Studying Novel Genes Affecting NAD+ Metabolism

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What is NAD+?

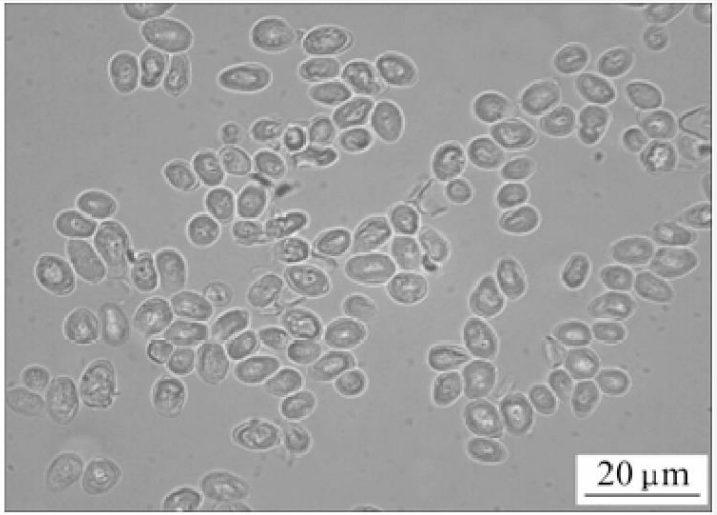
- Nicotinamide Adenine Dinucleotide
- Central to many biological functions
- Redox reactions, cellular respiration, protein modification, immune response

Figure 1: Summary of roles of NAD+ in metabolism pathways

Introduction

- As humans grow older -> less NAD+
- Abnormal levels of NAD+ is linked to disease
- Administration of NAD+ precursors is an emerging therapeutic target

