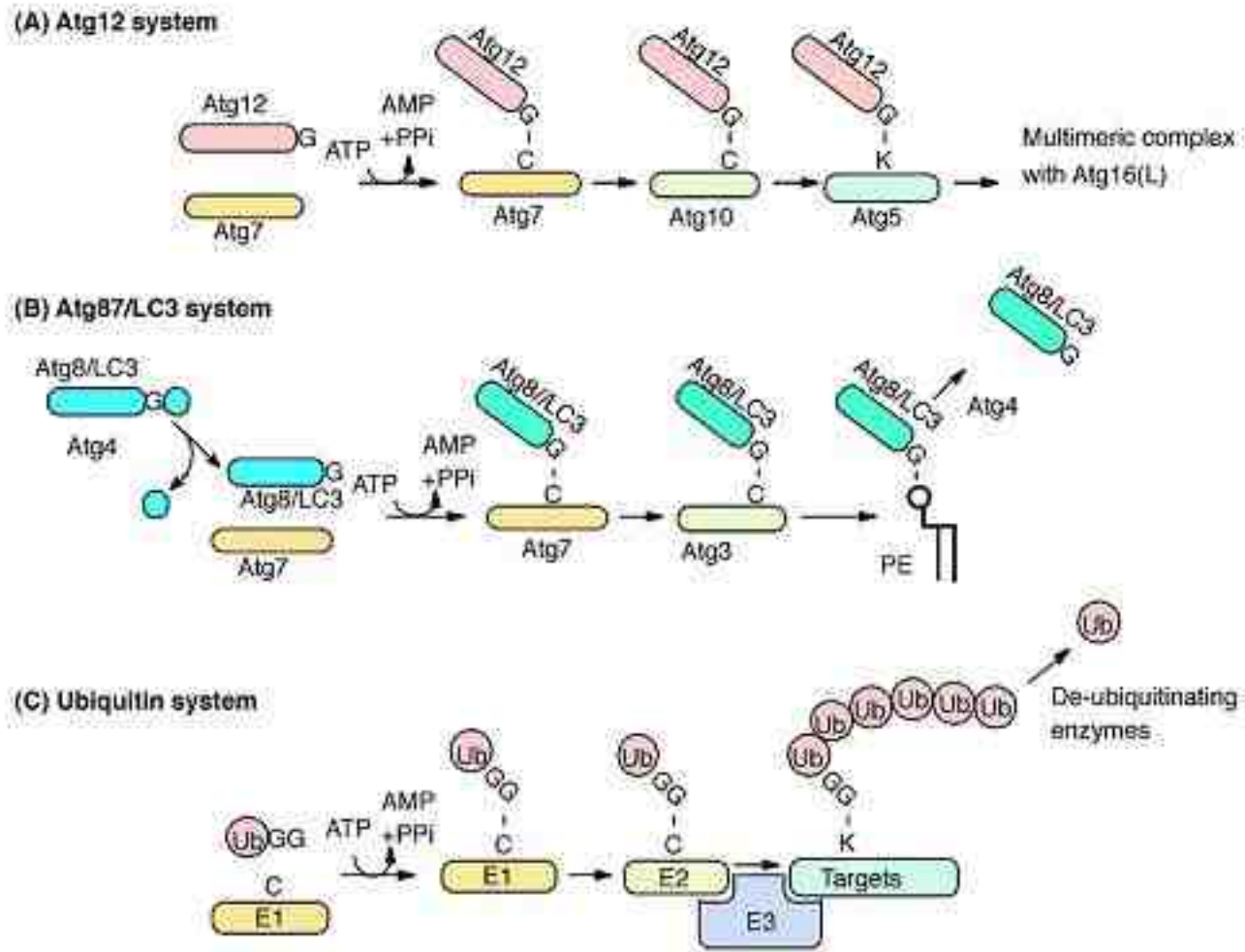
The ubiquitin-like attachment of Atg12 and Atg5 requires the activity of Atg7 and Atg10 which function in a similar manner to E1 and E2 enzymes in the ubiquitin system.

Atg5 is also a substrate for calpains. The truncated form of Atg5 generated by calpain-dependent cleavage at position Thr 193 is a pro-apoptotic molecule that translocates to mitochondria

