other pathological manifestations, under conditions where the oxidative phosphorylation system is limiting or unavailable e.g., due to inhibition by toxins, metabolic overload or genetic ablation of one or more of its components. The alternative enzymes are non-proton-motive, and are typically composed of just a single polypeptide, in contrast to the highly complex enzymes of the oxidative phosphorylation system. Vertebrates lack the alternative enzymes, leading to the idea that we could alleviate respiratory chain dysfunction such as those which occurs in pathological states, by expressing genes for these enzymes therapeutically or even prophylactically in humans. To this end, we have set about transferring the relevant genes to model organisms, using as our source material the urochordate Ciona intestinalis, a representative of the group of organisms phyletically closest to the vertebrates, but which has retained the alternative enzymes over the course of evolution. The Ciona alternative oxidase (AOX) can be expressed in both Drosophila and the mouse throughout the life cycle and across a wide range of tissues. Under standard conditions, it contributes hardly at all to mitochondrial electron flow, does not interfere with the oxidative phosphorylation system, and appears to cause no significant deleterious effects. However, under stress conditions, including both acute and chronic exposures to respiratory toxins, genetic deficiency of cytochrome oxidase, or treatments that create or potentiate overproduction of ROS, AOX can alleviate pathological phenotypes in cells, flies and in mice. If safe and efficient delivery of AOX can be achieved, it has enormous potential as a wide-spectrum therapy for conditions associated with primary or secondary mitochondrial dysfunction.

This work was funded by ERC, Academy of Finland, Tampere University Hospital Medical Research Fund and the Sigrid Juselius Foundation. I am indebted to a large team of laboratory scientists and collaborators who have brought the project to fruition, including Marten Szibor, Pierre Rustin, Thomas Braun, Eric Dufour, Riyad El-Khoury, Thomas Braun, Robert Voswinckel, Ilka Wittig, Alberto Sanz, Norbert Weissmann, who have brought the project to fruition, including Marten Szibor, Pierre Rustin, Thomas Braun, Eric Dufour, Riyad El-Khoury, Norbert Weissmann, Ilka Wittig, Alberto Sanz, Susi Vartiainen, Peter Rauschkolb, Frank Gellerich, Proveen Dhandapani, Ana Andjelkovic, Kumpainen, Frank Hirth, Gertjan Hakkaart, Emmanuel Dassa and Daniel Fernandez-Ayala.

doi:10.1016/j.bbabio.2014.05.226

S6.14

Predictive clustering of POLG disease mutations into functional modules in the human mitochondrial replicase

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We have established the genotype–phenotype correlations for the complete spectrum of POLG syndromes, by refining our previously described protocol for mapping pathogenic mutations in the human POLG gene to functional clusters in the catalytic core of the mitochondrial replicase, Pol γ. We assigned 136 mutations to five clusters and identify segments of primary sequence that can be used to delimit the boundaries of each cluster. We report that compound heterozygous patients with two mutations from different clusters manifested more severe, earlier onset POLG syndromes, whereas two mutations from the same cluster are less common and generally are associated with less severe, later onset POLG syndromes. We also show that specific cluster combinations are more severe than others, and have a higher likelihood to manifest at an earlier age. Our clustering method provides a powerful tool to predict the pathogenic potential and predicted disease phenotype of novel variants and mutations in POLG, the most common nuclear gene underlying mitochondrial disorders. We propose that such a prediction tool would be useful for routine diagnostics for mitochondrial disorders.

doi:10.1016/j.bbabio.2014.05.227

S6.01

The crystal structure of the NADH-bound form of the Escherichia coli Alkylhydroperoxide Reductase subunit AhpF and the basis for substrate specificity and alternating conformation during catalysis

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Reactive oxygen species (ROS) are toxic to the cells and there must be specific defense mechanism in the cells to alleviate oxidative stress. The primarily responsible enzyme for this reaction is the Alkylhydroperoxide Reductase (AhpF) enzyme, consisting of the 57 kDa flavo protein AhpF (PrxR) expressed only by eubacteria and the 21 kDa AhpC (Prx) protein expressed in all kingdoms [1,2]. The reduction of peroxides occurs via the redox active disulphide center of AhpF, whose reduced state becomes restored by AhpF, which transfers electrons from NADH onto AhpC. AhpF consists of three domains; thioredoxin (Trx) like N-terminal domain (NTD) attached to the thioredoxin reductase (TrxR) like C-terminal portion containing FAD- and NADH-binding domains. The proposed catalytic turnover of AhpF may require major domain motions, analogous to those deduced from bacterial Trx/TrxR system [3]. Here we are presenting for first time the crystal structure of E. coli AhpF complex with its substrate NADH at 2.5 Å resolution [4]. The structure reveals the basis of its substrate specificity for NADH over NADPH. In addition, it implies the alternating conformation required for the C-terminal NADH-binding domain to bring a bound NADH to the re-face of the isalloxazine ring of flavin, and enabling the electron transfer to occur as well as the oxidation of NADH-domain dithiol via the redox active disulphide centre of the NTD. The detailed structural model will open future research opportunities to design selective drug against bacterial AhpF as potential target.

References


doi:10.1016/j.bbabio.2014.05.228