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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 involves in not only protein synthesis, but also in various stress response in many organisms [1]. It was suggested that increase of intracellular proline level protects plants or yeast cell against stress via improvement vacuole biogenesis since vacuole is an important compartment for cell survive under various stress [2]. Autophagy is a conserved cellular process that mediates protein degradation in lysosomes called vacuoles in yeast [3]. It plays an important role in resource energy when cells undergoing nutrient starvation. Nitrogen starvation is a common used assay to test whether or not cells bear functional autophagy to nutrient starvation. Here we report the proline biosynthesis gene PRO3 null mutant of *Saccharomyces cerevisiae* BY4741 is hyper sensitive to nitrogen starvation, the survive rate under nitrogen starvation is similar to that of atg8 mutant strain in which autophagy is deficient. However other amino acid starvation such as leucine, histidine doses not affect yeast cells survive under shortage of nitrogen source. The autophagy bodies were further examined using transmission electron microscope. While growing in minimal medium without nitrogen for 4 hours, similar to atg8 mutant, the yeast cells of pro3 strain showed significant fewer autophagy bodies compared with wild-type and pep4 mutant. Our data suggests that proline may play an important role in autophagy formation.

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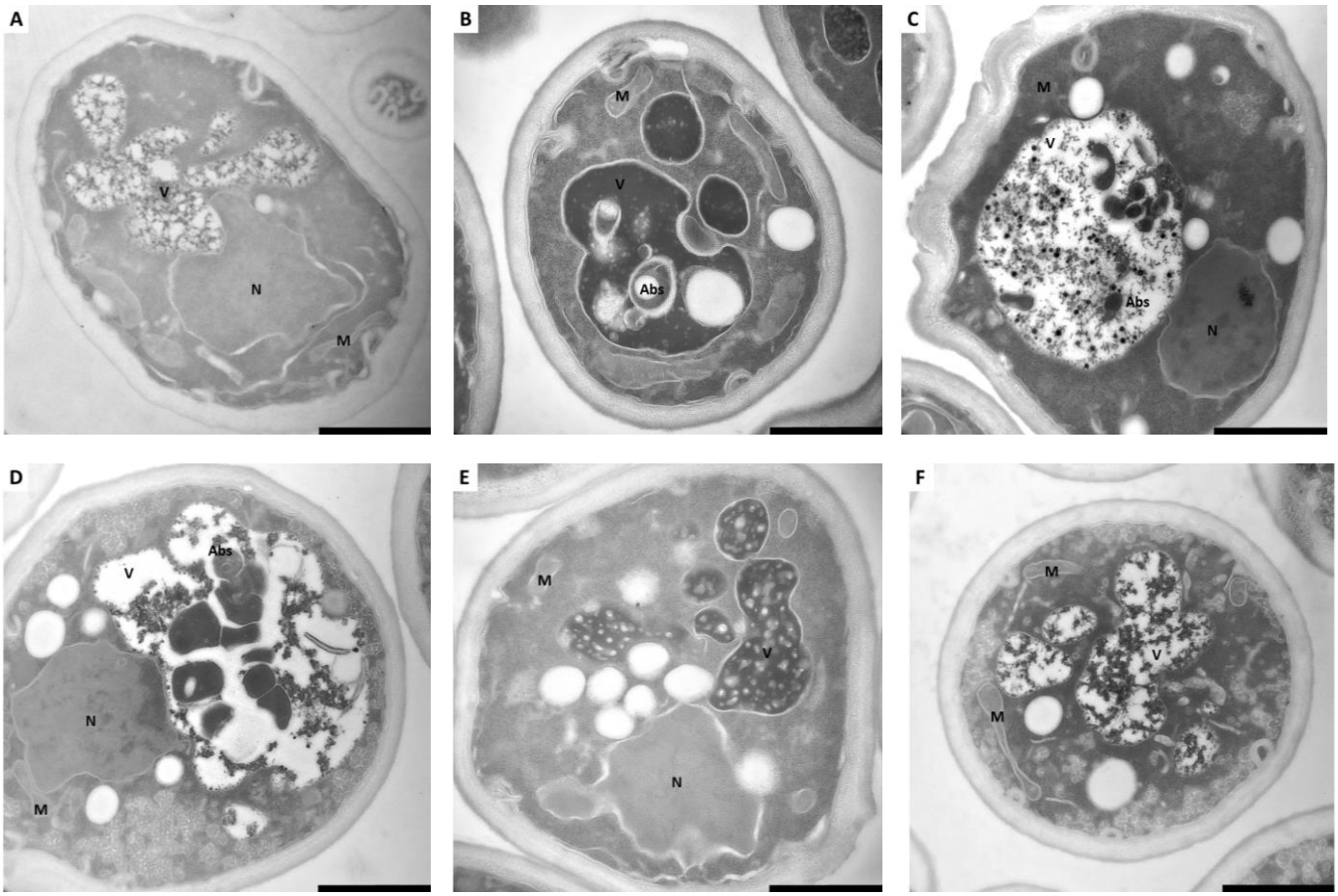

Figure 1. Ultrastructure of *Saccharomyces cerevisiae* BY 4741 cells grown in different medium. *Saccharomyces cerevisiae* BY 4741wild type, pep4, pro3, and atg8 mutants were growing at minimal synthetic minimal medium (SD) to log phase, then inoculated to minimal medium without nitrogen or without nitrogen and leucine, and with addition of 1mM PMSF for four hours, then subjected to transmission electron microscope photography [4]. Under nitrogen starvation (B,D-F) or nitrogen and leucine starvation (C) for 4 hours, wild type (B,C) and pep4 (D) mutant cells had accumulated multiple autophagic bodies with high-electron density in the vacuole, the bodies surrounded by a unit membrane, and the content of the bodies is morphologically indistinguishable from some cytosolic organelles. However, the autophagic bodies were not observed in pro3 (E) and atg8 (F) null mutant cells upon nitrogen starvation or wild type growing in SD medium (A). V: vacuole, Abs: autophagy bodies, M: mitochondria, N: nucleotide. Bars: 1μm.

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