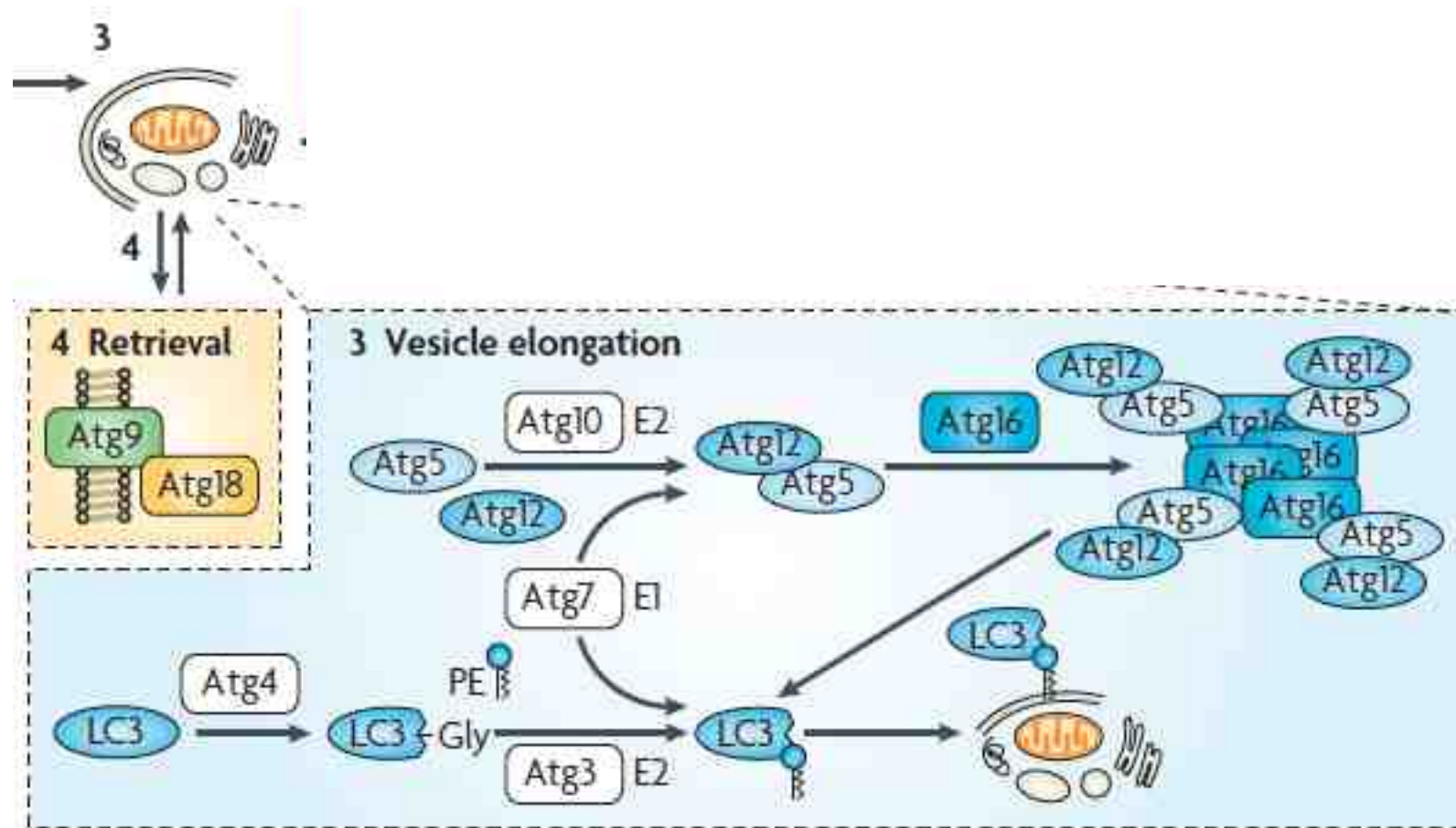
In response to interferon- γ (IFN- γ), Atg5-Atg12, through Atg5, interacts with FADD to trigger autophagic cell death.



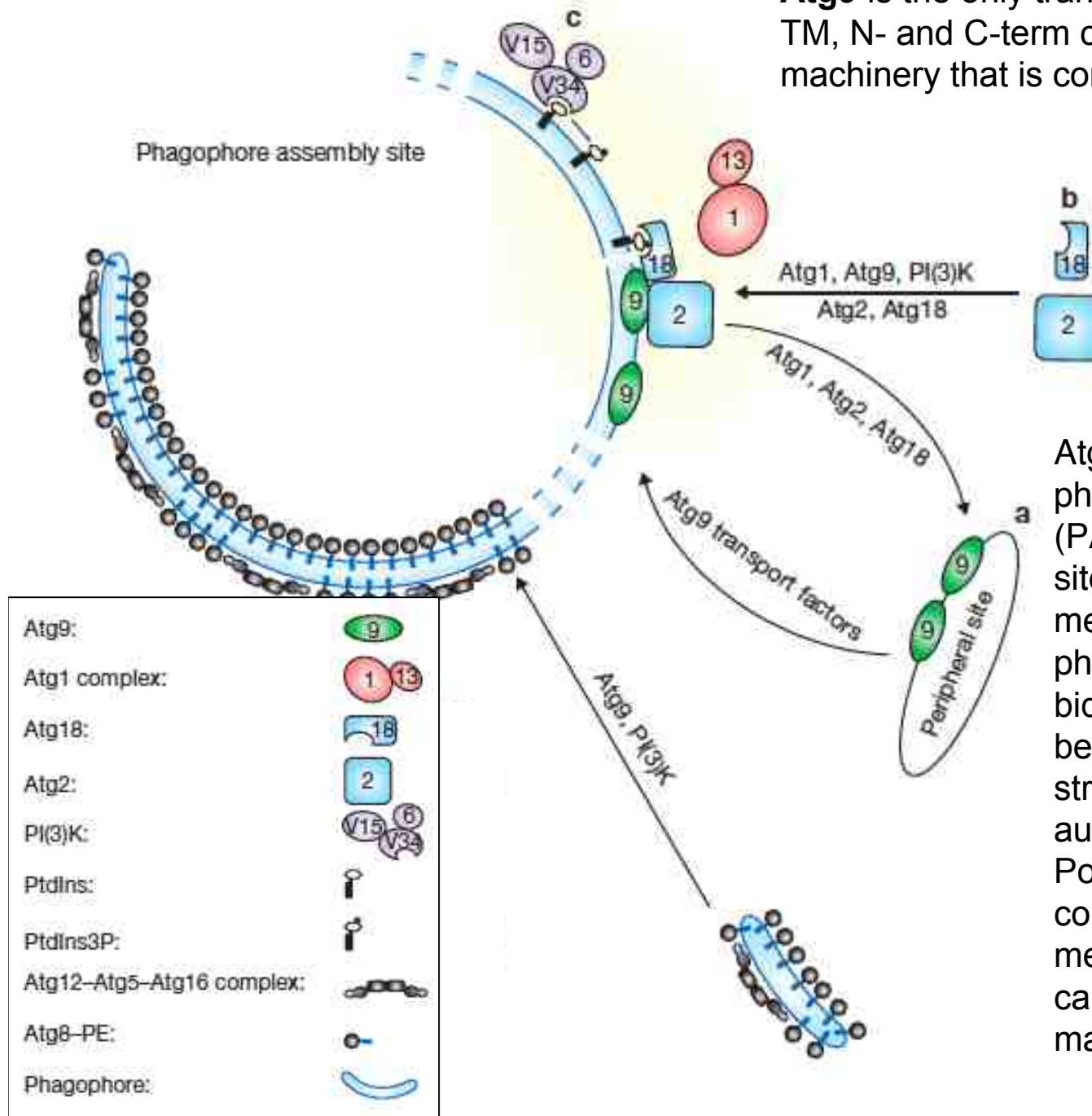
Two ubiquitin-like systems essential for autophagy



The mechanism of retrieval in which the Atg9 complex participates is poorly studied (**step 4**).



