

# Autophagy

⌚ Created	@April 13, 2024 3:46 PM
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## Cell Membrane

▼ How do we know that the cell membrane is dynamic ( how to prove the fluid mosaic model)

- FRAP: fluorescence recovery after photobleaching
- cell fusion experiments (mouse membrane GFP & RFP)

▼ Questions to pay attention

1. How variable are membranes in terms of their organisation and composition?
  - a. between different organelles
  - b. within the same membrane
2. How do different membranes interact
3. How does a cell build new membrane (in autophagy)

▼ Fluid mosaic model

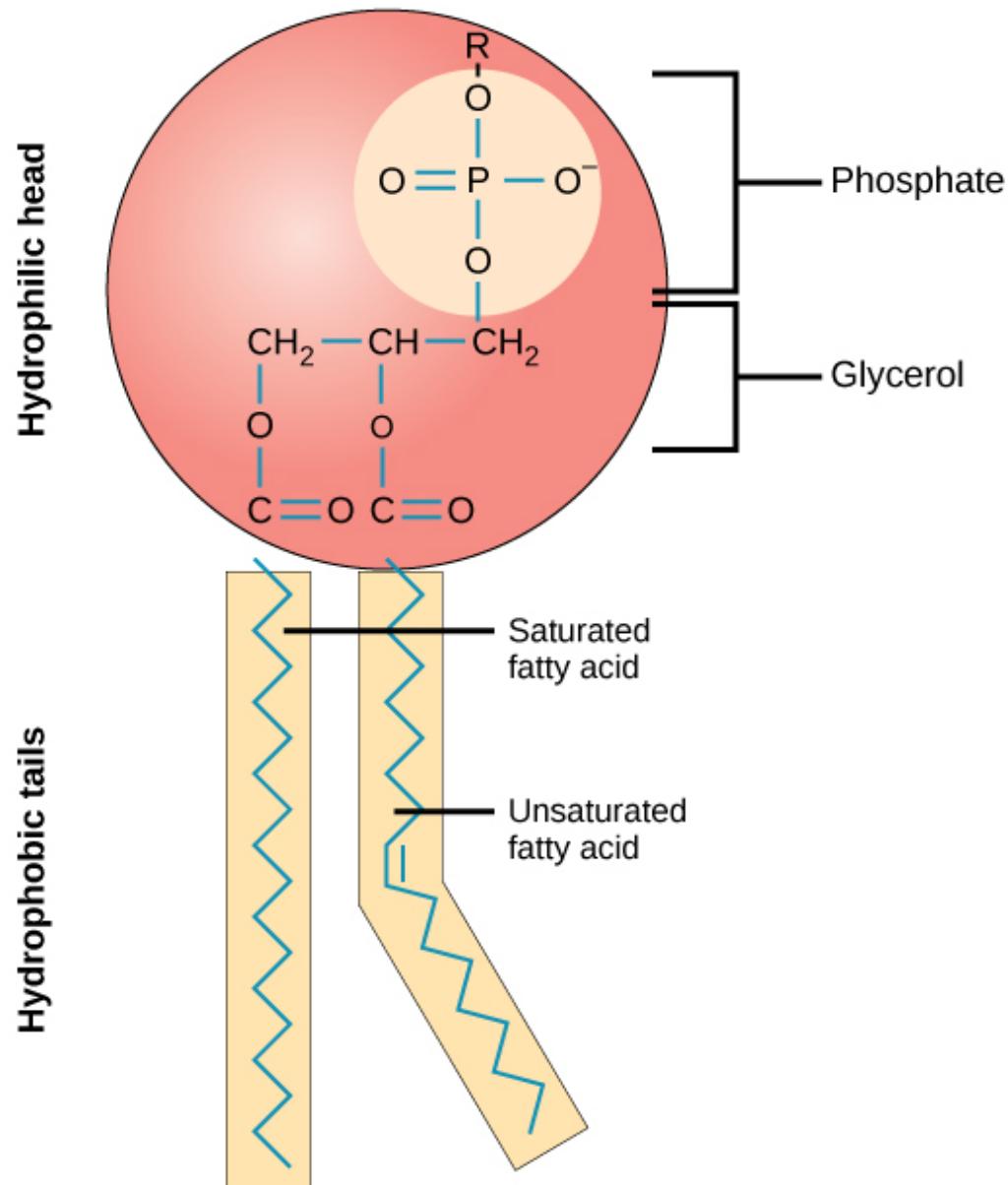
▼ size comparison

- 5-6 nm
- actin filament: 7nm

▼ Composition

- phospholipids
  - hydrophilic head: e.g. phosphate + other

- backbone (alcohol)
- hydrophobic tails — e.g. fatty acyl chains

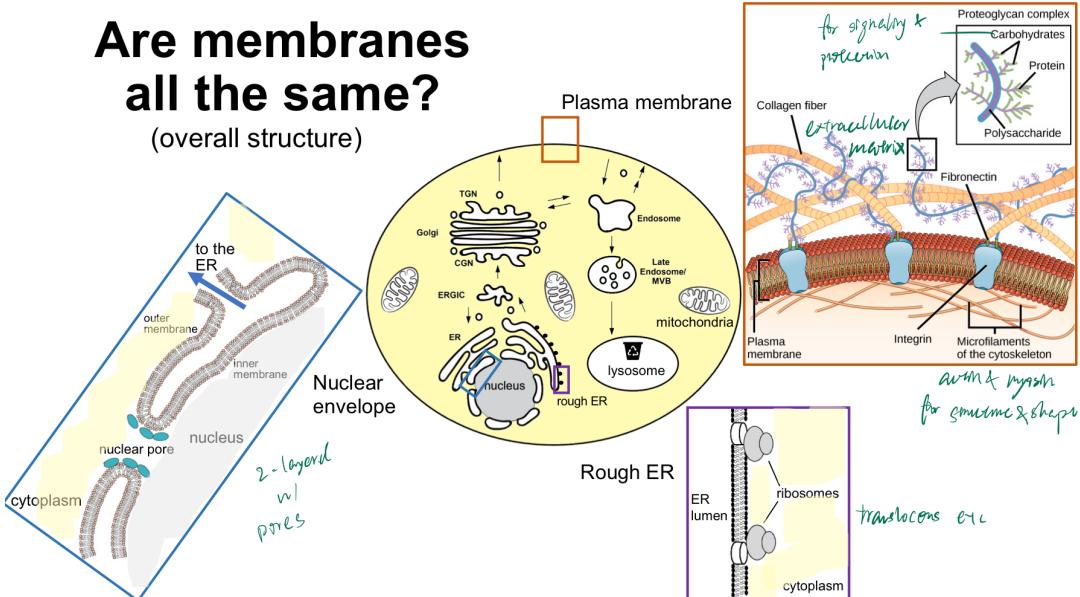


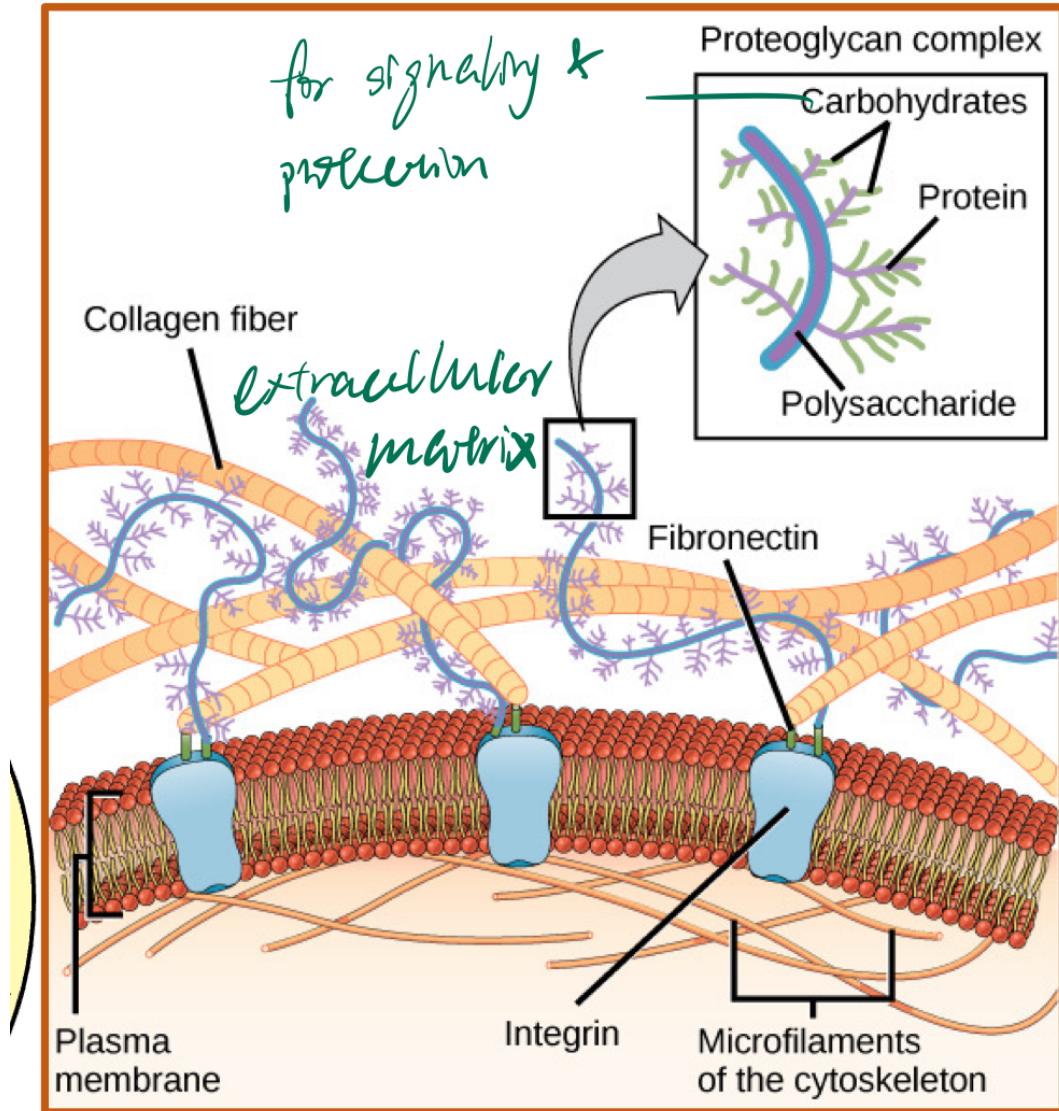
- Sphingolipids
  -
- **Glycerophospholipids** — **glycerol backbone**
  - **PC:** phosphatidylcholine

