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ORIGINAL ARTICLE

The SEACIT complex is involved in the maintenance of vacuole–mitochondria contact sites and controls mitophagy

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Abstract

The major signaling pathway that regulates cell growth and metabolism is under the control of the target of rapamycin complex 1 (TORC1). In *Saccharomyces cerevisiae* the SEA complex is one of the TORC1 upstream regulators involved in amino acid sensing and autophagy. Here, we performed analysis of the expression, interactions and localization of SEA complex proteins under different conditions, varying parameters such as sugar source, nitrogen availability and growth phase. Our results show that the SEA complex promotes mitochondria degradation either by mitophagy or by general autophagy. In addition, the SEACIT subcomplex is involved in the maintenance of the vacuole–mitochondria contact sites. Thus, the SEA complex appears to be an important link between the TORC1 pathway and regulation of mitochondria quality control.

Keywords Membrane contact sites · Vacuole · Mitochondria · SEA complex · TORC1 · Autophagy · Mitophagy

Abbreviations

ROS	Reactive oxygen species
SEACAT	SEA subcomplex activating TORC1
SEACIT	SEA subcomplex inhibiting TORC1
TORC1	Target of rapamycin complex 1
vCLAMPs	Vacuole–mitochondria contact sites

Introduction

The mechanistic target of rapamycin complex 1 (mTORC1) is a hallmark of the essential signaling pathway, which controls eukaryotic cell growth, senses oxygen and nutrient availability, responds to environmental stresses and regulates lifespan [1–3]. mTORC1 promotes anabolic processes when nutrients are abundant, while nutrient limitation or treatment with the macrolide drug rapamycin or its derivatives inhibits mTORC1 kinase and initiates autophagy—a catabolic process that mediates degradation and recycling of cytoplasmic components.

Among many functions exerted by mTORC1, one of the most complex involves regulation of mitochondrial homeostasis. Mitochondria play key roles in energy production, metabolism and cellular signaling. mTORC1 is essential for mitochondrial biogenesis, phosphorylation of mitochondrial proteins and regulation of mitophagy, the selective degradation of mitochondria by autophagy [4]. In addition, mitochondrial function feeds back on mTORC1. For example, reactive oxygen species (ROS), generated by mitochondrial depolarization, can affect mTORC1 signaling in a concentration-dependent manner, i.e., low level of ROS can induce mTORC1 activity, while the high level of ROS represses mTORC1 [5, 6]. In yeast (where the prefix "m" in the name of mTORC1 is dropped), retrograde signaling pathway (RTG) leads to TORC1 downregulation due to mitochondrial dysfunction caused by mitochondrial genomic effects [7, 8]. However, many details of the communication between the mTORC1 pathway and mitochondria remain elusive.

We have recently identified a novel upstream regulator of the TORC1 pathway in yeast—the multiprotein SEA complex [9, 10]. All eight members of the SEA complex

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are evolutionarily conserved and its mammalian homolog called GATOR [11]. The SEA complex consists of two subcomplexes, named the SEA subcomplex inhibiting TORC1 (SEACIT) and the SEA subcomplex activating TORC1 (SEACAT) [12]. In mammals, SEACIT and SEACAT are termed GATOR1 and GATOR2, respectively. The SEACAT is composed of five proteins (Sea2, Sea3, Sea4, Seh1, Sec13), containing structural elements present in intracellular trafficking complexes, including coated vesicles and tethers, which facilitate membrane proximity [9, 10]. Three proteins (Sea1/Iml1, Npr2, Npr3) with motifs typically found in GAPs (GTPase activating proteins) and GEFs (guanine-nucleotide-exchange factors) comprise the SEACIT [13]. Recent data demonstrate that the SEA/GATOR complex appears as a platform that can coordinate both structural and enzymatic activities necessary for the effective functioning of the TORC1 pathway [13–15].

Remarkably, the SEA complex also seems to be functionally associated with mitochondria: SEA genes exhibit synthetic genetic interactions with many mitochondria genes [16, 17]; Sea proteins interact with several mitochondria membrane proteins [18]; enriched mitochondrial fractions contain Sea proteins [19]; and the absence of the SEA2 gene leads to impaired respiration capacity [20]. Taken together, these data suggest a close functional and even physical connection between TORC1, its upstream regulator the SEA complex and mitochondria.

In the present work, we demonstrate that the SEA complex interacts with mitochondria and is involved in the maintenance of the vacuole–mitochondria contact sites (vCLAMPs). Moreover, the SEA complex promotes mitochondria degradation either by mitophagy or by general autophagy. The impaired TORC1 signaling and defective mitophagy in the cells with deletions of SEA components result in the changes in ROS production. To accommodate with its role in the mitochondria quality control and TORC1 regulation, the amount of the SEA components in the cells is regulated by metabolic changes. Specifically, the expression of SEA proteins increases during respiration growth and decreases when cells are in stationary phase and upon nitrogen starvation on glucose. In parallel, metabolic variations influence interactions between the SEA complex, TORC1 and mitochondria. In summary, the SEA complex appears as an important link between the TORC1 pathway and regulation of mitochondria quality control.

Materials and methods

Materials

The following materials were used in this study: Dynabeads M-270 Epoxy (LifeTechnologies, 143.02D); rabbit IgG (Sigma, 15006); protease inhibitor cocktail, PIC (Sigma, P-8340); HRP-mouse IgG (Jackson ImmunoResearch Laboratories); anti-GFP antibody (Roche, 11814460001), anti-PGK1 antibody (Sigma, 459250); anti-Porin1 antibody (Abcam, 16G9E6BC4), anti-Cox2 antibody (Abcam, 4B12A5), anti-Vma2 antibody (ThermoFisher Scientific, A-6427); concanavalin A (Sigma, C7275); glucose (Euromedex, UG3050), lactic acid (Sigma, L1250); Bacto-Peptone (BD LifeSciences, 2116770), yeast extract (BD LifeSciences, 212750), yeast nitrogen base without ammonium and amino acids (BD LifeSciences, 233520), yeast nitrogen base without amino acids and with ammonium sulfate (BD LifeSciences, 291940), drop-out mix complete without yeast nitrogen base (Euromedex UD9515), CSM-URA (MP Biomedicals, 4511-212), CSM-LEU (MP Biomedicals, 4510-512), CSM-URA, -LEU (MP Biomedicals, 4520-212).

Yeast strains and growth conditions

Yeast strains used in this study are listed in Table S1. To get yeast strains overexpressing Vps39-GFP, an integrative plasmid pRS406 TEFpr-VPS39-GFP ([21], a kind gift of Dr. Ungermann) was digested with StuI prior yeast transformation and subsequent clone selection.

The conditions for yeast growth are presented in Table 1. To test expression of the SEA proteins under different growth conditions (Fig. 1), yeast strains carrying SEA members genetically tagged with PrA [9] were first grown at 30 °C until mid-log phase (OD_{A600} = 0.6–0.8) in YPD (2% Bacto-Peptone, 1% yeast extract, and 2% glucose). The samples corresponded to "YPD log" phase were collected at this point. Cells were left growing for additional 2 days to collect samples corresponded to "YPD 2d" samples. An aliquot of the YPD log samples was used to inoculate media containing lactate (YPL: 2% Bacto-Peptone, 1% yeast extract, and 2% lactic acid). Lactic acid used for media preparation was adjusted with KOH till pH 5.6–5.8. "YPL log" samples were

Table 1 Growth conditions for yeast strains used in this study

Condition ID	Growth media	Growth state
YPD log	Rich medium, glucose	Logarithmic phase OD_{A600} = 0.6–0.8
YPD 2d	Rich medium, glucose	Stationary phase, 2 days of growth
YPL log	Rich medium, lactate	Logarithmic phase OD_{A600} = 0.6–0.8
YPL 2d	Rich medium, lactate	Stationary phase, 2 days of growth
SD-N 1d	No nitrogen, glucose	1 day of starvation
SD-N 7d	No nitrogen, glucose	7 days of starvation
SL-N 1d	No nitrogen, lactate	1 day of starvation
SL-N 7d	No nitrogen, lactate	7 days of starvation

collected when cells reached $OD_{A600} = 0.6–0.8$, "YPL 2d" samples were harvested 2 days later. Starvation experiments were conducted in synthetic media lacking nitrogen (SD-N: 0.17% yeast nitrogen base without ammonium and amino acids, 2% glucose; SL-N: 0.17% yeast nitrogen base without ammonium and amino acids, 2% lactate). For the starvation experiments, yeast grown on YPD or YPL were collected during log phase, washed once with water and once with the corresponding starvation media, and resuspended in the same amount of SD-N or SL-N media accordingly. Samples corresponding to "SD-N 1d" and "SL-N 1d" points were collected after 1 day from beginning of starvation; samples corresponding to "SD-N 7d" and "SL-N 7d" were collected 7 days later. For yeast cultivated during 2 or 7 days, 100 µg/mL (c.f.) of ampicillin was added to the media. Whole cell extracts prepared from the above samples were analyzed by Western blot with anti-Pgk1 antibody. Since IgG-HRP used as the secondary antibody can also recognize PrA moiety, both the SEA component and loading control (Pgk1) can be revealed simultaneously on the same blot.

For immunoprecipitation experiments, 6 L of yeast cells corresponding to each condition were grown, harvested and subjected to cryolysis as previously described [22]. To screen for optimal extraction conditions, 1 g of cell powder was used in each pull-out experiment with 10 extraction buffers, which varied in pH, salt and detergent types and concentration (Table S2, Figure S1 A). The goal of this screen was to optimize conditions of extraction to purify a stable complex, minimize post-extraction artifacts and maximize chances for capturing transient or weaker interactions. Immunoprecipitation experiments with Sea1-PrA and Npr3-PrA, two members of the SEACIT, gave nearly identical profiles of co-purified proteins (Figure S1 B and C). Once the optimal buffer was identified (buffer N3, Figure S1, Table S2), 2 g of cell powder was used for immunoprecipitations followed by mass-spectrometry analysis with extraction buffer composed of 20 mM HEPES, pH 7.4, 5 mM CHAPS, 150 mM NaCl, 1 mM DTT, 1:100 dilutions of solution P (2% PMSF, 0.04% pepstatin A in absolute ethanol), 1:200 dilutions of solution PIC. Affinity capturing of the SEA complex protein complexes from whole cell lysates using magnetic beads was performed as described previously [9, 18].

For imaging experiments (Figs. 3, 4, S2, S4, S5), cells transformed with a plasmid carrying mitochondria sensor mito-dsRED (or mito-mCherry) were grown in dropout (DO) media DO-URA (or DO-LEU) (0.17% yeast nitrogen base without ammonium and amino acids, 0.5% ammonium sulfate, 0.077% CSM-URA (or 0.069% CSM-LEU), 2% glucose (or 2% lactate)). Images were taken on a SP8 confocal microscope (Leica) and treated with LasX software. We have to emphasize here, however, that very low level of expression of the SEA components is the major obstacle during detection of the signal from endogenous proteins [9, 23]. In addition, during growth on glucose upon nitrogen starvation or when cells are in stationary phase, the level of the SEA proteins decreases (Fig. 1), which makes visualizing their localization even more challenging. High exposure times need to be applied to observe the signal, which might influence the signal-to-noise ratio. Therefore, we cannot exclude that some details and changes of the Sea1 and Sea4 localization were omitted due to technical limitations.