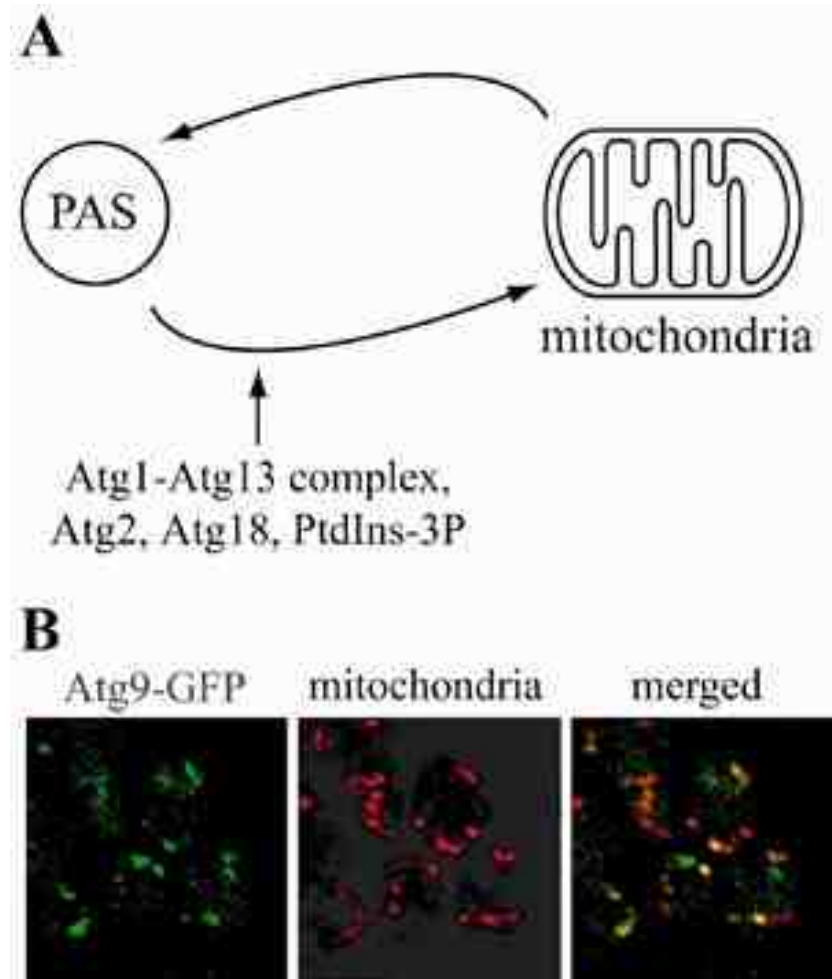
Atg9 is the only transmembrane protein (6-pass-TM, N- and C-term cytoplasmic) in the core machinery that is conserved across species.



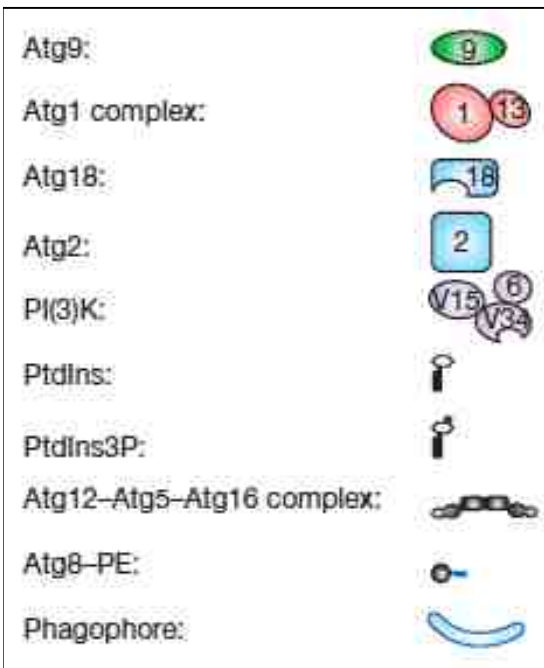
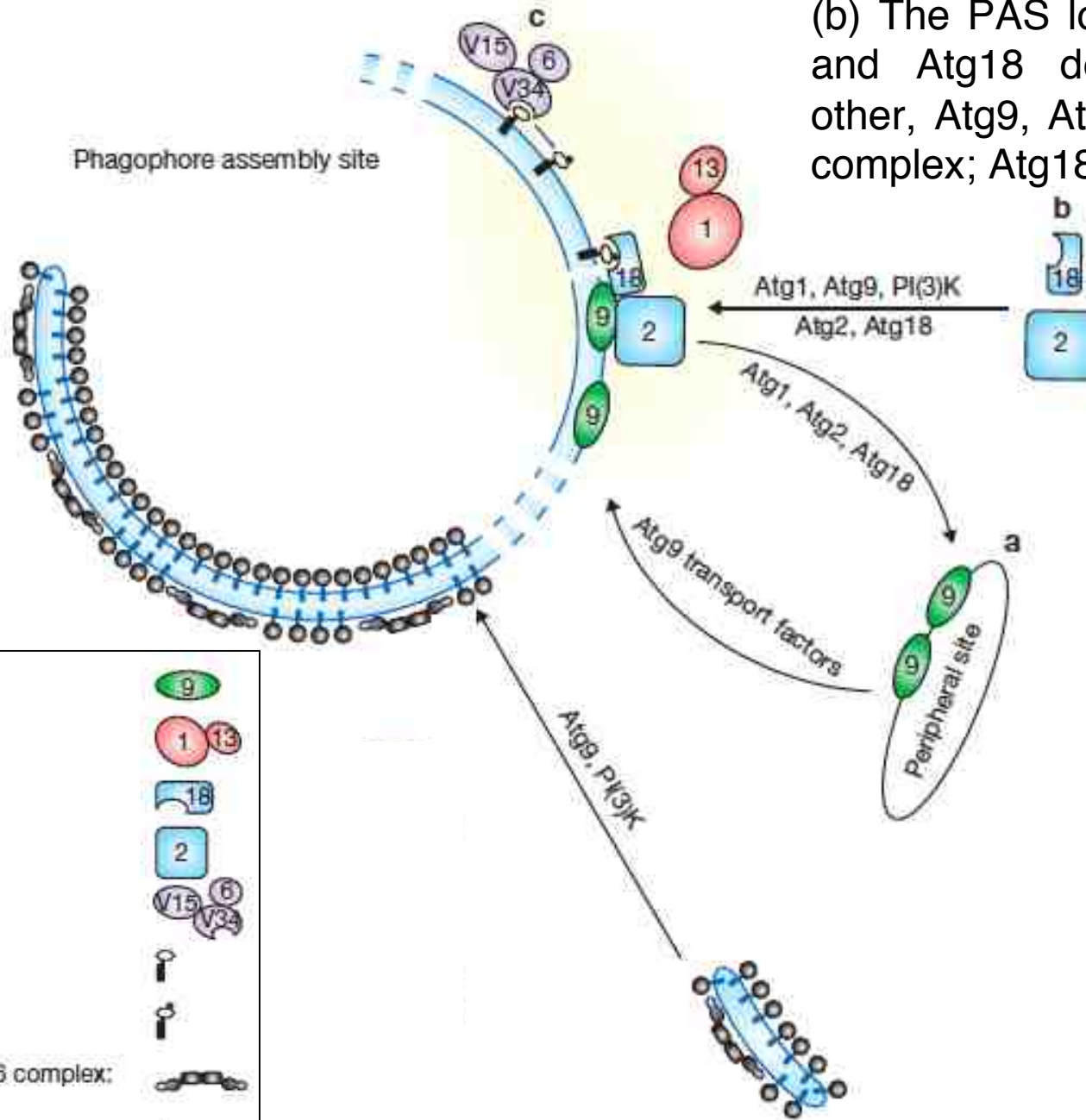
Atg9 cycles between the phagophore assembly site (PAS) and multiple peripheral sites, presumably carrying membrane required for phagophore expansion. The bidirectional movement of Atg9, between PAS and non-PAS structures, is necessary for autophagosome formation. Potentially, this shuttling could contribute to the delivery of membrane to the PAS. Atg9 is capable of self-interaction and may exist in a complex

