# As published in the 2024 European Congress on Biotechnology abstracts: Exploring yeast diversity for ethanol-fueled industrial biotechnology

Warmerdam, M.; Vieira Lara, M.A.; Mans, R.; Daran, J.G.; Pronk, J.T.

**DOI**
10.1016/j.bbabio.2024.149247

**Publication date**
2024

**Document Version**
Final published version

**Published in**
BBA - Bioenergetics

produced per substrate molecule. Aerobic processes are crucial in biotechnology for generating anabolic products such as biomass and proteins. These processes are intrinsically linked to ATP yield on substrate. Not only are yields on substrates essential, but yields on oxygen are also critical; large-scale aerobic processes often face the challenge of oxygen limitation, which impacts cellular metabolism and negatively affects production.

Yields are partially governed by the efficiency of electron transport chains, specifically P/O ratios (ATP molecules synthesized per electron pair used to reduce ½ $O_2$). On the one hand, the maximal theoretical P/O ratio in yeasts ranges from 1.4 to 2.3, whereas for mammalian cells it is accepted to be 2.7. On the other hand, yeasts and other microorganisms have faster growth rates and higher yields, and require less nutrient supplementation than mammalian cells, making them great hosts for large-scale production.

My newly established research group aims to address such energetic paradoxes in industrially relevant cells, focusing on both basic and applied science. Central to our approach is the quantitative analysis of cellular physiology, which is inherently growth-dependent. For this, we make use of bioreactor cultivation. Engineering energetic efficiency involves editing the mitochondria in eukaryotes. Although most proteins in the organelle are encoded by the nuclear genome, some are still retained in the mitochondrial genome. We are implementing biolistic transformation approaches for mitochondrial DNA editing, and respirometry systems for detailed mitochondrial characterization. We also make use of analytic tools together with modelling approaches to quantify cellular fluxes and control, and proteome efficiency in metabolic routes.

Current projects include exploring alternative feedstocks, investigating yeast diversity and enhancing biomass yields. A particular focus is on the composition of the electron transport chain in relation to cellular yields in yeasts [1]. In addition, we are initiating new projects on $F_1F_o$-ATP synthase engineering and also on the efficiency of mammalian cells in bioreactors for bioproduction.

[1] M. Warmerdam, Specific growth rates and growth stoichiometries of Saccharomycotina yeasts on ethanol as sole carbon and energy substrate (2024) Manuscript in preparation.

doi:10.1016/j.bbabio.2024.149245

**T6 P-6**

**The structure of a catalytically inactive Paracoccus denitrificans complex I mutant under turnover conditions**

Robert A. Waddell[a], Owen D. Jarman[a,b], Bozhidar S. Ivanov[a], Hannah R. Bridges[c], Judy Hirst[a]

[a]*Medical Research Council Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom*
[b]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[c]*Structura Biotechnology Inc., Toronto, Canada*
E-mail address: raw84@mrc-mbu.cam.ac.uk (R.A. Waddell)

Respiratory complex I is a key metabolic enzyme across many respiratory chains, pairing the reduction of NADH and oxidation of a quinone to pump four protons across an energy transducing membrane, maintaining a protonmotive force that drives the cell. The mechanisms of catalysis and regulation of complex I remain contentious, in particular the coupling of proton pumping in the membrane arm to redox reactions in the hydrophilic arm [1, 2]. This is in part due to the lack of structures of biochemically characterised intermediates on the complex I catalytic cycle. Paracoccus denitrificans provides a genetically tractable model organism, closely related to the mammalian mitochondrion, from which complex I can be purified and structurally characterised [3]. Mutations have been generated in P. denitrificans complex I which prevent turnover, offering an opportunity to capture a uniform population stalled during a turnover cycle, as opposed to a mixture of intermediates captured under normal turnover conditions. Here we elucidate the structure of a catalytically dead complex I mutant in P. denitrificans, stabilised in nanodiscs, in an attempt to capture a stalled intermediate in the turnover cycle of complex I.

[1] I. Chung, D.N. Grba, J. J. Wright, J. Hirst, Making the leap from structure to mechanism: are the open states of mammalian complex I identified by cryoEM resting states or catalytic
intermediates?, Curr Opin Struct Biol, 77 (2022) 102447.

[2] D. Kampjut, L. A. Sazanov, Structure of respiratory complex I – An emerging blueprint for the mechanism, Curr Opin Struct Biol, 74 (2022) 102350.

