

An investigation of subcellular localization of YlSnf1p-EGFP in response to a fatty acid in *Yarrowia lipolytica*

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Abstract: *Yarrowia lipolytica* is a hydrophobic substrate-assimilating yeast, breaking down substrates such as fatty acids and *n*-alkanes as sole carbon sources. Nonetheless, the control of the mechanisms of hydrophobic substrate utilization in the yeast has not been completely understood. From our previous finding, the deletion of *YLSNF1* in *Y. lipolytica*, encoding a sucrose non-fermenting 1 protein kinase, caused defective growth when the cells were cultured in fatty acid medium. As a result, the aim of this study was to elucidate the response of YlSnf1p to fatty acid utilization in *Y. lipolytica* by investigating the subcellular localization of YlSnf1p-EGFP. To begin with, pSNF1-EGFP was successfully constructed to express YlSnf1p-EGFP under the control of its own promoter in $\Delta Ylsnf1::ADE1$. Next, YlSnf1p-EGFP was expressed and intact inside the yeast cells, using western blot analysis. Moreover, YlSnf1p-EGFP could restore growth of YlSnf1p in $\Delta Ylsnf1::ADE1$. The subcellular localization of YlSnf1p-EGFP was then performed by observing the EGFP signal inside *Y. lipolytica* using fluorescence microscopic technique. The result showed that YlSnf1p-EGFP was mainly located in the whole cell body when glycerol was used as a carbon source. After the shift of carbon source from glycerol to other hydrophobic substrates, YlSnf1p-EGFP displayed distinct localization patterns, positioned in cytosol and nucleus for glucose and oleic acid media, respectively. These results suggested that the relocation of YlSnf1p-EGFP was associated with the control of fatty acid utilizations in *Y. lipolytica*.

Keywords: Snf1-protein kinase; protein relocation; control of fatty acid utilization



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