Link between neutral lipid and storage carbohydrate fluxes in S. cerevisiae revealed by Single Cell Synchrotron Fourier Tranform-Infrared Microspectroscopy

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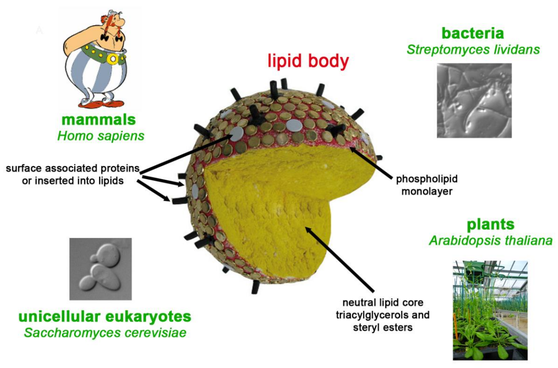
CONTEXT

Lipid body: a complex and dynamic organelle

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



Lipid bodies: not well known but with rising interest

From biologists

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

From medical field

➠ LBs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes) [2]

➠ **Oleosins** (from peanut and hazelnut), seed LB associated proteins are **allergens** [3].

From industrials

➠ **crushing** : oils for food and non food (biofuel and green chemistry) productions are extratcted from seed LBs

➠ **food processing industry, cosmetic and health** : oleosins harbor interfacial properties and could be use as **emulsifying agents or in drug delivery systems** [4]

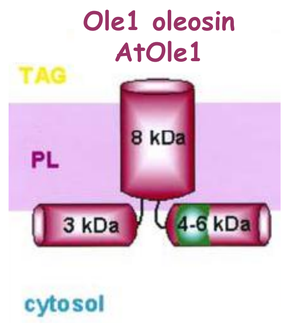
Oleosins, seed lipid body associated proteins

Oleosins are LB integral proteins

Predicted structure = tri-block organization :

➠ variable N-terminal and C-terminal part, exposed at the surface and in contact with the cytosol

➠ highly hydrophobic central part inserted into the phospholipid monolayer and/or the TAG core.



Questions and objectives

➠ Role on lipid filling

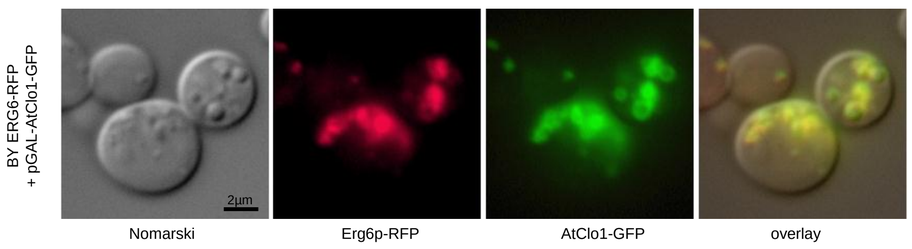
➠ Role on LB structure and stabilization

➠ Structural data on oleosins inserted into LB (natural environment)

RESULTS

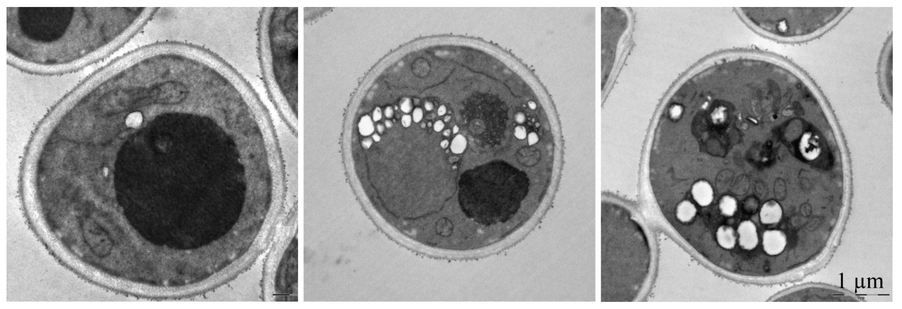
Oleosins are targeted to lipid bodies in *S. cerevisiae*

Photonic microscopy pictures (bright field and epifluorescence) of yeast expressing Erg6p-RFP (lipid body Delta(24)-sterol C-methyltransferase) and AtClo1-GFP [5].



