Lack of evidence for condensin or cohesin sequestration on lipid droplets with packing defects

Anaïs Mura\textsuperscript{1} and María Moriel-Carretero\textsuperscript{1§}
\textsuperscript{1}Centre de Recherche en Biologie cellulaire de Montpellier (CRBM), Université de Montpellier, Centre National de la Recherche Scientifique, 34293 Montpellier CEDEX 05, France
\textsuperscript{§}To whom correspondence should be addressed: maria.moriel@crbm.cnrs.fr

Abstract
Lipid droplets (LD) are organelles born from the endoplasmic reticulum that store fats and sterols in an apolar manner both as an energy reservoir and for protective purposes. The LD is delimited by a phospholipid monolayer covered by a rich proteome that dynamically evolves depending on the nutritional, genetic, pharmacological and environmental cues. Some of these contexts lead to discontinuities in the phospholipid monolayer, termed “packing defects”, that expose LD hydrophobic contents to the surrounding water environment. This triggers the unscheduled binding of proteins with affinity for hydrophobic surfaces, a thermodynamically favorable reaction. We have raised in the past the concern that this titration includes proteins with important roles in the nucleus, which entails a risk of genome instability. Analysis of previously published LD proteomes isolated from cells lacking the transcription factor Ino2p, a prototype of LD bearing packing defects, made us concentrate on two subunits of the cohesin (Smc1p and Smc3p) and one of the condensin (Smc2p) complexes, both essential to promote genome integrity by structuring chromosomes. We report that, in disagreement with the proteomic data, we find no evidence of titration of condensin or cohesin subunits onto LD in \textit{ino2Δ} cells. Importantly, during our analysis to label LD, we discovered that the addition of the widely used vital dye AUTODOT\textsuperscript{TM}, which emits in the blue range of the spectrum, leads, specifically in \textit{ino2Δ}, to the artefactual emission of signals in the green channel. We therefore take the opportunity to warn the community of this undesirable aspect when using this dye.
A

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</table>

C

![Image of images showing time since AUTODOT™ addition (min): 1, 3, 7, 10 for ino2Δ and fld1Δ for Smc1, Smc2, and Smc3-GFP](image)

D

![Image of images showing time since AUTODOT™ addition (min): 1, 5, 10 for WT and fld1Δ for DIC, LD, and GFP](image)
Figure 1. Assessment of the co-localization between cohesin or condensin complexes subunits and lipid droplets: (A) Table recapitulating the presence or absence, expressed as spectral counts, of Smc1p, Smc2p, Smc3p or Smc4p in the proteome of lipid droplets (LD) isolated from the indicated wild type (WT) and mutant strains. Data were extracted from (Fei et al. 2011b). (B) WT, ino2Δ and fld1Δ strains were modified to express a C-terminally GFP-tagged version of either Smc1p, Smc2p or Smc3p, grown in complete minimal medium until the exponential phase and imaged to observe GFP signals. To evaluate whether Smc-GFP signals co-localized with LD, the specific dye AUTODOT™, which emits in the blue channel, was added immediately prior to imaging. The shown pictures are representative of the patterns found in all acquired images out of three independent experiments. (C) ino2Δ cells expressing Smc1p-GFP were imaged at the indicated times since AUTODOT™ addition, and both Smc1p-GFP and LD signals monitored. Please note the progressive conversion of Smc1p-GFP-associated signals from nuclear to LD-like. (D) WT (left panel) and fld1Δ (right panel) strains bearing no GFP tagging were imaged at the indicated times since AUTODOT™ addition, and both GFP and blue (LD) channel signals monitored. For all acquisitions, exposure times were 50 ms for AUTODOT™ and 800 ms for GFP.

Description

Lipid droplets (LD) are organelles that form around neutral lipids that coalesce within the two leaflets of the endoplasmic reticulum (ER) membrane, more frequently the cytoplasmic one, and pop out towards the cytoplasm (Pol et al. 2014; Wilfling et al. 2014). They are the only organelle of the cell delimited by a monolayer of phospholipids and are present in virtually all species (Beller et al. 2010). LD act as an energy reservoir mostly in the shape of triacylglycerols (TAGs) and steryl esters, shelter lipids from unscheduled oxidation and protect the cell from lipotoxicity (Garbarino et al. 2009; Bailey et al. 2015). Mutations exist that modify the surface and the physico-chemical properties of the LD monolayer. For example, the absence of Cds1p, crucial for phosphatidylcholine synthesis (Klig et al. 1988), decreases the availability of phospholipids and, as a consequence, super-sized LD form to spare membrane by increasing the volume-to-surface area ratio (Fei et al. 2011a). Further, the monolayers of these LD bear discontinuities, named “packing defects”, that expose their hydrophobic contents to the surrounding water environment. The same defects can occur in mutants lacking Ino2p (Fei et al. 2011a), a transcription factor essential for the induction of multiple genes necessary for phospholipid and inositol synthesis (Carman and Henry 2007). Of note, not all mutations giving rise to giant LD are accompanied by packing defects. For example, mutations such as the lack of seipin (Fld1p), which give rise to the severe Berardinelli–Seip lipodystrophy congenital syndrome, support an aberrant flow of TAGs into forming LD, which become super-sized, yet are normally packed (Fei et al. 2011a; Wang et al. 2014; Wolinski et al. 2015). Importantly, the LD surface is covered by a rich proteome under constant evolution (Cermelli et al. 2006; Kory et al. 2016; Bersuker et al. 2018). Part of this proteome supports the function of the LD itself, while the rest constitutes a selective reservoir for other proteins that, this way, can be made available elsewhere in the cell in a regulated manner (Welte 2015).
data published previously when using similarly tagged strains (Bachellier-Bassi et al. 2008; Yeh et al. 2008; Yahya et al. 2020). In agreement with the proteomic data, no signals coming from the cytoplasm or that would co-localize with LD signals could be detected either in WT or in cells bearing super-sized LD but no packing defects, such as fld1Δ cells (Fig. 1B). Yet, in disagreement with the LD proteome data (Fei et al. 2011b), we also failed to detect any cytoplasmic fluorescent signals emitted by the GFP-tagged Smc proteins in ino2Δ cells (Fig. 1B). Thus, at least under our experimental conditions, the evaluated cohesin and condensin subunits do not seem to be titrated by the LD bearing packing defects of ino2Δ cells.