- **PE**: Phosphatidylethanolamine
  - **PS**: phosphatidylserine
  - **PI**: phosphatidylinositol
- Sterols
  - e.g. cholesterol
  - very small hydrophilic head
  - hydrophobic major components
  - **hydrocarbon ring**
- Proteins:
  - transmembrane
  - integral
  - peripheral
- Glycolipids
  - Oligosaccharide chain exposed to cell / organelle exterior
  - lipid component
- Glycoproteins
  - Oligosaccharide chain
  - polypeptide
- The function of membranes
  - barrier for foreign pathogens
  - signal amplification & transduction
  - control waste exit & nutrient intake
  - sense the environment & stress etc
  - produce energy — mitochondrial membrane

▼ The difference in composition between membranes of different organelles

- Nuclear pore:
  - two layers of membrane & formation of nuclear pore & the FG mesh
  - link to ER
- ER
  - as form of **sac / tubules**
  - active exocytosis & formation of coated vesicles
  - have ribosomes bound
- Plasma membrane
  - Other than the usual components...



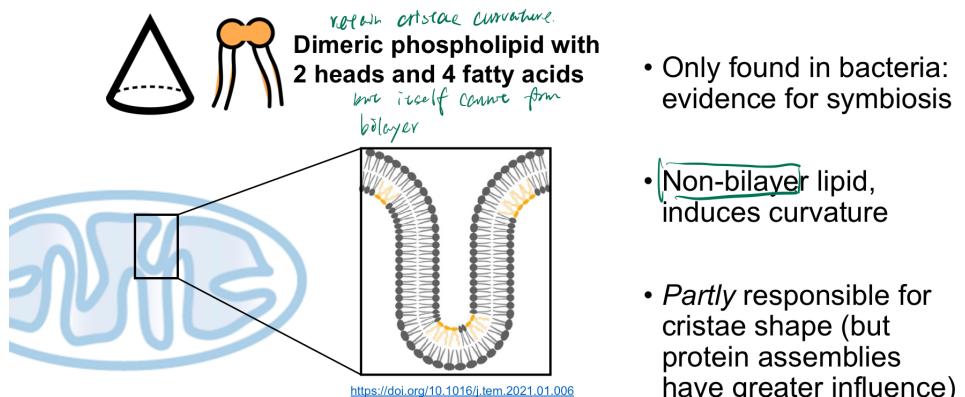


*with mesh*  
*for guidance & shape*

- Extracellular matrix!!
  - collagen fibre
  - polysaccharide chains containing carbohydrates & proteins
  - **fibronectin** — connecting to **integrin** located at the bilayer phospholipids

- Beneath the plasma membrane, underlies acto-myosin network — modulate cell shape & migration
- Mitochondrial membrane
  - inner & outer membrane
  - different permeability
  - electron transport chain at the inner — produce energy
  - **inner membrane forms invagination: cristae**
    - Partly results from **cardiolipin**
    - dimeric phospholipid: two hydrophilic heads and 4 fatty acid tails
    - cannot itself form a bilayer
    - The bulky structure & non-bilayer structure can induce curvature
    - only found in bacteria

## Mitochondria membranes exclusively contain the unusual lipid **cardiolipin**



- Overall composition in different membranes:

## In addition to overall structure, membranes also vary in **lipid composition**

Membrane:	% of total lipid content by weight						mostly found in organelle cells	mostly found in plasma membrane
	Sterol:	Sphingolipid:	Glycerophospholipid:	PE	PS	PC		
<b>Plasma membrane</b> (liver cell)	17	19	7	4	24	7	22	22
<b>Plasma membrane</b> (red blood cell)	23	18	18	7	17	3	14	14
<b>Mitochondria</b> (inner & outer membrane)	3	0	28	2	44	Trace	23	23
<b>Endoplasmic reticulum</b> (ER)	6	5	17	5	40	Trace	27	27
Bacteria ( <i>E. coli</i> )	0	0	70	Trace	0	0	30	30

- Bacteria membrane is mostly composed of **PE**
  - It is the only major type of glycerophospholipids (**no PC PS**)
  - **no sterol / sphingolipid** in bacteria
  - **no glycolipids**
- Glycolipid is often used for cellular signalling → **scarcely found in organelle membrane, mostly found in plasma membrane**
- More than **50% of plasma membrane composed of Sterol + Sphingolipids**
- Organelle membrane can also hardly find sterol & sphingolipid

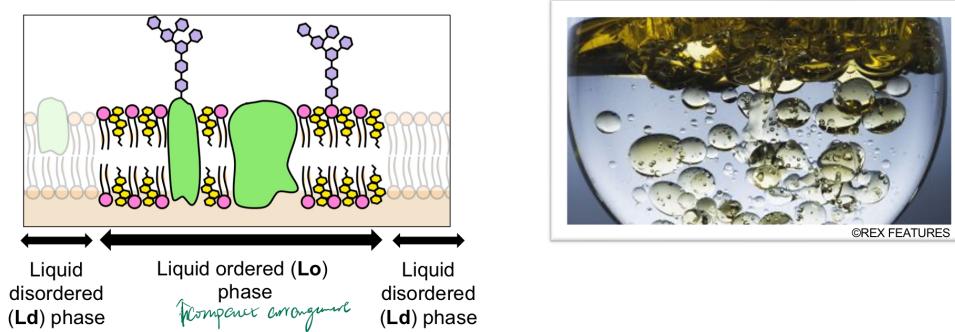
### ▼ The function of sterol & sphingolipid in plasma membrane

