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Study on the function of sphingolipids during the starvation response

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Research aims

Under nutrient-starved conditions, cells draw on a variety of responses to survive. In yeast, starvation causes a shift from fermentation to aerobic respiration. It also causes large changes in membrane dynamics, as several secreted proteins are redirected to vacuoles for degradation and autophagy is induced to recycle nutrients. We are currently studying the role of sphingolipids in the starvation response in yeast. We previously found that 1) synthesis of a specific sphingolipid species is required for autophagy[1] and 2) cells rapidly die under nitrogen starvation (-N) conditions if sphingolipid metabolism is perturbed. In this study, we focused on elucidating the importance of sphingolipid metabolism under -N conditions.

Methods

Sphingolipid synthesis (Fig. 1A) was inhibited at each step, and cell viability was calculated by counting dead cells that were intensely stained with phloxine B. Vacuole lumens were visualized by using 2′,7′-Dichlorofluorescein diacetate (DCFDA), which fluoresces green under acidic conditions.

Results

A mannosylinositol phosphorylceramide synthase mutant rapidly dies under -N conditions

Mutant cells (*csg1*Δ *csh1*Δ) that are defective in synthesizing mannosylinositol phosphorylceramide (MIPC) rapidly die under -N conditions (Fig. 1B). Interestingly, *csg1*Δ *csh1*Δ cells are resistant to carbon and nitrogen starvation (-NC). This result suggests that some energy-consuming process takes place during the -N response and that during this response, sphingolipid synthesis/metabolism becomes extremely important for cell survival. We examined the effect of myriocin, an inhibitor of *de novo* sphingolipid synthesis, on *csg1*Δ *csh1*Δ cells under -N conditions, and found that it greatly reduced cell death. This indicates that it is the accumulation of the MIPC precursor inositol phosphorylceramide (IPC), rather than the absence of MIPC, that has a role in the rapid cell death that occurs under -N conditions. Sphingolipids are composed of sphingoid long-chain bases (LCBs) and fatty acids, both of which undergo hydroxylation. *Saccharomyces cerevisiae* contains five IPC species, each differing in the number and/or positions of hydroxyl groups on the LCB and fatty acid moieties. MIPC-deficient cells defective in hydroxylation of the LCB moiety (*csg1*Δ *csh1*Δ *sur2*Δ cells) were resistant to -N (Fig. 1C), indicating that the accumulation of IPCs with hydroxyl groups on the LCB causes cell death under -N conditions.

Intracellular Ca^{2+} is responsible for MIPC synthase mutant cell death under -N conditions

IPCs with hydroxyl groups on the LCB moiety are thought to cluster through binding to Ca^{2+} and hydrogen bonding between their hydroxyl groups, leading to local destabilization of membranes.[2] Interestingly, *csg1*Δ *csh1*Δ cells cultured in medium lacking Ca^{2+} did not die after being transferred to -N medium. This result indicates that intracellular Ca^{2+} accumulated before undergoing -N is involved in *csg1*Δ *csh1*Δ cell death. Since the vacuole is the major Ca^{2+} storage organelle in yeast, we visualized the vacuoles of *csg1*Δ *csh1*Δ cells under -N conditions. In approximately half of the dying cells, vacuolar contents leaked prior to the death and shrinkage of the cells. Thus, at least in half of the dead cells, the leakage of vacuolar contents seems to be the trigger for cell death. Topologically, IPC is localized to the luminal (extracytosolic) leaflet of the lipid bilayers of the vacuolar membrane. Therefore, Ca^{2+} might directly bind to IPC in the luminal leaflet, leading to the destabilization of the vacuolar membrane. Secretory vesicles are redirected to the vacuole under -N conditions.[3] We speculate that, because of this rearrangement in membrane dynamics, IPC over-accumulates at the vacuolar membrane in *csg1*Δ *csh1*Δ cells under -N conditions. The accumulated IPC may then cluster excessively through binding to Ca^{2+} and hydrogen binding between the hydroxyl groups, and this clustering culminates in a local disruption of the vacuolar membrane (Fig. 2).[2]

The inactivation of TOR complex 2 leads to MIPC synthase mutant cell death

TOR complexes (TORC1 and TORC2) regulate various processes including protein synthesis and nutrient starvation. Therefore, we examined whether the rapid death of *csg1*Δ *csh1*Δ cells under nutrient starvation occurs through the inactivation of TOR complexes. Specific inactivation of TORC2 but not TORC1 caused the rapid death of *csg1*Δ *csh1*Δ cells, even in nutrient-rich medium. This result

