Box 1

Mitochondria are dynamic organelles contained within eukaryotic cells, with a double membrane. The inner mitochondrial membrane shows dynamic tubular and disk-shaped cristae that separate the mitochondrial matrix, i.e. the internal mitochondrial compartment, and the intermembrane space; the latter being enclosed by the outer mitochondrial membrane. Mitochondria were described for the first time in 1857 by Rudolph Albert von Kölliker as granular structures or 'sarkosomes'. In 1886 Richard Altman called them 'bioblasts' (published 1894). The word 'mitochondrium' (Greek mitos: thread; chondros: granule) was introduced by Carl Benda (1898). Mitochondria are the oxygen consuming electrochemical generators which evolved from endosymbiotic bacteria (Margulis 1970). The bioblasts of Richard Altmann (1894) include not only the mitochondria as presently defined, but also symbiotic and free-living bacteria. Mitochondria are the structural and functional elemental units of cell respiration, where cell respiration is defined as the consumption of oxygen coupled to electrochemical proton translocation across the inner mitochondrial membrane. In the process of oxidative phosphorylation (OXPHOS), the reduction of O_2 is electrochemically coupled to conservation of energy in the form of ATP (Mitchell 2011). As part of the OXPHOS system, these powerhouses of the cell contain the transmembrane respiratory complexes (i.e. FMN, Fe-S and cytochrome b, c, aa3 redox systems), alternative dehydrogenases and oxidases, the coenzyme ubiquinone (coenzyme Q) and ATP synthase together with the enzymes of the tricarboxylic acid cycle and the fatty acid oxidation enzymes, ion transporters, including substrate, co-factor and metabolite transporters, as well as proton pumps, and mitochondrial kinases related to energy transfer pathways. The mitochondrial proteome comprises over 1,200 proteins (Mitocharta), mostly encoded by nuclear DNA (nDNA), with a variety of functions, many of which are relatively well known (e.g. apoptosis-regulating proteins~~),~~); while others are still under investigation, or need to be identified (alanine transporter). Mitochondria maintain several copies of their own genome (hundred to thousands per cell) which is maternally inherited and known as mitochondrial DNA (mtDNA). mtDNA is 16.5 Kb in length, contains 13 protein-coding genes for subunits of the transmembrane respiratory Complexes CI, CIII, CIV and ATP synthase, and also encodes 22 tRNAs and the mitochondrial 16S and 12S rRNA. The mitochondrial genome is both regulated and supplemented by nuclear-encoded mitochondrial targeted proteins. Evidence has accumulated that additional gene content is encoded in the mitochondrial genome, e.g. microRNAs, smithRNAs, and even additional proteins. The inner mitochondrial membrane contains the non-bilayer phospholipid cardiolipin, which is not present in any other eukaryotic cellular membrane. Cardiolipin promotes the formation of respiratory supercomplexes, which are supramolecular assemblies based upon specific, though dynamic, interactions between individual respiratory complexes (Lenaz et al. 2017).
Mitochondria are highly mobile organelles, while recent studies indicate that there is an extensive wiring and functional crosstalk ~~There is a constant crosstalk~~ between mitochondria related processes that maintain cellular mitostasis and most (if not all) ~~the~~ other cellular components and signalling pathways including proteostatic (e.g. the ubiquitin-proteasome and autophagy-lysosome pathways) and genome stability modules. This functional crosstalk can occur at both the ~~at the~~ transcriptional and~~or~~ post-translational level~~, and~~ through cell signalling in response to varying energy demands (Quiros et al. 2016) or as a result of cellular adaptations to (among others) differentiation, different cell cycle phases or even cell death. Mitochondria are highly dynamic organelles and thus m~~M~~itochondrial morphology can change e.g. in response to energy requirements of the cell via processes that constitute the so-called mitochondrial dynamics and are known as fusion and fission. Through these processes ~~through which~~ mitochondria can communicate within a network, and in various pathological states which cause swelling or dysregulation of fission and fusion. Mitochondrial dysfunction is associated with aging and a wide variety of genetic and degenerative diseases. Therefore, a better understanding of mitochondrial physiology and functional crosstalk with other cellular components will improve our understanding of the etiology of aging, disease and the diagnostic repertoire of mitochondrial medicine. Furthermore, *in vivo* studies in model organisms (e.g. *C. elegans* or *D. melanogaster*) from MitoEAGLE partners will enhance current knowledge on mitochondria physiology at the tissue and whole organism level.
Abbreviation: mt, as generally used in mtDNA. Mitochondrion is singular and mitochondria is plural.