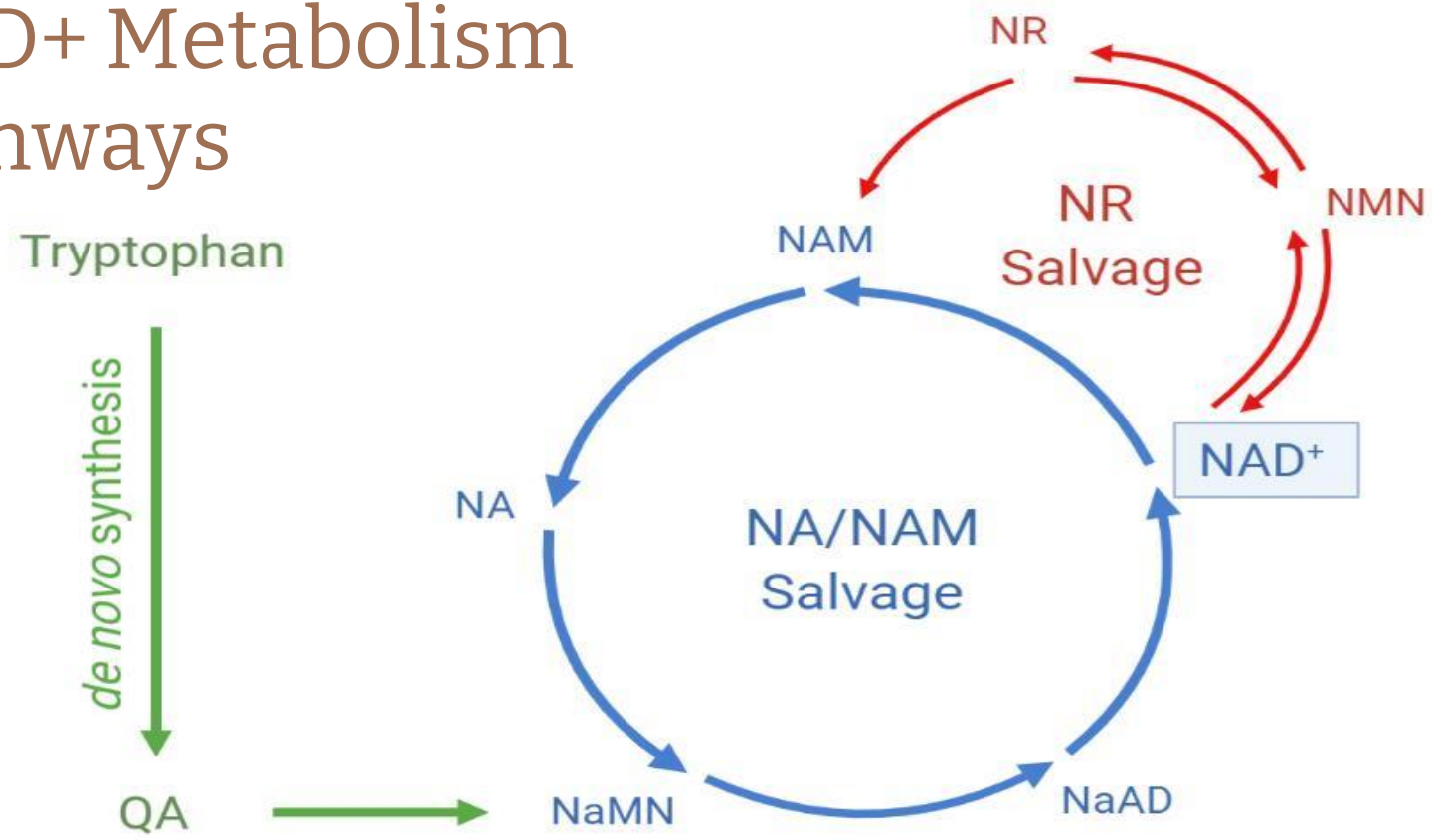
The Model System



- *Saccharomyces cerevisiae* or “budding yeast”
- Factors regulating NAD+ homeostasis are unclear because interconnections of pathways

Figure 2: Microscopic picture of Saccharomyces cerevisiae
Microscopic Image of Waste Saccharomyces Cerevisiae [31].
https://www.researchgate.net/figure/Microscopic-image-of-waste-Saccharomyces-cerevisiae-31_fig2_314207518.