[3] O.D. Jarman, O. Biner, J.J. Wright, J. Hirst, Paracoccus denitrificans: a genetically tractable model system for studying. respiratory complex I, Sci Rep, 11(1) (2021) 10143.

doi:10.1016/j.bbabio.2024.149246

**T6 P-7**

**As published in the 2024 European Congress on Biotechnology abstracts: Exploring yeast diversity for ethanol-fueled industrial biotechnology**

Marieke Warmerdam, Marcel Viera-Lara, Robert Mans, Jean Marc Daran, Jack Pronk

*Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, 2629, HZ, Delft, The Netherlands*
E-mail address: M.Warmerdam@tudelft.nl (M. Warmerdam)

Ethanol is produced at industrial scale from non-agricultural feedstocks by gas fermentation, while research on other low-emission processes for ethanol production is accelerating. In view of its degree of reduction, water solubility and relatively low toxicity, ethanol is an interesting candidate to replace sugars in aerobic, zero-emission processes for yeast-based production of whole-cell protein and low-molecular-weight compounds. Currently, little information is available on specific growth rates, biomass yields and biomass composition of yeast species during growth on synthetic medium with ethanol as sole carbon source. In this study, strains of 52 Saccharomycotina yeasts were screened for their growth characteristics on ethanol. After first screening in microtiter plates, 21 fast-growing strains that were further analysed in aerobic shake-flask cultures showed specific growth rates of 0.12-0.46 $h^{-1}$. Five fast-growing strains were further studied in aerobic, ethanol-limited chemostats (dilution rate 0.10 $h^{-1}$). Strains of the industrial yeasts Saccharomyces cerevisiae and Kluyveromyces lactis, whose genomes lack genes for a proton-coupled Complex-I NADH dehydrogenase, both showed biomass yields of 0.6 g biomass (g ethanol)$^{-1}$. Of three yeasts whose genome does contain Complex-I genes, Phaffomyces thermotolerans, showed the same biomass yield as S. cerevisiae, while Ogataea parapolymorpha and Cyberlindnera jadinii showed biomass yields of 0.67 ± 0.01 and 0.73 ± 0.00 g $g^{-1}$, respectively. The biomass yield of C. jadinii, which also showed the highest protein content of the 5 yeasts tested in chemostats, corresponded to 88% of the theoretical biomass yield in a scenario where growth is limited by assimilation rather than by energy metabolism.

doi:10.1016/j.bbabio.2024.149247

## T7 Respiratory complexes and supercomplexes - structure and function

### T7 P-1

### Structure of respiratory Complex I from *Yarrowia lipolytica* under turnover conditions

Jakob Andersson, Olga Petrova, Leonid Sazanov

*Institute of Science and Technology Austria, Klosterneuburg, Austria*
E-mail address: leonid.sazanov@ista.ac.at (L. Sazanov)

Respiratory complex I (NADH:ubiquinone oxidoreductase) is the starting point for biochemical energy conversion in bacteria and mitochondria and maintains approximately 40% of the proton gradient driving ATP synthesis by oxidising NADH and reducing ubiquinone. Deficiency of complex I causes a wide range of diseases such as cardiomyopathy and lactic acidosis[1] and alterations in complex I function are implicated in the proliferation of cancer cells [2]. A better understanding of its structure, function and assembly are essential to develop treatments for these conditions and due to its structural similarity, yeast can serve as a model organism for mammalian complex I.

We show here four distinct conformational states of the aerobic yeast Yarrowia lipolytica under turnover conditions, including closed, open and intermediate states at resolutions up to 2.3 Å. Similar to E. coli complex I, we found closed state only under turnover conditions while in the apo-state, the majority of the protein adopts the open-ready conformation that was previously observed only in E. coli [3]. Moreover, while mammalian complex I shows a strong deactive conformational state that is thought to exist primarily to protect from ischemia/reperfusion injury and reduce damaging ROS generation, we did not find this state in Yarrowia lipolytica.

[1] H. Swalwell, D. Kirby, E. Blakely, et al., Respiratory Chain Complex I Deficiency Caused by Mitochondrial DNA Mutations, Eur J Hum Genet, 19 (2011) 769-775.

[2] M. Sollazzo, M. De Luise, S. Lemma, L. Bressi, M. Iorio, S. Miglietta, S. Milioni, I. Kurelac, L. Iommarini, G. Gasparre, A.M. Porcelli, Respiratory Complex I Dysfunction in Cancer: From a Maze of Cellular Adaptive Responses to Potential Therapeutic Strategies, FEBS J, 289 (2022) 8003-8019.

