ER-TO-PLASMA MEMBRANE TETHERING PROTEINS REGULATE THE ASYMMETRIC DISTRIBUTION OF PMA1P DURING CELL DIVISION IN *SACCHAROMYCES CEREVISIAE*

[1]YUNSU BANG, [2]YOUNGSOO JUN

[1,2]Gwangju Institute of Science and Technology, Gwangju 61005 South Korea
E-mail: [1]junys@gist.ac.kr

Abstract - The endoplasmic reticulum (ER) makes a physical contact with various intracellular organelles, such as mitochondria and lysosomes, as well as the plasma membrane (PM) via membrane contact sites. Although their cellular functions remain largely unknown, the membrane contact site between ER and the PM is thought to mediate lipid exchange between the two membranes. Here, we found that deletion of genes encoding ER-to-plasma membrane tethering proteins markedly destroyed the asymmetric distribution of Pma1p, which regulates replicative lifespan in the budding yeast *Saccharomyces cerevisiae*. Thus, our results suggest that ER-PM membrane contact sites regulate cell aging in yeast.

Index terms - Endoplasmic reticulum, Membrane contact site, Pma1p, Aging, *Saccharomyces cerevisiae.*

I. INTRODUCTION

The budding yeast *Saccharomyces cerevisiae* has been used as an experimental model to study cell aging, because yeast cells undergo a process termed replicative aging: yeast cells produce a limited number of daughter cells in their lifetime[1]. However, the molecular mechanism by which yeast cells stop producing their daughter cells remains poorly understood. Recent studies[2,3]showed that increased cytosolic pH in the mother cell establishes cytosolic pH asymmetry between the mother cell and daughter cell during cell division, contributing to aging process in budding yeast[4]. Furthermore, Pma1p, a proton ATPase in the plasma membrane, is a key contributing protein to this cytosolic pH asymmetry by being retained in the mother cell instead of being evenly separated into the daughter cell. However, the molecular mechanism underlying how Pma1p is asymmetrically distributed during yeast budding is completely unknown. The membrane contact site between the endoplasmic reticulum (ER) and the plasma membrane (PM) is a region of the ER that tightly associates with the PM, yet its function is largely undefined. A recently study[5]identified three families of ER-PM tethering proteins in the budding yeast: Ist2p, the tricalbins Tcb1p, Tcb2p, Tcb3p, and Scs2 and Scs22. Loss of all these tethering proteins causes the separation of the ER form the PM. In this present study, we identified a potential role of the ER-PM tethering proteins for regulating Pma1p asymmetric distribution during cell division and thus presumably cell aging in the budding yeast.

II. GENERATION OF YEAST STRAINS THAT LACK ER-PM TETHERING PROTEINS

To explore the biological function of the ER-PM membrane contact site, the six genes encoding ER-PM tethering proteins (Ist2p, Tcb1p, Tcb2p, Tcb3p, Scs2p, and Scs22p) were sequentially deleted in BY4742 yeast strain through a homologous recombination-mediated gene disruption method, and their deletion was confirmed by PCR using yeast genomic DNA as template (Fig. 1).