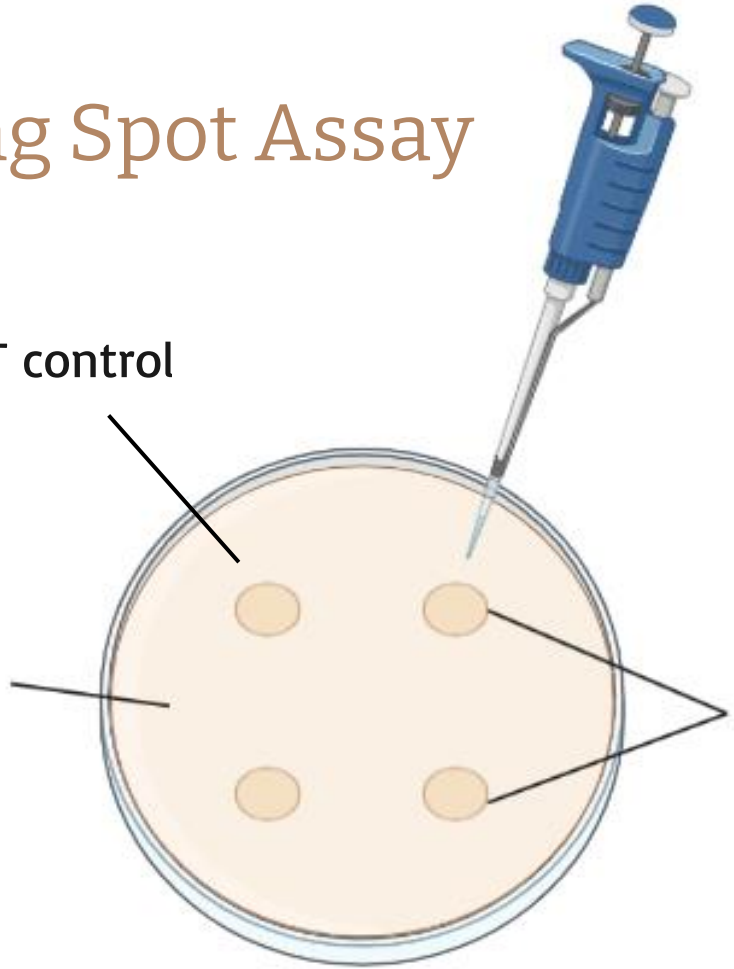
NAD+ Metabolism Pathways



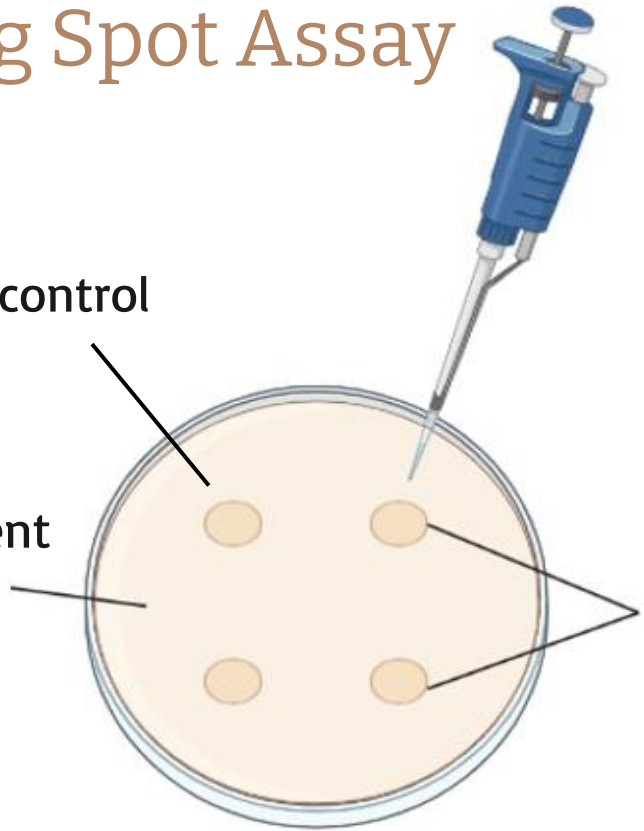
Methods

1. Previous genetic screen using Molecular Barcoded Yeast ORF 2.0 library (Horizon)
    - Selected 8 genes with strong intermediate phenotypes
2. Current secondary-screening
    - Cross-feeding spot assay
3. Transform into bacteria to amplify and isolate yeast plasmid
4. Sequence plasmid

