Involvement of the Rab GTPase Ypt7p, the HOPS complex and the V1-ATPase in the dynamics of Saccharomyces cerevisiae lipid droplets

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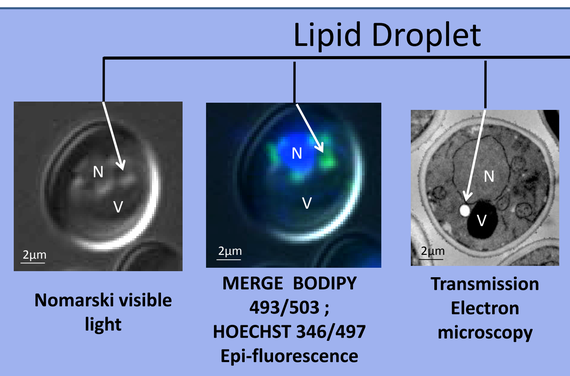
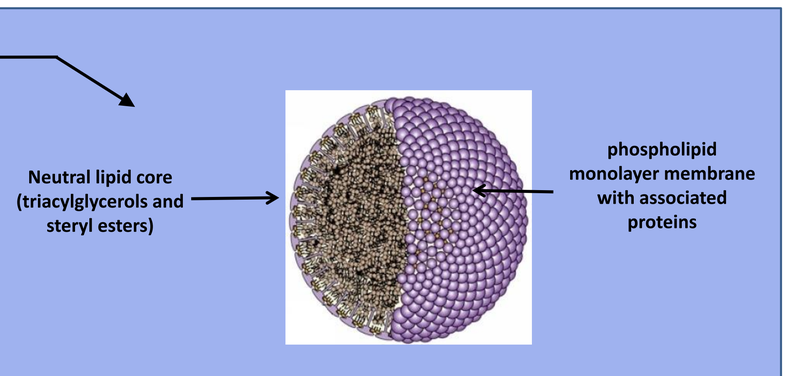
Institut Jean-Pierre Bourgin, UMR 1318 INRA-AgroParisTech, route de St Cyr (RD 10), 78026, Versailles cedex, France. Email : isabelle.bouchez@versailles.inra.fr

BACKGROUND

In yeast, storage lipids are maintained in the cytoplasm in specialized organelles called lipid droplets (LDs) (Murphy *et al.,* 2012). These structures consist mainly of a core of neutral lipids (triacylglycerols and/or steryl esters) enclosed in a monolayer of phospholipids. They contain a number of associated proteins, involved in many metabolisms, signaling and trafficking. Indeed, LD is now known to be a complex dynamic organelle. Many studies have reported interactions between LDs and intracellular organelles, like endoplasmic reticulum, early endosome, peroxisome and mitochondria (Zehmer *et al*., 2009). We have previously shown, in *S. cerevisiae*, the relationship between vacuole and LDs, and described first evidences of the involvement of the Rab GTpase Ypt7p in the dynamics of LDs.



Microscopic observations of LDs from *S. cerevisiae* (BY4741) ; V : vacuole ; N : Nucleus



Schematic organization of LD from yeast (Buchanan *et al*., 2000)

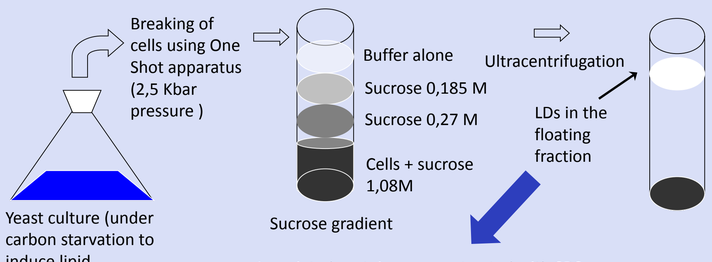
OBJECTIVES

The aim of this study was to investigate the involvement of Ypt7p in LD dynamics by identifying its potential partners on LDs and by exploring the trafficking pathways which could be involved in these dynamics.

RESULTS

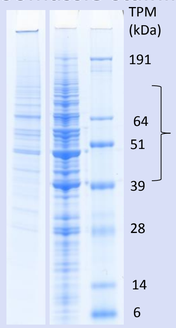
Proteins associated with LDs

Cellular fractionation



Control of LD purity

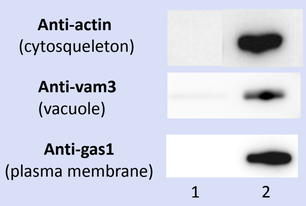
Coomassie-staining



1 : Purified LDs
2 : Total yeast proteins

The increase of proteins with a molecular weight higher than 39 Kda showed LDs were highly purified.