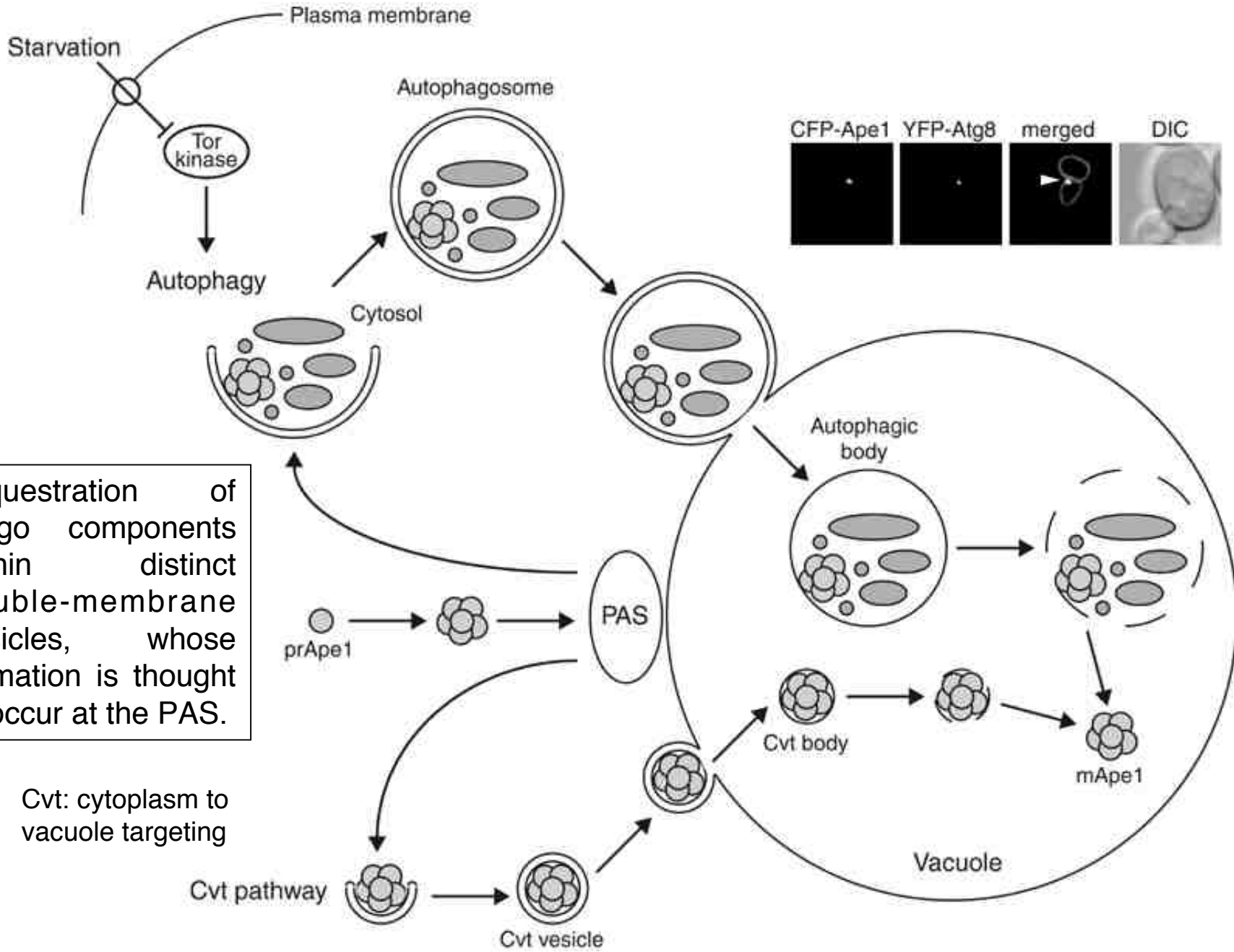
In yeast, some non-PAS Atg9 puncta are found to be adjacent to or at the surface of mitochondria. By contrast, human Atg9 homologues localize to the trans-Golgi network and late endosomes but not mitochondria.

