Molecular BioSystems

RSC Publishing

REVIEW

View Article Online
View Journal | View Issue



Cite this: *Mol. BioSyst.*, 2013, **9**, 1984

# The mitochondrial Italian Human Proteome Project initiative (mt-HPP)†

Andrea Urbani,*[ab] Michele De Canio,[bc] Ferdinando Palmieri,[d] Salvatore Sechi,[e] Luca Bini,[f] Massimo Castagnola,[g] Mauro Fasano,[h] Alessandra Modesti,[i] Paola Roncada,[cj] Anna Maria Timperio,[k] Luigi Bonizzi,[c] Maurizio Brunori,[l] Francesca Cutruzzolà,[l] Vito De Pinto,[m] Carmine Di Ilio,[no] Giorgio Federici,[p] Franco Folli,[q] Salvatore Foti,[r] Cecilia Gelfi,[st] Davide Lauro,[u] Antonio Lucacchini,[v] Fulvio Magni,[w] Irene Messana,[x] Pier Paolo Pandolfi,[y] Sergio Papa,[z] Piero Pucci,[aa] Paolo Sacchetta[ab] and the Italian mt-HPP Study Group-Italian Proteomics Association (www.itpa.it)

Received 15th February 2013,
Accepted 24th April 2013

DOI: 10.1039/c3mb70065h

www.rsc.org/molecularbiosystems

Mitochondria carry maternally inherited genetic material, called the mitochondrial genome (mtDNA), which can be defined as the 25th human chromosome. The chromosome-centric Human Proteome Project (c-HPP) has initially focused its activities addressing the characterization and quantification of the nuclear encoded proteins. Following the last International HUPO Congress in Boston (September 2012) it was clear that however small the mitochondrial chromosome is, it plays an important role in many biological and physiopathological functions. Mutations in the mtDNA have been shown to be associated with dozens of unexplained disorders and the information contained in the mtDNA should be of major relevance to the understanding of many human diseases. Within this paper we describe the Italian initiative of the Human Proteome Project dedicated to mitochondria as part of both programs:

[a] *Department of Experimental Medicine and Surgery, University of Rome "Tor Vergata", Via Montpellier 1, 00133 Rome, Italy. E-mail: andrea.urbani@uniroma2.it; Fax: +39-06-501703332*
[b] *IRCCS-Santa Lucia Foundation, Rome, Italy*
[c] *Dipartimento di Scienze Veterinarie e Sanità Pubblica, Università degli Studi di Milano, Milano, Italy*
[d] *Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy*
[e] *National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, USA*
[f] *Department of Biotechnologies, University of Siena, Siena, Italy*
[g] *Biochemistry and Clinical Biochemistry Institute, "Cattolica" University, Rome, Italy*
[h] *Department of Theoretical and Applied Sciences, University of Insubria, Busto Arsizio, Italy*
[i] *Department of Biochemistry, University of Firenze, Firenze, Italy*
[j] *Istituto Sperimentale Italiano L. Spallanzani, Milano, Italy*
[k] *Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy*
[l] *Department of Biochemical Sciences, University of Rome "La Sapienza", Rome, Italy*
[m] *Department of Biology, Geology and Environmental Sciences, University of Catania, Catania, Italy*
[n] *Ce.S.I. Center of Excellence on Aging, Chieti, Italy*
[o] *Department of Experimental and Clinical Sciences, University of Chieti "G. D'Annunzio", Chieti, Italy*
[p] *IRCCS-Children Hospital "Bambino Gesù", Rome, Italy*
[q] *Department of Medicine, the University of Texas Health Science Center San Antonio, San Antonio, TX, USA*
[r] *Department of Chemical Science, University of Catania, Catania, Italy*
[s] *SysBio Centre for Systems Biology, Milan and Rome, Italy*
[t] *IBFM-CNR, Milan, Italy*
[u] *Department of System Medicine, University of Rome "Tor Vergata", Rome, Italy*
[v] *Department of Pharmacy, University of Pisa, Pisa, Italy*
[w] *Department of Experimental Medicine, "Bicocca" University, Milan, Italy*
[x] *Department of Life and Environment Sciences, University of Cagliari, Cagliari, Italy*
[y] *Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA*
[z] *Institute of Biomembranes and Bioenergetics, National Research Council (CNR), Bari, Italy*
[aa] *Dipartimento di Scienze Chimiche, Università di Napoli "Federico II", Napoli, Italy*
[ab] *Department of Biomedical Sciences, University of Chieti "G. D'Annunzio", Chieti, Italy*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3mb70065h

chromosome-centric (c-HPP) and Biology/Disease (B/D-HPP). The mt-HPP has finally shifted the attention of the HUPO community outside the nuclear chromosomes with the general purpose to highlight the mitochondrial processes influencing the human health. Following this vision and considering the large interest and evidence collected on the non-Mendelian heredity of *Homo sapiens* associated with mt-chromosome and with the microbial commensal ecosystem constituting our organism we may speculate that this program will represent an initial step toward other HPP initiatives focusing on human phenotypic heredity.

## 1. Introduction

Mitochondria are essential organelles for cell life and death. A plethora of pathologies, including neurodegenerative diseases, cancer, diabetes but also aging, have been reported to be associated with mitochondrial dysfunction. Mitochondria carry maternally inherited genetic material, called the mitochondrial genome (mtDNA), which can be defined as the 25th human chromosome. Mutations in the mtDNA have been shown to be associated with dozens of unexplained disorders and the information contained in the mtDNA should be of major relevance for the understanding of many human diseases. Moreover, a systematic investigation of the proteins encoded by the mtDNA (mtProteins) and their relationships with nuclear Mendelian gene products will significantly improve our understanding of these diseases.

