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# Gene deletion of deoxyhypusine hydroxylase in *S. pombe* resulted in a metabolic shift from OXPHOS to glycolysis

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**Abstract:** The eukaryotic initiation factor 5A (eIF5A) is the only know protein to undergo hypusination. Much of the studies have been devoted to decipher the role of hypusination, but little is known about the precise role of another eIF5A post-translational modification which is acetylation. Deletion of the the deoxyhypusine hydroxylase, one of the enzymes responsible in hypusination, resulted in heavily acetylated eIF5A. Furthermore, acetylation of eIF5A impaired cellular growth, decreased respiration and increased ethanol production. These phenotypes were reversed when the site for acetylation was blocked as in the K49R mutant. This implies that eIF5A acetylation plays a role in the cell's metabolic shift from OXPHOS to glycolysis.

**Key Words:** cellular respiration; acetylation; cell metabolism; ethanol production

## 1. INTRODUCTION

The central dogma of molecular biology describes the flow of genetic information in all living cells. Translation is a fundamental process which converts the genetic information stored in the DNA into functional proteins which are necessary for cellular functions. Translation is a tightly regulated process and any slight defects can cause abnormalities in a cell's physiology. Aside from the ribosome, translation factors play very important roles in ensuring that the steps in protein translation are carried out in a precise manner. One such factor is the eukaryotic initiation factor 5A (eIF5A) which is a highly ubiquitous, essential and conserved protein (Schnier et al. 1991; Kim et al. 1998). eIF5A is the only known cellular protein to undergo a unique post-translational modification called hypusination. Hypusination is accomplished by addition of deoxyhypusine to a specific lysine residue in eIF5A by deoxyhypusine synthase (Dhs1) and successive hydroxylation of the intermediate to the fully mature and functional hypusine by deoxyhypusine hydroxylase (Lia1 in *S. cerevisiae*, Mmd1 in *S. pombe*).

Aside from hypusination, eIF5A is also subject to another post-translational modification which is acetylation. Sequence alignment of eIF5A in human and yeasts showed that the hypusinated and

acetylated lysines are well conserved and are in close proximity to each other. This led us to hypothesize that hypusination of eIF5A may block the access site for acetylases. It was previously demonstrated that GC7, a Dhs1 inhibitor, resulted to a decrease in hypusination and heavy acetylation of eIF5A in mammalian cells (Ishfaq et al. 2012). Furthermore, Ishfaq et al. also demonstrated that the acetylation state of eIF5A affects its cellular localization. In normal physiological conditions, eIF5A is localized in the cytoplasm to comply with its role in protein translation. However, upon acetylation, eIF5A is transported from the cytoplasm to the nucleus (S. B. Lee et al. 2009; Ishfaq et al. 2012). This shuttling of eIF5A between these two cellular compartments is proposed to be a mechanism to control the function of eIF5A.

The role of eIF5A hypusination has been extensively studied, however, studies on the precise function of eIF5A acetylation has been lacking. To describe the role of eIF5A acetylation in cellular growth and metabolism, we used *S. pombe* and *S. cerevisiae* strains lacking the genes for deoxyhypusine hydroxylase.

## 2. METHODOLOGY

### *2.1 Growth rate measurement*

Mid-log phase cells cultured overnight in minimal media (MM) were used in the growth rate assay. The $OD_{500}$ of triplicate samples grown in a 96-well plate were measured to generate the growth curve.

### *2.2 Oxygen consumption rate (OCR) assay*

The oxygen consumption rate was measured using the Agilent Seahorse XF Technology. Mid-log phase cells cultured in MM media overnight at 30 °C were diluted to 1.0 x $10^6$ cells/mL using fresh MM media. Triplicate samples using 200 uL aliquots were transferred into each sample wells and incubated at 30 °C for 30 min before measurement. MM media was used as the blank. The OCRs were measured at three cycles in triplicates and the average OCRs were reported.