Immunoblotting

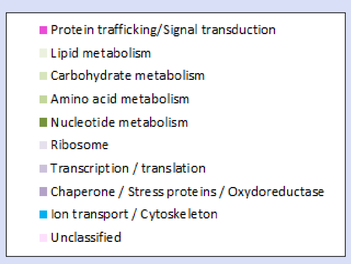


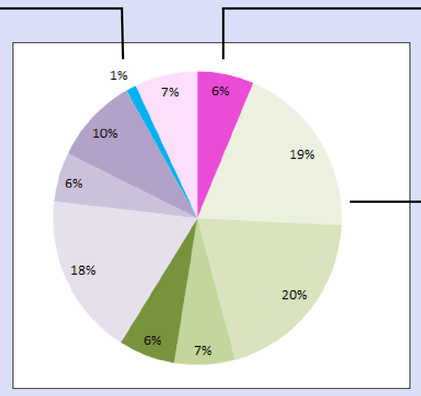
Antibodies did not react with LD proteins. It showed that no or few intrinsec proteins from other organelles than LD were present in the purified fraction.

Proteomic of purified LDs from WT *S. cerevisiae*

The 151 LD-associated proteins were classified according to their functionality (SGB database : http://www.yeastgenome.org). Many metabolism enzymes, especially lipid metabolism, were found showing important biological processes were carried at its surface. Several membrane-trafficking proteins were also identified showing that these proteins were functionally associated with LDs.

Subunits of the V1 peripheral membrane domain of the vacuolar H+-ATPase (Vma2p ; Vma5p ; Vma1p) ; Por1p





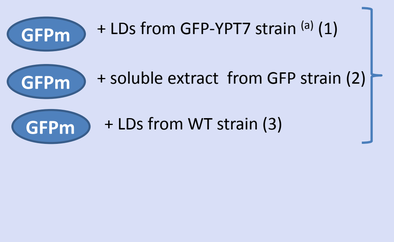
Small GTPase Rab Family (**Ypt7p/Rab7** ; Ypt1p/Rab1 ; Ypt31p/Rab11 ; Sec4p/Rab8) ; **GTPase family** (Arf1p ; Arf2p ; Vps1p, Sar1p) ; Nus1p, Gvp36p (vacuole biogenesis) ; Rer2p (ER protein sorting)

Transferase (erg6p ; Slc1p ; Loa1p ; Pdr16p ; Eht1p) ; **Lipase** (Tgl1p ; Tgl3p ; Tgl4p ; Tgl5p ; Pgc1p ; Yju3p) ; **Synthetase** (Faa4p ; Faa1p ; Faa3p ; Fat1p ; Fas1p ; Fas2p ; Erg7p ; Erg20p) ; **Esterase** (Tgl1p) ; **Reductase** (Ayr1p ; Tsc10p ; Erg27p) ; **Epoxidase** (Erg1p) ; **Hydrolase** (Yeh1p) ; **Deshydrogenase** (Hfd1p) ; **Deacetylase** (Say1p)

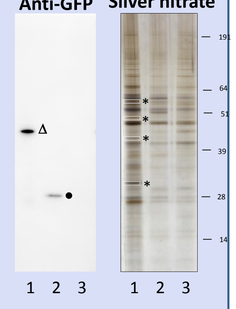
Relative abundance (%) of LD proteins from each class (based on PAI calculation for protein identified at least by 2 mass-spectra in LC-MS/MS analysis). Yeasts were at the exponential state.

Potential partners of Ypt7p

Co-immunoprecipitation



SDS-PAGE



Selected list of potential Ypt7p partners identified by LC/MS-MS

Potential partners	Function	Main localization
Lsp1p	BAR-domain protein ; Lipid binding ; Endocytose	Eisosome
Pet10p	Unknown	LD
Vma13p	Subunit H of the V1 peripheral membrane domain of the vacuolar H+-ATPase	Vacuole, mb
Kes1p	Ergosterol biosynthesis	Golgi, cytoplasm
Gdi1p	**GDP dissociating inhibitor ; known partner of Ypt7p**	**Secretory pathway**

The identification of **Gdi1p, a known partner of Ypt7p**, validated the experiment.