The chromosome-centric Human Proteome Project (c-HPP) started its activities pursuing the characterization of the proteins encoded by the nuclear 24 chromosomes (see Fig. 1 for a list of countries and the chromosomes they are pursuing).[1] However, many of these proteins, encoded at the nuclear level, play important physiopathological functions in the mitochondria and interact with other proteins that are encoded by the mtDNA. The functional and pathological correlation of the mtProteins with the nuclear encoded proteins (nProteins) is a fundamental task of the Mitochondrial Proteome Initiative (mt-HPP). Furthermore, the information collected in the mt-HPP program will be compared to the information that will be generated within the Biology/Disease Human Proteome Project (B/D-HPP), which consists of the most recent initiatives launched by the Human Proteome Organization (Fig. 2).[2] An integrated view of the molecular makeup of the human mitochondria will represent a strong base for studying the mitochondrial alterations which occur in many human diseases.

Within this article we present the concept and the mission of mt-HPP, a new HUPO initiative led by the Italian Proteomics Association (ItPA) focused on human mitochondrial proteins.

## 2. Mitochondrial basic biology and chromosome coding

Mitochondria are cellular organelles delegated to the generation of energy through oxidative phosphorylation (OXPHOS), although they are involved in many different functions such as pyruvate oxidation, the tricarboxylic acid cycle, and metabolism of amino acids, fatty acids, and steroids. These tasks are performed by mitochondrial structural and functional specializations, which allow the spatial separation of different compartments. In fact, the two mitochondrial membranes (outer and inner) delimit two chemically different spaces, the intermembrane space and the mitochondrial matrix. The inner membrane accommodates five multimeric protein complexes, NADH dehydrogenase–ubiquinone oxidoreductase (complex I), succinate dehydrogenase–ubiquinone oxidoreductase (complex II), ubiquinone–cytochrome *c* oxidoreductase (complex III), cytochrome *c* oxidase (complex IV), ATP synthase (complex V) and two small electron carriers (ubiquinone and cytochrome *c*) which altogether constitute the respiratory chain. The role of the respiratory chain is to produce metabolic energy in the form of ATP, by using the reducing substrates NADH and $FADH_2$ produced in intermediary metabolism pathways. Two coordinated processes make this possible: (1) oxidation of substrates in which the transfer of electrons from reduced dinucleotides to the ultimate acceptor, the molecular oxygen, is coupled to the active extrusion of protons in the intermembrane space by complexes I, III, and IV; and (2) phosphorylation of ADP plus phosphate in which the proton electrochemical gradient generated across the inner membrane is used by the ATPase (complex V) to drive back the protons in the matrix, thus providing the energy to support ATP synthesis.

Because mtDNA has only 37 genes, most of the approximately 900 gene products reported so far in the organelle are encoded by nuclear DNA (nDNA) and imported from the cytoplasm.[3] An example is provided by the 53 human gene products (*i.e.*, the mitochondrial carrier family) that transport metabolites, nucleotides and cofactors across the inner mitochondrial membrane providing a link between enzymatic reactions/metabolic pathways located in the mitochondria and other cellular compartments.[4] Human mtDNA is a 16 569 bp, double-stranded, circular molecule containing 37 genes (Fig. 1). Among them, 24 are needed for mtDNA translation (2 ribosomal RNAs [rRNAs] and 22 transfer RNAs [tRNAs]), while 13 encode subunits of the respiratory chain: seven subunits of complex I (ND1, 2, 3, 4, 4L, 5, and 6), one subunit of complex III (CYB), three subunits of cytochrome *c* oxidase (CO1, 2, and 3), and two subunits of ATP synthase (ATP6 and 8). Mitochondrial genetics differs from Mendelian genetics in three major aspects: maternal inheritance, heteroplasmy, and mitotic segregation. Nuclear gene mutations can affect not only mtDNA structure (deletions) and content (depletions), but also the assembly and maintenance of respiratory chain complexes, causing secondary respiratory chain defects. These mutations affect many mitochondrial functions and can have secondary effects on oxidative phosphorylation and other processes.