### *2.3 Ethanol production assay*

*S. cerevisiae* cells were grown in yeast extract peptone dextrose (YPD) media at 30°C until mid-log phase. Triplicate samples were harvested for the WT and *lia1*Δ every 3 h for a period of 9 h. Right after harvest, the samples were clarified by centrifugation at 15,000 rpm. The clear supernatant liquid was transferred in a 96-well plate and the ethanol produced by the alcohol fermentation was measured using the Ethanol UV-method kit (Roche) following the manufacturer's protocol. The kit contains two enzymes: (1) alcohol dehydrogenase which catalyzes the following reaction: ethanol + $NAD^+$ ⇌ acetaldehyde + NADH + $H^+$; and (2) aldehyde dehydrogenase which catalyzes the following reaction: acetaldehyde + $NAD^+$ ⇌ acetic acid + NADH + $H^+$. The NADH produced is measured spectrophotometrically at 340 nm. A calibration curve using standard ethanol diluted with YPD media was constructed as reference. The absorbance of the samples was interpolated from the calibration curve to calculate the ethanol concentration.

## 3. RESULTS AND DISCUSSION

When cells are grown in low glucose conditions, the canonical response is to shift the cellular metabolism from glycolysis to OXPHOS to maintain energetic states. Acetylation of eIF5A is blocked in the eIF5A-K49R strain due to the replacement of lysine residue with non-acetylatable arginine. The eIF5A-K49R mutant strains were used in this study to demonstrate the role of eIF5A acetylation in cellular growth.

The growth assay revealed that the growth defect phenotype caused by *mmd1*Δ can be partially rescued in the eIF5A-K49R mutant (Figure 1). This suggests that eIF5A acetylation in *mmd1*Δ cells may inhibit cell growth and blocking eIF5A acetylation in the eIF5A-K49R strain can rescue this defective phenotype.

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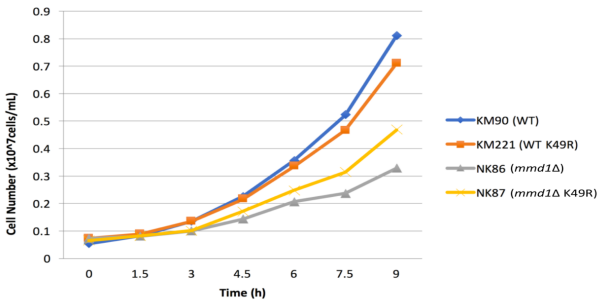


Figure 1. Growth curve showed that blocking eIF5A acetylation in the eIF5A-K49R mutant can partially rescue the growth defect caused by *mmd1*Δ.

Measurement of the oxygen consumption rate revealed that the respiration defect caused by *mmd1*Δ can be rescued in the eIF5A-K49R mutant strain (Figure 2). This imply a possible role of acetylated eIF5A in *mmd1*Δ cells in the inactivation of respiration which results in a metabolic shift from OXPHOS to glycolysis. Taken together, our results demonstrate that suppression of eIF5A acetylation by blocking the acetylation site in eIF5A-K49R mutant can rescue the growth and respiration defect phenotype caused by *mmd1*Δ.

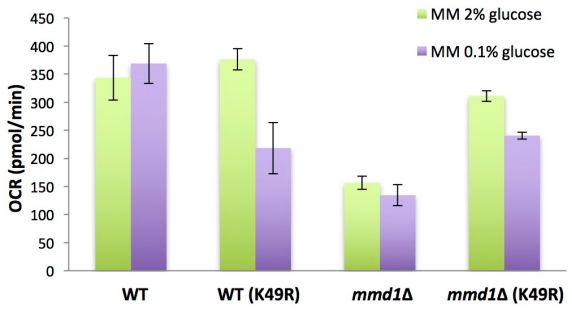


Figure 2. Oxygen consumption rate measured by the Seahorse assay. The respiration defect caused by *mmd1*Δ was partially rescued in eIF5A-K49R strain.

