Principal Investigator: Motohiro Tani
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PURPOSE Glycosphingolipids are important membrane lipids for eukaryotes that are involved in maintaining various cellular functions. The multifunctionality of glycosphingolipids is thought to be supported by their structural diversity, but the details are not clear. In addition, while glycosphingolipids exhibit extremely complex structural diversity within an organism, their basic structures clearly differ between species. Thus, in order to investigate the significance of glycosphingolipid diversity, it is important to consider the existence of both "structural diversity within a single organism" and "structural diversity between different species". In this study, we aimed to elucidate the significance of the diversity of glycosphingolipids and the significance of the existence of glycosphingolipids themselves using *Saccharomyces cerevisiae*. Specifically, we tried to elucidate the cellular functions supported by the structural diversity of glycosphingolipids and the reasons why glycosphingolipid structures differ between different species.

METHODS In order to investigate the significance of sphingolipid diversity within an organism, we used a yeast mutant strain (*csg1*Δ *csh1*Δ *sur2*Δ *scs7*Δ (*ccss*Δ) cells) that has only one type of glycosphingolipid. *ccss*Δ cells exhibited hypersensitivity to pleiotropic stress, and we screened for suppressor mutations that can suppress this abnormal phenotype. We also established a yeast strain in which the sphingoid base structure of glycosphingolipids was replaced with a mammalian type (sphingosine). Furthermore, we focused on sterols that are functionally related to glycosphingolipids, and established a yeast strain in which both sterols and sphingoid bases were structurally replaced with mammalian types (spingosine and cholesterol).

RESULTS We identified *TRS85*, which is involved in intracellular vesicular transport, and the plasma membrane phospholipid flippase gene *DNF2* as suppressor mutations that can suppress the stress sensitivities of *ccss*Δ cells. The deletion of *TRS85* suppresses the increase in plasma membrane permeability and restores the damage to cell wall integrity observed in *ccss*Δ cells, and it was suggested that these are also related to the rescue of *ccss*Δ cells from pleiotropic stress. Furthermore, we examined effects of the deletion of *LEM3*, which encodes the regulatory subunit of *DNF2* and its paralog *DNF1*, on *ccss*Δ cells, and it was suggested that lack of plasma-membrane flippase confers stress resistance through a completely different mechanism than that of *TRS85*.

Yeast in which the sphingoid base structure has been replaced with sphingosine (SPH cells) is highly sensitive to various stresses such as high osmotic pressure, pH, high temperature, organic acids, and SDS. It was also suggested that these hypersensitivities are partly due to a decrease in the integrity of plasma membranes and cell walls. We considered that the abnormal phenotype observed in SPH cells may be due to the fact that the sterol involved in the formation of microdomains together with glycosphingolipids is yeast-type (ergosterol). Thus, a yeast strain with both sphingoid bases and sterols of mammalian type was established (SPH/Chol cells). The abnormal phenotypes observed in the SPH cells were also observed in the SPH/Chol cells. To our knowledge, this is the first study showing that *S. cerevisiae* can grow even if sphingoid base and sterol structures are simultaneously replaced with mammalian types.