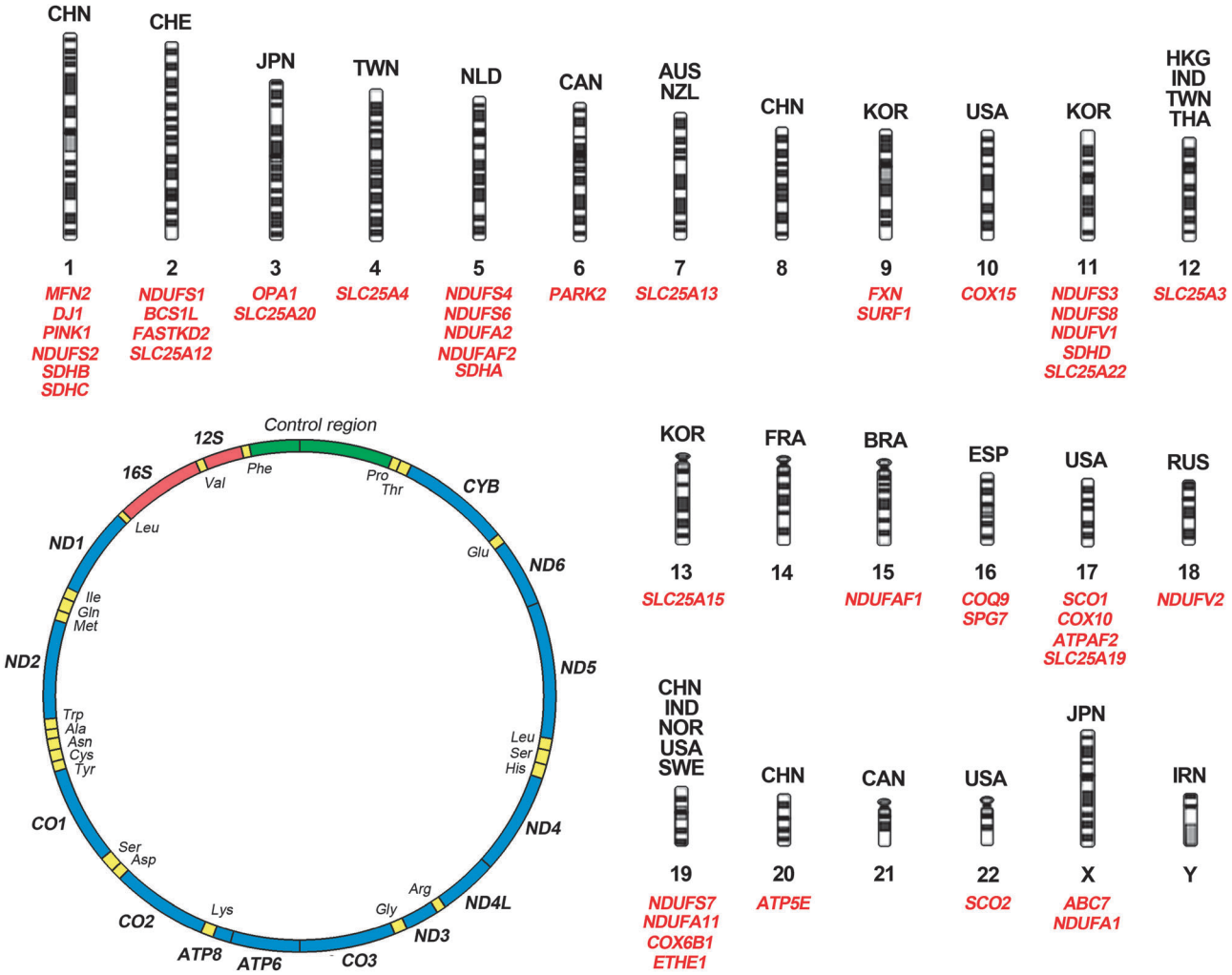


**Fig. 1** Map of the mitochondrial DNA (mtDNA) and chromosome array of the nuclear DNA (nDNA). Human mtDNA codifies 2 ribosomal RNAs (12S and 16S), 22 transfer RNAs (amino acid symbols) and 13 protein subunits of the respiratory chain: seven subunits of complex I (ND1, 2, 3, 4, 4L, 5, and 6), one subunit of complex III (CYB), three subunits of cytochrome *c* oxidase (CO1, 2, and 3), and two subunits of ATP synthase (ATP6 and 8). The remaining mitochondrial proteins are codified by the nDNA. The panel shows some genes affecting mitochondria-related diseases (in red) sorted by the chromosome location (1–22, *X* and *Y*). The nations or the international consortia participating in c-HPP are indicated (three-letter country codes).

Aside from providing the cell with most of its energy, mitochondria buffer cytosolic calcium ($Ca^{2+}$), generate and control Reactive Oxygen Species (ROS) production and regulate apoptosis through the mitochondrial Permeability Transition Pore (mtPTP). Moreover, mitochondria regulate several signalling cascades through ATP and acetyl-CoA production: ATP and acetyl-CoA are co-reactants in the phosphorylation and acetylation pathways of histones as well as of many other proteins.[5] Furthermore, mitochondrial metabolism regulates the redox status of small molecules such as NADP/NADPH, glutathione and other thiol/disulfide couples and, in this way, the activity of several enzymes and transcription factors.

Optimal mitochondrial function is ensured by a quality-control system tightly coupled to fusion and fission processes (*i.e.* MFN2, OPA1 and DRP1 gene products).[6] A huge decrease of Mnf2 and Drp1 has been reported in old rat liver suggesting age-correlated reduced mitochondrial dynamics in liver tissue.[7] Several factors have been described to convert hormonal, neuronal, environmental and metabolic stimuli into transcriptional adaptations leading to enhanced mitochondrial turnover and content. Modulation of mitochondrial content is exerted through the orchestrated expression of nuclear and mitochondrial genomes. Although mitochondria do not contain histones, mtDNA is coated by several proteins and packaged into aggregates called nucleoids or mitochromosome. Among these proteins, mitochondrial transcription factor A (TFAM) is the most abundant. Similar to histones TFAM could be acetylated.[8] Moreover, the presence of three sirtuins, SIRT3, 4 and 5, $NAD^+$-dependent protein deacetylase, which couple their biochemical and biological functions to the organism's energetic state *via* their dependency on $NAD^+$, have been reported. The mitochondrial sirtuins act to control basic mitochondrial biology, including energy production, metabolism, apoptosis, and intracellular signalling.[9] Cytosine methylation of mtDNA, restricted to CpG dinucleotides, was reported in the mitochondria of several species. The mechanisms establishing

 


**Fig. 2** The position of the mitochondrial Italian Human Proteome Project Initiative (mt-HPP) in the Human Proteome Project (HPP). The verge diagram shows how the mt-HPP is connected to both the investigative arms of HPP: mt-HPP participates with the chromosome-centric HPP (c-HPP) in the characterization of mtDNA-encoded proteins, but it is also related to the other biology and disease driven projects (B/D-HPP) being focused on the proteins involved in mitochondria-associated diseases.

and maintaining mtDNA methylation and the functional significance of this modification in mtDNA are not known; however, for this epigenetic modification a role in the regulation of mitochondrial transcription has been hypothesized.[10]

In Table 1, the proteins encoded in the mtDNA are reported together with the number of theoretical SRM proteotypic peptides observed by different MS platforms. However the number of total mtProteins is relatively low, few proteotypic transitions have been reported (ESI,† Table S1). Moreover, the distribution of the peptide descriptors is not homogenous along the protein sequences. As an example, we can evaluate the distribution of proteotypic peptides for NADH-ubiquinone oxidoreductase chain 1 (Fig. 3), taken from PeptideAtlas (www.peptideatlas.org), where only the N-terminus and the C-terminus of the protein are experimentally accessible by SRM. Such a distribution matches the soluble domain of the protein sequence without covering the trans-membrane region.