In yeast



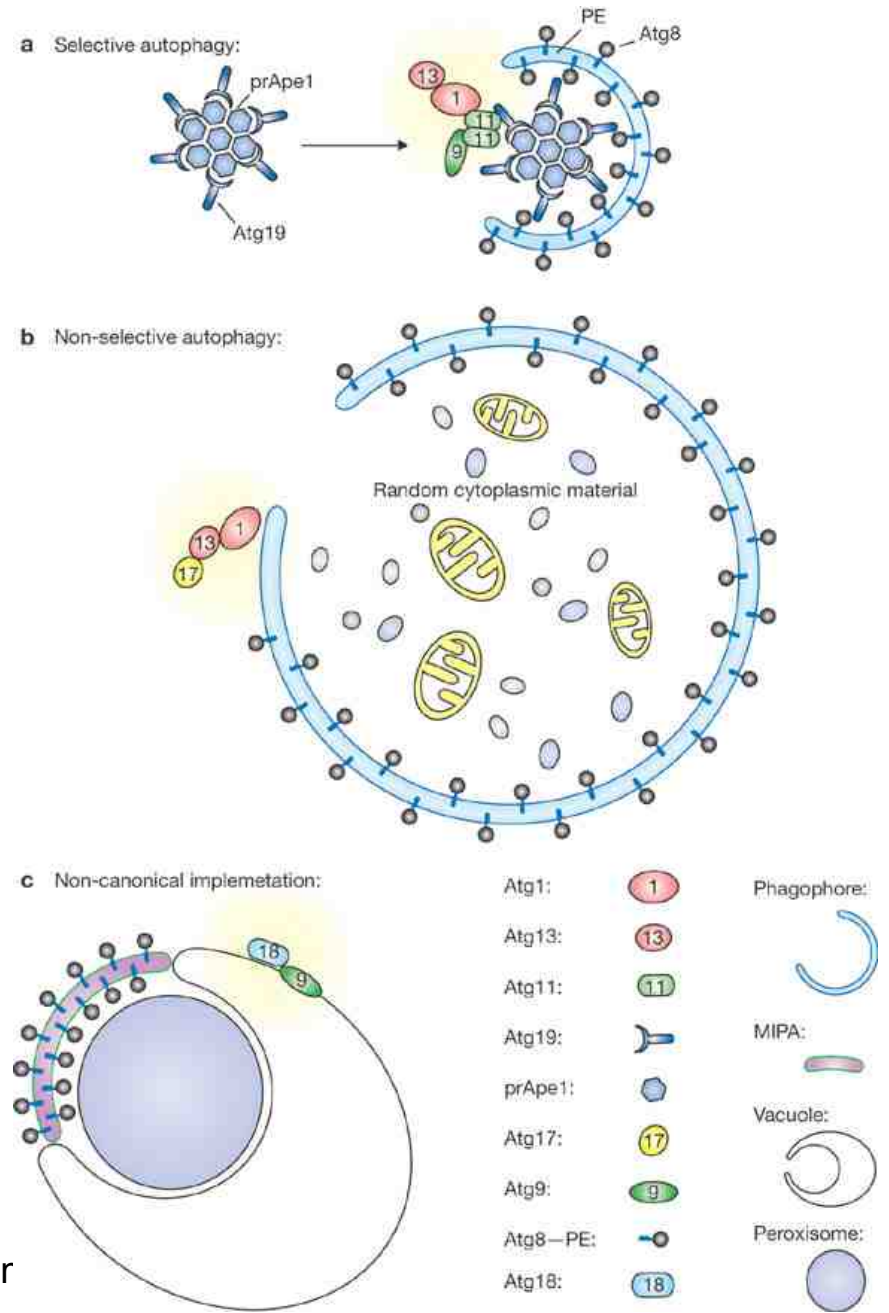
(b) The PAS localization of Atg2 and Atg18 depends on each other, Atg9, Atg1 and the PI(3)K complex; Atg18 binds PtdIns(3)P.





sequestration of cargo components within distinct double-membrane vesicles, whose formation is thought to occur at the PAS.

Cvt: cytoplasm to vacuole targeting

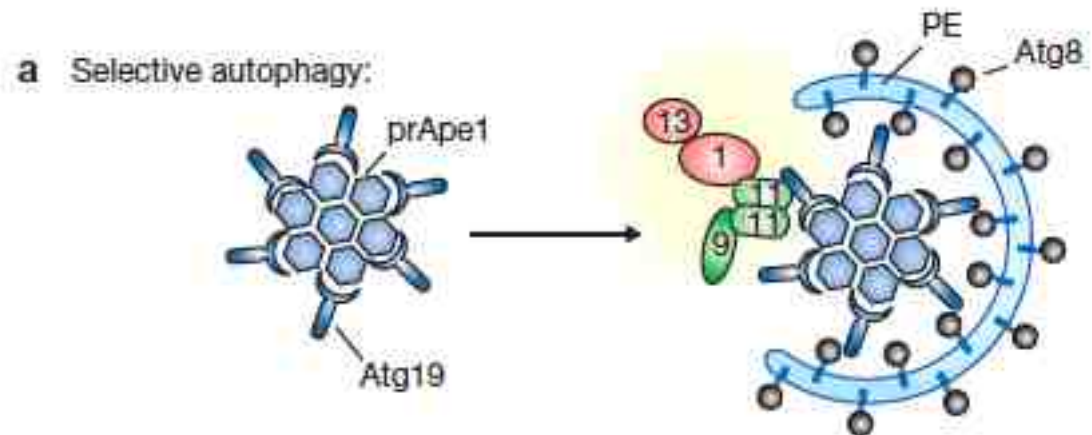
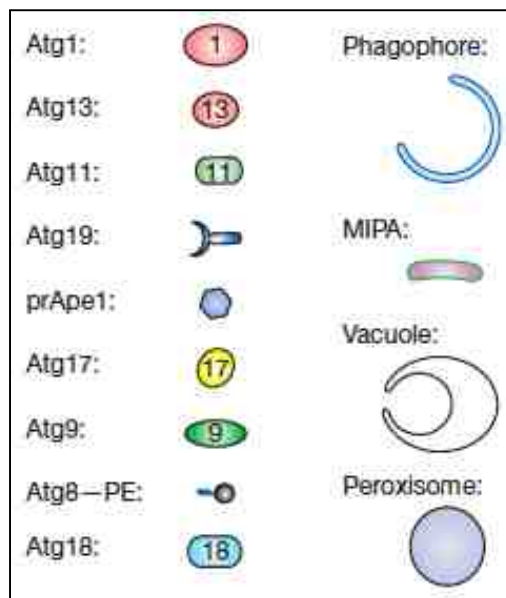