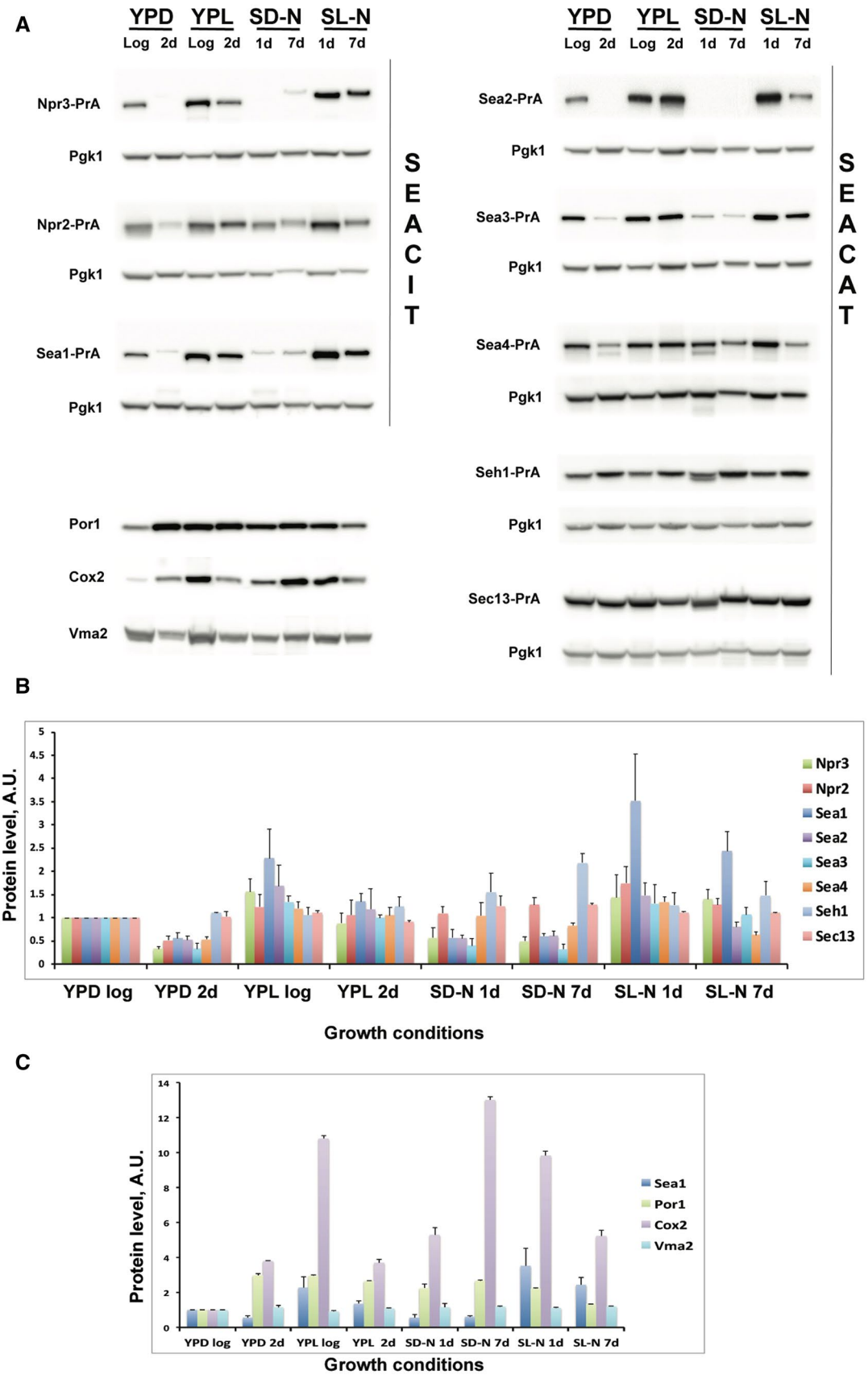
Mass spectrometry analysis of immunoprecipitations

The entire elution from the IP was loaded on 4–12% Bis–Tris gels (Novex/Thermofisher) and run only 4–6 mm into the gel to produce a "gel plug" [24]. This small area of gel, containing all proteins present in immunoprecipitate, was excised and subjected to in-gel digest with trypsin essentially as described earlier [18]. Two technical replicates were performed for each condition.

Mass spectra acquisition: LC/MS analysis was performed on EASY-nLC 1000 (Thermo Scientific) paired with Q Exactive quadrupole-orbitrap hybrid mass spectrometer (Thermo Scientific). The peptide mixture was separated on an EASY-Spray 15 cm × 75 µm 3 µm 100A C18 PepMapR reverse-phase column (Thermo Scientific) using a 75-min three-step water–acetonitrile gradient (0–60 min, 0 → 30% LC buffer B (0.1% formic acid in acetonitrile); 60–65 min, 30 → 90%; hold for 10 min) at 300 nL/min flow rate. The intensities of precursor ions were gauged in positive mode at scan range 400–2000 *m/z*, resolution 70,000, automatic gain control (AGC) target 1E6, maximum injection time 100 ms, followed by forwarding 10 most intense ions of a spectrum for MS2 fragmentation and measurement at resolution 17,500, AGC target 5E4, maximum injection time 100 ms, isolation window 2 *m/z* with 30 s dynamic exclusion.

Discovery analysis: Raw mass spectrometric data were analyzed by Proteome Discoverer v.1.4.0.288. MS2 spectra were searched against the *Saccharomyces cerevisiae* Swiss-Prot database using Mascot engine set for 10 ppm precursor mass and 0.02 Da fragment mass tolerances with 2 allowed missed cleavage sites. Oxidation on methionine, phosphorylation on serine, threonine and tyrosine, deamidated on asparagine and glutamine and GlyGly on lysine as dynamic modification, cysteine carbamidomethylation on cysteine were indicated as static modification. False discovery rate (FDR) was calculated using Percolator [25] with 0.01 strict and 0.05 relaxed target cutoff values.

To retain high confidence interacting partners of the SEA complex at eight different growth conditions, several steps of filtering were performed. First, given localization of the endogenous SEA complex at limiting membrane of the vacuole [9], proteins with a nuclear, nucleoplasm


A
YPD YPL SD-N SL-N
Log 2d Log 2d 1d 7d 1d 7d
Npr3-PrA
Pgk1
Npr2-PrA
Pgk1
Sea1-PrA
Pgk1
SEACIT
Por1
Cox2
Vma2
YPD YPL SD-N SL-N
Log 2d Log 2d 1d 7d 1d 7d
Sea2-PrA
Pgk1
Sea3-PrA
Pgk1
Sea4-PrA
Pgk1
Seh1-PrA
Pgk1
Sec13-PrA
Pgk1
SEACAT
B
Protein level, A.U.
Npr3
Npr2
Sea1
Sea2
Sea3
Sea4
Seh1
Sec13
YPD log
YPD 2d
YPL log
YPL 2d
SD-N 1d
SD-N 7d
SL-N 1d
SL-N 7d
Growth conditions
C
Protein level, A.U.
Sea1
Por1
Cox2
Vma2
YPD log
YPD 2d
YPL log
YPL 2d
SD-N 1d
SD-N 7d
SL-N 1d
SL-N 7d
Growth conditions

◂**Fig. 1** Stability of the SEA complex components at different growth conditions. **a** Yeast cells carrying an SEA member tagged with PrA were grown under different conditions (see Table 1); samples were collected, whole cell extracts prepared and analyzed by Western blotting with anti-Pgk1 (primary) and IgG-HRP (secondary) antibodies (note, that IgG-HRP recognizes PrA moiety). Immunoblottings with anti-Por1, anti-Cox2 and anti-Vma2 were done on Sea1-PrA samples. Samples corresponding to SEACIT and SEACAT subcomplexes are indicated to the right of the blot panels. **b** The quantification of the SEA proteins' signals on the Western blots from (**a**) was done by Image J program. The protein level in arbitrary units (AU) was calculated by normalizing the PrA signal to the corresponding Pgk1 signal from blots shown in **a**. The signal at "YPD log" was set at 1. Error bars represent the standard deviation in three independent experiments. **c** Expression of Por1, Cox2, Vma2 compared to Sea1-PrA. Quantification is done as in **b**

or nucleolus gene ontology terms were excluded. We then considered only those proteins whose total peptide spectrum match (PSM) across all conditions and replicas was higher than 50. This selection would automatically exclude proteins, which were identified only at one replica and proteins with 1-3 PSMs at any given condition. At the same time, a protein will be retained if it was identified with low PSM in one condition, but was detected with high PSM in another condition.

Mitophagy and autophagy analysis

To test autophagy or mitophagy, wild type and various deletion strains of the SEA complex members were transformed with a plasmid coding for GFP-Atg8 (for autophagy) or with Idp1-GFP (for mitophagy), grown in drop-out media without uracil until mid-log phase and shifted to SD-N or SL-N media as described above. Samples were taken between 2 and 24 h of starvation to check nitrogen starvation-induced autophagy and mitophagy. For stationary phase-induced mitophagy, yeast were grown on SL media (0.67% yeast nitrogen base without amino acids and with ammonium sulfate; 0.2% drop-out mix complete without yeast nitrogen base; 0.1% KH_2PO_4; 2% lactate, pH 5.7) and samples were collected 24 and 72 h after cells were reached log phase. Whole cell lysates were prepared from above samples and used for the Western blotting with anti-GFP and anti-Pgk1 antibodies. Imaging experiments were performed after 24 h of starvation. To prevent cell movement yeast were placed on a slide covered with 1 mg/mL concanavalin A and visualized at room temperature. Steady-state images for Idp1-GFP and GFP-Atg8 localization were obtained on a custom confocal microscope, comprising a Leica TCS SpE with a 63X ON 1,3 oil objective, and 491 nm solid-state laser. All components were driven by Leica software (LAS AF).

Analysis of ROS production

1 mL of cultured cells was pelleted at 4000 rpm for 2 min. Cell pellets were resuspended in 1 mL of phosphate-buffered saline (pH 7.4) containing 50 μM of dihydroethidium (DHE; Molecular Probes) and incubated at 30 °C for 10 min in the dark. Cells were pelleted again and resuspended in 1 mL of PBS without dye. Flow cytometry was carried out on BD LRSFortessa cytometer. The DHE fluorescence indicated is the direct output of the PerCP-Cy5-5-A (red fluorescence detecting) channel without compensation. A total of 20,000 cells were analyzed for each curve.

Results

Expression of SEA proteins is induced during respiratory growth

In our previous study [18], we reported that inhibition of TORC1 either by nitrogen starvation or by rapamycin changes the stability of SEA complex members. Given that TORC1 controls multiple cellular processes, including various types of autophagy and aging, we decided to extend our survey and monitored expression of the SEA complex proteins during logarithmic growth and in stationary phase in a variety of growth conditions: during fermentable growth in rich media containing glucose (YPD) or under respiratory conditions in the presence of non-fermentable carbon source lactate (YPL), and in "starvation" media lacking nitrogen (SD-N; SL-N). Growth in starvation media promotes autophagy (SD-N) or autophagy and mitophagy (SL-N) (Table 1).