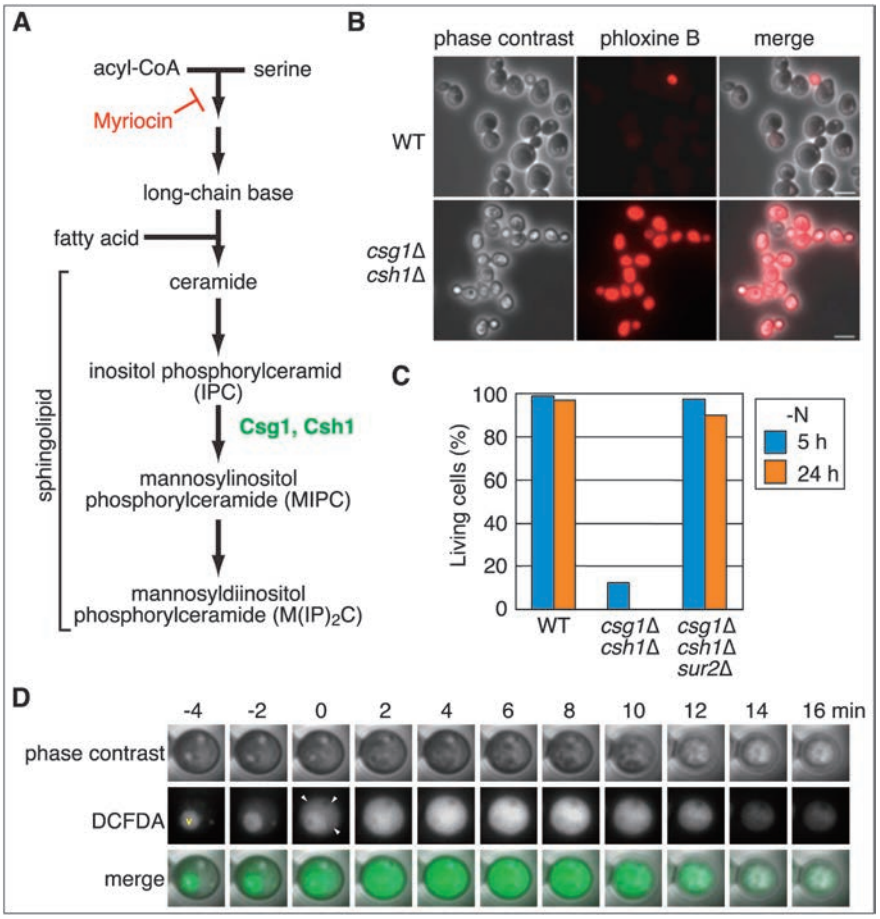

Fig. 1. Accumulation of IPC leads to cell death through leakage of vacuolar contents. A. Sphingolipid synthesis in yeast. B. Cells at log phase were transferred to -N medium and cultured for 4 h. Dead cells were stained with phloxine B. Bar, 5 μm. C. MIPC-deficient cells defective in hydroxylation of LCB (*csg1*Δ *csh1*Δ *sur2*Δ), as well as wild-type (WT) and *csg1*Δ *csh1*Δ cells, were cultured in -N medium, and their viability was calculated at the indicated time. D. Vacuoles of *csg1*Δ *csh1*Δ mutant cells (v) were visualized with DCFDA and observed successively at 2 h of -N. In approximately half of the dying cells, vacuolar contents leaked (arrowhead) prior to the death and shrinkage of the cell. The time when the leakage of vacuolar contents became obvious was defined as 0 min.

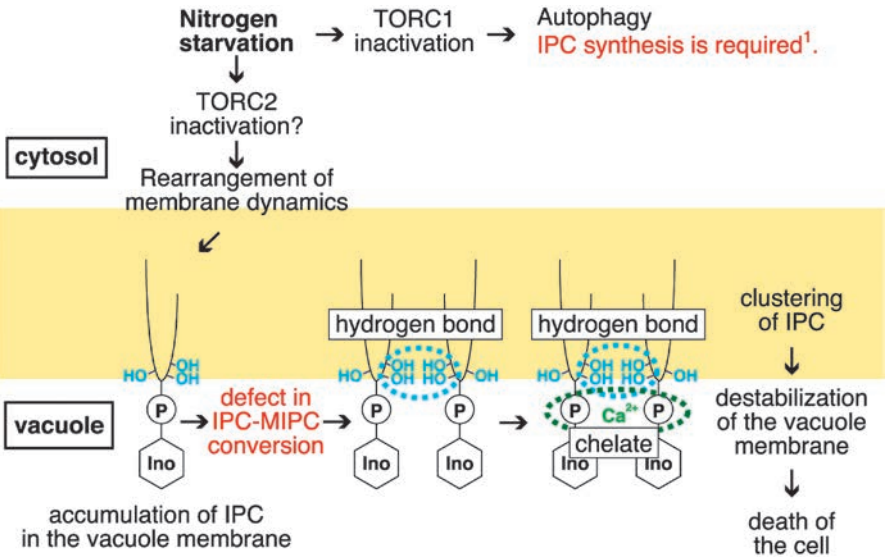

Fig. 2. Working hypothesis. -N invokes certain cellular responses, some of which are induced through the inactivation of TOR complexes. In the early starvation responses that are downstream of TORC2 inactivation, the conversion of IPC to MIPC is essential for cell survival. Perturbation of IPC–MIPC conversion results in accumulation in the vacuolar membrane of IPC species that have hydroxyl groups on their LCB moieties. This causes the disruption of the vacuolar membrane through excessive clustering of IPC via binding to Ca^{2+} and hydrogen bonding between hydroxyl groups. On the other hand, *de novo* synthesis of IPC is required for autophagy,[1)] which is induced through TORC1 inactivation. In summary, the appropriate flow of sphingolipid synthesis and metabolism is extremely important for starvation responses occurring through TOR complexes.

strongly suggests that the rapid death of *csg1*Δ *csh1*Δ cells under -N conditions is a downstream event of TORC2 inactivation.

Conclusion

Under -N conditions, the conversion of IPC to MIPC is a critical step for cell survival.[2] On the other hand, *de novo* synthesis of IPC is required for autophagy.[1] Thus, the synthesis and metabolism of sphingolipids is extremely important for early starvation responses. Processes that require normal IPC–MIPC conversion are likely downstream of TORC2 inactivation. During such processes, excessive accumulation of IPC at the vacuolar membrane may have a fatal effect, *i.e.,* the leakage of vacuolar contents to the whole cell through some mechanism involving Ca^{2+}.

References

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