Study of organelle size control in yeast

Kazunori KUME

Graduate School of Integrated Sciences for Life, Hiroshima University

Research objective

Membrane-bound organelles are compartmentalized by membranes in eukaryotic cells and are of an appropriate size. Eukaryotic cells are thought to have acquired multiple cell functions through the uptake of organelles; however, it is unclear how cells control organelle size and how this affects cell function. Therefore, the purpose of this study was to elucidate the mechanism of organelle size control and to investigate the effect of its perturbation on cell function using fission yeast as a model organism. To understand the mechanism of nuclear size control, we screened a fission yeast deletion library for nuclear size mutants.[1,2)] The analysis of these mutants revealed that, in addition to nuclear enlargement, they presented defects in the structure of other organelles. Therefore, we focused on the nucleus and other organelles of the nuclear size mutants to elucidate the mechanism of organelle size control. Furthermore, to clarify the effect of organelle size control on cell function, we performed transcriptome analysis and cell proliferation assays using these organelle size mutants.

Methods

1. Observation of organelles in living cells and image analysis

Organelles were visualized using fluorescent probes that specifically stain certain organelles in a yeast strain whose nucleus is labeled with a fluorescent protein. We used Cut11 as a nuclear membrane marker. ImageJ free software was used to analyze organelle structures. For any organelle that could not be clearly stained with a fluorescent probe, we used yeast strains in which GFP or mCherry was fused with a protein localized at that specific organelle.

2. Transcriptome analysis and cell proliferation assay

After growing cells until the exponential growth phase, RNA was extracted and subjected to RNA sequencing (RNA-seq) for transcriptome analysis. Cell proliferation was assessed by spot assay on an agar medium using several inhibitors, including a DNA synthesis inhibitor, a DNA replication inhibitor, a protein synthesis inhibitor, and a lipid synthesis inhibitor. Yeast cells were suspended in sterile water at a concentration of 5×10^6 cells/mL and spotted on synthetic medium containing yeast extract. Yeast cell growth was evaluated at different temperatures (25–36 °C).

Results

1. Analysis of the mechanism of organelle size control

To investigate the size of the nucleus and that of other organelles in living cells, we searched for an experimental condition allowing simultaneous visualization of the nucleus and other organelles, such as lipid droplets, vacuoles, mitochondria, and the endoplasmic reticulum. We tested commercially available fluorescent probes and found that probes for lipid droplets (BODIPY, Thermo Fisher Scientific), vacuoles (FM4-64, Thermo Fisher Scientific), and mitochondria (MitoTracker, Thermo Fisher Scientific) were suitable for the experimental conditions. As for the endoplasmic reticulum, the signal of the fluorescent probe ER-Tracker (Thermo Fisher Scientific) was weak; therefore, a strain in which a fluorescent protein was fused to a protein localized in the endoplasmic reticulum was constructed. We examined multiple proteins localized in the endoplasmic reticulum (i.e., Sec63, Elo2, and Rtn1) and found that a strain in which the fatty acid elongase Elo2 was fused with a fluorescent protein was suitable for the experimental conditions. Under these conditions, we observed nuclei and other organelles in nuclear size mutants,[1,2)] and found that the structures of vacuoles and lipid droplets in these mutants were different from those in the wild-type strain. In particular, the vacuole size in the nuclear size mutants was reduced to almost half of that in the wild-type strain. Conversely, the size of lipid droplets in the nuclear size mutants was similar to that in the wild-type strain, but their number was lower. These results suggest that the size control mechanism of the nucleus might be associated with that of vacuoles and lipid droplets. To examine this possibility, we examined the effect of the deletion of the gene mediating the conversion of lipid droplets, which are lipid storage organelles, into structural lipids on the nuclear size of the mutants. Nuclear enlargement was partially suppressed in the double mutant, indicating that the size control mechanism of the nucleus and that of lipid droplets were associated. We are currently investigating the possible relationship between the size control mechanism of the nucleus and that of vacuoles.

2. Effects of the perturbation of organelle size control on cell function

Next, we examined the growth of organelle size mutants, in which the sizes of the nucleus and other organelles were altered, on agar medium containing various inhibitors. We found that the mutants showed severe growth defects in presence of a protein synthesis inhibitor. Next, we performed transcriptome analysis on an organelle size mutant strain and a wild-type strain. A total of 434 genes were upregulated and 281 genes were downregulated in the mutant strain compared to the wild-type strain. Gene Ontology (GO) enrichment analysis revealed that genes related to transcription and translation were enriched among the downregulated genes. We will soon examine the relationship between the perturbation of organelle size control and these phenotypes.

Conclusion

Our study revealed that mutants displaying enlarged nuclear size presented defects also in the size of other organelles. This suggests the existence of a size control mechanism shared among multiple organelles. In addition, organelle size mutants showed growth defects in presence of a protein synthesis inhibitor and fluctuations in the expression of genes involved in transcription and translation, indicating a functional linkage between organelle size control and these phenotypes.

References

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