In accordance with our previous results [18], we observed that the amount of the SEA proteins is significantly decreased during nitrogen starvation on glucose 24 h after induction of the starvation and is not altered further after 7 days of starvation. In contrast, when cells were cultured under respiratory conditions, in the presence of lactate, we detected that the expression of the SEA complex proteins is significantly increased, with or without starvation (Fig. 1a, b). The expression of SEA proteins is generally reduced when cells are in the stationary phase, but this difference is less pronounced for cells grown on lactate. The expression of Seh1 and Sec13 does not change significantly in all the conditions tested, most probably due to the fact that these two proteins are also present in other abundant protein complexes (nuclear pore complex and COPII vesicles), where their quantity is not altered by these growth conditions. Similarly, the expressions of Pgk1 and vacuole membrane protein Vma2 were also practically not affected by growth conditions. In contrast, mitochondrial outer membrane protein Por1 and inner membrane protein Cox2 do respond to

the metabolic changes, used in our experiments. Although these proteins do not behave exactly in the same way, when compared to each other, their amount is significantly elevated in respiratory conditions (especially for Cox2), which corresponds to increased mitochondria proliferation. In agreement with previous observations, Por1 and Cox2 signals are reduced during starvation conditions, as a result of mitophagy [26].

Taken together we found that cellular amount of the SEA proteins is influenced by metabolic changes. Overall the level of the SEA components increased during respiratory growth (cultivation on lactate) and decreased during stationary phase and upon nitrogen starvation on glucose. Accordingly, there will be more of the SEA complex in the cells under conditions that trigger mitophagy than in cells cultivated in the rich media during fermentable growth.

Interactome of the SEA complex varies depending on growth conditions

We next decided to investigate how the metabolic changes influence the SEA complex proteome. We, therefore, performed immunoprecipitations (IPs) for mass spectrometry analysis with the yeast strain expressing Npr3-PrA, because this protein (together with Sea3), connects SEACIT to SEACAT [18]. Note, that C-terminal PrA tag does not interfere with the ability of the Npr3 to interact with other members of the SEA complex [9, 18, 27]. Yeast cells were grown in eight different conditions (Table 1) and immunoprecipitations of Npr3-PrA were performed using standard procedures that were applied previously for the SEA complex proteome study [9, 18].

The resulting list of proteins in the SEA complex interactome contained 331 proteins (Table S3), which were classified according to their localization and function (Fig. 2). The analysis of the SEA complex proteome across different conditions revealed that the strongest interactions are detected between the members of the SEA complex itself (Table S4). These interactions seem to be enhanced during growth on lactate in accordance with the overall increase of the SEA complex expression level under respiratory growth. Nitrogen starvation on glucose, and even more on lactate, significantly increases amount of peptide score matches (PSMs) of TORC1 proteins in the SEA complex proteome. The highest amount of PSMs corresponding to TORC1 proteins is observed when cells are in stationary phase during growth on lactate with and without starvation.

In addition to TORC1, the SEA complex network also includes cytoplasmic and vacuole proteins as well as components of the mitochondria, ER and plasma membrane (Fig. 2a). Vacuole membrane proteins in the SEA complex proteome are represented by surprisingly narrow set of proteins, which belong to two large complexes: vacuolar H+ translocating ATPase (V-ATPase) and polyphosphate polymerase (or vacuolar transporter chaperone, VTC). Cytoplasmic components of the SEA complex interactome include ribosomes and proteins involved in a variety of metabolic processes, including amino acid, fatty acid, sterol synthesis together with components of glycolysis, gluconeogenesis and glycogen metabolism (Table S3). Accordingly, 70% of proteins in the SEA complex interactome are involved in the processes of translation and metabolism (Fig. 2b).

We noticed that about 20% of proteins co-purifying with the SEA complex belong to mitochondria (Fig. 2a). The amount of mitochondria proteins in immunoprecipitates increases 20–30%, when cells are grown during 7 days on respiratory conditions (Table S4). The majority of the mitochondrial proteins identified in immunoprecipitates reside on the inner or outer membranes and are the components of the electron transport chain (cytochrome c, cytochrome bc1, F_1/F_0 ATP synthase), prohibitins, translocase of outer membrane (Tom40, Tom70), voltage-dependent anion channel (porin 1, Om45, Om14) and a protein found at the vacuole–mitochondria contact sites (Vps13). Even though the mitochondrial mass is increased during respiratory conditions, the amount of mitochondrial PSMs in our proteome does not follow exactly the same path. For example, we find the maximum of mitochondrial proteins in the conditions "SL-N 7d" (nitrogen starvation at lactate during 7 days), yet the mitochondrial mass is only slightly increased at these conditions in comparison to the wild type cells (Fig. 1, Table S4). Alternatively, in the conditions of nitrogen starvation on glucose, when the mitochondrial mass is doubled in comparison to "YPD log" condition, we detected twice as less of mitochondrial proteins in the pullouts. Analysis of proteomic data by STRING database revealed a highly interconnected network of mitochondria membrane proteins with the SEA complex and TORC1 (Fig. 2c).

Taken together, the SEA complex interactome represents a network of the proteins related to the metabolic processes and the profile of the interactome is changing depending on growth conditions.

The SEA complex co-localizes with mitochondria

To confirm the data of the proteomics, we verified whether SEA proteins can be co-localized with mitochondria and how the SEA complex localization is affected under various growth conditions. To address this, we observed fluorescence of one SEACIT member (Sea1) and one SEACAT member (Sea4) genomically tagged at the C-terminus with GFP in the corresponding strains transformed with a plasmid expressing mito-DsRed (Fig. 3, Figures S2–S4).

Both Sea1-GFP and Sea4-GFP are predominantly localized to the limiting membrane of the vacuole in all the conditions tested. For cells subjected to nitrogen starvation and

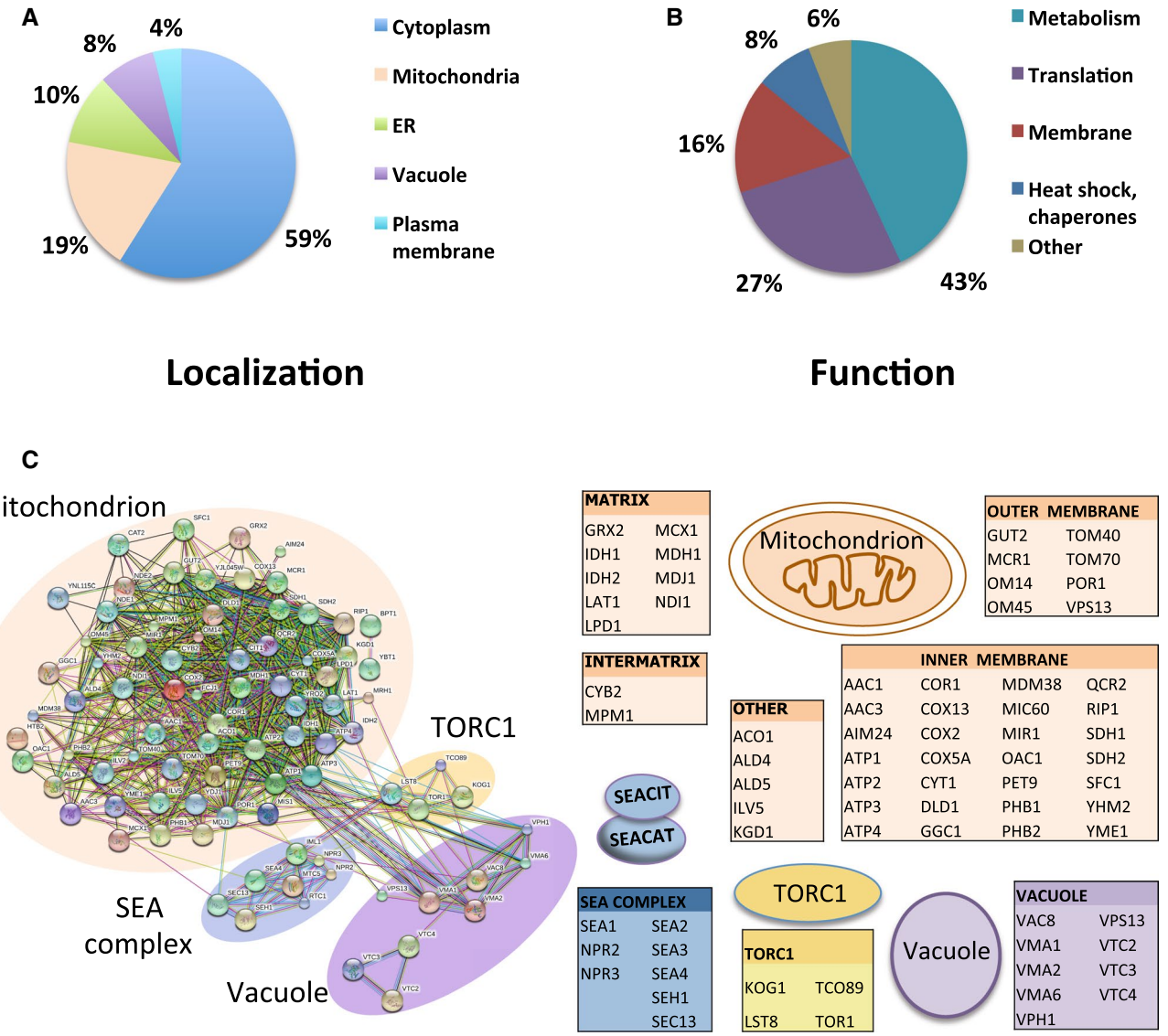
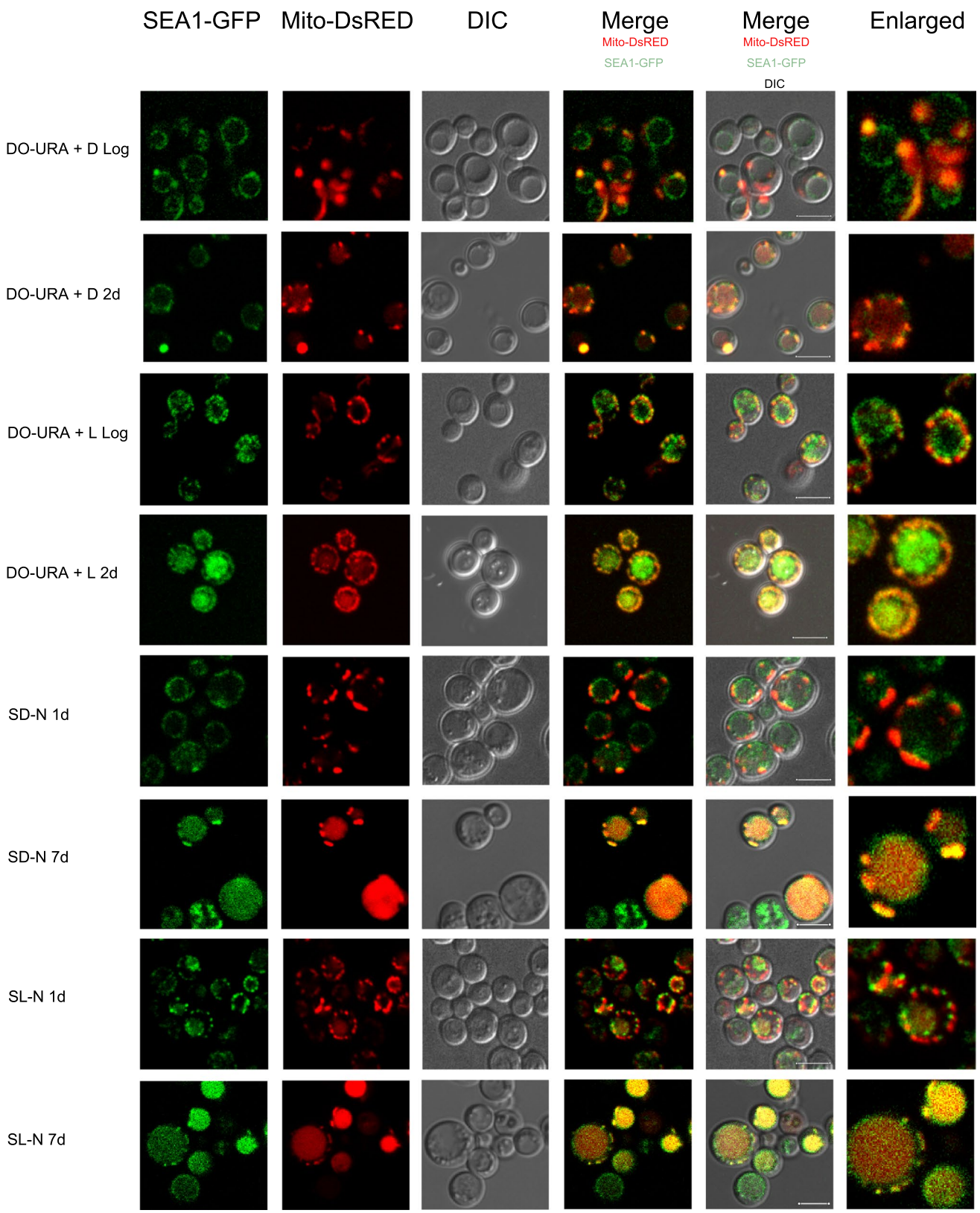


