Shortening of average acyl chain length enables yeast to grow without the major membrane lipid phosphatidylcholine

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Phosphatidylcholine (PC) is the most abundant phospholipid in most eukaryotes and generally considered essential. PC is synthesized by the triple methylation of phosphatidylethanolamine (PE) mediated by the methyltransferases Cho2p and Opi3p, and by the CDP-choline (or Kennedy) pathway that relies on the supply of choline. As a consequence, a *cho2opi3* double mutant is strictly auxotrophic for choline. However, we isolated *cho2opi3* suppressors (*cho2opi3***S**), which grow robustly without PC or PC substitute and present the first eukaryotes that survive without this abundant membrane lipid.

Analysis of the lipidome of *cho2opi3***S** clones revealed that PC is below the detection limit after culture without choline; instead PE has become the most abundant phospholipid. The neutral lipid triacylglycerol strongly accumulates in most PC-free *cho2opi3* suppressors, and accordingly increased number and size of lipid droplets were observed by electron microscopy. Importantly, the lipidome of the PC-free *cho2opi3* suppressor strains reveals an overall shift to shorter average acyl chain length, which is thought to play a key role in maintaining membrane physical properties.

Whole genome sequencing of a subset of *cho2opi3***S** clones revealed 2N-1 aneuploidy as the most common genetic adaptation underlying the suppression of choline auxotrophy. In addition, a suppressor with a homozygous point mutation was identified that provides important clues as to the mechanism of suppression.