- raise **fluidity & permeability**
- distribution — **lipid raft hypothesis** vs random distribution
  - form a subdomain of the plasma membrane
  - because composed of **sterol and sphingolipids + transmembrane proteins** — more **saturated fatty acid tail** — can be **thicker** than the rest of the plasma membrane
  - can float within the membrane & integrate with other LR

▼ LLPS (liquid-liquid phase separation): potential mechanism for LR formation

- The LR integrated into a large cluster & more compact & less flexible
- isolate out the other phospholipid components (just like oil cannot mix with water)
- LR is in **liquid-ordered phase**

## Lipid rafts form through liquid-liquid phase separation (LLPS)



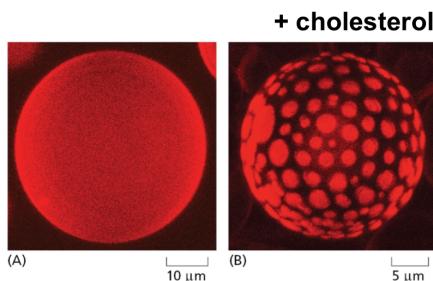
- Potential function of LR

might recruit certain types of transmembrane protein to interact in close proximity — involved in pathway of immunity

▼ The idea of LR is controversial!!

# The evidence for lipid rafts is controversial

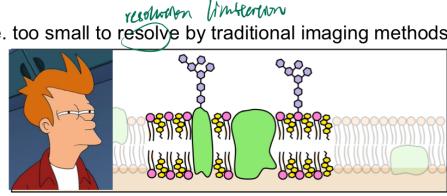
Artificial vesicles (liposomes) composed of PC + Sphingomyelin



Alberts et al. Molecular Biology of the Cell, 7<sup>th</sup> Edition  
Chapter 10 Figure 10-12  
(from from N. Kahya et al., J. Struct. Biol. 147:77-89, 2004)

...But in cells, lipid rafts are suggested to be much smaller

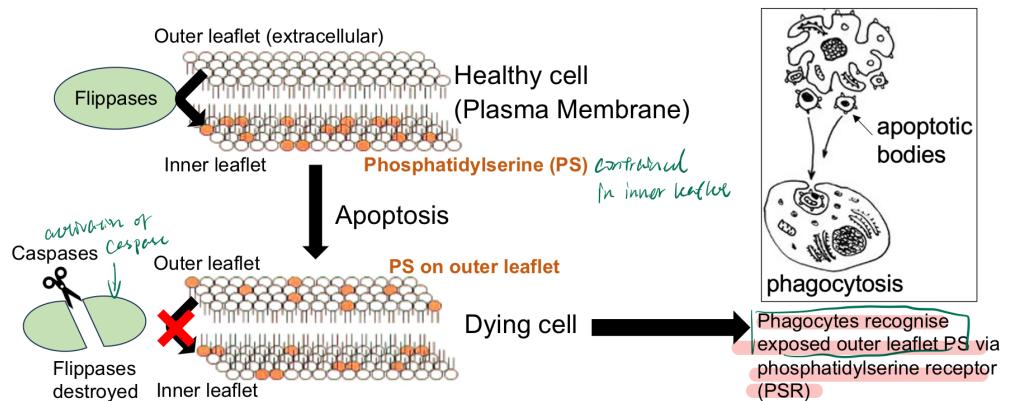
i.e. too small to resolve by traditional imaging methods!



too small to be resolved by microscopy  
... also, experimentally disrupting lipid rafts (by altering cholesterol levels) likely affects other aspects of membrane function.

## ▼ Uneven distribution — PS in outer leaflet

### Asymmetry between leaflets of the same bilayer – apoptosis example



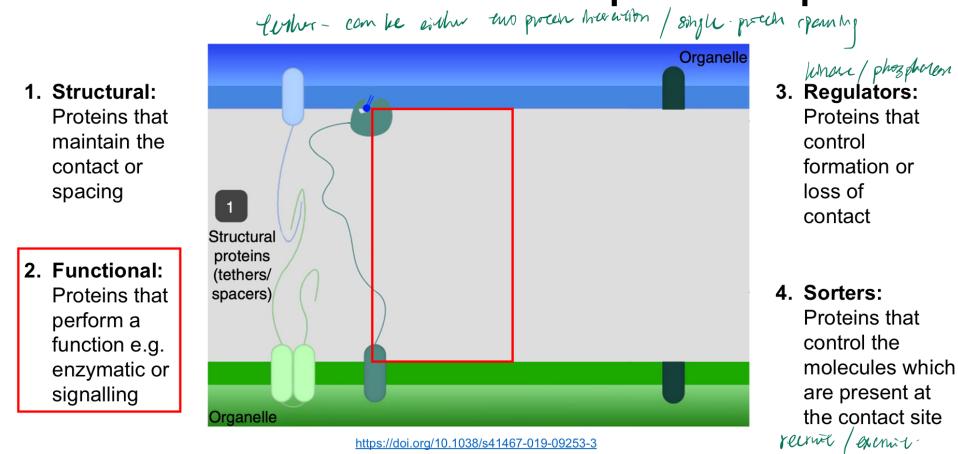
[https://doi.org/10.1002/\(SICI\)1097-0320\(19980101\)31:1<1::AID-CYTO1>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1097-0320(19980101)31:1<1::AID-CYTO1>3.0.CO;2-R)