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Autophagy can be selective or non-selective

During **selective autophagy**, only pertinent cargoes are sequestered into autophagosomes.

The autophagosomes generated under these conditions, including Cvt vesicles, pexophagosomes and bacteria-containing autophagosomes, have contours that resemble those of the cargoes, and they contain little bulk cytosol between the cargo and the vesicle inner membrane.

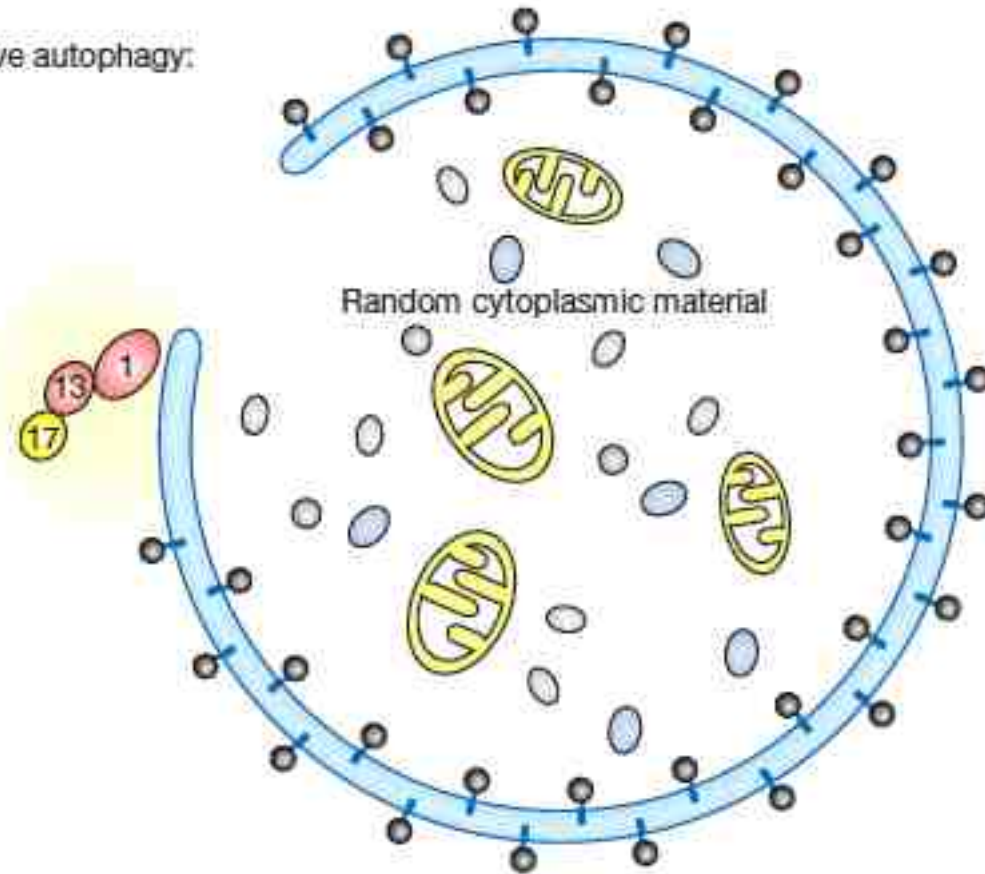


The vesicles assemble directly around these cargoes using them as a scaffold.

By contrast, during **non-selective autophagy**, autophagosomes primarily contain bulk cytoplasmic material. Owing to the cargo's soluble nature, the core machinery conceivably needs additional mechanisms to determine its own workload.

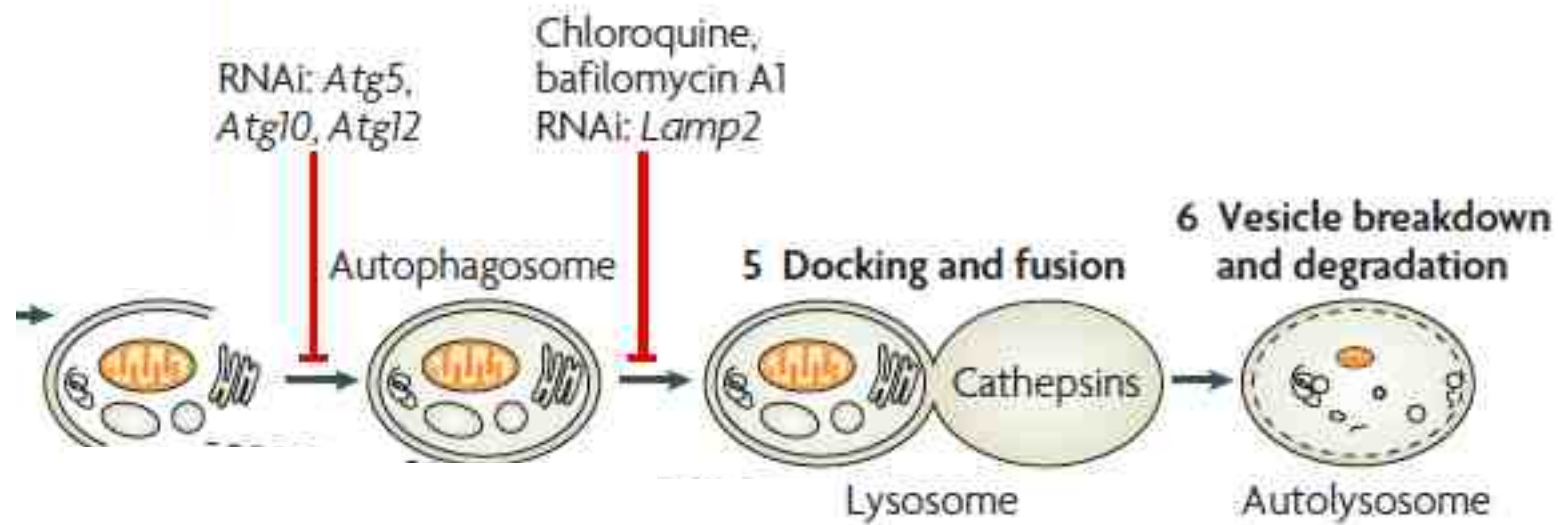
Non-selective autophagy has been observed in all eukaryotes analysed

