investigate the physiological role of the three terminal oxidases under aerobic and microaerobic conditions. Our data revealed that the cbh1-type oxidase is the major terminal oxidase under aerobic conditions while both cbh2-type and bd-type oxidases are involved in respiration at low-O2 tensions. On the contrary, the low O2-affinity A-type cytochrome c oxidase was not detected in our experimental conditions even under aerobic conditions and would therefore not be required for aerobic respiration in \textit{S. oneidensis} MR-1. In addition, the deduced amino acid sequence suggests that the A-type cytochrome c oxidase is a cacaa-type oxidase since an uncommon extra-C-terminal domain contains two c-type heme-binding motifs, an uncommon feature among A-type oxidases.

Reference
[1] S. Le Laz, A. Kpebe, M. Bauzan, S. Lignon, M. Rousset, M. Brugna, P. Hildebrandt, M. Yamazaki, F. Amano, S. Harada, K. Kita, K. Ito, Unraveling of diiron-containing oxygen reductases. The nuclear-encoded subunit 5 of \textit{Saccharomyces cerevisiae} cytochrome c oxidase (COX) has two isoforms, 5A and 5B. Their expression is differentially modulated by oxygen concentration. COX5A is expressed under aerobic conditions while COX5B is expressed only at oxygen tensions below 1 \(\mu\)M. Since subunit 5 is essential for enzyme assembly, under aerobic growth conditions a COX5A-deleted strain contains no or very low level of COx and so is respiratory deficient [1]. Previously, respiratory growth was restored by combining mutations of \textit{ROX1} that encodes a transcriptional repressor of \textit{COX5B} expression with \textit{ΔcoxA} [1]. The level of 5B isoform expression in these mutants was 30–50% of wildtype (5A isoform) and its maximum turnover number was up to 3 fold greater than that of the 5A isoform [2]. To assess the structural basis of this elevated activity, a mutant strain was constructed in which \textit{COX5B} was inserted downstream of the \textit{COX5A} promoter. This allowed the 5B isoform to be expressed at wildtype levels without the complications of additional mutation in a transcription factor. When expressed in this manner, the isoforms displayed no significant differences in their maximum catalytic activities or in their affinities for cytochrome c or oxygen. Hence, the elevated activity of the 5B isoform in the \textit{rox1} mutant is not caused simply by exchange of isoforms and must arise from a secondary effect that is still to be resolved.

References

S9.P5
Diiron four-helix-bundle proteins in oxygen and hydrogen peroxide detoxification
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Oxygen and derived reactive species pose a huge challenge to present day living forms, and specialized enzymes exist to deal with their toxicity. Among those, are the large family of diiron-containing enzymes, such as the so-called alternative oxidase, a quinol:oxygen oxidoreductase [1], and the rubrerythrin sub-family, putative hydrogen peroxide reductases [2–3]. Both rubrerythrins and AOX share a common four-helix-bundle structural fold, harboring a catalytic diiron center bound to histidines and aspartates/glutamates and a μ-(hydr)oxo bridge in the d ferric state. Rubrerythrins may contain additional domains (one or two), namely possessing single mononuclear rubredoxin-type [FeCys₄] centers, located either before or after the four-helix bundle (e.g. [3]).

The most consensual activity for rubrerythrins is that of \(H_2O_2\) reductase, linked to NADH oxidation by redox partner enzymes. Rubrerythrin-like enzymes are present in the three life domains. Biochemical and enzymatic, spectroscopic, and structural studies on several rubrerythrins, of the simplest one-domain enzyme (from the hyperthermoacidophilic Archaeon \textit{Acidianus ambivalens} erythrin), of \textit{Cl} \textit{dium difficile} (a bacterial human pathogen) two-domain rubrerythrin, and the more complex three-domain \textit{Campylobacter jejuni} (also a human bacterial pathogen), desulfuruberythrin, will be described. A detailed comparison of the 3D structures of AOX and rubrerythrins will be presented, which reinforce our previous hypothesis [4] that rubrerythrins may have been primitive ancestors of diiron-containing oxygen reductases.

References

S9.P6
Kinetic comparisons of 5A and 5B isozymes of yeast cytochrome c oxidase
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The nuclear-encoded subunit 5 of \textit{Saccharomyces cerevisiae} cytochrome c oxidase (COX) has two isoforms, 5A and 5B. Their expression is differentially modulated by oxygen concentration. COX5A is expressed under aerobic conditions while COX5B is expressed only at oxygen tensions below 1 \(\mu\)M. Since subunit 5 is essential for enzyme assembly, under aerobic growth conditions a COX5A-deleted strain contains no or very low level of COx and so is respiratory deficient [1]. Previously, respiratory growth was restored by combining mutations of \textit{ROX1} that encodes a transcriptional repressor of \textit{COX5B} expression with \textit{ΔcoxA} [1]. The level of 5B isoform expression in these mutants was 30–50% of wildtype (5A isoform) and its maximum turnover number was up to 3 fold greater than that of the 5A isoform [2]. To assess the structural basis of this elevated activity, a mutant strain was constructed in which \textit{COX5B} was inserted downstream of the \textit{COX5A} promoter. This allowed the 5B isoform to be expressed at wildtype levels without the complications of additional mutation in a transcription factor. When expressed in this manner, the isoforms displayed no significant differences in their maximum catalytic activities or in their affinities for cytochrome c or oxygen. Hence, the elevated activity of the 5B isoform in the \textit{rox1} mutant is not caused simply by exchange of isoforms and must arise from a secondary effect that is still to be resolved.

References

S9.P7
Revisiting individual absorption spectra of reduced hemes \(α\) and \(α_5\) in bovine-heart cytochrome c oxidase
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Cytochrome c oxidase (COX) is a terminal enzyme of the respiratory chain which catalyzes electron transport (ET) from cytochrome c to \(O_2\) coupled to transmembrane \(H^+\)-pumping. The ET is mediated by two
Flavodiiron enzymes as oxygen and/or nitric oxide reductases

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The flavodiiron proteins (FDPs) are present in all life domains, from unicellular microbes to higher eukaryotes. FDPs reduce oxygen to water and/or nitrous oxide to nitrous oxide, actively contributing to combat the toxicity of O2 or NO. The catalytic ability of FDPs is comparable to that of bona fide heme–copper/iron O2/NO transmembrane reductases. FDPs are multi-modular water soluble enzymes, exhibiting a two-domain catalytic core, whose the minimal functional unit is a ‘head-to-tail’ homodimer, each monomer being built by a beta-lactamase domain harbouring a diiron catalytic site, and a short-chain flavodoxin, binding FMN [1–3].

Despite extensive data collected on FDPs, the molecular determinants defining their substrate selectivity remain unclear. To clarify this issue, two FDPs with known and opposite substrate preferences were analysed and compared: the O2-reducing FDP from the eukaryote Entamoeba histolytica (EhFdp1) and the NO reductase FIRd from Escherichia coli. While the metal ligands are strictly conserved in these two enzymes, differences near the active site were observed. Single and double mutants of the EhFdp1 were produced by replacing the residues in these positions with their equivalent in the E. coli FIRd. The biochemical and biophysical features of the EhFdp1 WT and mutants were studied by potentiometric-coupled spectroscopic methods (UV-visible and EPR spectroscopies). The O2/NO reactivity was analysed by amperometric methods and stopped-flow absorption spectroscopy.

The reactivity of the mutants towards O2 was negatively affected, while their reactivity with NO was enhanced. These observations suggest that the residues mutated have a role in defining the substrate selectivity and reaction mechanism.

References