## Contact site

- Mostly found in ER (with MT)
- Criteria
  - membranes from two different organelles come close **without fusing**

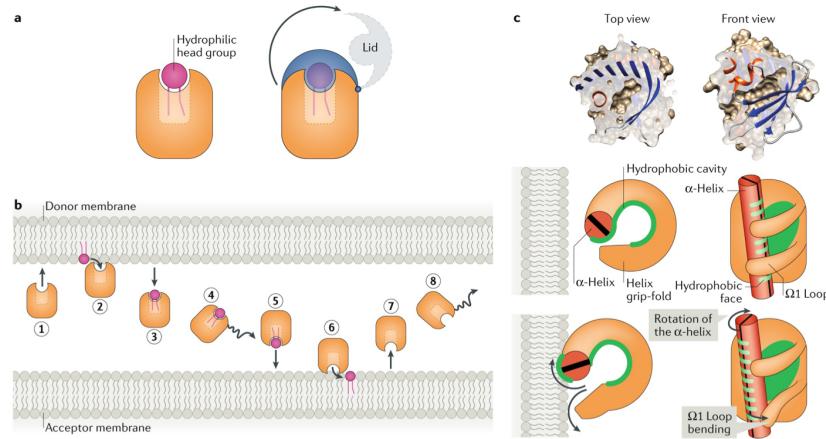
- tethering: direct interaction between molecules (lipids and proteins)
  - Structural
  - Functional: e.g lipid transfer
  - Regulators (GEF, GAP)
  - Sorters (recruit / exclud)

## Membrane contact sites contain specialised proteins



- Unique and distinct composition of protein and lipids
- perform specific functions
- unique protein - phospholipid content
- Function of contact sites — lipid transfer
  - When ER vesicles are blocked — lipid transfer still happens
  - Lipid transfer proteins locating on the contact sites
  - Those proteins have **hydrophobic cavity** which can bind single lipid
  - and a alpha-helix which can **rotate the hydrophobic cavity**

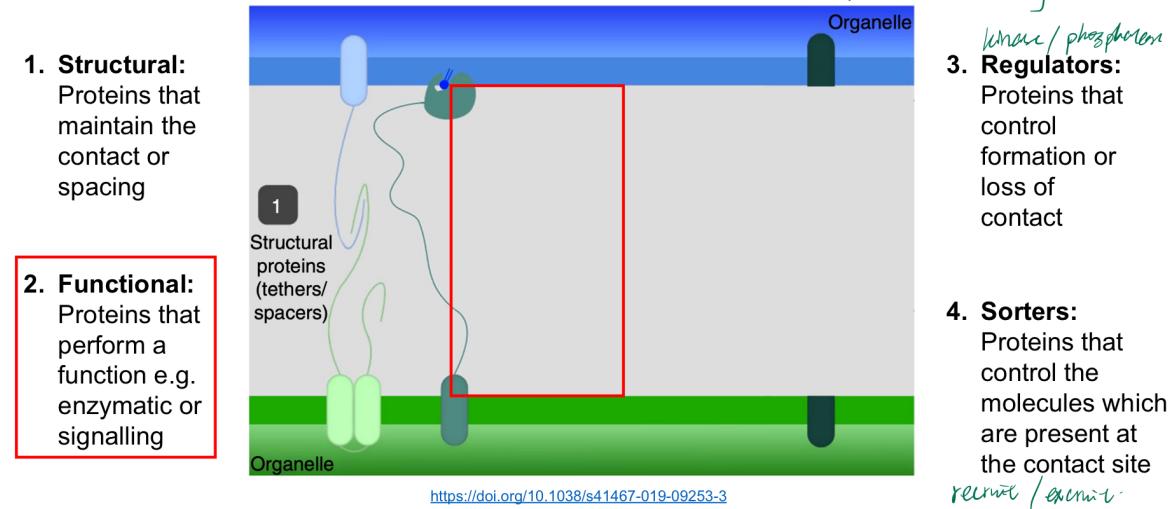
# Reminder: lipid transfer proteins



- Or they form channel-like transmembrane proteins to allow lipid transfer

## Membrane contact sites contain specialised proteins

*tether - can be either two protein interaction / single protein spanning*



- ER-MT contact site protein mutations may link to neurological disorders
  - But these proteins have other functions, so requires further specification
  - VAPB — ALS
  - MFN2: mitofusin2 — Chacot-Marie -Tooth disease Type 2A

## Autophagy

- transfer of cellular materials to lysosomes for degradation & recycling
- ▼ 3 routes of autophagy
1. Macroautophagy
    - a. formation of autophagosome: new **double membrane** organelle, containing large portions of cytoplasm
    - b. then the autophagosome fuses with lysosome
  2. Chaperone-mediated autophagy
    - a. Chaperone proteins bind to the **client protein** in cytoplasm
    - b. translocate it to specialised channel on lysosome (**LAMP-2A**)—**engulfment**
  3. Microautophagy  
**Invagination** of lysosomal membrane