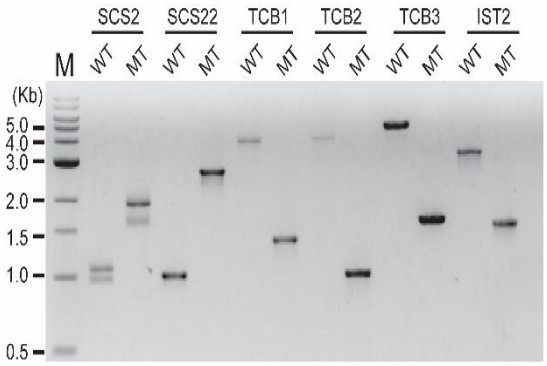


Figure 1. Generation of ER-PM tether mutants

III. ER MORPHOLOGY OF YEAST STRAINS THAT LACK ER-PM TETHERING PROTEINS

To analyze the ER morphology of yeast cells that lack one or more ER-PM tethering proteins, EGFP-HDEL was expressed, and its fluorescence was analyzed by fluorescent microscopy (Fig. 2). While cortical and perinuclear ER were clearly observed in wild-type and *scs2*□ yeast cells (Fig. 2A-B), deletion of both Scs2p and Scs22p markedly disrupted cortical ER (Fig. 2C). Furthermore, deletion of tricalbins in the *scs2*□ *scs22*□ yeast cells further destroyed ER-PM contacts (Fig. 2D-E). Finally, deletion of all the six ER-PM tethering proteins completely abolished ER-PM contacts, resulting in accumulation of cytosolic ER in the cytoplasm (Fig. 3). These results not only confirmed the previous result[5], but also revealed that each ER-PM tethering protein contributes to formation of normal ER-PM contacts.

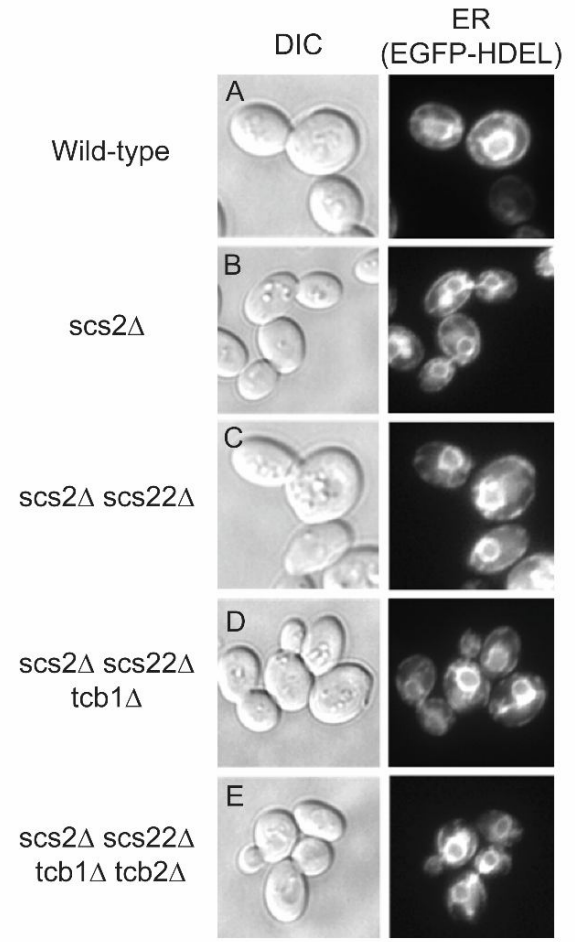


Figure 2. ER morphology of ER-PM tether mutants

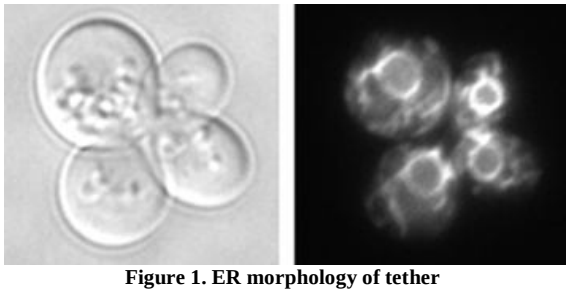
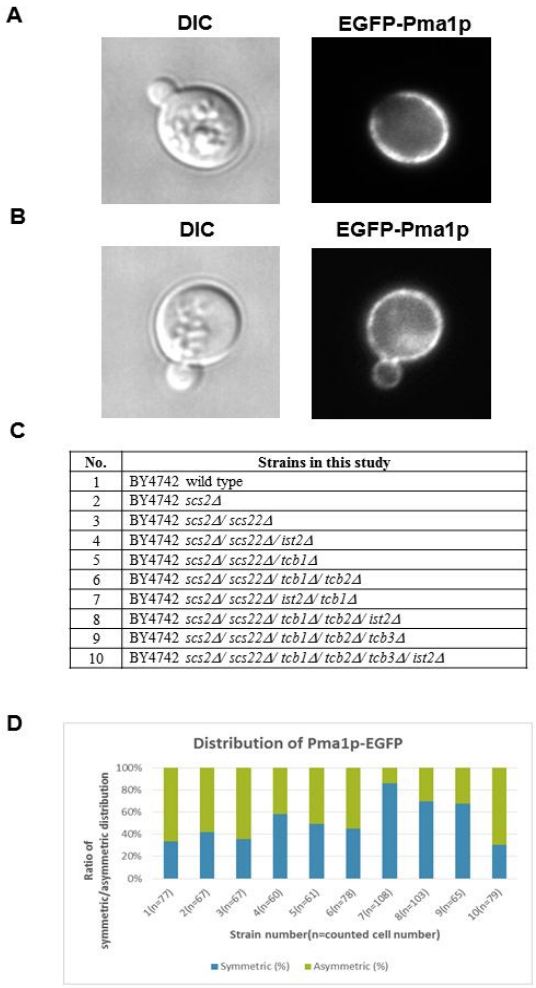