The development of novel strategies to extend region coverage is a key step for exploring potential modifications of mtProteins and to correlate them to a specific function. Such modifications have been recently explored in the NDUFS4 subunit of complex I of the mammalian respiratory chain which has a conserved carboxy-terminus with a canonical RVSTK phosphorylation site. Immunochemical analysis with specific antibodies shows that the serine in this site of the protein is natively present in complex I in both the phosphorylated and non-phosphorylated states.[11]

## 3. Mitochondria and disease

Almost 200 different mutations of mtDNA have been reported in man. Advances in genetics and cell biology have provided valuable insights into the function of mitochondria and the contribution of mitochondrial metabolism defects in human diseases. mtDNA mutations are a primary cause and can be due to inherited sequence changes in the mitochondrial genome (*i.e.* deletions, rearrangements, point mutations). However, mitochondrial-related diseases can be associated with several primary genetic causes affecting also the nuclear genome. Some of these mutations are consistently associated with specific phenotypes (*e.g.* parkin and PINK1 with Parkinson's disease, MFN2 with Charcot–Marie–Tooth disease type 2A, frataxin with Friedreich's ataxia and SLC25A20 encoding the carnitine/acylcarnitine carrier (CAC) with CAC deficiency) (Table 2).

Several lines of evidence suggest that mitochondrial dysfunction is an early event in most late-onset neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, *etc.* Several studies and reviews have reported that age-related, mitochondria generated ROS are factors in the development and progression of late-onset neurodegenerative diseases.[12–18] The fascinating possibility to delay the progression of these diseases may involve the reformatting of mitochondrial functions before symptoms appear.[19] In this respect, the understanding of the interactions of both the mtDNA and the respiratory chain complexes with nitric oxide (NO) appears crucial. NO is released enzymatically in the body and also at

**Table 1** Mitochondrial DNA genes

| Gene name | Protein name | UniprotKB | Sequence length (aa) | Distinct peptides* | Total observations* | Protein coverage* (%) | Likely observable sequence* (%) |
|---|---|---|---|---|---|---|---|
| MT-ND1 | NADH-ubiquinone oxidoreductase chain 1 | P03886 | 318 | 4 | 309 | 9.4 | 29.4 |
| MT-ND2 | NADH-ubiquinone oxidoreductase chain 2 | P03891 | 347 | 1 | 102 | 2.5 | 6.1 |
| MT-ND3 | NADH-ubiquinone oxidoreductase chain 3 | P03897 | 115 | 1 | 50 | 13.0 | 25.0 |
| MT-ND4 | NADH-ubiquinone oxidoreductase chain 4 | P03905 | 459 | 4 | 101 | 12.4 | 43.5 |
| MT-ND4L | NADH-ubiquinone oxidoreductase chain 4L | P03901 | 98 | — | — | — | — |
| MT-ND5 | NADH-ubiquinone oxidoreductase chain 5 | P03915 | 603 | 5 | 215 | 12.6 | 16.7 |
| MT-ND6 | NADH-ubiquinone oxidoreductase chain 6 | P03923 | 174 | 1 | 2 | 8.0 | 21.5 |
| MT-CYB | Cytochrome *b* | P00156 | 380 | 1 | 64 | 2.3 | 8.2 |
| MT-CO1 | Cytochrome *c* oxidase subunit 1 | P00395 | 513 | 2 | 25 | 7.6 | 19.5 |
| MT-CO2 | Cytochrome *c* oxidase subunit 2 | P00403 | 227 | 18 | 2326 | 63.8 | 81.4 |
| MT-CO3 | Cytochrome *c* oxidase subunit 3 | P00414 | 261 | 2 | 3 | 8.8 | 42.5 |
| MT-ATP6 | ATP synthase subunit a | P00846 | 226 | 1 | 150 | 4.4 | 10.3 |
| MT-ATP8 | ATP synthase protein 8 | P03928 | 68 | 2 | 18 | 29.4 | 31.7 |

*Information obtained by PeptideAtlas (www.peptideatlas.org).