Fig. 2 Analysis of the SEA complex proteome. Distribution of co-purified cytoplasmic proteins according to localization (**a**) and function (**b**). **c** Protein network analysis. Protein network for the SEA complex and its interactors from TORC1, vacuole and mitochondria was assembled by STRING (left). Each group of proteins is marked by color. Mitochondrial proteins found in the proteome are classified according to their localization on different parts of the organelle and indicated in the text boxes along with co-purifying proteins from the SEACIT, SEACAT, TORC1 and vacuole (right). Note that in the STRING map Sea1 is marked as Iml1, Sea2—as Rtc1 and Sea3—as Mtc5

grown on lactate, GFP signal can also be observed inside the vacuole in both logarithmic and stationary phases, which suggest that a portion of cellular pool of the SEA complex is degraded by the vacuole under these conditions.

The partial co-localization between SEA members and mito-dsRED was observed in all conditions tested. Starvation under respiratory growth, which corresponds to the induction of mitophagy and autophagy (see below), results in almost complete accumulation of mitochondria signal in the vacuole. SEA components seem to be accompanying mitochondria during this process, since fluorescence signal from Sea1-GFP and Sea4-GFP is also detected inside the vacuole (Fig. 3, Figure S4). At the same time, accumulation of the mitochondria in the vacuole after 1 day of nitrogen starvation on glucose is not so efficient in comparison with the nitrogen starvation under respiratory growth.

Our co-localization data are in agreement with the results from recently published survey of yeast proteome dynamics [28]. In this study, a high-throughput imaging followed by automated analysis allowed quantification of the abundance and localization of GFP-tagged yeast ORFeome, including the observation of protein dynamics in the yeast subjected



to rapamycin treatment. Because in our experiments, we used Sea1-GFP strain from the same yeast collection [29] (Table S1), we retrieved the information from data published in [28] and CYCLoPS database about Sea1-GFP localization in wild-type cells and cells subjected to rapamycin treatment for 60 min and 220 min (Figure S3). A computational

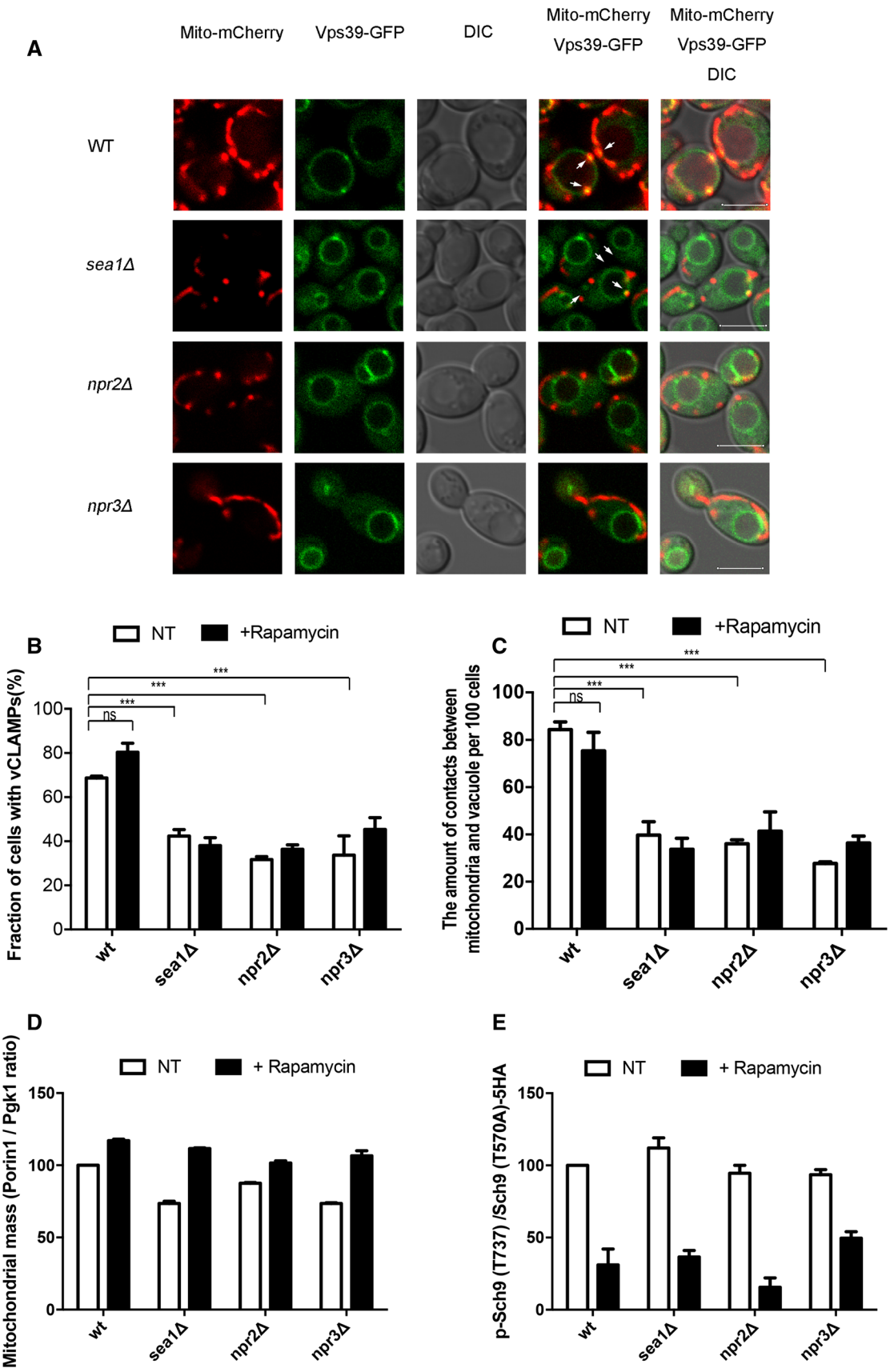
◂**Fig. 3** The SEA complex co-localizes with mitochondria. Fluorescence images of yeast cells co-expressing Sea1-GFP and mito-DsRED at different growth conditions (indicated at the left). Scale bar is 5 μm. Yeast cells were grown in the media without uracil (DO-URA) to maintain the expression of mito-DsRED plasmid either on glucose (D) or on lactate (L) and observed either on logarithmic phase (log) or after 2 days of cultivation (2d). For the starvation experiments, yeast grown on YPD or YPL were collected during corresponding log phase, washed once with water and once with the corresponding starvation media and resuspended in the same amount of SD-N or SL-N media accordingly. Samples corresponded to "SD-N 1d" and "SL-N 1d" points were observed after 1 day since the beginning of starvation; samples corresponded to "SD-N 7d" and "SL-N 7d" points were observed after 7 days

analysis of Sea1-GFP localization shows that Sea1-GFP is located at the vacuole membrane in yeast grown to early logarithmic phase. Treatment with rapamycin significantly increases the amount of cells with cytoplasmic and mitochondrial localizations of Sea1-GFP, yet a fraction of Sea1-GFP can still be observed at the vacuole ([28], CYCLoPS database, Figure S3).

SEA complex is required for the maintenance of the vacuole–mitochondria contact sites

Recently, several laboratories identified that yeast vacuole and mitochondria interact at the contact sites called vCLAMPs (vacuole and mitochondria patches) [19, 21, 30–32]. There are at least two functionally distinct vCLAMPs. Vps13-containing vCLAMPs include besides Vps13 itself a mitochondrial receptor Mcp1 protein [30–32]. Vps39-containing vCLAMPs are formed by a member of the vacuole tethering HOPS complex Vps39 protein, the Rab GTPase Ypt7 and protein translocase of the outer mitochondria membrane Tom40 [19, 21, 30]. Overexpression of Vps39 is used to induce and enlarge the contacts between two organelles, which are otherwise quite rare in the cells [21, 30, 32]. Remarkably, the proteomic analysis of Vps39 interactome at the contact sites revealed enrichment for the SEA complex proteins (Sea4, Sea2, Sea1, Npr2, Npr3) [19]. These data together with our localization results prompted us to investigate whether the SEA complex can be involved in the vCLAMPs organization. To do so, we compared vCLAMPs formation in the cells overexpressing Vps39-GFP in wild type and SEACIT deletions strains (Fig. 4a). Deletion of any of SEACIT member reduced vCLAMPs number by 30–40% (Fig. 4b, c). We further checked whether the reduction of the vCLAMPs can be related to the changes in the mitochondrial mass in the deletion strains. Our results demonstrate that when Vps39 is overexpressed the mitochondrial mass is decreased by 10–20% in SEACIT deletion strains in comparison with the wild type strains (Fig. 4d). Therefore, the mitochondrial mass reduction cannot be the only explanation of the vCLAMPs reduction. Interestingly, in the yeast strains without Vps39 overexpression the mitochondrial mass decrease in SEACIT strains is more significant: 30–50% (Figure S5). We next verified whether vCLAMP reduction can be due to an elevated TORC1 activity in SEACIT deletion strains. Surprisingly, we found that in the Vps39 overexpression strains the TORC1 activity is already increased and deletions of SEACIT components practically do not have any further impact (Fig. 4e, Figure S6). This is most probably due to the fact that Vps39 (also known as Vam6) is also a GEF for yeast Rag GTPase homolog Gtr1, which is in its GTP- bound form strongly induces TORC1 activity [33]. Treatment with rapamycin inhibits TORC1, but does not restore the Vps39-containing vCLAMPs in SEACIT deletion strains (Fig. 4, Figure S6), meaning that the function of the SEA complex components in the maintenance of vCLAMPs is probably not due to their role in TORC1 regulation. Thus, the SEACIT is important for the vCLAMPs formation.