Cross-feeding Spot Assay



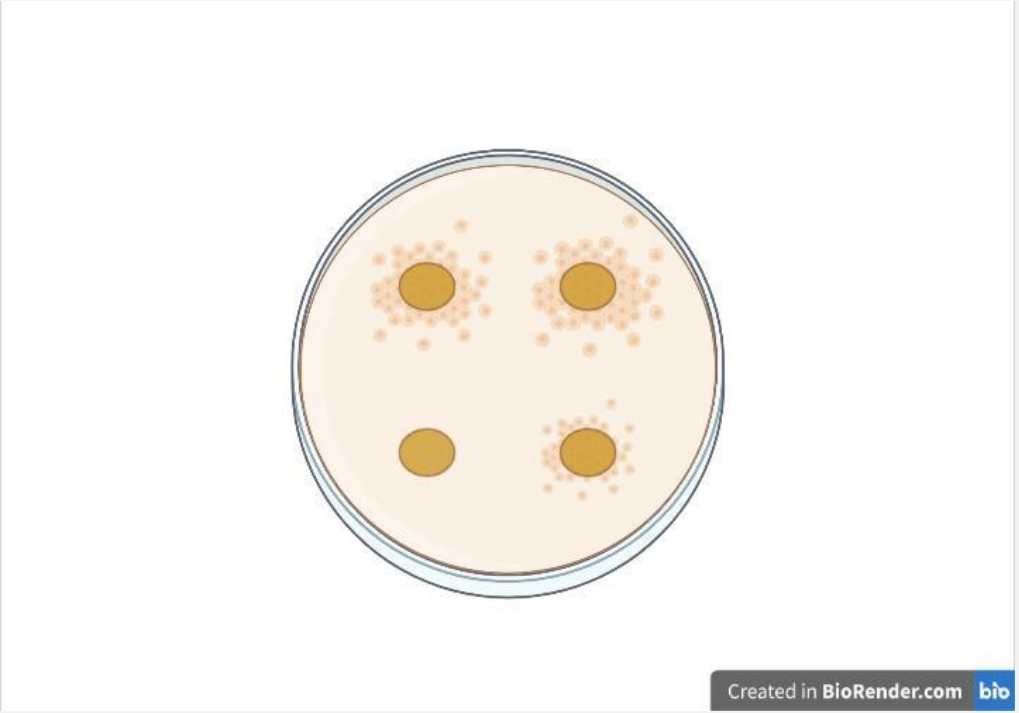
Cross-feeding Spot Assay



NEED: QA, NA/NAM, NR

RELEASE: QA, NA/NAM, NR

Cross-feeding Spot Assay

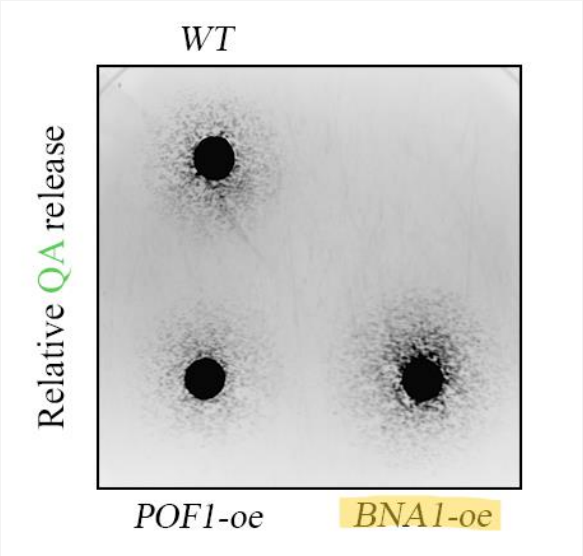


Greater release of intermediates = greater diameter of growth.

Little to no release of intermediates = smaller diameter of growth.

Fig 3: Example of yeast growth after cross-feeding

Results: *BNA1* overexpression



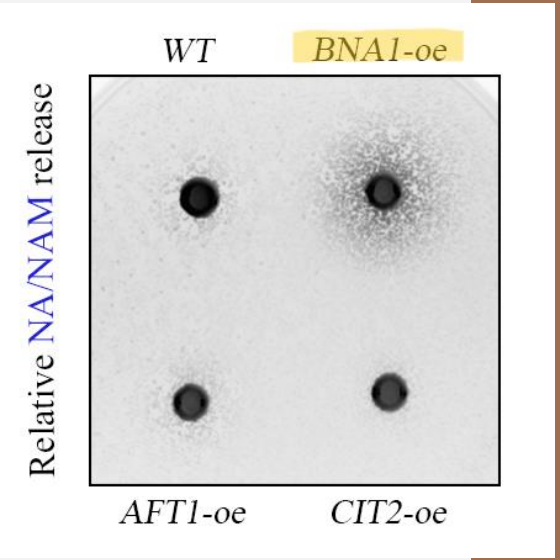


Figure 4: Relative QA release (modelling *de novo* pathway) and relative NA/NAM release of recipient yeast cells

Analysis

- *BNA1* is directly correlated with the production of QA

- Large release of NA/NAM could hint to unknown secondary mechanisms or connection to other genes in salvage pathway