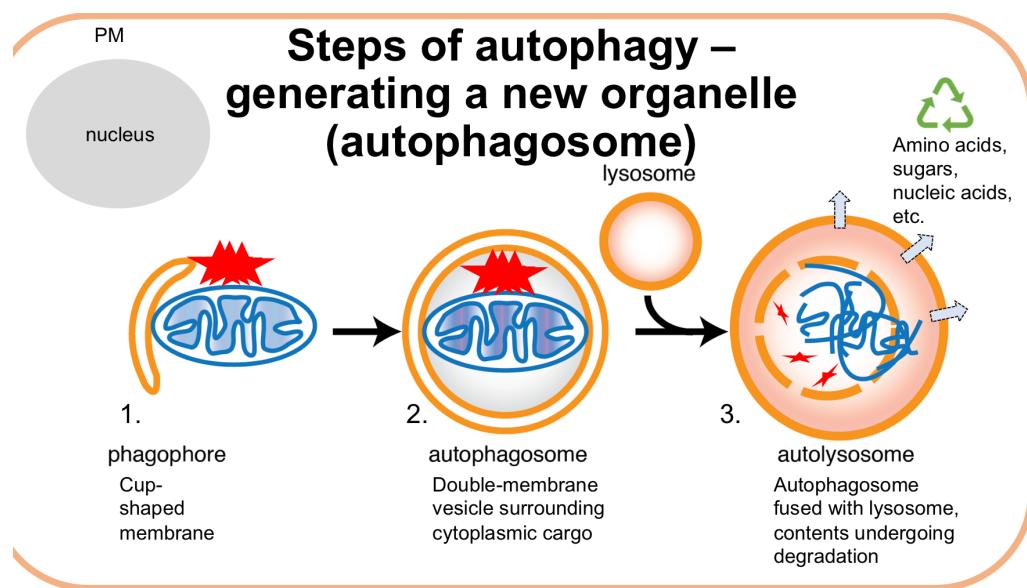
▼ Characteristics of macroautophagy

- efficient: a lot of materials in once
- can be selective or non-selective
- autophagosome formation in cytoplasm

# (Macro)autophagy



- Occurs in the cytoplasm
  - Degrades in **bulk** (a lot of material at once)
  - Material degraded ranges in size and type:
    - Protein/DNA/lipids/sugars
    - Whole organelles (e.g. mitochondria)
    - Whole organisms (bacteria/virus)
  - Can be non-selective or selective (targeted)
  - **Involves formation of new organelle (autophagosome)** *double membrane*
  - **Material degraded is recycled!**
- 
- Steps of macroautophagy



Autophagosome can range from 500nm - 2μm in diameter — can be slightly larger than MT

▼ Stimulation and the corresponding function of autophagy

- **Stress & starvation**
  - autophagy can then mobilise the energy source in cells via recycling nutrients
- **Cell damage**
  - can then remove the damaged organelles & protein aggregates — promote longevity
- **Infection**
  - remove cytosolic pathogens
- Cellular **remodelling** during development
- Cell **differentiation**: haematopoiesis, immune system
- Maintenance: **protect neurons from neurodegeneration & ageing**

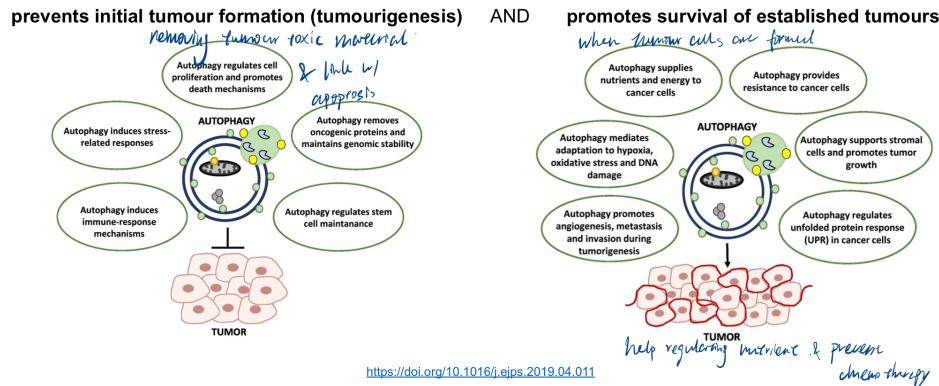
With autophagy deficiency:

Postnatal mice can die from: **failure of development / starvation**

▼ Autophagy role in diseases

- in cancer:
  - autophagy can prevent tumourigenesis by responding to cellular stress and clearance of oncogenic proteins
  - also when tumour is formed: can provide available nutrients to tumours & promotes angiogenesis

- Cancer: complex role, autophagy likely both:

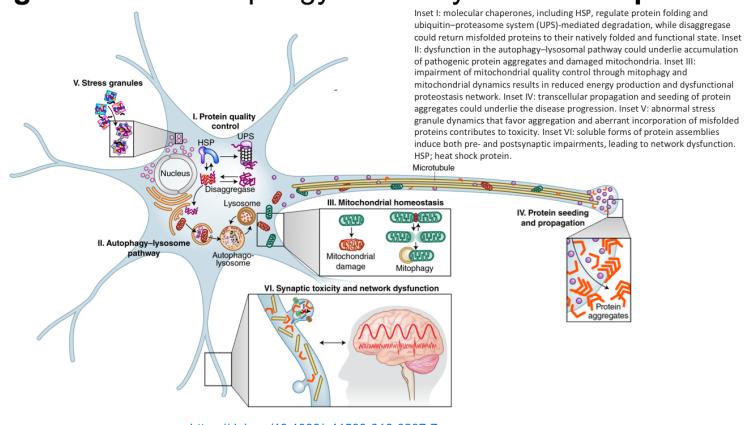


- In neurodegenerative disease: often protective
  - protein aggregates
  - mitophagy of damaged mitochondria
  - protein quality control

## Role of autophagy in diseases

*removing MT, & protein aggregation*

- Neurodegeneration: autophagy is usually considered protective.