We further evaluated genetic interactions (GIs) between genes of SEA complex components, vCLAMPs and members of another complex, ERMES, which is involved in the formation of contact sites between endoplasmic reticulum (ER) and mitochondria. It has been demonstrated that deletion mutants between Vps39-vCLAMPs and ERMES are synthetically lethal, which is related to the impaired transport of mitochondrial phospholipids [19, 21]. We reasoned that if the SEACIT is important for the vCLAMPs maintenance it might as well be involved in the genetic interactions with ERMES. To follow the profile of GIs between SEA, vCLAMPs and ERMES, we took an advantage of recently published whole-genome studies of synthetic genetic interactions in yeast [17, 34, 35]. Many of these data are now summarized in a web-accessible database TheCellMap.org [36], from which we retrieved the GI data for SEA, vCLAMPs and ERMES (Figure S7A). These data demonstrate that SEACAT genes show positive GIs with both vCLAMPs and ERMES, while SEACIT proteins are preferentially involved in significant negative GIs with ERMES. This is another remarkable demonstration of the opposite roles of two subcomplexes of the same protein complex, with SEACAT properties as TORC1 activator and SEACIT functions as TORC1 inhibitor [11, 37]. We next created double deletion strain *mdm34*Δ*npr3*Δ carrying a deletion of one ERMES component (*mdm34*Δ) and one SEACIT component (*npr3*Δ) and compared its fitness with a wild type and single deletion strains (Figure S7B). Our results show that double deletion strain has reduced fitness both at 30 °C at 37 °C in comparison with any single deletion strains (note that mdm34 single deletion strain grow much slower at 37 °C than at 30 °C). Therefore, these data indicate that the SEACIT is probably not a "bona-fide" vCLAMPs component (although we cannot completely exclude such a


A
Mito-mCherry
Vps39-GFP
DIC
Mito-mCherry Vps39-GFP
Mito-mCherry Vps39-GFP DIC
WT
sea1Δ
npr2Δ
npr3Δ
B
NT
+Rapamycin
Fraction of cells with vCLAMPs(%)
ns
***
wt
sea1Δ
npr2Δ
npr3Δ
C
The amount of contacts between mitochondria and vacuole per 100 cells
D
+ Rapamycin
Mitochondrial mass (Porin1 / Pgk1 ratio)
E
p-Sch9 (T737) /Sch9 (T570A)-5HA

◂**Fig. 4** The SEACIT is required for vCLAMP maintenance. **a** Overexpressed Vps39-GFP and mitochondria marked by mito-mCherry were visualized by fluorescence microscopy. W303a cells without deletions ("wild type", WT) and those carrying various deletions of SEACIT components were grown overnight in the media without uracil and leucine (DO-URA, -LEU) to maintain the expression of mito-mCherry plasmid and Vps39-GFP. The yeast cultures were then diluted, shifted to DO-URA media and allowed to grow for 3–4 additional doubling times till mid-logarithmic phase ($OD_{A600}=0.6–0.8$) prior visualization. vCLAMPs are marked with arrows. Scale bar is 5 µm. (B) Percentage of cells (grown as in **a**), containing vCLAMPs. Yeast were grown as in **a**, 150 cells in each strain were observed and amount of cells containing vCLAMPs was counted. The data are represented as a percentage of the cells with vCLAMPs. Error bars correspond to data from three independent experiments. $***p<0.001$. **c** Quantification of vCLAMPs number in 100 cells. In wild-type strains, two–three vCLAMPs per cell in average can be observed. In some of the deletion strains, this amount is lower. An average amount of vCLAMPs per 100 cells was calculated. 150 cells of each strain were observed. The data are represented as a percentage of vCLAMPs per 100 cells. Error bars correspond to data from three independent experiments. $***p<0.001$. **d** Evaluation of mitochondria mass as a ratio of Porin1 to Pgk1 in the wild type and SEACIT deletion strains (see also Figure S6). **e** Estimation of TORC1 activity as ratio of p-Sch9-Thr^{737} to full-length Sch9-T570A-5HA in the wild type and SEACIT deletion strains, overexpressing Vps39-GFP (see also Figure S6)

possibility at this stage), but rather plays an essential regulatory role in the maintenance of these contact sites.

The SEACIT complex regulates mitochondria quality control via general autophagy and mitophagy

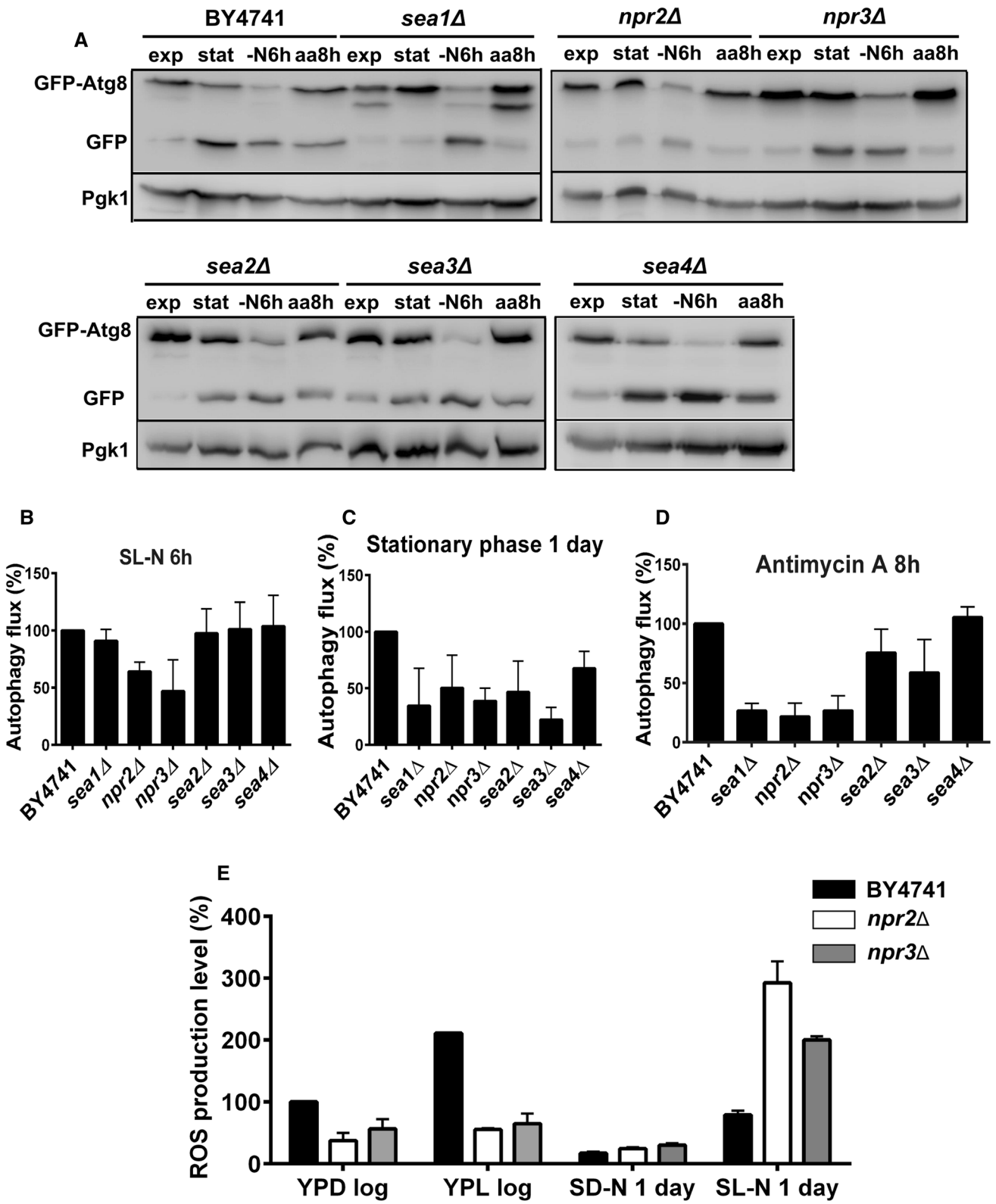
Our proteomic data demonstrate that a half of the mitochondrial proteins found in the SEA complex proteome belongs to inner mitochondrial membrane (IMM) or mitochondrial matrix (Table S4, Table S5). The amount of these proteins increases in the conditions that provoke the appearance of damaged mitochondria, especially in chronologically aged cells and in the cells grown during prolonged respiratory conditions. One of the explanations of this phenomenon could be that the SEA complex interacts not only with healthy mitochondria but also with damaged organelle. To check this, we provoked mitochondrial dysfunction by treating cells with antimycin A—a respiratory chain inhibitor that blocks the reduction of semi-ubiquinone by cytochrome *b*, resulting in the increased production of ROS. Antimycin A does not induce mitophagy, but triggers nonspecific autophagy [38]. Treatment with antimycin A failed to trigger autophagy in deletion strains of SEA components, once again with more severe effects for the SEACIT deletions than for SEACAT deletions (Fig. 5a–d).

Mitochondrial dysfunction is often related to the increased production of reactive oxygen species [26, 39]. ROS production can also be promoted in the actively grown cells with reduced TORC1 signaling [40]. We, therefore, checked how deletion of the SEACIT components Npr2 and Npr3 influences ROS production. Deletions of *NPR2* and *NPR3* activate TORC1 in actively grown yeast (Fig. 4, [12, 37]). In such deletion, strains ROS production is induced (Fig. 5e, Figure S8). This effect is more pronounced during respiratory growth on lactate. Inversely, in the cells subjected to nitrogen starvation deletion of SEACIT components increased ROS production. Because in such cells mitophagy is impaired, the accumulation of damaged mitochondria is probably the reason of elevated ROS. Of note, deletions of Atg32 and Atg11, two proteins essential for mitophagy, also lead to increased ROS production during nitrogen starvation [26].

Next we decided to check whether the SEA complex is involved in the regulation of mitophagy. We and others have already observed that all three members of the SEACIT control general autophagy, which is not surprising because SEACIT inhibits TORC1, and TORC1 inhibition induces autophagy [9, 18, 27, 41–44]. SEACIT is also engaged in the regulation of a specific form of autophagy, non-nitrogen-starvation (NNS)-induced autophagy, which happens in yeast when cells are switched from a rich to a minimal media with lactate as a carbon source [27, 42, 45]. However the effect of the SEA complex on other forms of selective autophagy mitophagy has not yet been intensively explored.

In yeast, mitophagy serves primarily as a mechanism for the elimination of excess mitochondria and is triggered mainly in two ways [46]. "Starvation induced mitophagy" is activated when yeast is cultured under respiratory conditions, in the presence of a non-fermentable carbon source (e.g., lactate), which will induce proliferation of mitochondria essential to metabolize this nutrient. Yeast must also be subjected to nitrogen starvation because if growth continues, excess mitochondria are segregated into daughter cells during cell division. This type of mitophagy can be observed in SL-N media. The second approach to trigger mitophagy consists of culturing of cells under respiratory conditions to post-logarithmic phase. Accordingly, "stationary phase mitophagy" occurs because cells in a post-logarithmic phase have low energy requirements and, therefore, mitochondria are in excess. This type of mitophagy might be a part of a quality-control process that targets damaged mitochondria accumulating in non-dividing cells. Conditions corresponding to stationary phase mitophagy could be achieved in YPL or SL-N media with the cells grown to post-logarithmic phase.