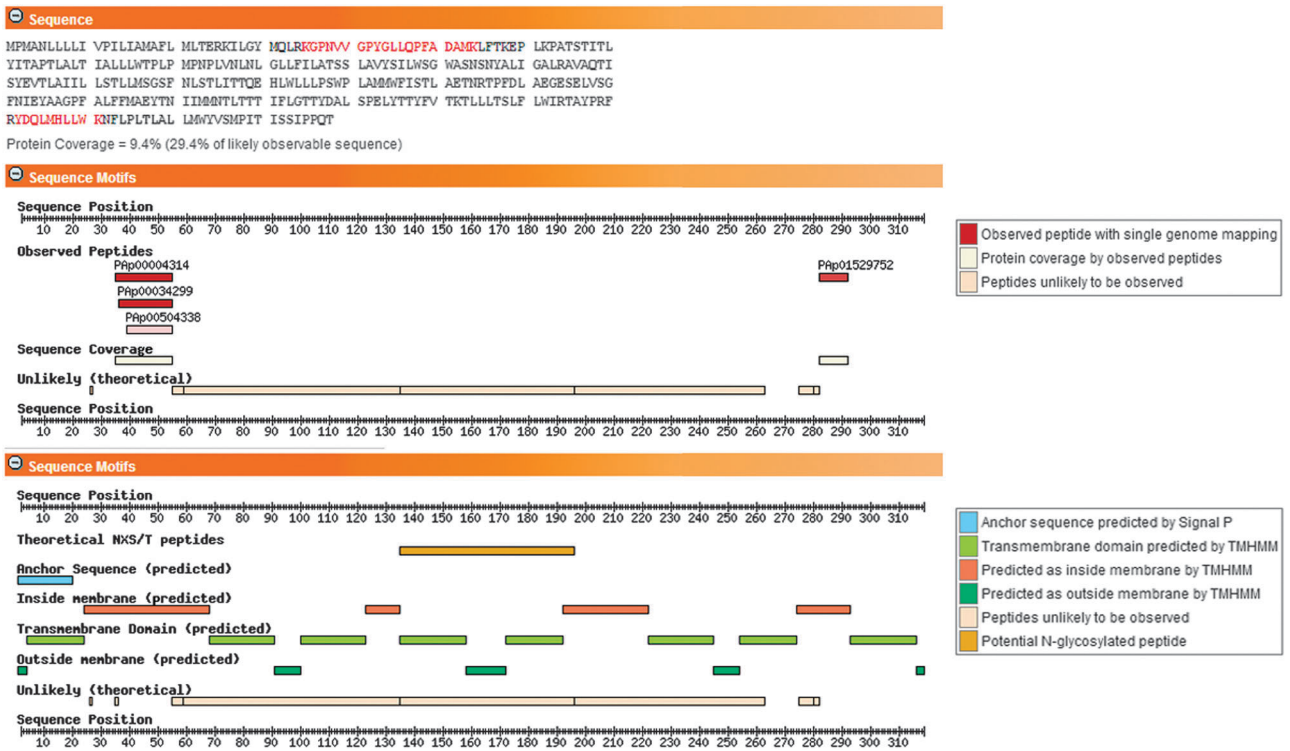


**Fig. 3** The distribution of putative proteotypic peptides along the amino acid sequence of the ND1 subunit of complex I. A PeptideAtlas (www.peptideatlas.org) elaboration on the availability of tryptic peptides for SRM quantitation of ND1 subunit of complex I. The four observed peptides match two short traits of the ND1 sequence with 9.4% total sequence coverage (upper and middle panels). The prediction of likely observable sequences indicates that the accessible regions are restricted to the N- and C-termini of the amino acid chain (bottom panel).

the mitochondrial level by specific NO synthases (mtNOS); moreover, the highly oxidizing peroxynitrite (reactive oxygen and nitrogen species, RONS) is produced by the NO and the superoxide ion, accounting for severe degradation of proteins and DNA in the mitochondrion. Interestingly, carbon monoxide and hydrogen sulfide have also been reported to crosstalk with mitochondria controlling their function.[20]

A common involvement of mitochondria in the oxidant imbalance was documented in fibromyalgia and chronic fatigue, two chronic pain syndromes with unknown etiology and pathophysiology. Several groups of investigators suggested that biochemical dysfunction in metabolism of ATP and OXPHOS and consequent free radical production and oxidative stress might play an important role in the pathophysiology of CFS.[21,22] On the other hand morphological and numerical changes of mitochondria have been observed in skeletal muscle from fibromyalgia patients[23,24] along with reduced levels of coenzyme $Q_{10}$, a decrease in mitochondrial membrane potential and an increase in levels of mitochondrial superoxide in blood mononuclear cells.[25] The study of mitochondrial proteins, their characterization and involvement in these diseases may contribute to the understanding of the pathophysiology of fibromyalgia[26] and chronic fatigue syndrome and allow us to identify new therapeutic approaches.

Mitochondrial metabolism impairment may play a central role in the pathophysiology of common chronic non-transmissible diseases such as type 2 diabetes. Limited evidence is available on the impact of active-foods and nutritional modulation on mitochondria and lifespan regulation as much as on the development of potential treatments for mitochondrial dysfunction-related diseases. Dietary interventions, especially caloric restriction, have been shown to improve the course of chronic non-transmissible disorders and to extend life expectancy; however, a subtle integrative model of mitochondrial function is still not available.[27]

Mitochondria were first proposed to be relevant in cancer by Otto Warburg who reported that cancer cells exhibited "aerobic-glycolysis".[28] Although this aspect was originally interpreted as indicating that the function of mitochondria was defective, we now understand that cancer cells are in an altered metabolic state.[29] The resurgence of interest in metabolism in cancer cells has started to focus attention back on the mitochondria and there is increasing evidence that mutations in mtDNA encoded genes can contribute to the development of cancer.[30] It is possible that such mutations provide metabolic adaptivity to the cancer cells. A number of cancer-related mitochondrial defects have been identified and described in the literature. These defects include altered expression and activity of respiratory chain subunits and glycolytic enzymes, decreased oxidation of NADH-linked substrates, as well as mtDNA mutations. However, DNA mutations affecting mitochondrial function in cancer can also occur in nuclear DNA genes, such as fumarate hydratase (FH) gene, which is associated with a rare cancer syndrome, the hereditary

 