## Mechanism of macroautophagy — dynamics of membrane

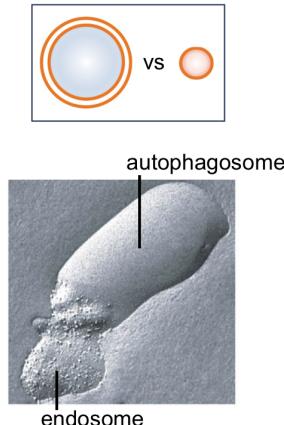
- Autophagosome
  - smooth membrane comparing to endosome!

- lack vesicle coat
- unclear membrane source

## Autophagosomes are a unique type of vesicle

- 1. Double membrane**
- 2. Large** (bigger than transport vesicles, peroxisomes, most lysosomes)
- 3. Smooth appearance:**
  - lack of visible “vesicle coat” e.g. *clathrin*
  - few membrane proteins
- 4. Membrane source is widely debated**

*not mainly derived by pre-existing*  
Therefore, autophagosomes must have a highly **specialised** formation mechanism

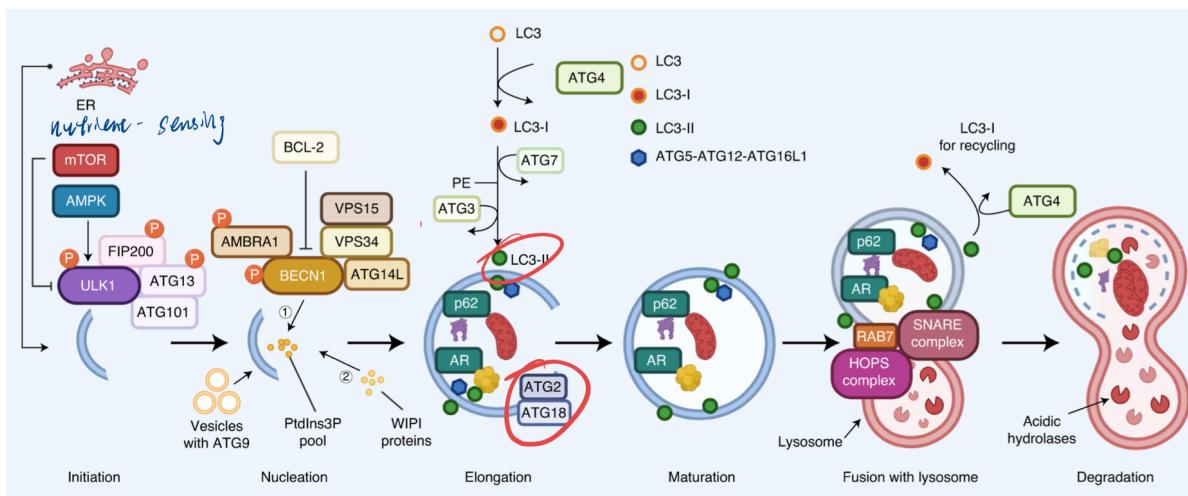


<https://doi.org/10.1038/nrm2245>

- Process

Initiation (ER stress) → Nucleation → Elongation → Maturation → fusion of lysosome → lysosomal acidic degradation

## The autophagy “core machinery” (no need to memorise all...)



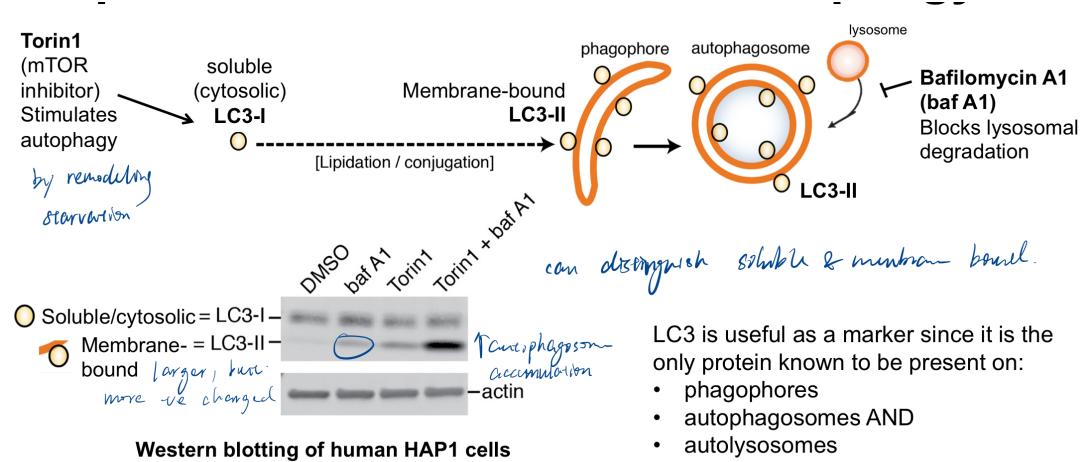
- LC3 & ATG2 at elongation

▼ LC3

- Function
  - membrane & expansion
  - cargo & effector binding
- LC3 is the only protein known to be associated to autophagosomes even after lysosome fusion
- Signalling process