Functional validation with mutants

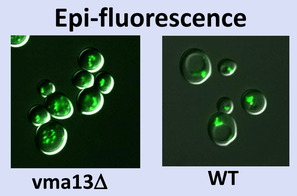
Fatty-acid (FA) analysis of mutant strains of selected Ypt7p partners

Strain	% of variation of total FA / WT (GC)
kes1Δ	+ 6 %
lsp1Δ	- 6 %
pet10Δ	- 8 %
vma13Δ	**+ 25 %**

Vma13p, good candidate?

Staining of neutral lipids with Bodipy 493/503

Epi-fluorescence



Strain	WT	*vma13Δ*
Fluorescent signal (485/518) recorded using Fluoroskan	187 +/- 3.5	310 +/- 30.2 (**+ 65 %**)
Average number of LDs per cell (EPI-fluorescence observation)	3.71 +/- 1.69	6.36 +/- 2.57 (**+ 71 %**)*

* : pvalue < 0,05 calculated on 173 cells using student test

Vma13p : Potential partner of Ypt7p, involves in LD dynamics

Is HOPS complex involved in LD dynamics ?

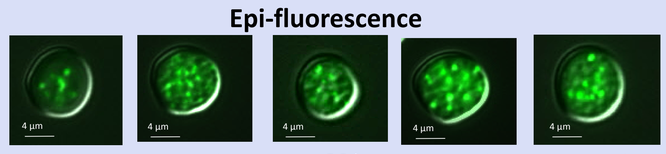
Ypt7p is the Rab effector of the multisubunit tethering HOPS complex, which involves specifically Vps41p, Vps39p and the interacting SNAREs Vam3p and Vam7p. It acts in late endosome/vacuole or vacuole/vacuole membrane fusion.

FA analysis of mutant strains of HOPS pathway

Strain	% of variation of total FA / WT (GC)
vps41Δ	+ 10 %
vps39Δ	+ 27 %
vam3Δ	+ 30 %
vam7Δ	+ 19 %

Staining of neutral lipids with Bodipy 493/503

Epi-fluorescence



Strain	WT	*vps41Δ*	*vps39Δ*	*vam3Δ*	*vam7Δ*
Fluorescent signal (485/518)	655 +/- 0.9	820 +/- 87 (**+ 24 %**)	809 +/- 57 (**+ 23 %**)	812 +/- 91 (**+ 24 %**)	764 +/- 0.7 (**+ 17 %**)

HOPS complex involves in LD dynamics

CONCLUSION and ACKNOWLEDGMENT

The proteome of purified LDs from WT *S. cerevisiae* showed several membrane-trafficking proteins, whom Ypt7p, and many subunits of the V1 peripheral membrane domain of the vacuolar H+ ATPase. One other V1 subunit of this pump, Vma13p, was identified by Co-IP as a potential partner of Ypt7p. Its deletion had a great effect on LD dynamics. As its deletion is also known to abolish the homeostasis and the acidification of the vacuole, the effect on LDs could be a consequence of these dysfunctionments. Anyway, this showed a tight link between LD dynamics and vacuole. Thus, we hypothesized that accumulation of LDs in the cytosol of *VMA13* mutants could be due to modifications of interactions between LDs and vacuole, involving Ypt7p and Vma13p, in the context of defects in vacuole-linked homeostasis. This remains to be confirmed by other interacting tools, like yeast double-hybrid.

We also showed that deletion of subunits of the HOPS complex lead to accumulation of LDs in the cells. Thus, this showed this tethering complex, known to be involved in fusion processes, is involved in LD dynamics.

Altogether, these results lead us to hypothesize that LDs could interact with vacuole, via a hemi-fusion mechanism involving Ypt7p, as Rab effector, the HOPS complex and the V-ATPase.

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(a) : GFP-MYC-YPT7 strain was provided by C. Ungermann, Dept of Biology/Chemistry, University of Osnabrück, Osnabrück, Germany. Murphy *et al*., (2012), Protoplasma, **249** : 541. ; Zehmer *et al*., (2009), proteomics, **9**: 914. ; Buchanan *et al.,* (2000), Biochem. Mol. Biol. Plants.