The Rab7-like protein Ypt7p is involved in Saccharomyces cerevisiae lipid droplet dynamics

Isabelle Bouchez, Marie Pouteaux, Michel Canonge, Mélanie Genet, Thierry Chardot, Alain Guillot, Marine Froissard

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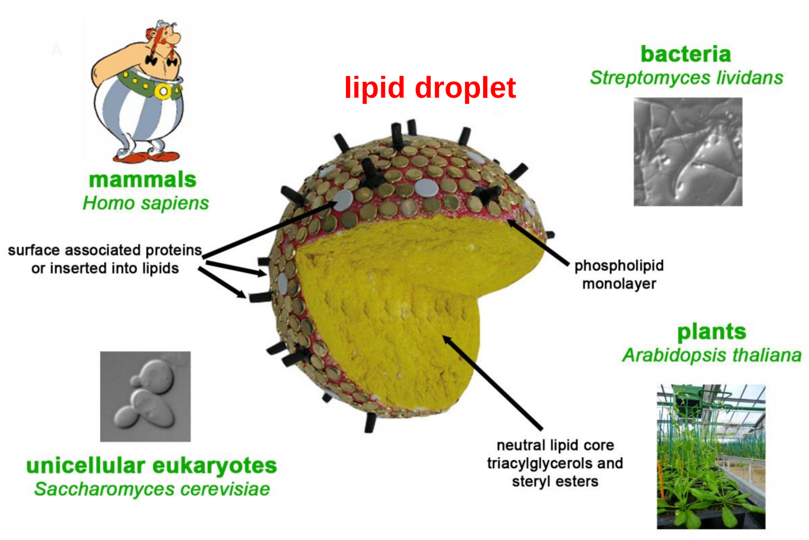
CONTEXT

Lipid droplet: a complex and dynamic organelle

In cells, neutral lipids (triglycerides and steryl esters) are stored in organelles called **lipid droplets (LD)** [1]. They are present in all organisms, from bacteria to plants and animals.



Lipid droplets: not well known but with rising interest

From biologists

- LD is not an inert fat depot but a **dynamic organelle** which regulates cell metabolism and signaling

From medical field

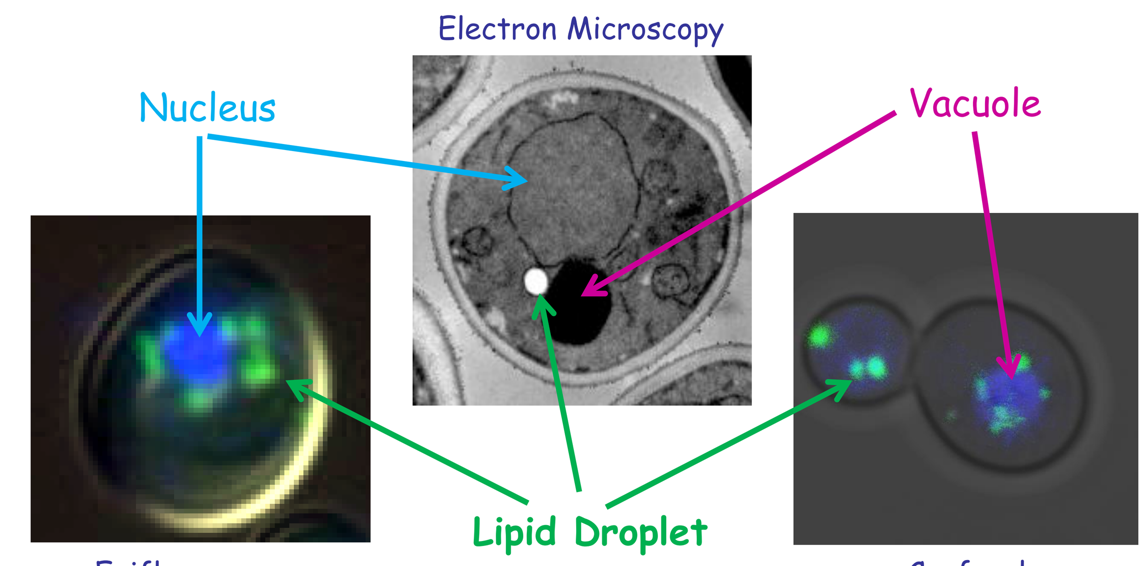
- LDs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes) [2]
- **Oleosins** (from peanut and hazelnut), seed LD associated proteins are **allergens** [3].