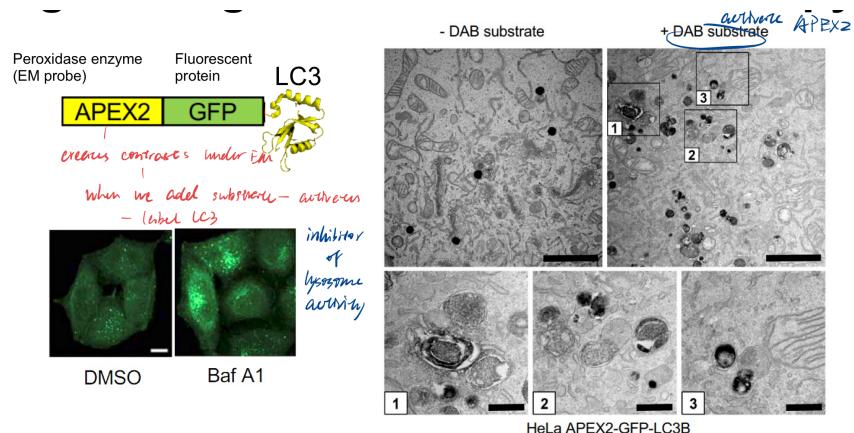
Soluble LC3-I → (lipidation / conjugation PE) → membrane-bound LC3II

- Experiments



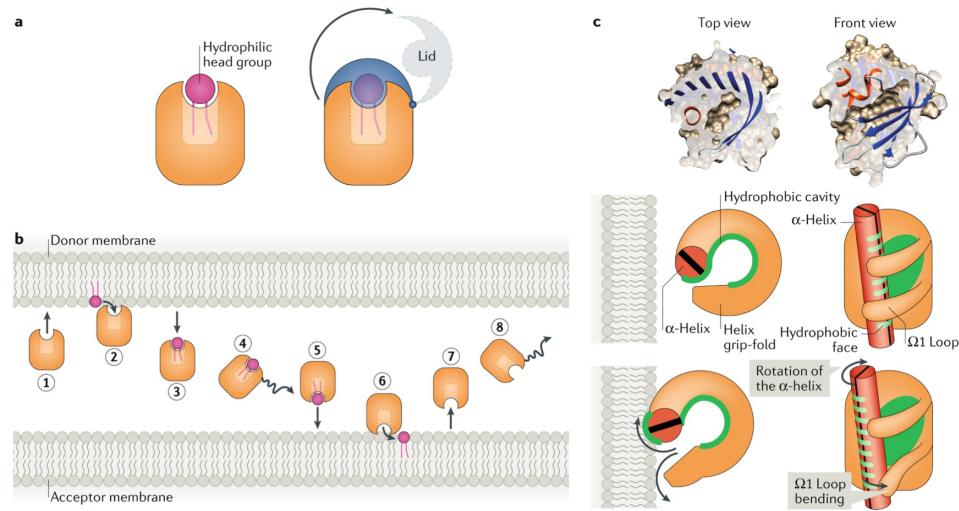
LC3 is useful as a marker since it is the only protein known to be present on:

- phagophores
- autophagosomes AND
- autolysosomes



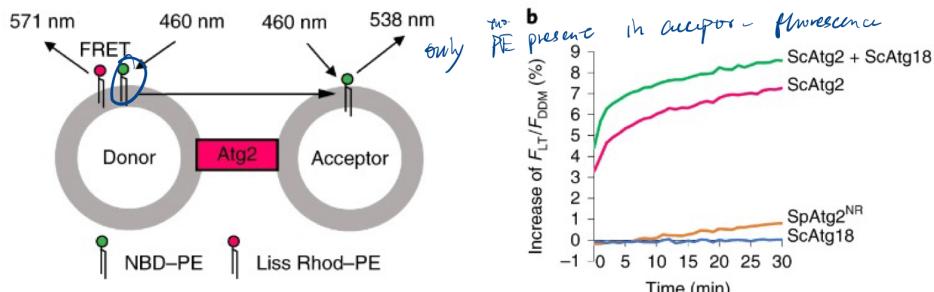
## ▼ ATG2

- transiently localise to phagophores
- present in ER
- lipid transfer protein



- ATG2 can bind to PE
- Experiment
-

## Atg2 can transfer PE phospholipid between membranes



"To confirm whether Atg2 can transfer phospholipids between membranes, we developed an assay to detect the LT activity of Atg2 in vitro by modifying previously reported methods (Fig. 3a<sup>14,15</sup>). This LT assay uses two kinds of liposomes: one containing 8% N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine (NBD-PE) and 2% L- $\alpha$ -phosphatidylethanolamine-N-(lissamine rhodamine B sulfonyl) (Liss Rhod-PE; the donor liposome) and the other without fluorescently labeled lipids (the acceptor liposome). The NBD fluorescence intensity in donor liposomes is decreased by fluorescence resonance energy transfer (FRET) between NBD and Rhod. If Atg2 possesses LT activity, transfer of NBD-PE from donor to acceptor liposomes results in reduced NBD-Rhod FRET efficiency and a concomitant increase in NBD-PE fluorescence intensity (dequenching)."

- FRET: Fluorescence energy transfer
- Transfer of NBD-PE from donor to acceptor liposome would increase the fluorescent activity
- The greatest increase would be the activation of Atg2 and Atg18

## Atg2 helps autophagosomes form by establishing membrane contact and transferring lipids from the ER

