Transformation mechanism of budding yeast *Saccharomyces cerevisiae*

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Abstract

Transformation is an indispensable method for the genetic manipulation of cells. Budding yeast *Saccharomyces cerevisiae* can be transformed by incubating intact cells and plasmid DNA in the presence of polyethylene glycol (PEG) alone. Lithium acetate (LiAc) and single-stranded carrier DNA (ssDNA) enhance the transformation efficiency (number of transformants per μg plasmid DNA). The mechanism underlying transformation itself as well as this enhancement has remained elusive.

To elucidate the mechanism for transformation, the transformation efficiency of each of nonessential gene mutants (about 5,000 strains) of *S. cerevisiae* was initially analyzed. As a result, it was revealed that the Arp2/3 activation machinery involving the Myo3/5p, Vrp1p, Las17p, Pan1p, and Arp2/3 complex is crucial to the transformation; DNA possibly enters into the cell via an endocytosis-like event, being at least partially different from well-known endocytosis. The effect of PEG was also examined and it was concluded that the physical effect of PEG on cell membrane, rather than the effect of PEG itself on the intracellular response, could cause high transformation efficiency of *S. cerevisiae*. Moreover, the obtained data strongly suggested that during the transformation of intact cell, plasmid DNA is initially absorbed on the cell wall, passes through the cell wall with the aid of heat shock, reaches to the membrane, and enters into the cell together with the membrane structure. Thus, it was proposed that that the capacity of the cell wall to absorb DNA is at least one of the determinants of transformation efficiency. Finally, it was revealed that LiAc and ssDNA synergistically enhance transformation efficiency and the synergistic effect of LiAc and ssDNA on transformation of *S. cerevisiae* was for the first time visualized, which would explain the mechanism underlying the enhancement of transformation efficiency by LiAc and ssDNA.