**Table 2** Nuclear-encoded genes which affect mitochondrial related diseases

| Gene name | Location | Protein name | Disease | OMIM |
|---|---|---|---|---|
| MFN2 | 1p36.22 | Mitofusin-2 | Charcot–Marie–Tooth disease, type 2A2 | 609 260 |
| | | | Hereditary motor and sensory neuropathy VI | 601 152 |
| COQ9 | 16q21 | Ubiquinone biosynthesis protein COQ9 | Coenzyme Q10 deficiency, primary, 5 | 614 654 |
| ABC7 | Xq13.3 | ATP-binding cassette sub-family B member 7 | Anemia, sideroblastic, with ataxia | 301 310 |
| FXN | 9q21.11 | Frataxin | Friedreich ataxia | 229 300 |
| SPG7 | 16q24.3 | Paraplegin | Spastic paraplegia 7, autosomal recessive | 607 259 |
| OPA1 | 3q29 | Dynamin-like 120 kDa protein | Optic atrophy 1 | 165 500 |
| | | | Optic atrophy with or without deafness, ophthalmoplegia, myopathy, ataxia, and neuropathy | 125 250 |
| PARK7 | 1p36.23 | Protein DJ-1 | Parkinson disease 7, autosomal recessive early-onset | 606 324 |
| PINK1 | 1p36.12 | Serine/threonine-protein kinase PINK1 | Parkinson disease 6, early onset | 605 909 |
| PARK2 | 6q26 | E3 ubiquitin-protein ligase parkin | Adenocarcinoma of lung, somatic | 211 980 |
| | | | Adenocarcinoma, ovarian, somatic | 167 000 |
| | | | Parkinson disease, juvenile, type 2 | 600 116 |
| SLC25A20 | 3p21.31 | Mitochondrial carnitine/acylcarnitine carrier protein | Carnitine-acylcarnitine translocase deficiency | 212 138 |
| SLC25A4 | 4q35.1 | ADP/ATP translocase 1 | Cardiomyopathy, familial hypertrophic | 192 600 |
| | | | Progressive external ophthalmoplegia with mitochondrial DNA deletions 3 | 609 283 |
| SLC25A3 | 12q23.1 | Phosphate carrier protein | Mitochondrial phosphate carrier deficiency | 610 773 |
| SLC25A15 | 13q14.11 | Mitochondrial ornithine transporter 1 | Hyperornithinemia–hyperammonemia–homocitrullinemia syndrome | 238 970 |
| SLC25A22 | 11p15.5 | Mitochondrial glutamate carrier 1 | Epileptic encephalopathy, early infantile, 3 | 609 304 |
| SLC25A19 | 17q25.1 | Mitochondrial thiamine pyrophosphate carrier | Microcephaly, Amish type | 607 196 |
| | | | Thiamine metabolism dysfunction syndrome 4 (progressive polyneuropathy type) | 613 710 |
| SLC25A12 | 2q31.1 | Calcium-binding mitochondrial carrier protein Aralar1 | Hypomyelination, global cerebral | 612 949 |
| SLC25A13 | 7q21.3 | Calcium-binding mitochondrial carrier protein Aralar2 | Citrullinemia, adult-onset type II | 603 471 |
| | | | Citrullinemia, type II, neonatal-onset | 605 814 |
| NDUFS1 | 2q33.3 | NADH-ubiquinone oxidoreductase 75 kDa subunit | Mitochondrial complex I deficiency | 252 010 |
| NDUFS2 | 1q23.3 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 2 | Mitochondrial complex I deficiency | 252 010 |
| NDUFS3 | 11p11.2 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 3 | Leigh syndrome | 256 000 |
| NDUFS4 | 5q11.2 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 4 | Leigh syndrome | 256 000 |
| | | | Mitochondrial complex I deficiency | 252 010 |
| NDUFS6 | 5p15.33 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 6 | Mitochondrial complex I deficiency | 252 010 |
| NDUFS7 | 19p13.3 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 7 | Leigh syndrome | 256 000 |
| NDUFS8 | 11q13.2 | NADH dehydrogenase [ubiquinone] iron–sulfur protein 8 | Leigh syndrome | 256 000 |
| NDUFV1 | 11q13.2 | NADH dehydrogenase [ubiquinone] flavoprotein 1 | Mitochondrial complex I deficiency | 252 010 |
| NDUFV2 | 18p11.22 | NADH dehydrogenase [ubiquinone] flavoprotein 2 | Mitochondrial complex I deficiency | 252 010 |
| NDUFA1 | Xq24 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 1 | Mitochondrial complex I deficiency | 252 010 |
| NDUFA2 | 5q31.3 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2 | Leigh syndrome | 256 000 |
| NDUFA11 | 19p13.3 | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 11 | Mitochondrial complex I deficiency | 252 010 |
| NDUFAF1 | 15q15.1 | Complex I intermediate-associated protein 30 | Mitochondrial complex I deficiency | 252 010 |
| NDUFAF2 | 5q12.1 | Mimitin | Leigh syndrome | 256 000 |
| | | | Mitochondrial complex I deficiency | 252 010 |
| SDHA | 5p15.33 | Succinate dehydrogenase [ubiquinone] flavoprotein subunit | Cardiomyopathy, dilated, 1GG | 613 642 |
| | | | Leigh syndrome | 256 000 |
| | | | Mitochondrial respiratory chain complex II deficiency | 252 011 |
| | | | Paragangliomas 5 | 614 165 |
| SDHB | 1p36.13 | Succinate dehydrogenase [ubiquinone] iron–sulfur subunit | Cowden-like syndrome | 612 359 |
| | | | Gastrointestinal stromal tumor | 606 764 |
| | | | Paraganglioma and gastric stromal sarcoma | 606 864 |
| | | | Paragangliomas 4 | 115 310 |
| | | | Pheochromocytoma | 171 300 |
| SDHC | 1q23.3 | Succinate dehydrogenase cytochrome b560 subunit | Gastrointestinal stromal tumor | 606 764 |
| | | | Paraganglioma and gastric stromal sarcoma | 606 864 |
| | | | Paragangliomas 3 | 605 373 |
| SDHD | 11q23.1 | Succinate dehydrogenase [ubiquinone] cytochrome *b* small subunit | Carcinoid tumors, intestinal | 114 900 |
| | | | Cowden-like syndrome | 612 359 |

Table 2 (*continued*)