To follow mitophagy, wild-type yeast and cells with deleted SEA components were transformed with Idp1-GFP, a mitochondrial matrix protein often used for mitophagy monitoring [38, 47]. The fluorescence analysis of these cells grown in rich media showed that deletions of the SEA components did not significantly alter mitochondrial morphology in comparison with wild-type cells, although we noticed slightly increased level of mitochondria fragmentation in the



deletion strains (Fig. 6). Yeast cells were further subjected to nitrogen starvation in the presence of lactate to trigger starvation-induced mitophagy or were cultured in SL media for 2 days to activate stationary phase mitophagy.

Our results indicate that starvation-induced mitophagy is decreased in all SEA mutation strains with more severe effects for SEACIT deletions than for SEACAT deletions (Fig. 6). At the same time, general autophagy, which was

◂**Fig. 5** The SEA complex controls general autophagy induced by nitrogen starvation at respiratory conditions and non-specific autophagy provoked by antimycin A treatment. **a** Western blots showing the analysis of autophagy in the wild type (BY4741) yeast and indicated deletion strains of SEA components expressing GFP-Atg8. Cells were grown in SL media, harvested at indicated time points, whole cell extracts prepared and analyzed by Western blots with anti-GFP and anti-Pgk1 antibodies. "t0"—exponential (logarithmic) phase; "stat"—stationary phase corresponding to 1 day of growth; "-N6 h"—cells grown in the absence of nitrogen during 6 h; "aa8 h"—antimycin A (2 µg/mL) was added at time "0" and cells were grown during additional 8 h. **b**–**d** The autophagy flux was calculated as a ratio (%) of free GFP to total GFP signal (combined free GFP and GFP-Atg8) in corresponding Western blots from **a**. The quantification of the signals on the Western blots from three independent experiments was done with Image J. **e** ROS levels in the indicated wild type (BY4741) and SEACIT deletion strains under different conditions of growth were measured with ROS-sensitive dye DHE and FACS (see "Materials and methods" and Figure S6)

monitored by observing the degradation of GFP-Atg8 protein, was only affected in the SEACIT deletions strains and practically was not changed in the strains with SEACAT deletions (Fig. 5). Stationary phase mitophagy is strongly repressed upon deletion of SEACIT members; however, it is not significantly affected in the strains with SEACAT deletions and even slightly enhanced in *sea2*Δ strain. Interestingly, general autophagy observed during stationary phase under respiratory growth was not very efficient in all SEA deletion strains.

Taken together our data indicate that the SEA complex interacts with mitochondria and is involved in the control of mitochondria's quality. Damaged organelles that appeared after antimycin A treatment and excess mitochondria that accumulate in the aging cells or cells cultivated under respiration conditions can be eliminated either by non-specific autophagy or by mitophagy. Thus, the SEA complex controls mitophagy and non-specific autophagy induced by damaged mitochondria and, therefore, appears as an essential regulator of cellular homeostasis.

Discussion

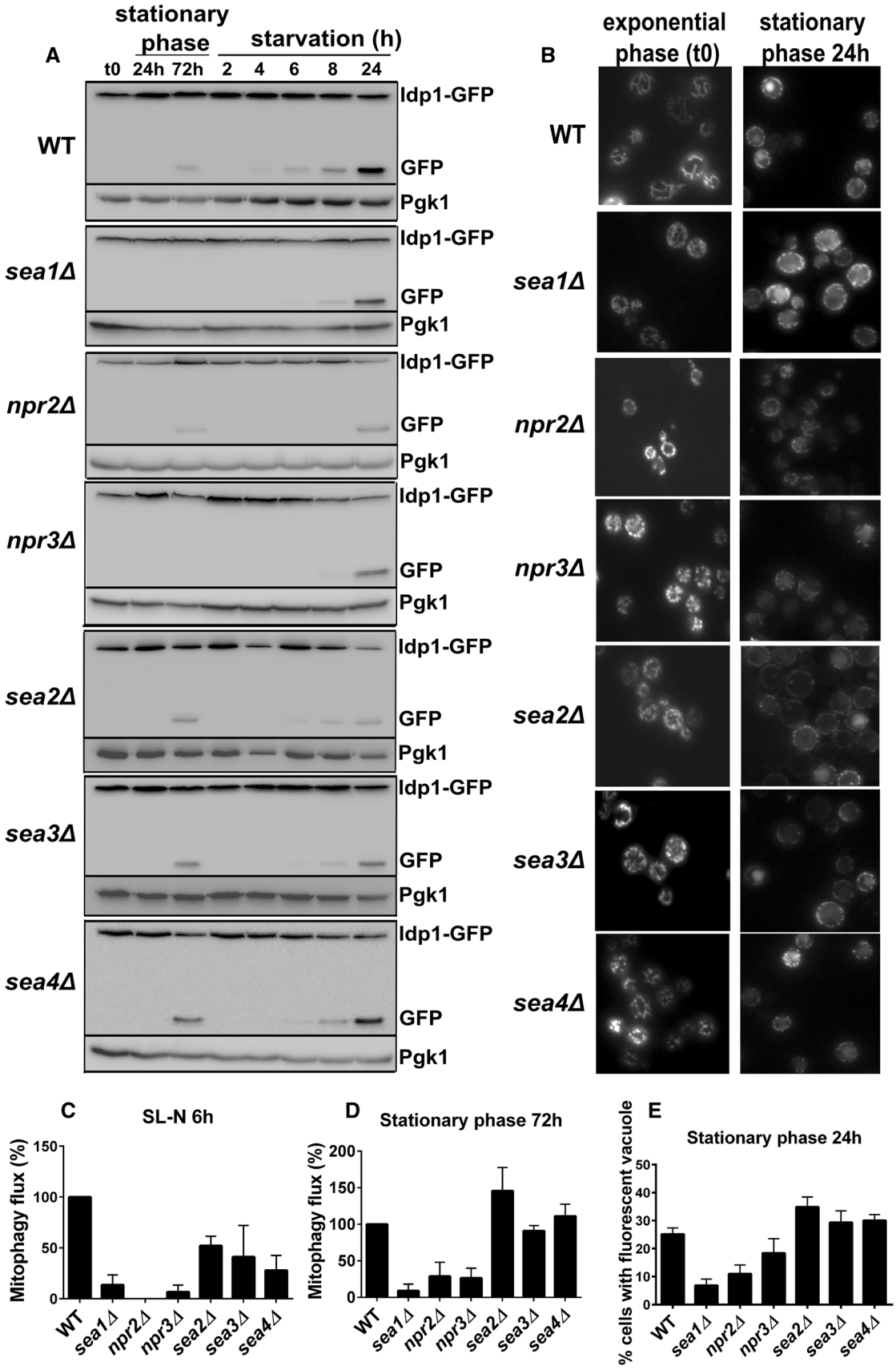
Mitochondria are implicated in the multiple processes, including amino acid and lipid synthesis, energy and hormone production. Mitochondria are also a primary source of reactive oxygen species and, consequently, can produce deleterious effects for cells. Therefore, it is crucial for cells to be able to detect and eliminate damaged mitochondria.

Mitochondria mostly rely on direct transport through membrane contacts with other organelles in order to recruit the substrates for multiple biochemical reactions and export resulting products, although vesicular transport from the mitochondria has also been described [48]. Recently, discovered mitochondria–vacuole contact sites (vCLAMPs) play an important role in the exchange of lipids between these two organelles [19, 21, 30, 32] and also serve as an alternative transport route for the lipid transport to the endoplasmic reticulum. Contacts between vacuole and mitochondria during the first hours of nitrogen starvation when grown on fermentable sugars have also been reported [49]. So far only five proteins have been characterized as bona fide components of vCLAMPs—Vps39, Ypt7 and Tom40 for Vps39-vCLAMP; Vps13 and Mcp1 for Vps13-vCLAMP [19, 21, 30, 32].

Here, we propose that the SEA complex regulates vCLAMPs formation (Fig. 7). Indeed, deletions of any SEACIT members drastically reduce the amount of vCLAMPs in the cells (Fig. 4). Moreover, pull-downs of vCLAMP member Vps39 from cellular fractions enriched for mitochondria revealed interactions with SEACAT and SEACIT members [19]. In our study, the SEA complex component Npr3 pulled down only traces of Vps39 (data not shown), most probably because endogenous levels of Vps39 were too low and overexpression would be required for Vps39 detection in the context of vCLAMPs. Interestingly, Vps13 protein, which was suggested to be a marker for naturally occurring vCLAMPs [31], was found with very good scores in the Npr3 immunoprecipitations, especially when cells are in logarithmic growth in rich media (Table S4).

Before this study, vCLAMPs were thought to be involved mainly in lipid exchange [19]. Our results suggest a possible novel function for vCLAMPs—sensing the integrity and functionality of mitochondria. Given that the degradation of damaged organelles takes place in the vacuole via the processes of autophagy and mitophagy, and that the key players of this process—TORC1 and a number of its upstream regulators including the SEA complex—reside at the vacuole membrane, it would be "spatially beneficial" for the cells to attribute this function (at least partially) to vCLAMPs. When detected by a vCLAMP, information about a dysfunctional organelle can be immediately transferred to the adjacent TORC1 network in order to initiate degradation (Fig. 7). In addition, it is not impossible that the SEA complex might share the "sensing" activity with vCLAMPs or can have a potential regulatory function in this process, given that the SEA complex interacts with mitochondria membrane proteins and maintains vCLAMPs.

Our proteomics data show that more than a half of mitochondrial proteins found in immunoprecipitations belong to the mitochondrial inner membrane or mitochondrial matrix (Table S4, Table S5). Interactions with these proteins are greatly induced in the conditions, which provoke mitochondrial damage (e.g., growth till stationary phase). Interestingly, recent data by Levine's laboratory [50] describe inner mitochondrial protein Prohibitin 2 as mitophagy receptor, indicating that membrane rupture may represent a common feature of specific autophagy. Prohibitins 1 and 2 are also


A
stationary phase
starvation (h)
t0 24h 72h 2 4 6 8 24
WT
sea1Δ
npr2Δ
npr3Δ
sea2Δ
sea3Δ
sea4Δ
Idp1-GFP
GFP
Pgk1
B
exponential phase (t0)
stationary phase 24h
C
SL-N 6h
Mitophagy flux (%)
D
Stationary phase 72h
E
Stationary phase 24h
% cells with fluorescent vacuole

◂**Fig. 6** The SEA complex controls mitophagy. **a** Wild type (BY4741) and indicated deletion strains of SEA components expressing Idp1-GFP were grown in SL media. Cells were harvested at indicated time points and conditions (t0—exponential (logarithmic) phase). Whole cell extracts prepared and analyzed by Western blot with antibodies against GFP and Pgk1. **b** Cells were observed by fluorescence microscopy at t0 and at the stationary phase after 24 h of growth. **c**, **d** The mitophagy flux was calculated as a ratio (%) of free GFP to total GFP signal (combined free GFP and Idp1-GFP) in corresponding Western blots from **a**. The quantification of the signals on the Western blots from three independent experiments was done with Image J. **e** Calculation of % of cells with fluorescent signal in the vacuole from **b**. 150 cells were observed for each strain

found in the SEA complex immunoprecipitations and once again this interaction is greatly induced (3–4 times more PSMs) in the conditions, which stimulate mitochondria damage (Table S4, Table S5).

