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The Observation of *Saccharomyces cerevisiae* Ultrastructure Changes under Proline Limitation

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Proline is an important amino acid, which not only participates in protein synthesis, but also is involved in various stress responses in many organisms. The possible mechanisms by which proline protects cells against stress are proposed as the following: osmo protection, ROS scavenging, redox regulation [1, 2]. It was also suggested that increases of intracellular proline levels protects plants or yeast cells against stress via improvement of vacuole biogenesis since the vacuole is an important compartment for cell survive under various stress[3, 4].

The phenotypes of ultrastructure of proline biosynthesis gene pro2 null mutant of *Saccharomyces cerevisiae* BY4741 was examined. Under proline limitation condition, yeast cells displayed stress-sensitive phenotypes similar to that related to vacuole dysfunction including being sensitive to osmotic changes, metal and stressed by PH change . The ultrastructure of pro2 null mutan cells was examined. Cells were prepared using a modification of the procedure of Mulholland and Botstein[5].Ultrathin sections were observed on a Hitachi H7500 Transmission Electron Microscope. Cells were grown in complete synthetic medium (SD) without proline, the pro2 mutant cells showed larger vacuoles visualized by confocal microscopy, and abnormal vacuole structures visualized by transmission electron microscopy. The morphology of pro2 mutants were similar to wild type while growing in the SD medium with addition of 10 mM proline.

Our data suggests that appropriate proline level maintained by proline biosynthesis pathway may be required for normal function of yeast vacuole.

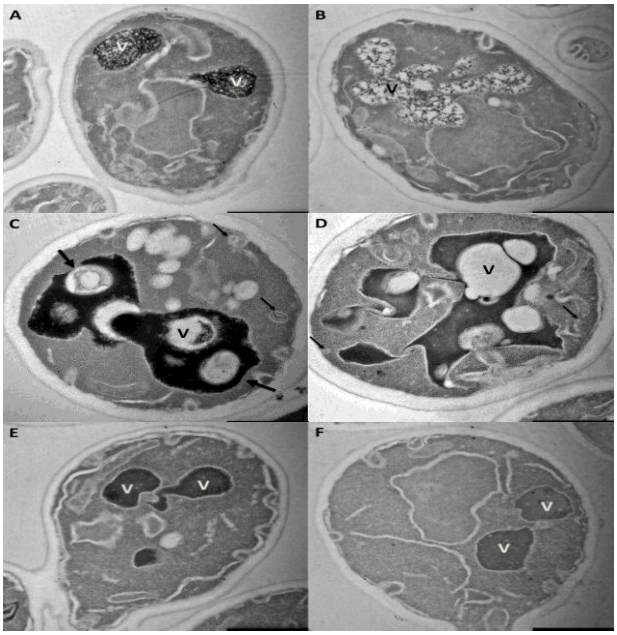

Figure 1. *Saccharomyces cerevisiae* BY 4741 wild type (A, B, E) and pro2 mutant (C, D, F) were growing in complete synthetic media (SD) without proline (A, B, C, D) or with 10 mM proline (E, F) to exponential phase. Under SD medium without proline condition, the vacuole of wild-type (A, B) appeared as a single large spot (B) or 2-3 spots of approximately equal size (A). However the vacuoles in pro2 mutant cells (C, D) contained inclusions (large arrow, C), the bodies in the vacuoles were surrounded by a unit membrane, the contents of the bodies were electron transparent, suggesting that they do not contain significant amount of nuclei acid, glycoproteins and sugars, those might be autophagic bodies. A variety of novel membrane-enclosed organelles, including vesicles and Berkeley bodies were also observed (small arrow, C and D). When growing at SD medium with 10 mM proline, the vacuoles of pro2 mutant (F) and wild type (E) are similar to that of wild type growing in SD medium without 10 mM proline (A, B). V: vacuole, Bars: 1um

References:
[1] Szabados, L. and A. Savoure, Proline: a multifunctional amino acid. Trends Plant Sci, 2010. 15(2): p. 89-97.
[2] Phang, J.M., W. Liu, and O. Zabirnyk, Proline metabolism and microenvironmental stress. Annu Rev Nutr, 2010. 30: p. 441-63.
[3] Maggio, A., et al., Does proline accumulation play an active role in stress-induced growth reduction? Plant J, 2002. 31(6): p. 699-712.
[4] Matsuura, K. and H. Takagi, Vacuolar functions are involved in stress-protective effect of intracellular proline in Saccharomyces cerevisiae. J Biosci Bioeng, 2005. 100(5): p. 538-44.
[5] Mulholland, J. and D. Botstein, Immunoelectron microscopy of aldehyde-fixed yeast cells. Methods Enzymol, 2002. 351: p. 50-81.
[6] The authors acknowledge funding from National Institutes of Health, Grant Number: GM079393.