b Non-selective autophagy:



Atg1:		Phagophore:	
Atg13:		MIPA:	
Atg11:		Vacuole:	
Atg19:		Peroxisome:	
prApe1:			
Atg17:			
Atg9:			
Atg8-PE:			
Atg18:			

Autophagosomes undergo maturation by fusion with lysosomes to create autolysosomes (**steps 5 and 6**). In the autolysosomes, the inner membrane as well as the luminal content of the autophagic vacuoles is degraded by lysosomal enzymes that act optimally within this acidic compartment. Lamp2, lysosome-associated membrane glycoprotein-2.



Cell Migration

Single cell migration

Collective cell migration

Cell Migration

Single cell migration

Collective cell migration

Why is Cell Migration Important?

Embryonic development

You wouldn't be here without it.

Tissue repair

Wound healing and regeneration.

Angiogenesis

Formation of new blood vessels in development, tissue repair and tumor growth.

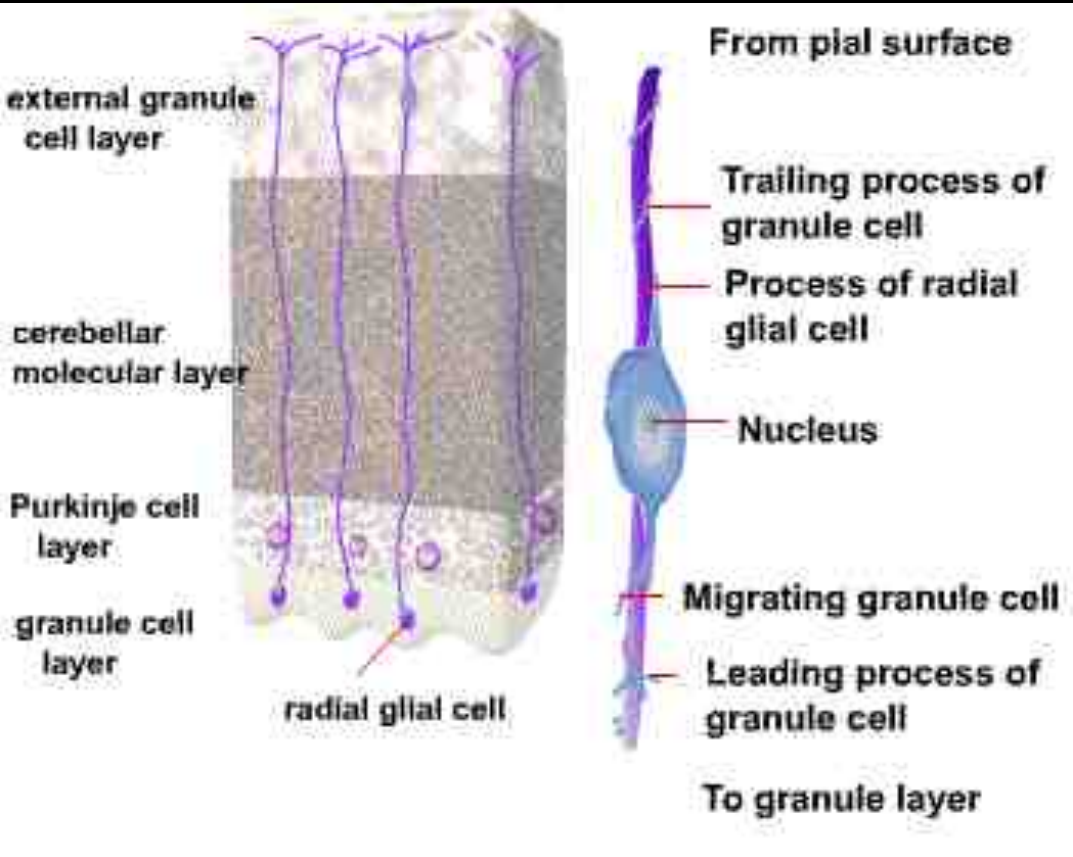
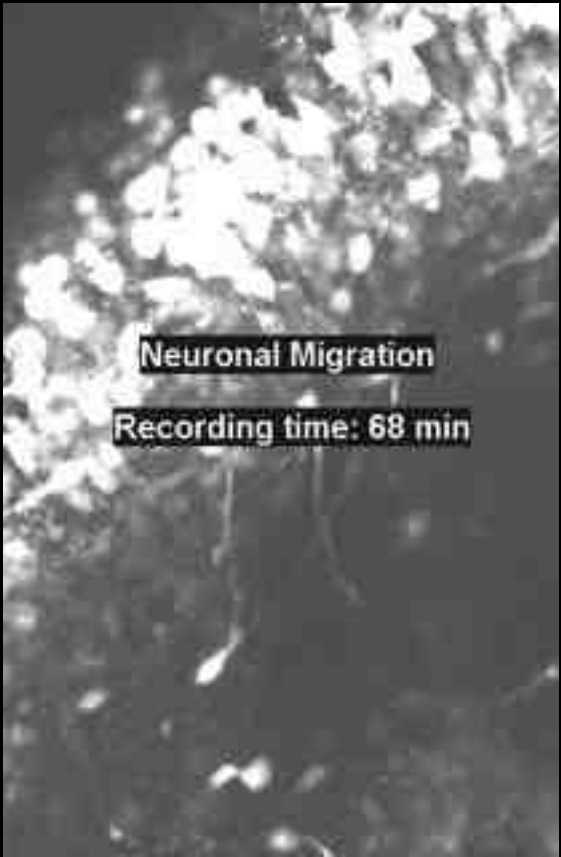
Cancer invasion and metastasis

Spreading of tumors.