Figure 1. ER morphology of tether

IV. THE DELETION OF ER-PM TETHERING PROTEINS DISRUPTS THE ASYMMETRIC DISTRIBUTION OF Pma1p DURING CELL DIVISION

To examine of the cellular function of ER-PM tethering proteins, we first analyzed Pma1p distribution during cell division by expressing GFP-tagged Pma1p in the mutants lacking ER-PM tethering proteins. As shown in Fig. 4A, GFP-Pma1p was mostly retained in the mother cell during cell division, while the bud barely expressed GFP-Pma1p, which is consistent with the previous report[3]. Deletion of Scs2p alone or both Scs2p and Scs22plittle affected the Pma1p asymmetric distribution (Fig. 4E, bars 2-3).



No.	Strains in this study
1	BY4742 wild type
2	BY4742 *scs2Δ*
3	BY4742 *scs2Δ/ scs22Δ*
4	BY4742 *scs2Δ/ scs22Δ/ ist2Δ*
5	BY4742 *scs2Δ/ scs22Δ/ tcb1Δ*
6	BY4742 *scs2Δ/ scs22Δ/ tcb1Δ/ tcb2Δ*
7	BY4742 *scs2Δ/ scs22Δ/ ist2Δ/ tcb1Δ*
8	BY4742 *scs2Δ/ scs22Δ/ tcb1Δ/ tcb2Δ/ ist2Δ*
9	BY4742 *scs2Δ/ scs22Δ/ tcb1Δ/ tcb2Δ/ tcb3Δ*
10	BY4742 *scs2Δ/ scs22Δ/ tcb1Δ/ tcb2Δ/ tcb3Δ/ ist2Δ*

Figure 2. ER-PM tethering proteins regulate Pma1p asymmetric distribution during cell division.

Deletion of Scs2p/Scs22p/Ist2p/Tcb1p almost completely abolished the asymmetric distribution of Pma1p (Fig. 4B and Fig. 4D, bar 7), suggesting that ER-PM tethering proteins regulate Pma1p distribution. Interestingly, however, further deletion of Tcb2p in the mutant lacking Scs2p/Scs22p/Ist2p/Tcb1p largely restored the Pma1p asymmetric distribution. This unexpected result suggests that tricalbins antagonize with each other for regulating Pma1p distribution. Furthermore, the mutant lacking all the ER-PM tethering proteins showed Pma1p asymmetry almost identically with wild-type cells. Although further studies will be required to clearly explain this unexpected result, it is possible that an unknown compensatory pathway is initiated to maintain the replicative lifespan of the mutant cells.