Yet, during our analysis, we realized that addition of AUTODOT™ to ino2Δ cells to visualize LD led to the artefactual emission of signals in the GFP channel (Fig. 1C). This was evidenced by a progressive transformation of the initial Smc1-GFP nuclear signals into cytoplasmic dots until, at 10 minutes, GFP signals fully colocalized with LD ones (Fig. 1C). We add AUTODOT™ at a 20 µM final concentration to our cells immediately prior to mounting and imaging. This procedure allows visualization of LD in the blue channel without major bleed through the GFP one, at least in the 10 minutes-frame needed for image acquisition (Yang et al. 2012; Kumanski et al. 2021). To reinforce this notion, we evaluated the phenomenon in a WT strain that does not express any GFP fluorophore, and recapitulated minor or no bleed through (Fig. 1D, left panel). It was possible that the smaller size of LD in WT cells makes this artifact less apparent. Yet, fld1Δ cells, bearing super-sized LD, did not display any major promiscuous GFP signals either (Fig. 1D, right panel). Thus, we warn that the use of AUTODOT™ specifically in ino2Δ cells, and probably in cells with LD bearing packing defects more generally, leads to artefactual signals in the GFP channel shortly after addition.

Our work highlights the importance of additional experimental validation when considering data obtained from wide proteomic studies. Contrary to our confirmation and characterization of the presence of nucleoporins onto LD (Kumanski et al. 2021), we do not confirm the presence of either cohesin nor condensin subunits onto the super-sized LD of cells lacking the transcription factor Ino2p. This argues against further focusing on these factors’ titration as a possible trigger of genome instability in cells bearing LD with packing defects. The study of these potential titration events is relevant because conditions such as obesity are prone to the development of genome instability syndromes like cancer (Deng et al. 2016), yet the underlying links are poorly understood. Further, we want to raise awareness that the dye AUTODOT™, known for its violet absorbance and its blue emission in lipophilic environments, and accompanied by a negligible emission in other channels (Yang et al. 2012), promiscuously permeates into the green emission channel in ino2Δ cells. One could imagine that the increased local concentration of apolar molecules as occurring in super-sized LD may trigger a transition justifying this phenomenon, but the same was not observed at the giant LD in fld1Δ cells. Perhaps the insufficient packing provided by the incomplete monolayer of ino2Δ LD forces the disorganization of the stored apolar lipids thus altering the emission properties of the intercalated dye molecules.

Methods
Request a detailed protocol

Saccharomyces cerevisiae cells were grown at 25°C in complete YNB liquid medium supplemented with 2% glucose. All experiments were performed with exponentially growing cells. For microscopy analyses, 1 mL of the culture of interest was centrifuged; then, the supernatant was thrown away and the pellet was resuspended in the remaining 50 µL. 1 µL of a 1 mM stock AUTODOT™ was added to this volume and, immediately, 3 µL of this cell suspension was directly mounted on a coverslip for imaging at the indicated channels. Fluorescent signals were detected using the adequate wavelength and acquired with a Zeiss Axioimager Z2 microscope and Metamorph software. Subsequent image visualization and analysis were performed with Image J v2.0.0-rc-69/1.52i. The determination of the eventual co-localization of Smc-GFP and LD signals in all cells was done through visual inspection by the experimenter.

Reagents

The otherwise wild-type strains bearing the GFP-tagged Smc proteins (MM-285, Smc1-GFP; MM-284, Smc2-GFP; MM-283; Smc3-GFP) have been reported in (Huh et al. 2003) and were kindly provided by Alenka Čopič, Montpellier. The ino2Δ and the fld1Δ deletions were built by classical gene disruption using the G418 resistance cassette kanMX6 and the hygromycin-resistance cassette hphMX4, respectively. We also used AUTODOT™ (SM1000a, Abcepta, San Diego, CA, USA).

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References


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