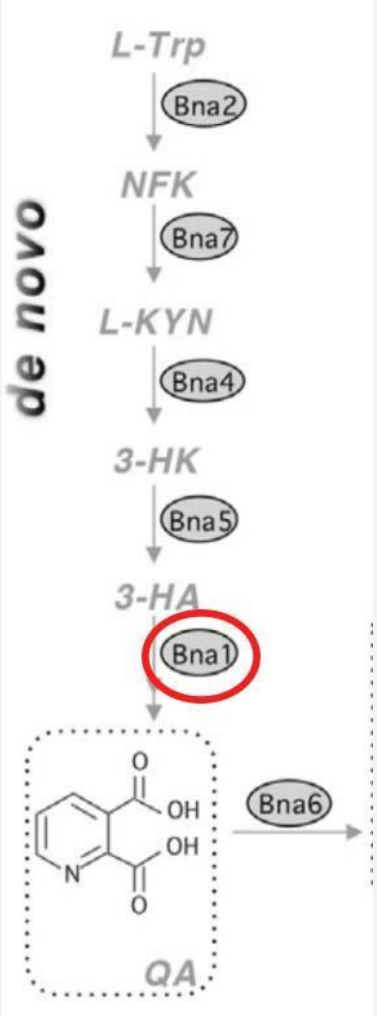
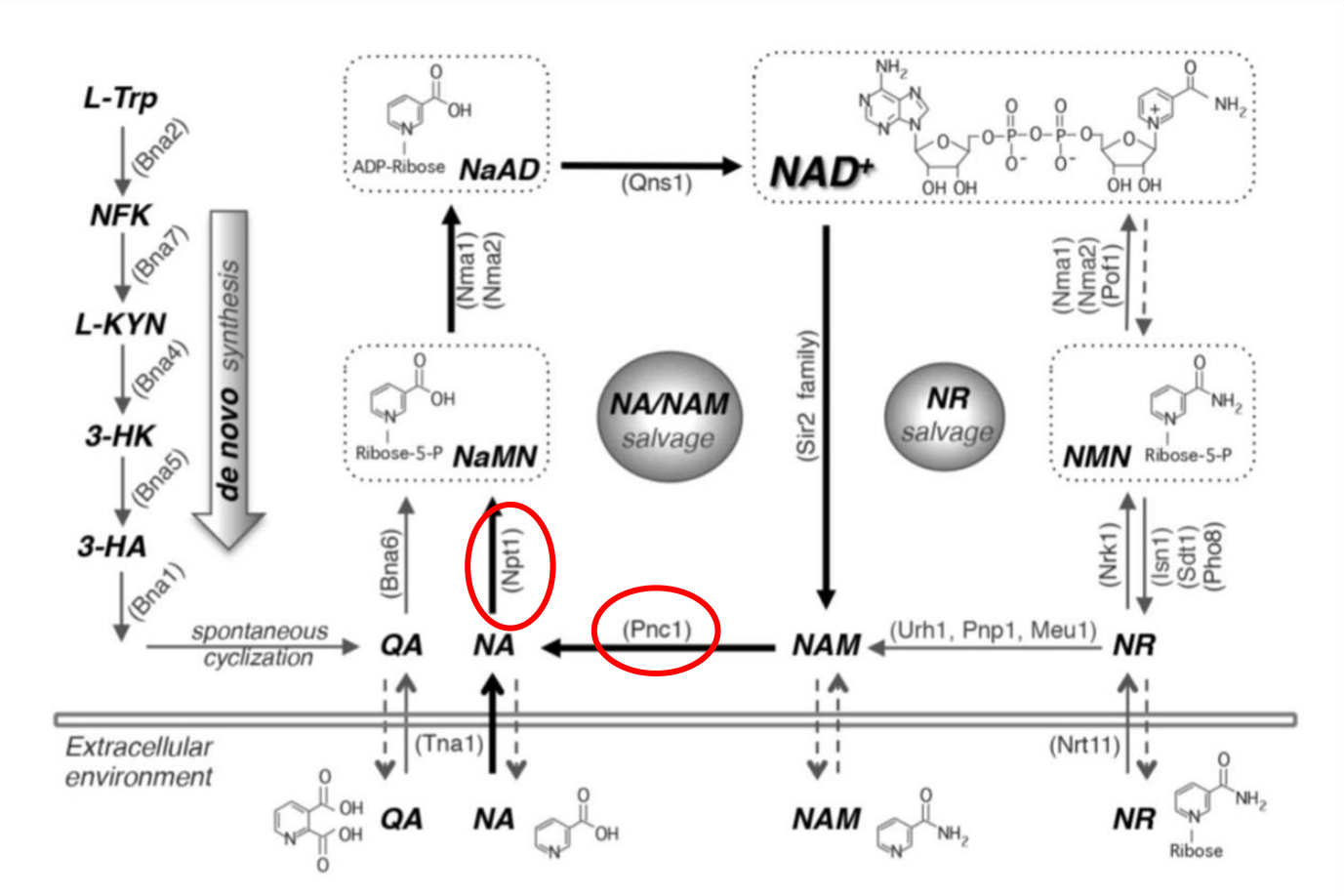
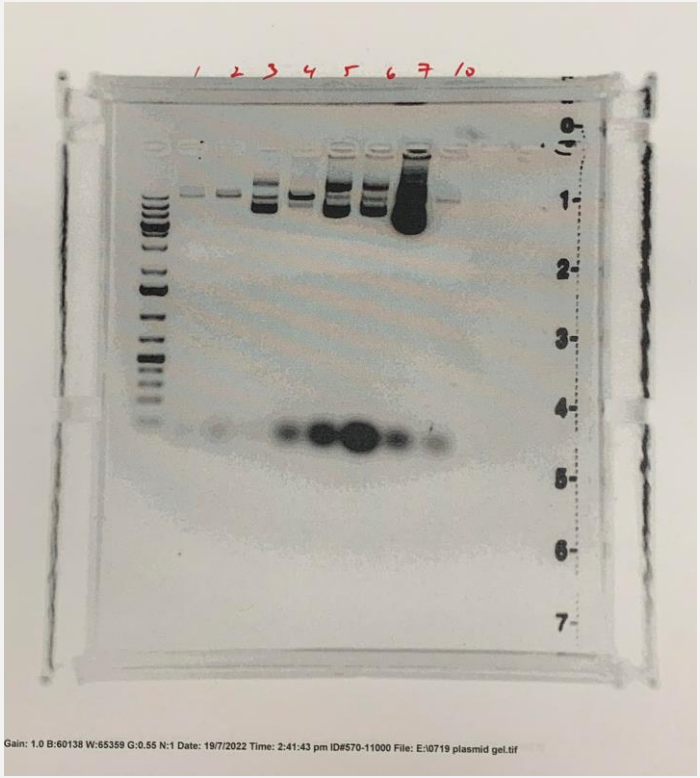