The SEA complex affects both nitrogen starvation-induced mitophagy and stationary phase mitophagy. Moreover, the SEA complex may play additional roles in the mitochondria quality control. Our experiments with the respiratory chain inhibitor antimycin A demonstrate that the SEA complex is also involved in the regulation of non-specific autophagy that is induced by dysfunctional mitochondria. These effects seem to be ultimately related to the role of the SEA complex in TORC1 regulation. The suppression of mitophagy is stronger in the deletion strains of the SEACIT components compared to the deletions of SEACAT members. This observation can be explained because deletions of TORC1 inhibitors from SEACIT complex induce hyperactivation of TORC1 and, therefore, inhibit mitophagy. When this manuscript was under revision, a study completely in agreement with this conclusion demonstrated that TORC1 regulates mitophagy via SEACIT [51].

So far a search for modulators of mitophagy in yeast has been reported in three genome-wide studies. Mitophagy was induced either by growth until stationary phase and under respiratory conditions [52, 53] or by treatment with the rapamycin [54]. Only three proteins were common in these studies, probably due to the fact that each screen used different setups and growth conditions to induce autophagy. This might also explain why the involvement of the SEA complex in mitophagy was not discovered until now (see also [51]). There are other examples of proteins that were overlooked in these surveys and yet appeared important for mitophagy. This is the case of MAP kinase Slt2 and its partner Bck1, which were shown to be required for the mitophagy in two recent independent studies [38, 55]. Yet Bck1 was detected in only one genome-wide screen of mitophagy modulators [53], while Slt2 was not found in any. Remarkably, five SEA complex genes appeared in the genome-wide survey of synthetic genetic arrays exhibit genetic interactions with Bck1 and Slt2 [16, 56]. At the same time, one of the three proteins identified in all three genome-wide mitophagy screens was Vps41—a member of the HOPS tethering complex that controls membrane fusion at the vacuole. Another HOPS and vCLAMPs member—Ypt7 was found in two screens [52, 53] and Vps39—in one [53]. Therefore, it will be tempting to revisit the role of the HOPS complex in mitophagy.

In humans, a growing body of data illustrates the impact of the GATOR–mTORC1 regulatory pathway in diseases, especially in cancer [13]. GATOR1 components, notably NPRL2, were suggested to be tumor suppressors in various types of cancers [11, 57]. The molecular basis of the tumor suppression by GATOR1 members is largely unknown, although it is reasonable to suggest that they might be associated with GATOR1's function in the regulation of the mTORC1 pathway. Our results demonstrate that the GATOR homologs in yeast are upregulated during cultivation on lactate. It is well known that tumor cells are characterized by elevated glucose uptake and lactic acid release—a process known as Warburg effect [58]. It is, therefore, conceivable that during cultivation on lactate and in the absence of the SEACIT/GATOR1 hyperactivated mTORC1 will promote cell mass growth. Future experiments will be needed to confirm this hypothesis, as well as the potential role of lysosome–mitochondria contact sites in tumor development.

In summary, our data indicate that the SEA complex responds to changes in growth conditions, interacts with mitochondria and plays an important role in mitophagy regulation.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests

References

1. Loewith R, Hall MN (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 189(4):1177–1201. https://doi.org/10.1534/genetics.111.133363
2. Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. Cell 149(2):274–293. https://doi.org/10.1016/j.cell.2012.03.017
3. Huang K, Fingar DC (2014) Growing knowledge of the mTOR signaling network. Semin Cell Dev Biol 36:79–90. https://doi.org/10.1016/j.semcdb.2014.09.011

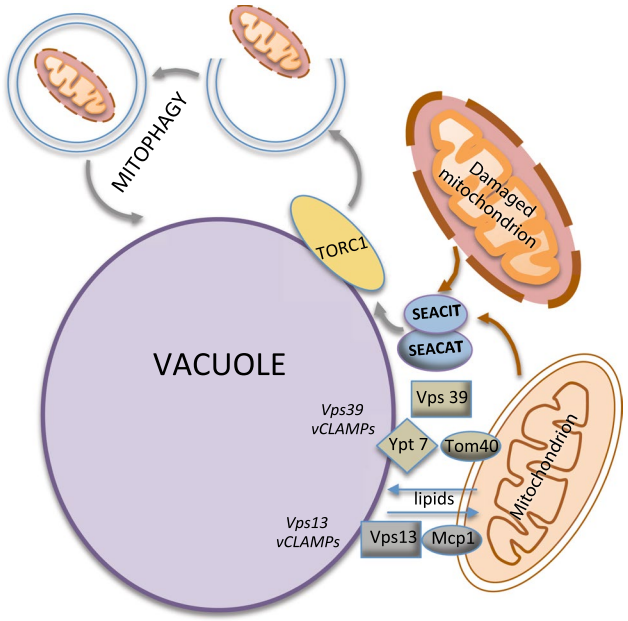

Fig. 7 A model for the interaction between the vacuole and mitochondria. The contacts between two organelles are maintained through the vacuole–mitochondria contact sites. Vps39-vCLAMPs contain Vps39, Ypt7, Tom40; Vps13-vCLAMPs contain Vps13, Mcp1. vCLAMPs are required for lipid exchange (blue arrows). The SEA complex, dynamically associated with the vacuole membrane, interacts with both healthy and damaged mitochondria (brown arrows) and is required for the maintenance of vCLAMPs. The signal of dysfunctional mitochondria is transmitted via the SEA complex to TORC1 (grey arrows), which in turn promotes mitophagy. Other contact sites both for the vacuole and mitochondria are not labeled for simplicity

4. Ruetenik A, Barrientos A (2015) Dietary restriction, mitochondrial function and aging: from yeast to humans. Biochem Biophys Acta 1847 11:1434–1447. https://doi.org/10.1016/j.bbabio.2015.05.005
5. Groenewoud MJ, Zwartkruis FJ (2013) Rheb and mammalian target of rapamycin in mitochondrial homoeostasis. Open Biol 3(12):130185. https://doi.org/10.1098/rsob.130185
6. Li M, Zhao L, Liu J, Liu A, Jia C, Ma D, Jiang Y, Bai X (2010) Multi-mechanisms are involved in reactive oxygen species regulation of mTORC1 signaling. Cell Signal 22(10):1469–1476. https://doi.org/10.1016/j.cellsig.2010.05.015
7. Kawai S, Urban J, Piccolis M, Panchaud N, De Virgilio C, Loewith R (2011) Mitochondrial genomic dysfunction causes dephosphorylation of Sch9 in the yeast *Saccharomyces cerevisiae*. Eukaryot Cell 10(10):1367–1369. https://doi.org/10.1128/EC.05157-11
8. Jazwinski SM, Kriete A (2012) The yeast retrograde response as a model of intracellular signaling of mitochondrial dysfunction. Front Physiol 3:139. https://doi.org/10.3389/fphys.2012.00139
9. Dokudovskaya S, Waharte F, Schlessinger A, Pieper U, Devos DP, Cristea IM, Williams R, Salamero J, Chait BT, Sali A, Field MC, Rout MP, Dargemont C (2011) A conserved coatomer-related complex containing Sec13 and Seh1 dynamically associates with the vacuole in *Saccharomyces cerevisiae*. Mol Cell Proteomics 10(6):M110006478. https://doi.org/10.1074/mcp.m110.006478
10. Dokudovskaya S, Rout MP (2011) A novel coatomer-related SEA complex dynamically associates with the vacuole in yeast and is implicated in the response to nitrogen starvation. Autophagy 7(11):1392–1393. https://doi.org/10.4161/auto.7.11.17347
11. Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED, Carter SL, Meyerson M, Sabatini DM (2013) A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science 340(6136):1100–1106. https://doi.org/10.1126/science.1232044
12. Panchaud N, Peli-Gulli MP, De Virgilio C (2013) SEACing the GAP that nEGOCiates TORC1 activation: evolutionary conservation of Rag GTPase regulation. Cell Cycle 12(18):1–5. https://doi.org/10.4161/cc.26000
13. Dokudovskaya S, Rout MP (2015) SEA you later alli-GATOR—a dynamic regulator of the TORC1 stress response pathway. J Cell Sci 128(12):2219–2228. https://doi.org/10.1242/jcs.168922
14. Bar-Peled L, Sabatini DM (2014) Regulation of mTORC1 by amino acids. Trends Cell Biol 24(7):400–406. https://doi.org/10.1016/j.tcb.2014.03.003
15. Powis K, De Virgilio C (2016) Conserved regulators of Rag GTPases orchestrate amino acid-dependent TORC1 signaling. Cell Discov 2:15049. https://doi.org/10.1038/celldisc.2015.49
16. Costanzo M, Baryshnikova A, Bellay J, Kim Y, Spear ED, Sevier CS, Ding H, Koh JL, Toufighi K, Mostafavi S, Prinz J, St Onge RP, VanderSluis B, Makhnevych T, Vizeacoumar FJ, Alizadeh S, Bahr S, Brost RL, Chen Y, Cokol M, Deshpande R, Li Z, Lin ZY, Liang W, Marback M, Paw J, San Luis BJ, Shuteriqi E, Tong AH, van Dyk N, Wallace IM, Whitney JA, Weirauch MT, Zhong G, Zhu H, Houry WA, Brudno M, Ragibizadeh S, Papp B, Pal C, Roth FP, Giaever G, Nislow C, Troyanskaya OG, Bussey H, Bader GD, Gingras AC, Morris QD, Kim PM, Kaiser CA, Myers CL, Andrews BJ, Boone C (2010) The genetic landscape of a cell. Science 327(5964):425–431. https://doi.org/10.1126/science.1180823
17. Costanzo M, VanderSluis B, Koch EN, Baryshnikova A, Pons C, Tan G, Wang W, Usaj M, Hanchard J, Lee SD, Pelechano V, Styles EB, Billmann M, van Leeuwen J, van Dyk N, Lin ZY, Kuzmin E, Nelson J, Piotrowski JS, Srikumar T, Bahr S, Chen Y, Deshpande R, Kurat CF, Li SC, Li Z, Usaj MM, Okada H, Pascoe N, San Luis BJ, Sharifpoor S, Shuteriqi E, Simpkins SW, Snider J, Suresh HG, Tan Y, Zhu H, Malod-Dognin N, Janjic V, Przulj N, Troyanskaya OG, Stagljar I, Xia T, Ohya Y, Gingras AC, Raught B, Boutros M, Steinmetz LM, Moore CL, Rosebrock AP, Caudy AA, Myers CL, Andrews B, Boone C (2016) A global genetic interaction network maps a wiring diagram of cellular function. Science. https://doi.org/10.1126/science.aaf1420
18. Algret R, Fernandez-Martinez J, Shi Y, Kim SJ, Pellarin R, Cimermancic P, Cochet E, Sali A, Chait BT, Rout MP, Dokudovskaya S (2014) Molecular architecture and function of the SEA complex, a modulator of the TORC1 pathway. Mol Cell Proteomics 13(11):2855–2870. https://doi.org/10.1074/mcp.M114.039388
19. Elbaz-Alon Y, Rosenfeld-Gur E, Shinder V, Futerman AH, Geiger T, Schuldiner M (2014) A dynamic interface between vacuoles and mitochondria in yeast. Dev Cell 30(1):95–102. https://doi.org/10.1016/j.devcel.2014.06.007
20. Merz S, Westermann B (2009) Genome-wide deletion mutant analysis reveals genes required for respiratory growth, mitochondrial genome maintenance and mitochondrial protein synthesis in Saccharomyces cerevisiae. Genome Biol 10(9):R95. https://doi.org/10.1186/gb-2009-10-9-r95
21. Honscher C, Mari M, Auffarth K, Bohnert M, Griffith J, Geerts W, van der Laan M, Cabrera M, Reggiori F, Ungermann C (2014) Cellular metabolism regulates contact sites between vacuoles and mitochondria. Dev Cell 30(1):86–94. https://doi.org/10.1016/j.devcel.2014.06.006

22. Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Rout MP, Sali A (2007) Determining the architectures of macromolecular assemblies. Nature 450(7170):683–694. https://doi.org/10.1038/nature06404
23. Neklesa TK, Davis RW (2009) A genome-wide screen for regulators of TORC1 in response to amino acid starvation reveals a conserved Npr2/3 complex. PLoS Genet 5(6):e1000515. https://doi.org/10.1371/journal.pgen.1000515
24. LaCava J, Molloy KR, Taylor MS, Domanski M, Chait BT, Rout MP (2015) Affinity proteomics to study endogenous protein complexes: pointers, pitfalls, preferences and perspectives. Biotechniques 58(3):103–119. https://doi.org/10.2144/000114262
25. Brosch M, Yu L, Hubbard T, Choudhary J (2009) Accurate and sensitive peptide identification with Mascot Percolator. J Proteome Res 8(6):3176–3181. https://doi.org/10.1021/pr800982s
26. Kurihara Y, Kanki T, Aoki Y, Hirota Y, Saigusa T, Uchiumi T, Kang D (2012) Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. J Biol Chem 287(5):3265–3272. https://doi.org/10.1074/jbc.M111.280156
27. Wu X, Tu BP (2011) Selective regulation of autophagy by the Iml1-Npr2-Npr3 complex in the absence of nitrogen starvation. Mol Biol Cell 22(21):4124–4133. https://doi.org/10.1091/mbc.E11-06-0525
28. Chong YT, Koh JL, Friesen H, Duffy SK, Cox MJ, Moses A, Moffat J, Boone C, Andrews BJ (2015) Yeast proteome dynamics from single cell imaging and automated analysis. Cell 161(6):1413–1424. https://doi.org/10.1016/j.cell.2015.04.051
29. Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK (2003) Global analysis of protein localization in budding yeast. Nature 425(6959):686–691. https://doi.org/10.1038/nature02026nature02026
30. Gonzalez Montoro A, Auffarth K, Honscher C, Bohnert M, Becker T, Warscheid B, Reggiori F, van der Laan M, Frohlich F, Ungermann C (2018) Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. Dev cell 45(5):621-636 e627. https://doi.org/10.1016/j.devcel.2018.05.011
31. Lang AB, John Peter AT, Walter P, Kornmann B (2015) ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. J Cell Biol 210(6):883–890. https://doi.org/10.1083/jcb.201502105
32. John Peter AT, Herrmann B, Antunes D, Rapaport D, Dimmer KS, Kornmann B (2017) Vps13-Mcp1 interact at vacuole-mitochondria interfaces and bypass ER-mitochondria contact sites. J Cell Biol. https://doi.org/10.1083/jcb.201610055
33. Binda M, Peli-Gulli MP, Bonfils G, Panchaud N, Urban J, Sturgill TW, Loewith R, De Virgilio C (2009) The Vam6 GEF controls TORC1 by activating the EGO complex. Mol Cell 35(5):563–573. https://doi.org/10.1016/j.molcel.2009.06.033
34. Kuzmin E, VanderSluis B, Wang W, Tan G, Deshpande R, Chen Y, Usaj M, Balint A, Mattiazzi Usaj M, van Leeuwen J, Koch EN, Pons C, Dagilis AJ, Pryszlak M, Wang JZY, Hanchard J, Riggi M, Xu K, Heydari H, San Luis BJ, Shuteriqi E, Zhu H, Van Dyk N, Sharifpoor S, Costanzo M, Loewith R, Caudy A, Bolnick D, Brown GW, Andrews BJ, Boone C, Myers CL (2018) Systematic analysis of complex genetic interactions. Science. https://doi.org/10.1126/science.aao1729
35. van Leeuwen J, Pons C, Mellor JC, Yamaguchi TN, Friesen H, Koschwanez J, Usaj MM, Pechlaner M, Takar M, Usaj M, VanderSluis B, Andrusiak K, Bansal P, Baryshnikova A, Boone CE, Cao J, Cote A, Gebbia M, Horecka G, Horecka I, Kuzmin E, Legro N, Liang W, van Lieshout N, McNee M, San Luis BJ, Shaeri F, Shuteriqi E, Sun S, Yang L, Youn JY, Yuen M, Costanzo M, Gingras AC, Aloy P, Oostenbrink C, Murray A, Graham TR, Myers CL, Andrews BJ, Roth FP, Boone C (2016) Exploring genetic suppression interactions on a global scale. Science. https://doi.org/10.1126/science.aag0839
36. Usaj M, Tan Y, Wang W, VanderSluis B, Zou A, Myers CL, Costanzo M, Andrews B, Boone C (2017) TheCellMap.org: a web-accessible database for visualizing and mining the global yeast genetic interaction network. G3 (Bethesda) 7(5):1539–1549. https://doi.org/10.1534/g3.117.040220
37. Panchaud N, Peli-Gulli MP, De Virgilio C (2013) Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. Sci Signal 6(277):ra42. https://doi.org/10.1126/scisignal.2004112
38. Deffieu M, Bhatia-Kissova I, Salin B, Klionsky DJ, Pinson B, Manon S, Camougrand N (2013) Increased levels of reduced cytochrome b and mitophagy components are required to trigger nonspecific autophagy following induced mitochondrial dysfunction. J Cell Sci 126(Pt 2):415–426. https://doi.org/10.1242/jcs.103713
39. Lee J, Giordano S, Zhang J (2012) Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. Biochem J 441(2):523–540. https://doi.org/10.1042/BJ20111451
40. Pan Y, Schroeder EA, Ocampo A, Barrientos A, Shadel GS (2011) Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. Cell Metab 13(6):668–678. https://doi.org/10.1016/j.cmet.2011.03.018
41. Graef M, Nunnari J (2011) Mitochondria regulate autophagy by conserved signalling pathways. EMBO J 30(11):2101–2114. https://doi.org/10.1038/emboj.2011.104
42. Sutter BM, Wu X, Laxman S, Tu BP (2013) Methionine inhibits autophagy and promotes growth by inducing the SAM-responsive methylation of PP2A. Cell 154(2):403–415. https://doi.org/10.1016/j.cell.2013.06.041
43. Kira S, Tabata K, Shirahama-Noda K, Nozoe A, Yoshimori T, Noda T (2014) Reciprocal conversion of Gtr1 and Gtr2 nucleotide-binding states by Npr2-Npr3 inactivates TORC1 and induces autophagy. Autophagy 10(9):1565–1578. https://doi.org/10.4161/auto.29397
44. Laxman S, Sutter BM, Shi L, Tu BP (2014) Npr2 inhibits TORC1 to prevent inappropriate utilization of glutamine for biosynthesis of nitrogen-containing metabolites. Sci Signal 7(356):ra120. https://doi.org/10.1126/scisignal.2005948
45. Laxman S, Sutter BM, Wu X, Kumar S, Guo X, Trudgian DC, Mirzaei H, Tu BP (2013) Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. Cell 154(2):416–429. https://doi.org/10.1016/j.cell.2013.06.043
46. Klionsky DJ (2016) Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy 12(1):1–222. https://doi.org/10.1080/15548627.2015.1100356
47. Abeliovich H, Zarei M, Rigbolt KT, Youle RJ, Dengjel J (2013) Involvement of mitochondrial dynamics in the segregation of mitochondrial matrix proteins during stationary phase mitophagy. Nat Commun 4:2789. https://doi.org/10.1038/ncomms3789
48. Sugiura A, McLelland GL, Fon EA, McBride HM (2014) A new pathway for mitochondrial quality control: mitochondrial-derived vesicles. EMBO J 33(19):2142–2156. https://doi.org/10.15252/embj.201488104
49. Kissova I, Salin B, Schaeffer J, Bhatia S, Manon S, Camougrand N (2007) Selective and non-selective autophagic degradation of mitochondria in yeast. Autophagy 3(4):329–336
50. Wei Y, Chiang WC, Sumpter R Jr, Mishra P, Levine B (2017) Prohibitin 2 is an inner mitochondrial membrane mitophagy receptor. Cell 168(1–2):224-238 e210. https://doi.org/10.1016/j.cell.2016.11.042

51. Liu Y, Okamoto K (2018) The TORC1 signaling pathway regulates respiration-induced mitophagy in yeast. Biochem Biophys Res Commun 502(1):76–83. https://doi.org/10.1016/j.bbrc.2018.05.123
52. Okamoto K, Kondo-Okamoto N, Ohsumi Y (2009) Mitochondria- anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev Cell 17(1):87–97. https://doi.org/10.1016/j.devcel.2009.06.013
53. Kanki T, Wang K, Baba M, Bartholomew CR, Lynch-Day MA, Du Z, Geng J, Mao K, Yang Z, Yen WL, Klionsky DJ (2009) A genomic screen for yeast mutants defective in selective mitochondria autophagy. Mol Biol Cell 20(22):4730–4738. https://doi.org/10.1091/mbc.E09-03-0225
54. Muller M, Kotter P, Behrendt C, Walter E, Scheckhuber CQ, Entian KD, Reichert AS (2015) Synthetic quantitative array technology identifies the Ubp3-Bre5 deubiquitinase complex as a negative regulator of mitophagy. Cell Rep 10(7):1215–1225. https://doi.org/10.1016/j.celrep.2015.01.044
55. Mao K, Wang K, Zhao M, Xu T, Klionsky DJ (2011) Two MAPK-signaling pathways are required for mitophagy in Saccharomyces cerevisiae. J Cell Biol 193(4):755–767. https://doi.org/10.1083/jcb.201102092
56. Algret R, Dokudovskaya S (2012) The SEA complex—the beginning. Biopolym Cell 28(N4):281–284
57. Li F, Wang F, Haraldson K, Protopopov A, Duh FM, Geil L, Kuzmin I, Minna JD, Stanbridge E, Braga E, Kashuba VI, Klein G, Lerman MI, Zabarovsky ER (2004) Functional characterization of the candidate tumor suppressor gene NPRL2/G21 located in 3p21.3C. Cancer Res 64(18):6438–6443
58. Luc R, Tortorella SM, Ververis K, Karagiannis TC (2015) Lactate as an insidious metabolite due to the Warburg effect. Mol Biol Rep 42(4):835–840. https://doi.org/10.1007/s11033-015-3859-9