1. Introduction

Mitochondria are the powerhouses of the cell with numerous physiological, molecular, and genetic functions (Box 1). Every study of mitochondrial function and disease is faced with Evolution, Age,

Gender and sex, Lifestyle, and Environment (EAGLE) as essential background conditions characterizing the individual patient or subject, cohort, species, tissue and to some extent even cell line. As a large and highly coordinated group of laboratories and researchers, the global MitoEAGLE Network's mission is to generate the necessary scale, type, and quality of consistent data sets and conditions to address this intrinsic complexity. Harmonization of experimental protocols and implementation of a quality control and data management system is required to interrelate results gathered across a spectrum of cell based and *in vivo* studies and to generate a rigorously monitored database focused on mitochondrial respiratory function. In this way, researchers within the same and across different disciplines will be positioned to compare their findings to an agreed upon set of clearly defined and accepted international standards. Reliability and comparability of quantitative results depend on the accuracy of measurements under strictly-defined conditions. A conceptually clearly-defined framework is also required to warrant meaningful interpretation and comparability of experimental outcomes carried out by research groups at different institutes. With an emphasis on quality of research, cell based or *in vivo* ~~collected~~ data can be useful far beyond the specific question of a specific experiment. Vague or ambiguous jargon can lead to confusion and may relegate valuable signals to wasteful noise. For this reason, measured values must be expressed in standardized units for each parameter used to define mitochondrial respiratory function. Standardization of nomenclature and technical terms is essential to improve the awareness of the intricate meaning of divergent scientific vocabulary. The focus on coupling states in mitochondrial preparations is a first step in the attempt to generate a harmonized and conceptually oriented nomenclature in bioenergetics and mitochondrial physiology. Coupling states of intact cells and respiratory control by fuel substrates and specific inhibitors of respiratory enzymes will be reviewed in subsequent communications.

Conclusions

MitoEAGLE can serve as a gateway to better diagnose mitochondrial functionality and respiratory defects linked to genetic variation, aging, age-related health risks, sex-specific mitochondrial performance and ~~,~~ lifestyle along with its ~~with its~~ effects on degenerative diseases, and thermal ~~and~~ or chemical environment. The present recommendations on coupling control states and rates, linked to the concept of the protonmotive force (Part 1) will be extended in a series of reports on pathway control of mitochondrial respiration, respiratory states in intact cells, and harmonization of experimental procedures. The optimal choice for expressing mitochondrial and cell respiration (Box 5) as O_2 flow per biological system, and normalization for specific tissue ~~markers~~ (volume, mass, protein) and mitochondrial ~~markers~~ (volume, protein, content, mtDNA, activity of marker enzymes, respiratory reference state) markers is guided by the scientific question under study. Interpretation of the obtained data depends critically on appropriate normalization, and therefore reporting rates merely as nmol·s-1 is discouraged, since it restricts the analysis to intra-experimental comparison of relative (qualitative) differences. Expressing O_2 consumption per cell may not be possible when dealing with tissues. For studies with mitochondrial preparations, we recommend that normalizations be provided as far as possible: (1) on a per cell basis as O_2 flow (a biophysical normalization); (2) per g cell or tissue protein, or per cell or tissue mass as mass-specific O_2 flux (a cellular normalization); and (3) per mitochondrial marker as mt-specific flux (a mitochondrial normalization). With information on cell size and the use of multiple normalizations, maximum potential information is available (Renner et al. 2003; Wagner et al. 1257 2011; Gnaiger 2014). When using isolated mitochondria, mitochondrial protein is a frequently applied mitochondrial marker, the use of which is basically restricted to isolated mitochondria. Mitochondrial markers, such as citrate synthase activity as an enzymatic matrix marker, provide a link to the tissue of origin on the basis of calculating the mitochondrial yield, i.e., the fraction of mitochondrial marker obtained from a unit mass of tissue.