| Gene name | Location | Protein name | Disease | OMIM |
|---|---|---|---|---|
| | | | Paraganglioma and gastric stromal sarcoma | 606 864 |
| | | | Paragangliomas 1, with or without deafness | 168 000 |
| | | | Pheochromocytoma | 171 300 |
| BCS1L | 2q35 | Mitochondrial chaperone BCS1 | Bjornstad syndrome | 262 000 |
| | | | GRACILE syndrome | 603 358 |
| | | | Leigh syndrome | 256 000 |
| | | | Mitochondrial complex III deficiency | 124 000 |
| SURF1 | 9q34.2 | Surfeit locus protein 1 | Leigh syndrome, due to COX deficiency | 256 000 |
| SCO1 | 17p13.1 | Protein SCO1 homolog | Hepatic failure, early onset, and neurologic disorder | |
| SCO2 | 22q13.33 | Protein SCO2 homolog | Cardioencephalomyopathy, fatal infantile, due to COX deficiency | 604 377 |
| COX10 | 17p12 | Protoheme IX farnesyltransferase | Encephalopathy, progressive mitochondrial, with proximal renal tubulopathy due to COX deficiency | |
| COX15 | 10q24.2 | Cytochrome *c* oxidase assembly protein COX15 homolog | Cardiomyopathy, hypertrophic, early-onset fatal Leigh syndrome due to COX deficiency | 256 000 |
| COX6B1 | 19q13.12 | Cytochrome *c* oxidase subunit 6B1 | Cytochrome *c* oxidase deficiency | 220 110 |
| FASTKD2 | 2q33.3 | FAST kinase domain-containing protein 2 | Mitochondrial complex IV deficiency | 220 110 |
| ETHE1 | 19q13.31 | Protein ETHE1 | Ethylmalonic encephalopathy | 602 473 |
| ATPAF2 | 17p11.2 | ATP synthase mitochondrial F1 complex assembly factor 2 | Mitochondrial complex V (ATP synthase) deficiency, nuclear type 1 | 604 273 |
| ATP5E | 20q13.32 | ATP synthase subunit epsilon | Mitochondrial complex V (ATP synthase) deficiency, nuclear type 3 | 614 053 |

leiomyomatosis and renal cell carcinoma (HLRCC).[31] While there are many reports of these phenomena, the mechanisms responsible for the initiation and evolution of mtDNA mutations, and their roles in the development of cancer, drug resistance, and disease progression still remain unknown. Thus, a thorough proteomic investigation of mitochondria in different cancer cell models will be instrumental to identify novel protein targets/pathways relevant to define these aspects. This approach will eventually allow us to draw unique gene expression stress signatures leading to these adaptive responses, which potentially may provide molecular markers for treatment modality.

## 4. Further implications for human health

DNA mutations in the mitochondrial genome are the major contributing factors of aging and senescence. Accumulation of somatic mtDNA defects is responsible for ROS production and oxidative damage in aged tissues, even more in the presence of NO excess. Over-expression of mitochondrially targeted antioxidants, such as catalase, manganese superoxide dismutase, and methionine sulfoxide reductase, decreases oxidative damage and extends healthy lifespan. Mitochondria, in fact, utilize more than 90% of cellular oxygen and during normal respiration 0.1–0.2% of ROS are formed as by-products. ROS produce damage primarily to mtDNA, lipids and proteins, which lie in proximity to the main cellular ROS producer, the mitochondrial electron transport chain. The oxidative damage of mitochondrial lipids and proteins as well as the gradual accumulation of mtDNA mutations over time result in defective electron transport and increase of RONS production. Mitochondria, on the other hand, are endowed with several defense mechanisms, including RONS scavengers, such as glutathione binding NO and complex IV oxidizing NO to nitrite, and ROS converting enzymes, like manganese superoxide dismutase and peroxiredoxin 3.[32]

Interaction with other species could modulate the metabolic life of mitochondria. Microbiomes or single species of bacteria through the production of toxins (or effectors) and metabolites which specifically target mitochondria could modulate this organelle during an infection[33] or during the normal life.[34] Furthermore, it has been well documented that mitochondrial metabolism could also be regulated by bacterial presence. There are indeed some compounds of bacterial origin, which can interfere with mitochondria enhancing or reducing their activity. Probiotics, like lactic acid bacteria (*Lactobacillus* and *Bifidobacterium genera*, LAB), are found in the normal gut microflora of mammals and humans. There is evidence that LAB can modulate through mitochondria the apoptosis of cardiomyocytes.[35] Thus, a topic that will be explored is the relation of mitochondrial protein expression in immunocompetent cells (*i.e.* lymphocytes) during chronic infection. Chronic infection induces hyperactivation of the immune system and increases exposure to ROS. These phenomena could contribute to alter mitochondrial metabolism and protein expression. For instance, the changes induced by HIV infection on mtDNA have already been well documented.[36] To date, however, a systematic investigation of the mitochondrial proteome in immune cells during chronic infection has not been pursued. Because of the documented interaction between bacterial symbionts, human cells and mitochondrial protein expression, we can speculate on the development of a complete investigation which takes into account the overall proteomes of the superorganism. The superorganism is an organism resulting from symbiotic interactions among human and bacterial cells which contribute to regulate the overall protein expression and metabolism of every human tissue.

 

## 5. The framework for the Mitochondrial Proteome Initiative (mt-HPP)

The Mitochondrial Proteome Initiative (mt-HPP) has been initiated by the Italian Proteomics Association (ItPA) executive board with the aim to integrate the chromosome-centric Human Proteome Project (c-HPP) with the characterization of the mitochondrial DNA-encoded proteins. Moreover the development of such a program is directly providing the framework for the integration with the Biology and Disease Program of the Human Proteome Project (B/D-HPP).

Herein, we indicate the overall key milestones which we intend to pursue following the mt-HPP initiative.

Key milestones will include the following tasks:

(1) Definition of Proteomics-based technologies for the identification and quantitation of mtDNA-encoded proteins (mtProteins). This task will include an effort to develop separation, SRM strategies and top-down investigations to tackle:

(i) Single nucleotide polymorphism variants

(ii) Post-translational modifications

(iii) mtProtein biomarkers in specific diseases

(2) Definition of a comprehensive protein interaction map for the mtProteins. This task will include the following items:

(i) mtProtein–mtProtein interaction and oligomeric status in the membrane

(ii) mtProtein–nProtein (nuclear encoded protein) interaction map

(iii) mtProtein–p/scProtein (pathogen/symbiont coded protein) interaction map

(3) Definition of an integrated OMICS model with a particular focus on metabolite fingerprint – the proteome map. This action will include:

(i) Correlation of mtProtein profiles, metabolite fingerprints and RNA patterns

(ii) Correlation of nProtein localized in the mitochondria, metabolite fingerprints and RNA patterns

(iii) Correlation of mtProtein and nProtein localized in the mitochondria profiles with ionomics.