Oleosins induce lipid body accumulation in yeast

Thin sections of yeasts expressing AtOle1-GFP or AtClo1-GFP (transmitted electron microscopy)

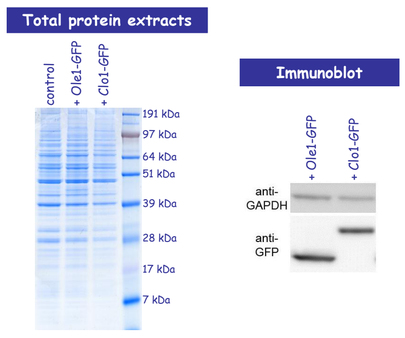


Lipid bodies, round and white structures, are more abundant in cells expressing oleosins when compared to the control cells.

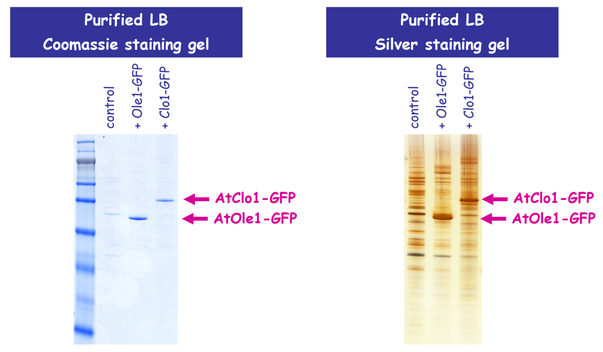
BIOCHEMICAL STUDIES

Oleosins become the major proteins of lipid bodies in yeast

Plant protein expression level was estimated using immunoblot experiments

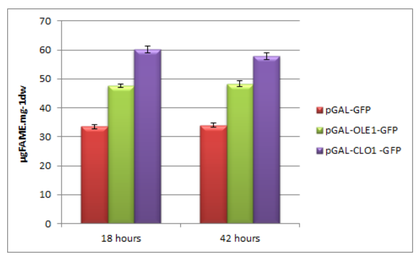


Association of plant proteins with lipid bodies was evaluated using cell fractionation experiments



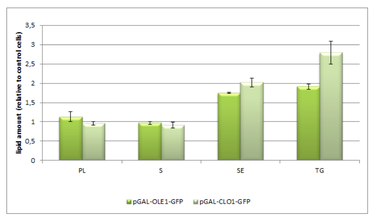
Oleosins induce neutral lipid accumulation in yeast

Total fatty acid content was determined using gas chromatography



AtClo1-GFP expressing cells accumulate more neutral lipids than AtOle1-GFP ones

Lipid classes were analyzed using thin layer chromatography after Folch extraction



Lipid accumulation is correlated with a decrease in glycogen

Trehalose **Glycogen**

trehalase at 37° C → **Free glucose**

α-amyloglucosidase at 57° C → **Free glucose**

high-performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD)

Conclusions and perspectives

➠ Using ZnSe hemisphere and synchrotron radiation, we obtained **yeast single cell FTIR spectra**.

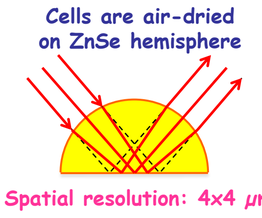
➠ We observed that lipid accumulation induces global metabolic modifications. These results were confirmed by biochemical analysis and revealed a **link between storage lipid and storage carbohydrate fluxes**

➠ We will determine if these metabolic modifications are the consequence of **pathway regulations at the transcription level** by conducting a transcriptomic analysis of yeast cells