[3] V. Kravchuk, O. Petrova, D. Kampjut, A. Wojclechowska-Bason, Z. Breese, L. Sazanov, A Universal Coupling Mechanism of Respiratory Complex I, Nature, 609 (2022) 808-837.

doi:10.1016/j.bbabio.2024.149248

### T7 P-2

### Respiratory supercomplexes act early to support complex I assembly

Maria G. Ayala-Hernandez[a], Claire Montgomery[b], Gino Cortopassi[b], James A. Letts[a]

[a]*Department of Molecular and Cellular Biology, University of California, Davis, United States*
[b]*Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, United States*
E-mail address: jaletts@ucdavis.edu (J.A. Letts)

The most common primary mitochondrial diseases result from mutations of complex I. Inheritance of mutant NDUFS4, thought to support complex I assembly, causes partial complex I deficiency and the severe neuromuscular disease Leigh syndrome. However, even with complete deletion of NDUFS4 residual complex I activity remains in mice. We have investigated the state of assembly of complex I and respiratory supercomplexes isolated from livers of NDUFS4 mutant mice. We confirm holo-complex I only exists in supercomplexes, as do incompletely assembled complex I subassemblies. We determine the structure of supercomplexes containing complex I subassemblies lacking: 1) the N-module (N-less); and 2) entire peripheral arm (membrane domain alone). Thus, we observe stalled complex I assembly intermediates bound to complex $III_2$ and complex IV. These findings support the view that supercomplexes are early scaffolds of complex I assembly and not a late stage higher-order product forming after all complexes are fully assembled and thus, enhancing supercomplex formation may help mitigate complex I deficiency.

doi:10.1016/j.bbabio.2024.149249

### T7 P-3

### Phosphate Regulates the Acute Changes in Complex V Association in Skeletal Muscle Mitochondria During Calcium Overload

Maria Canellas da Silva, Sarah Kuzmiak-Glancy

*Department of Kinesiology, University of Maryland, United States of America*
E-mail address: canellas@umd.edu (M.C. da Silva)

Skeletal muscle mitochondria are sensitive to intracellular signals to increase ATP production, with calcium (Ca++) being a key signaling molecule. A rise in cytosolic Ca++ results in mitochondrial uptake, with physiological Ca++ levels activating mitochondrial ATP production and supraphysiological levels leading mitochondrial permeability transition pore (MPTP) opening and loss of function. While these functional changes occur in seconds, it remains unknown if reorganization of mitochondrial complexes, particularly ATP synthase, can be acutely induced, and how intracellular signals affect this reorganization. **PURPOSE:** The goal of this study was to determine if activating and/or overload $Ca^{++}$ levels 1) induce changes in the organization of the mitochondrial complexes, specifically, the organization of CV into monomers ($CV_1$) and dimers ($CV_2$), 2) and if Pi plays a role in the regulation of $CV_1$ and $CV_2$ formation. **METHODS:** Mitochondria were isolated from skeletal muscle of Sprague-Dawley rats, then incubated in respiration media with glutamate + malate (sample aspirated), acutely subjected to activating Ca++ or overload Ca++ (samples aspirated), and then 10 mM Pi was added (samples aspirated). Aspirated mitochondria suspension samples were pelleted, solubilized (digitonin), and separated on a BN-PAGE gel to allow for the assessment of $CV_1$ & $CV_2$, analyzed via densitometry. **RESULTS:** Acute exposure of mitochondria to Ca++ resulted in a decrease in $CV_1$ and a concomitant increase in $CV_2$. Ca++ overload resulted in a larger decrease in $CV_1$ compared to Ca++ activation ($CV_1$ %76.34±5.4 vs 58.4±3.3), and a larger increase in $CV_2$ abundance ($CV_2$% 22.7+2.5 vs 35.3±3). $CV_1$ abundance decreased further when Pi was added after Ca++ overload; however, CV1 abundance increased when Pi was added after Ca++ activation. Cyclosporin A, an MPTP blocker, did not prevent the decrease in $CV_1$ percentage during Ca++ overload, with or without the presence of Pi ($CV_1$% 55.6±5 vs 47.6 ±7, without Pi, p=0.069; 54.6±5 vs 42.7±6.6, with Pi, p=0.058).

**CONCLUSION:** Mitochondrial complex associations can be acutely altered by intracellular signals. Calcium activation and overload both appear to induce the CV to dimerize, and Pi plays a regulatory role in this dimerization. This modifiable, dynamic complex reorganization may play a key role in the mechanisms involved in MPTP opening.

doi:10.1016/j.bbabio.2024.149250