From industrials

- **crushing** : oils for food and non food (biofuel and green chemistry) productions are extracted from seed LDs
- **food processing industry, cosmetic and health** : oleosins harbor interfacial properties and could be use as **emulsifying agents or in drug delivery systems** [4]

Lipid droplets interact with many organelles

LD interact with many organelles, including endoplasmic reticulum, mitochondria, peroxysome, **vacuole** …

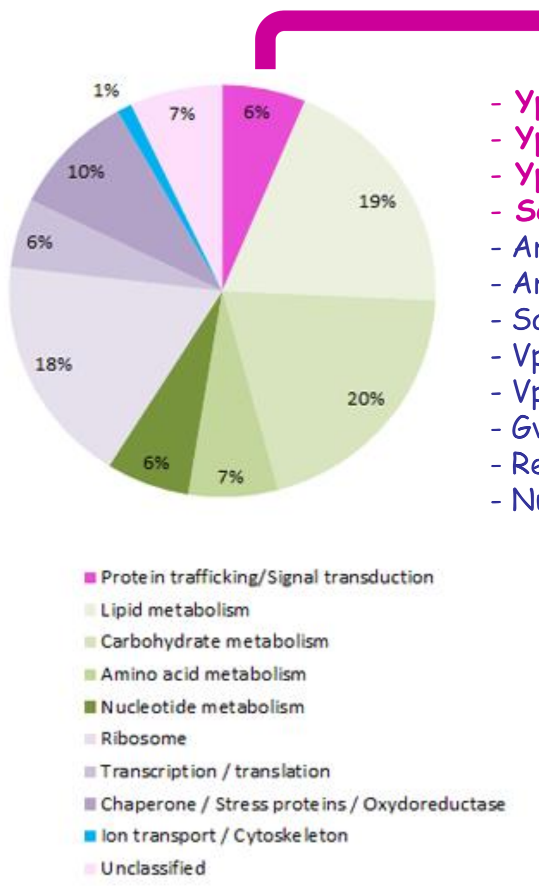


Epifluorescence Hoechst + Bodipy 493/503

Confocal CMAC + Bodipy 493/503

RESULTS

Lipid droplet proteome

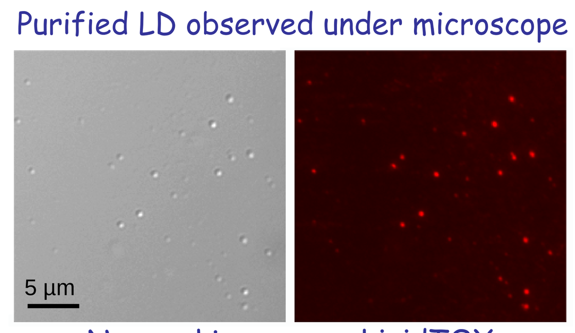


- Ypt7p (Rab7)
- Ypt1p (Rab1)
- Ypt31p (Rab11)
- Sec4p (Rab 8)

Small GTPase Rab family

- Arf1p
- Arf2p
- Sar1p
- Vps1p
- Vps66p
- Gvp36p
- Rer2p
- Nus1p

Purified LD observed under microscope



Nomarski LipidTOX

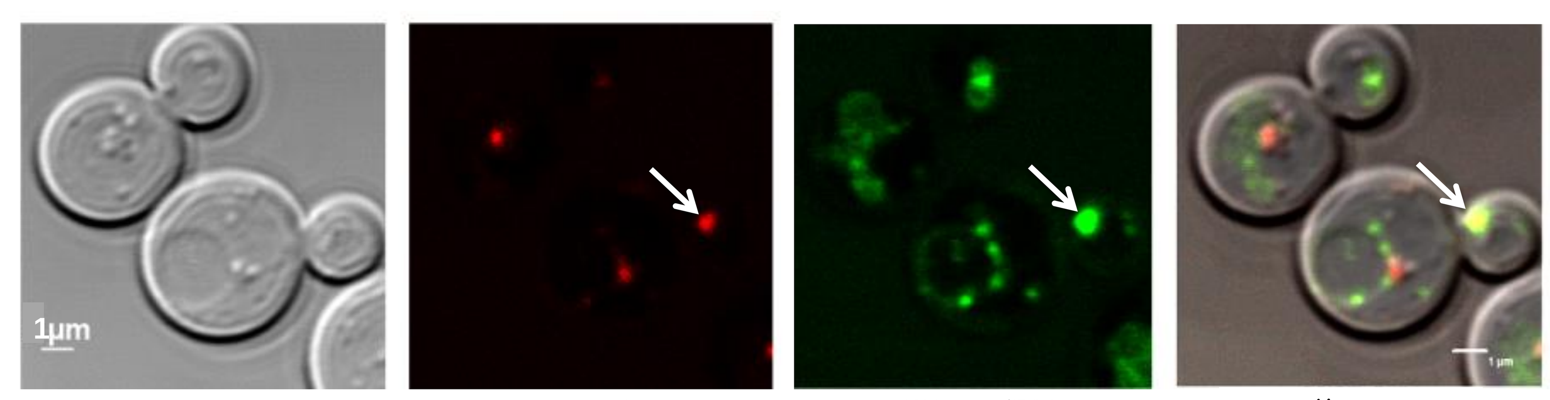
LD show a complex and dynamic proteome.