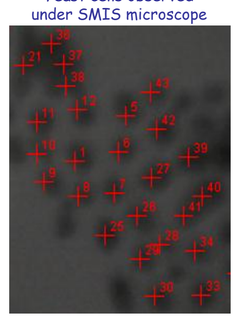
SINGLE CELL FTIR STUDIES

Neutral lipid increase is also revealed by single cell FTIR analysis

Cells are air-dried on ZnSe hemisphere



Yeast cells observed under SMIS microscope



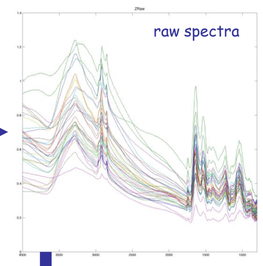
Spatial resolution: 4x4 µm

= Single cell spectra acquisition on cells expressing

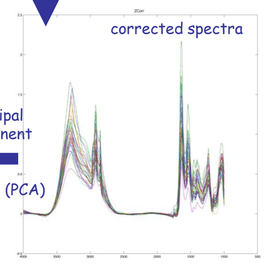
➠ GFP

➠ AtOle1-GFP

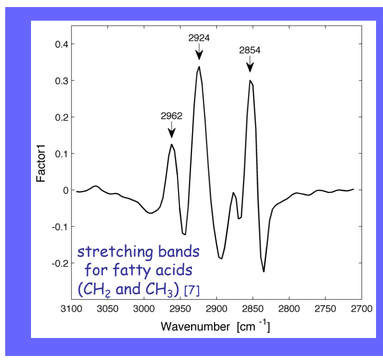
➠ AtClo1-GFP

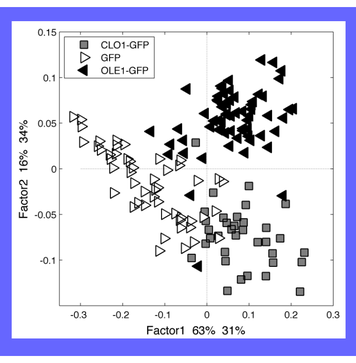


Matlab pretreatment RMieS-EMSC correction [6]

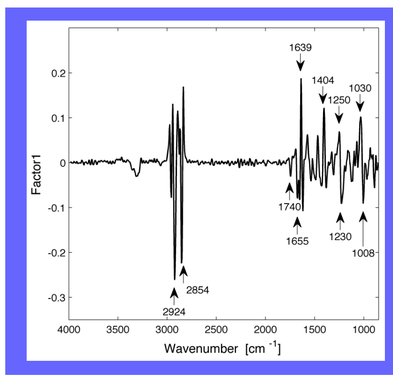


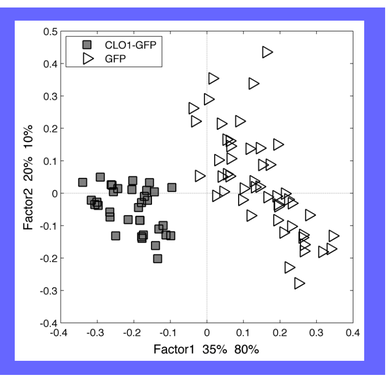
Principal Component Analysis (PCA)





Neutral lipid increase is correlated with global metabolic modifications





1030 cm^{-1}: glycogen
1230 cm^{-1}: amide III
1250 cm^{-1}: amide III
1404 cm^{-1}: CH_3
1639 cm^{-1}: amide I
1655 cm^{-1}: amide I
1740 cm^{-1}: ester (lipides)
2854 cm^{-1}:CH_2 lipides
2924 cm^{-1}:CH_2 lipides

Metabolic modifications were detected using FTIR [7] and confirmed using biochemical analysis

BIBLIOGRAPHIE [1] Brasaemle (2012). J. Biol. Chem. 287, 2273. [2] Bostrom et al. (2007). Nat. Cell Biol. 9, 1286. [3] Pons et al. (2002). Allergy. 57, 88. [4] Capuano et al. (2007). Biotechnol. Adv. 25, 203. [5] Froissard et al. (2009). FEMS Yeast Res. 9, 428. [6] Bassan et al. (2010). The Analyst. 135, 268. [7] Movasaghi et al. (2008). Aplied Spectroscopy Reviews. 43,, 134.