under anaerobic conditions and it enables anaerobic growth with urocanic acid as a sole terminal electron acceptor. The latter’s capability can provide the cells of UrdA-containing bacteria with a niche where no other bacteria can compete and survive.

Reference

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S8.P6

**Flavin based electron bifurcation: A mechanistic approach**

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Energy conservation in strict anaerobic bacteria and archaea was thought to be only mediated by substrate level phosphorylation (SLP). However, their energetics could not be completely understood, until in clostridia a flavin-based electron bifurcation process was discovered in 2008. Its later detection in methanogenic archaea and acetogenic bacteria as well as probably in sulfate reducing bacteria and benzoate degrading anaerobes demonstrates its fundamental importance in bioenergetics since the origin of life [1]. In butyric acid forming bacteria, an electron bifurcating reaction is catalyzed by electron transferring flavoprotein (Etf) and butyryl CoA-dehydrogenase (Bcd); the two electrons of NADH (E' = –280 mV) bifurcate to the high potential crotonyl-CoA (E' = –10 mV) and the low potential ferredoxin (E' = –500 mV). Reduced ferredoxin, the preferred electron donor of anaerobic bacteria and archaea, reduces protons to H2 or generates ΔψNa+ via the membrane bound ferredoxin-NAD+ reductase (Rfn). Using crystallographic and UV-visible spectroscopic methods, we gained profound mechanistic insights into electron bifurcating process of Etf and Bcd of *Acidaminococcus fermentans* [2]. The heterodimeric Etf contains two FAD, α-FAD in subunit α and β-FAD in subunit β. The Etf-NAD+ complex structure revealed β-FAD as acceptor of the hydride of NADH and as bifurcating FAD. α-FAD is able to approach β-FADH– and takes up one electron yielding a stable anionic semiquinone, α-FAD2–, which donates this electron further to D-FAD of Bcd most likely after a large-scale conformational change. The remaining non-stabilized neutral semiquinone, β-FADH, positioned close to the protein surface, immediately reduces ferredoxin. Repetition of this process affords a second reduced ferredoxin and D-FADH of Bcd that converts crotonyl-CoA to butyryl-CoA [2].

References

S8.P7

**In vivo imaging of the supramolecular organization of *Escherichia coli* OXPhos complexes**

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Aerobic respiring bacteria gain ATP predominantly by substrate level phosphorylation (SLP) and by electron transport phosphorylation (ETP). In many of these organisms ETP is assumed to be the main contributor to ATP synthesis. This qualifies the ETP as a promising metabolic engineering target aiming at the design of energetically superior strains.

In this study, we use a data-driven model-based approach to achieve, for the first time, a quantitative characterization of energy formation in the industrial platform organism *Corynebacterium glutamicum*. This superior amino-acid producer possesses a respiratory type of energy metabolism with oxygen or nitrate as terminal electron acceptors. ATP can either be synthesized by SLP or by ETP with the membrane-bound F1F0-ATP synthase using the proton motive force (PMF) as driving force [1]. The respiratory chain of *C. glutamicum* contains two terminal oxidases for oxygen as terminal electron acceptor differing in proton translocation efficiency by a factor of three.

13C-labeling experiments were performed with the wild type and a mutant that is solely dependent on ATP generated by SLP (ΔΔF0) [2]. Data were evaluated with 13C metabolic flux analysis [3]. A comparison of the in vivo metabolic reaction rates revealed that SLP and ETP contribute equally to ATP generation. Additionally, the results predicted that 65% of the PMF is actually not used for ATP synthesis, possibly leaving room for improving the efficiency of the ETP and the product formation, e.g. by overexpressing the ATP synthase to increase the portion of PMF used by the ETP.

References

S8.P8

**Protein–protein interaction in *Rhodothermus marinus* respiratory chain studied by NMR spectroscopy**

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References

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References

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