- **151 proteins** associated to S. cerevisiae purified LD.
- lipid metabolism proteins but also **trafficking protein**

Identified Rab homologue on LD	traffic	Yeast	Mammals [5]	Drosophila [6]	Arabidopsis
Ypt1p/Rab1	ER and Golgi	×	×	×	×
Ypt7p/Rab7	Endosome and vacuole	×	×	×	×
Ypt31p/Rab11	Intra-Golgi transport	×	×	×	
Sec4p/Rab8	Golgi to plasma membrane	×	×	×	×

Colocalization of Ypt7p with lipid droplet protein

Confocal microscopy pictures (bright field and epifluorescence) of yeasts expressing Erg6p-RFP (lipid body Delta(24)-sterol C-methyltransferase) and GFP-Ypt7p [7].



Nomarski RFP 556/583 GFP 488/509 Merge

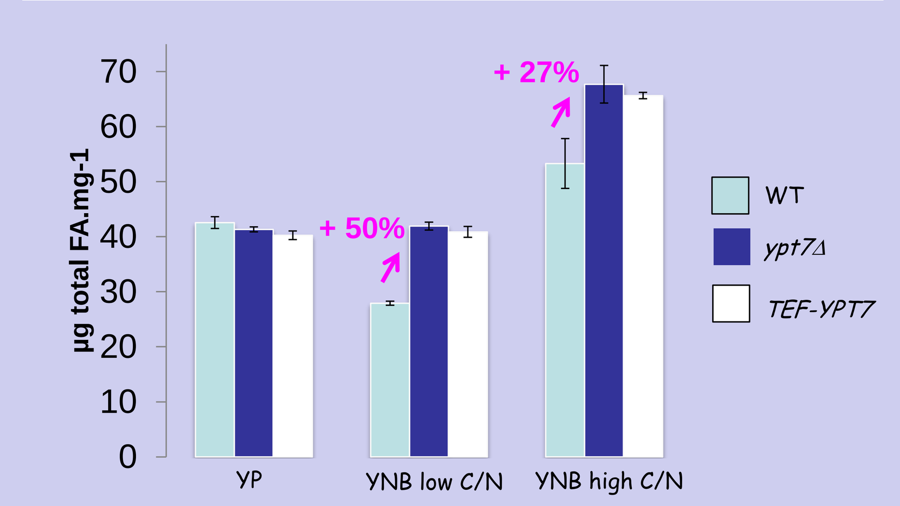
GFP-Ypt7p colocalized with Erg6p-RFP revealing that Ypt7p are associated with LD.
Close contacts between LD, endosomes and vacuole are observed

Ypt7p is involved in LD dynamics

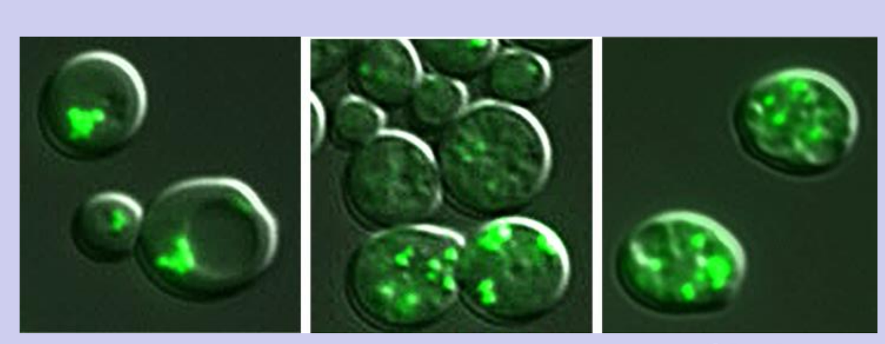
Genetic studies

- **Using mutant strains**
  - ✓ *ypt7Δ*
  - ✓ *TEF-YPT7*
- **In various media**
  - ✓ YP (complete)
  - ✓ YNB low C/N (synthetic)
  - ✓ YNB high C/N (synthetic)

Gas chromatography revealed that total fatty acid content is increased in *ypt7* mutants



Fluorescence quantification of cell Bodipy staining revealed that neutral lipid content is increased in *ypt7* mutants