V. Ist2p PLAYS A CRITICAL ROLE IN Pma1p ASYMMETRIC DISTRIBUION.

As shown in Fig. 4D, deletion of Ist2p appears to markedly affect Pma1p asymmetric distribution during cell division. Thus, we asked if Ist2p deletion

alone was sufficient to disrupt Pma1p asymmetric distribution by generating yeast cells deleted for Ist2p (Fig. 5A) and analyzing EGFP-Pma1p (Fig. 5B). Deletion of Ist2p alone did not profoundly affect ER-PM contacts, but moderately influenced Pma1p asymmetric distribution (Fig. 5C). Taken together, our results collectively suggest that ER-PM membrane contact sites regulate the asymmetric distribution of Pma1p, and thereby the replicative lifespan of the budding yeast *Saccharomyces cerevisiae*.In addition, of the ER-PM tethering proteins, Ist2p appears to play a critical role in regulating Pma1p distribution during cell division.

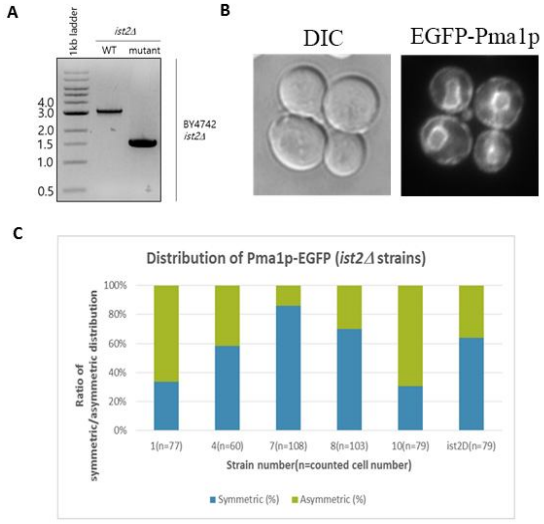


Figure 5. Ist2p regulates Pma1p distribution.

CONCLUSION

Here, we generated the budding yeast mutants that lacking ER-PM tethering proteins and examined whether ER-PM contacts play a role in regulating the asymmetric distribution of Pma1p during cell division and thereby replicative lifespan in yeast. Consistent with the previous result[5], the mutant lacking all six ER-PM tethering proteins showed almost no cortical ER. Instead, it accumulated considerable amount of ER membranes in the cytoplasm. Interestingly, this mutant did not have any clear defects in the Pma1p asymmetric distribution. Rather, it showed almost identical Pma1p distribution to wild-type cells. However, deletion of Scs2p/Scs22p/Ist2p/Tcb1p almost completely disrupted Pma1p asymmetric distribution during cell division. Thus, defects in ER-PM contacts do not directly correlate with defects in Pma1p asymmetric distribution. Nonetheless, it is clear that ER-PM tethering proteins have a role in regulating Pma1p asymmetric distribution during cell division, and therefore possibly in regulating yeast replicative lifespan. Intriguingly, Ist2p, related to mammalian TMEM16 ion channels, appears to play a critical role in controlling Pma1p distribution over the plasma membrane, suggesting that ER-PM tethering proteins have differential roles in establishing ER-PM contacts over regulating Pma1p distribution. Although further studies will be required to confirm that the replicative lifespan is indeed affected by deletion of ER-PM tethering proteins, our present study provides the first molecular insight into how membrane contact sites between intracellular organelles or membranes regulate cellular aging.

REFERENCES

[1] Steinkraus, K. A., Kaeberlein, M. & Kennedy, B. K. Replicative Aging in Yeast: The Means to the End. http://dx.doi.org/10.1146/annurev.cellbio.23.090506.12350924, 29–54 (2008).

[2] Thayer, N. H. et al. Identification of long-lived proteins retained in cells undergoing repeated asymmetric divisions. Proc. Natl. Acad. Sci. U.S.A.111, 14019–14026 (2014).

[3] Henderson, K. A., Hughes, A. L. & Gottschling, D. E. Mother-daughter asymmetry of pH underlies aging and rejuvenation in yeast. Elife3, e03504 (2014).

[4] Hughes, A. L. & Gottschling, D. E. An early age increase in vacuolar pH limits mitochondrial function and lifespan in yeast. Nature492, 261–265 (2012).

[5] Manford, A. G., Stefan, C. J., Yuan, H. L., Macgurn, J. A. & Emr, S. D. ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology. Dev. Cell23, 1129–1140 (2012).

★★★