To support our findings in *S. pombe*, we deleted the *lia1* which is an *mmd1* ortholog in *S. cerevisiae* cells. Despite the slower growth rate of *lia1*Δ strains compared to WT strains in YPD (Figure 3), *lia1*Δ cells are able to increase ethanol production by as much as two-folds relative to the WT cells (Figure 4).

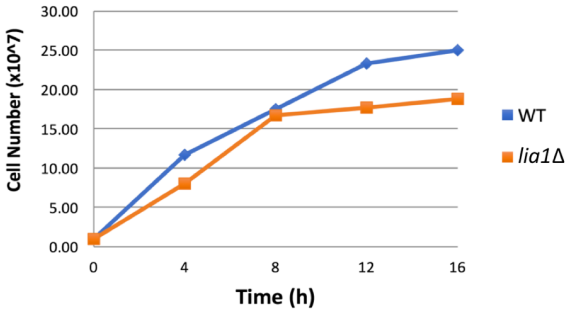


Figure 3. Growth Curve of WT and *lia1*Δ *S. cerevisiae* cells grown in YPD media at 30°C.

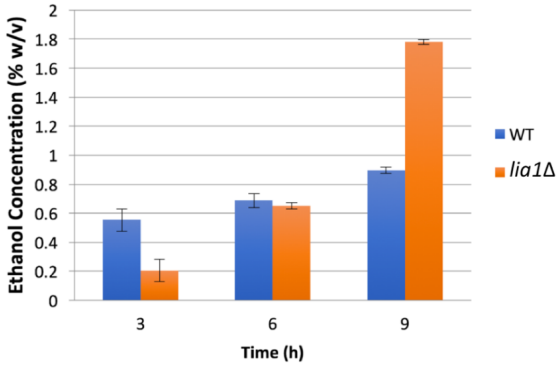


Figure 4. Time-course analysis of ethanol production of WT and *lia1*Δ *S. cerevisiae* cells grown in YPD media at 30°C.

Taken together, these results further support the hypothesis that in *mmd1*Δ or *lia1*Δ strains, the cell shifts from OXPHOS to glycolysis as demonstrated by the increased ethanol production and lower oxygen consumption in the mutant strains relative to the WT strains.

## 4. CONCLUSIONS

Deletion of the deoxyhypusine hydroxylase gene resulted in heavy acetylation of eIF5A which translates to slower growth rate, decreased oxygen consumption rate and increased ethanol production. These phenotypes were reversed in the K49R strain where the eIF5A site for acetylation was blocked by mutation of lysine to arginine. This implies that eIF5A acetylation may play a role in the cellular metabolic shift from OXPHOS to glycolysis.

## 5. ACKNOWLEDGMENTS

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## 6. REFERENCES

Ishfaq, Muhammad, Kazuhiro Maeta, Satoko Maeda, Toru Natsume, Akihiro Ito, and Minoru Yoshida. 2012. "Acetylation Regulates Subcellular Localization of Eukaryotic Translation Initiation Factor 5A (eIF5A)." *FEBS Letters* 586 (19):3236–41.

Kim, Kyeong Kyu, Li-Wei Hung, Hisao Yokota, Rosalind Kim, and Sung-Hou Kim. 1998. "Crystal Structures of Eukaryotic Translation Initiation Factor 5A from Methanococcus Jannaschii at 1.8 Å Resolution." *Proceedings of the National Academy of Sciences* 95 (18):10419–24.

Lee, Seung Bum, Jong Hwan Park, Jorn Kaevel, Monika Sramkova, Roberto Weigert, and Myung Hee Park. 2009. "The Effect of Hypusine Modification on the Intracellular Localization of eIF5A." *Biochem Biophys Res Commun.* 383 (4):497–502.

Schnier, J, H G Schwelberger, Z Smit-McBride, H A Kang, and J W Hershey. 1991. "Translation Initiation Factor 5A and Its Hypusine Modification Are Essential for Cell Viability in the Yeast Saccharomyces Cerevisiae." Molecular and Cellular Biology 11 (6). *American Society for Microbiology*:3105–14.