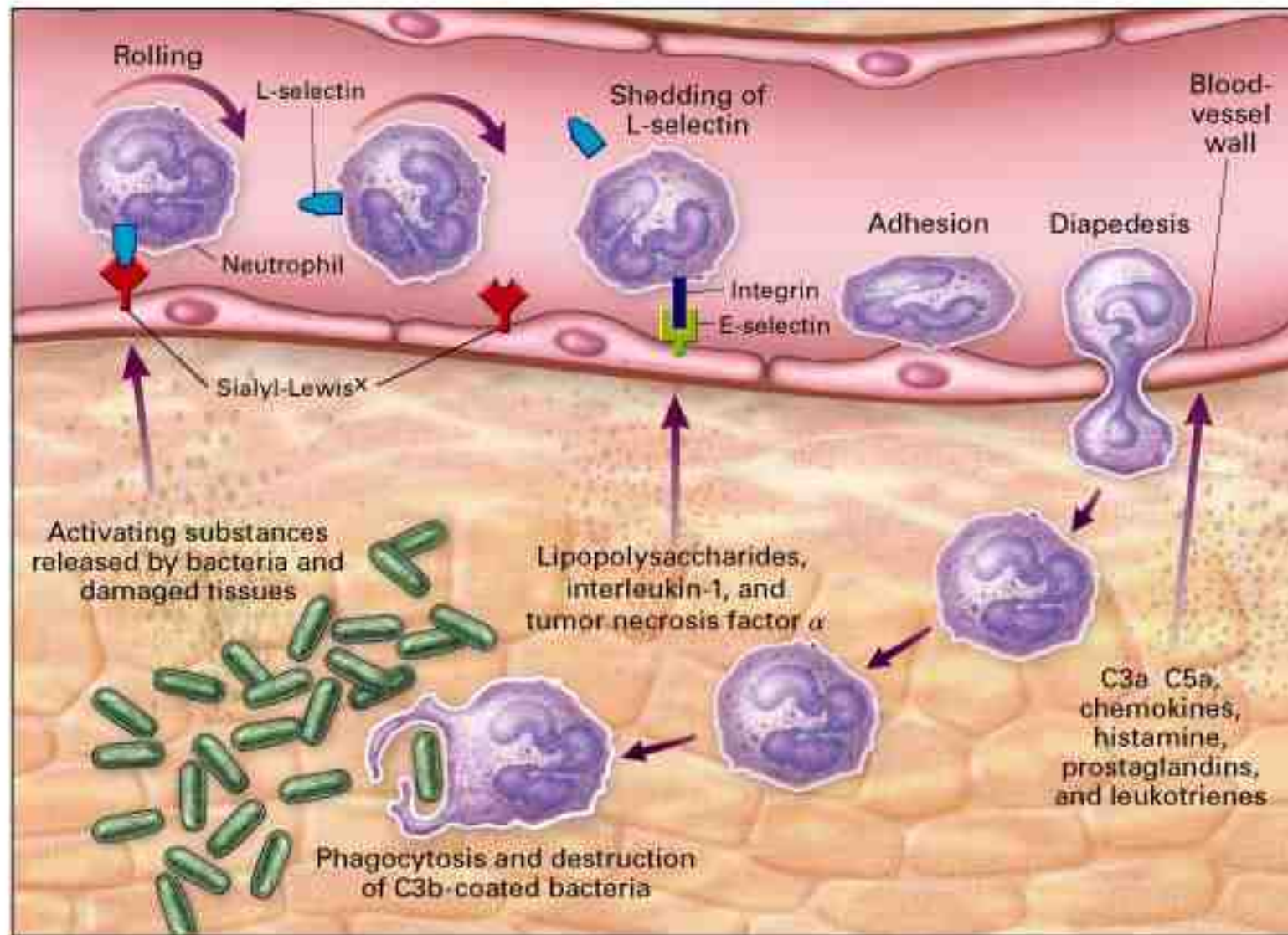
Immune function and inflammation

Find and catch invaders.

Embryonic development



Immune function and inflammation: “*find and catch invaders*”



Regolazione della chemiotassi

neutrophil



Dictyostelium



Che cosa é la chemiotassi?

Motilità orientata delle cellule in risposta a segnali chimici (chemiotattici) rilasciati da altre cellule

La chemiotassi è un importante meccanismo di comunicazione cellulare:

- (1) Nei processi morfogenetici durante lo sviluppo embrionale
- (2) Nella rimarginazione di ferite e rigenerazione di tessuti
- (3) Nelle infezioni e infiammazioni (i leucociti sono attratti da prodotti catabolici di batteri (f-Met-Leu-Phe) o protozoi)
- (4) Nella risposta immunologica
- (5) Nella metastasi (invasività e migrazione) di cellule tumorali

PRINCIPALI MODELLI DI STUDIO: NEUTROFILI E DICTYOSTELIUM

Fasi nel processo chemiotattico

Ceppo selvatico



- La cellula:
- (1) "sente" il gradiente chemiotattico
- (2) propaga il segnale alla periferia (signal relay)
- (3) **si polarizza (pseudopodio nel fronte guida; pseudopodi laterali inibiti)**
- (4) si muove col fronte guida verso la fonte del gradiente
- (5) ritrae la parte posteriore

Il citoscheletro di actina controlla filopodi, pseudopodi e lamellipodi