Figure 6: The *de novo* pathway

More in-depth NAD+ metabolism pathways diagram





Example of gel electrophoresis

- Isolated yeast plasmid to send for sequencing and future experiments

Figure 5: *CIT2* overexpression

Discussion and Next Steps

- Confirmed 6 out of 8 genes
- Inconsistencies from initial to secondary screening
- Fine-tuning for further experiments
- NAD+ assay
    - Do overexpression genes lead to different production levels of NAD+?

Future Application

- Contributes to existing research with new information about NAD+ genes
- Help elucidate the mechanisms of the regulation of NAD+ pathways
- Provides more knowledge for eventual clinical studies

Acknowledgements

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Resources

Belenky, P., Racette, F. G., Bogan, K. L., McClure, J. M., Smith, J. S., & Brenner, C. (2007). Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD+. Cell, 129(3), 473-484.

Camacho-Pereira, J., Tarragó, M. G., Chini, C. C., Nin, V., Escande, C., Warner, G. M., ... & Chini, E. N. (2016). CD38 dictates age-related NAD decline and mitochondrial dysfunction through an SIRT3-dependent mechanism. Cell metabolism, 23(6), 1127-1139.

Cantó, C., Menzies, K. J., & Auwerx, J. (2015). NAD+ metabolism and the control of energy homeostasis: a balancing act between mitochondria and the nucleus. Cell metabolism, 22(1), 31-53.

Croft, Trevor, et al. "A functional link between NAD+ homeostasis and N-terminal protein acetylation in Saccharomyces cerevisiae." *Journal of Biological Chemistry* 293.8 (2018): 2927-2938

Faivre, Anna, et al. "Differential role of nicotinamide adenine dinucleotide deficiency in acute and chronic kidney disease." *Nephrology Dialysis Transplantation* 36.1 (2021): 60-68.

Groth, Benjamin, Padmaja Venkatakrishnan, and Su-Ju Lin. "NAD+ metabolism, metabolic stress, and infection." Frontiers in Molecular Biosciences (2021): 446.

Imai, Shin-ichiro, and Leonard Guarente. "NAD+ and sirtuins in aging and disease." *Trends in cell biology* 24.8 (2014): 464-471.

James Theoga Raj, C., & Lin, S. J. (2019). Cross-talk in NAD+ metabolism: insights from Saccharomyces cerevisiae. Current Genetics, 65(5), 1113-1119.

Lautrup, Sofie et al. “NAD+ in Brain Aging and Neurodegenerative Disorders.” Cell metabolism vol. 30,4 (2019): 630-655. doi:10.1016/j.cmet.2019.09.001

Raj, Christol James Theoga, et al. "The copper-sensing transcription factor Mac1, the histone deacetylase Hst1, and nicotinic acid regulate de novo NAD+ biosynthesis in budding yeast." *Journal of Biological Chemistry* 294.14 (2019): 5562-5575.

Thank you!

Questions?