4. Murphy, M. P., Bayir, H., Belousov, V. V., Chang, C. J., Davies, K. J. A., Davies, M. J., Dick, T. P., Finkel, T., Forman, H. J., Janssen-Heininger, Y., & Gems, D. (2022). Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nature Metabolism, 4*, 651–662.
5. Prokopiv, T., Stasyuk, N., & Gonchar, M. (2023). Nanozyme can substitute a natural *Ogataea polymorpha* catalase enzyme in vivo. *Microchimica Acta, 190*, 174. https://doi.org/ 10.1007/ s00604-023-05753-8
6. Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., Qin, L., & Wei, H. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). *Chemical Society Reviews, 48*, 1004–1020.
7. Xu, D., Wu, L., Yao, H., & Zhao, L. (2022). Catalase-like nanozymes: Classification, catalytic mechanisms, and their applications. *Small, 18*(37), 2203400.
8. Yang, W., Yang, X., Zhu, L., Chu, H., Li, X., & Xu, W. (2021). Nanozymes: Activity origin, catalytic mechanism, and biological application. *Coordination Chemistry Reviews, 448*, 214170.

https://doi.org/10.30970/apmicro.2026.40

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METABOLIC REMODELING DURING THE EVOLUTIONARY TRANSITION TO MACROSCOPIC MULTICELLULARITY

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Abstract. The evolution of macroscopic body size in multicellular organisms is a key but poorly understood step in the history of life. Using the yeast long-term evolution experiment, in which selection for larger size drove clonal clusters of the budding yeast *Saccharomyces cerevisiae* to become macroscopic over more than 1,000 daily rounds of selection, we combined steady-state metabolomics, ^{13}C metabolic flux analysis, and transcriptomics to characterize the accompanying metabolic changes. We found a system-wide metabolic reallocation from growth toward maintenance, marked by depletion of amino acids, lipids, nucleotides, and central-carbon metabolites, reduced growth rate, and a sharp rise in the protective sugar trehalose. These results indicate that metabolic remodeling, and trehalose accumulation in particular, might be a major driver of the evolutionary transition to macroscopic multicellularity in this system.
Keywords: multicellularity; snowflake yeast; metabolic remodeling; trehalose; proteostasis

The evolution of multicellularity was a major step in the rise of complex life on Earth, yet our understanding of this transition is still incomplete. Progress has been hindered by the ancient origins of extant multicellular lineages, making it difficult to reconstruct the changes that accompanied the emergence of multicellularity. In particular, it remains largely unknown whether metabolic changes facilitated early multicellular evolution.

To test whether metabolism reorganizes during the transition to multicellularity, we used yeast evolved in the Multicellularity Long-Term Evolution Experiment (MuLTEE), an experimental evolution system that lets us examine this transition as it unfolds. In the MuLTEE, replicate populations of *Saccharomyces cerevisiae* founded from a common ancestor undergo daily selection for larger size through rapid settling, driving the evolution

of increasingly large and complex multicellular clusters. Over 1,000 daily rounds of selection, this yeast evolved from microscopic snowflake clusters into macroscopic groups containing hundreds of thousands of clonally related cells (Bozdag et al., 2023). Using steady-state metabolomics, metabolic flux analysis, and transcriptomics, we mapped metabolic changes across this evolutionary course to determine how metabolism is reorganized as multicellular size and complexity evolve.

Macroscopic snowflake yeast shows a broad metabolic transition from growth toward maintenance and structural reinforcement: amino acids, lipids, and TCA intermediates decline while trehalose accumulates massively alongside NAD^+ biosynthesis and cell wall precursors, with the serine-glycine-one-carbon axis showing the strongest evolutionary signal. This transition is highly convergent across independently evolved populations, with metabolic change concentrated around the point at which macroscopic size is reached and then largely plateauing. This global metabolic downshift correlated with a reduced growth rate in the macroscopic multicellular state. Interestingly, we found that trehalose, a storage sugar and chemical chaperone, rose sharply during the transition. Our prior work found that chaperones, particularly Hsp90, declined (Montrose et al., 2024), facilitating the transition toward macroscopic multicellularity and suggesting that trehalose upregulation may compensate for proteostasis. Transcriptomics supports these shifts, and upregulation of the NAD^+/kynurenine pathway reflects the added fermentative burden of larger anaerobic clusters.

Together, our results show that metabolism is broadly reoriented from growth toward maintenance and structural reinforcement in evolved macroscopic clusters, supporting the progression of an evolutionary transition in individuality from small to macroscopic group sizes. Our results also suggest that trehalose – potentially by stabilizing protein folding and modulating cytoplasmic viscosity – is a major driver of this shift.

References

1. Bozdag, G. O., Zamani-Dahaj, S. A., Day, T. C., Kahn, P. C., Burnetti, A. J., Lac, D. T., Tong, K., Conlin, P. L., Balwani, A. H., Dyer, E. L., Yunker, P. J., & Ratcliff, W. C. (2023). De novo evolution of macroscopic multicellularity. *Nature*, *617*(7962), 747–754. https://doi.org/10.1038/s41586-023-06052-1
2. Montrose, K., Lac, D. T., Burnetti, A. J., Tong, K., Bozdag, G. O., Hukkanen, M., Ratcliff, W. C., & Saarikangas, J. (2024). Proteostatic tuning underpins the evolution of novel multicellular traits. Science Advances, 10, eadn2706. https://doi.org/10.1126/sciadv.adn2706