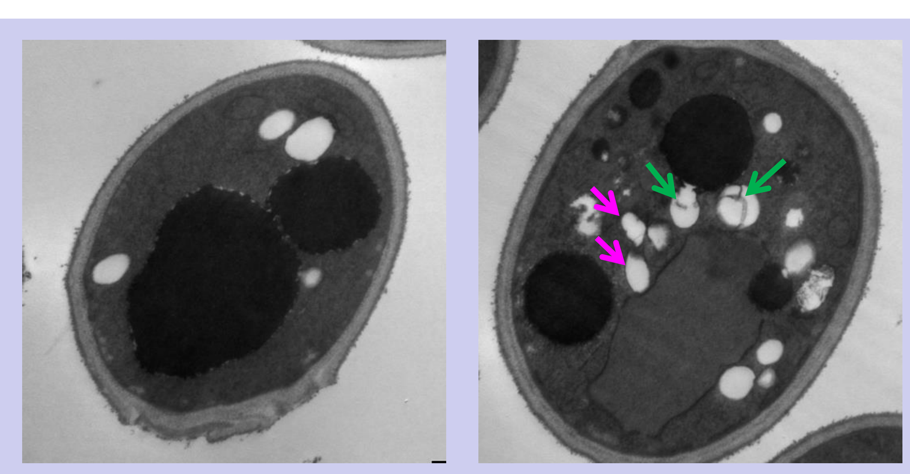


WT *ypt7Δ* *TEF-YPT7*

STRAIN	BODIPY FLUORESCENCE	% WT
WT	187 ± 3.5	
ypt7Δ	371 ± 36.7	+ 98 %
TEF-YPT7	345 ± 16.4	+ 84 %

Transmission Electron Microscopy (TEM) revealed LD morphology defects in *ypt7* mutants

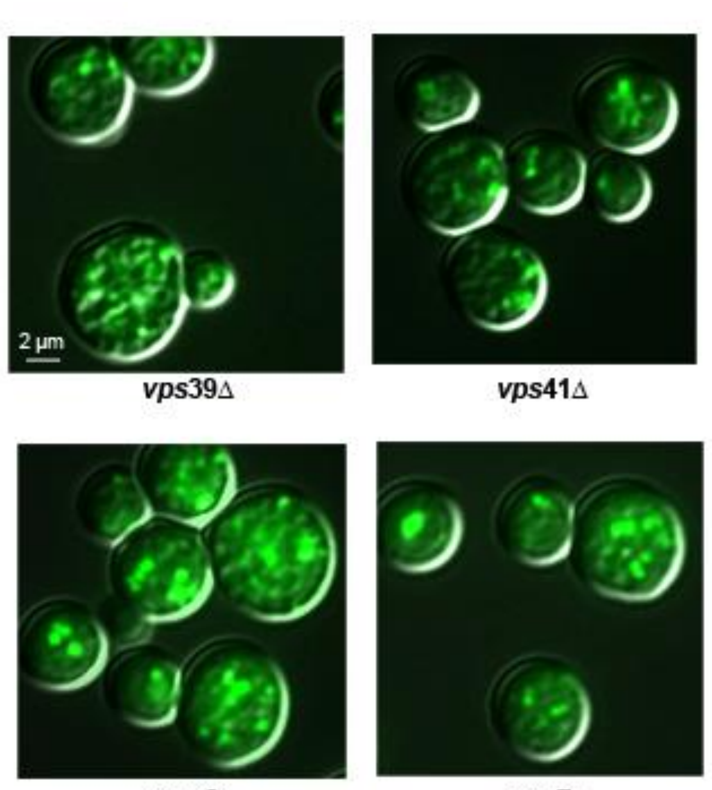
- LD accumulation
- LD morphology altered
- LD fusion process altered



WT *TEF-YPT7*

Defect in HOPS complex induces LD accumulation

Fluorescence quantification of cell Bodipy staining and gas chromatography analysis revealed that lipid content is increased in HOPS tethering complex mutants



vps39Δ vps41Δ vam3Δ vam7Δ

STRAIN	COMPLEX	% FA / WT	% BODIPY / WT
vam3Δ	vSNARE	+ 30 %	+ 24 %
vam7Δ	vSNARE	+ 19 %	+ 17 %
vps39Δ	HOPS	+ 23 %	+ 23 %
vps41Δ	HOPS	+ 24 %	+24 %
apl5Δ	ALP/AP-3	+ 4 %	
apm3Δ	ALP/AP-3	- 6 %	

Conclusions and perspectives

- Proteomics revealed that trafficking proteins are associated with LD.
- LDs partly co-localize with endosomes and vacuole and **Ypt7p is present in LD**.
- in ***ypt7* mutants** we observed an **increase in lipid content** and **alteration of LD morphology** revealing a **role for Ypt7p in LD dynamics**.
- Mutants of the homotypic fusion and vacuole protein sorting (HOPS) complex show similar phenotypes.

Role of other trafficking proteins in LD dynamics? Role of the vacuole in LD dynamics?

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