A detailed description of the specific project plans and their associated methodologies will follow the kick-off meeting of the first project proposals which will be held in Padua (June 18th–21st, 2013) during the next Italian Proteomics Association National Congress.

## 6. Future perspectives

Since the launching of the mt-HPP initiative, this has collected the spontaneous adhesion of many Italian scientists engaged in Italy and abroad in the investigation of structure–function relationships of the mitochondrial proteins and their physiological/pathological implications for cellular metabolism. The wide participation leads us to extend our field of interest to all the proteins operating in the mitochondrion, thus including the nuclear DNA-encoded proteins involved in mitochondrial processes which have a relevant impact on human health. In a future perspective we wish to draw into the initiative a larger number of contributors looking with special attention over the national borders in accordance with the international connotation of the HPP.

Mitochondrial proteins are involved in a broad range of pathologies, therefore the mt-HPP offers the valued opportunities to cooperate with different Biology and Disease driven Projects (B/D-HPP) launched by HUPO (*e.g.* cancers and diabetes). Moreover, we consider particularly relevant the development of partnerships with the HUPO initiatives devoted to specific diseases or organs such as the Human Brain Proteome Project (HBPP) and the initiative on Model Organism Proteomes (iMOP). The HBPP workgroup shares common objectives as a deeper understanding of proteome modifications underlying the neurodegenerative diseases and aging or the identification of prognostic and diagnostic biomarkers. The Initiative on Model Organism Proteomes (iMOP) has a fundamental role given the possibility to compare the proteomics data, pathways and mechanisms conserved between models and humans.

mt-HPP has finally shifted the attention of the HUPO c-HPP community outside the nuclear chromosome by proposing and pursuing the specific aim to characterize and quantify the mtProteins, with the general purpose to highlight the mitochondrial processes influencing human health. Following this vision and considering the large interest and evidence collected over the non-Mendelian heredity of *Homo sapiens* associated with mt-chromosome and with the microbial commensal consortia constituting our organism we may speculate that the next initiative of HPP will be focused on the proteome encoded by the chromosome of the human symbiotic microorganism community.

## References

1 G. Marko-Varga, G. S. Omenn, Y. K. Paik and W. S. Hancock, *J. Proteome Res.*, 2013, **12**, 1–5.
2 R. Aebersold, G. D. Bader, A. M. Edwards, J. E. van Eyk, M. Kussmann, J. Qin and G. S. Omenn, *J. Proteome Res.*, 2013, **12**, 23–27.
3 S. DiMauro and E. A. Schon, *New Engl. J. Med.*, 2003, **348**, 2656–2668.
4 F. Palmieri, *Mol. Aspects Med.*, 2013, **34**, 465–484.
5 D. C. Wallace and W. Fan, *Mitochondrion*, 2010, **10**, 12–31.
6 R. J. Youle and A. M. van der Bliek, *Science*, 2012, **337**, 1062–1065.
7 V. Pesce, A. Cormio, L. C. Marangi, F. W. Guglielmi, A. M. Lezza, A. Francavilla, P. Cantatore and M. N. Gadaleta, *Gene*, 2002, **286**, 143–148.
8 M. M. Dinardo, C. Musicco, F. Fracasso, F. Milella, M. N. Gadaleta, G. Gadaleta and P. Cantatore, *Biochem. Biophys. Res. Commun.*, 2003, **301**, 187–191.
9 E. Verdin, M. D. Hirschey, L. W. Finley and M. C. Haigis, *Trends Biochem. Sci.*, 2010, **35**, 669–675.
10 L. S. Shock, P. V. Thakkar, E. J. Peterson, R. G. Moran and S. M. Taylor, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 3630–3635.
11 D. De Rasmo, G. Palmisano, S. Scacco, Z. Technikova-Dobrova, D. Panelli, T. Cocco, A. M. Sardanelli, A. Gnoni,

L. Micelli, A. Trani, A. Di Luccia and S. Papa, *Mitochondrion*, 2010, **10**, 464–471.
12 R. H. Swerdlow and S. M. Khan, *Med. Hypotheses*, 2004, **63**, 8–20.
13 M. F. Beal, *Ann. Neurol.*, 2005, **58**, 495–505.
14 D. C. Wallace, *Cold Spring Harbor Symp. Quant. Biol.*, 2005, **70**, 363–374.
15 P. H. Reddy, *J. Biomed. Biotechnol.*, 2006, **2006**, 31372.
16 A. H. Schapira, *Lancet*, 2006, **368**, 70–82.
17 M. T. Lin and M. F. Beal, *Nature*, 2006, **443**, 787–795.
18 P. H. Reddy and M. F. Beal, *Trends Mol. Med.*, 2008, **14**, 45–53.
19 P. H. Reddy, *Neuromol. Med.*, 2008, **10**, 291–315.
20 P. C. Herrmann and E. C. Herrmann, *Methods Mol. Biol.*, 2012, **823**, 265–277.
21 S. Myhill, N. E. Booth and J. McLaren-Howard, *Int. J. Clin. Exp. Med.*, 2009, **2**, 1–16.
22 N. E. Booth, S. Myhill and J. McLaren-Howard, *Int. J. Clin. Exp. Med.*, 2012, **5**, 208–220.
23 J. H. Park, K. J. Niermann and N. Olsen, *Curr. Rheumatol. Rep.*, 2000, **2**, 131–140.
24 H. Sprott, S. Salemi, R. E. Gay, L. A. Bradley, G. S. Alarcón, S. J. Oh, B. A. Michel and S. Gay, *Ann. Rheum. Dis.*, 2004, **63**, 245–251.
25 M. D. Cordero, M. de Miguel and J. A. Sanchez-Alcazar, in *New insights into fibromyalgia*, ed. W. S. Wilke, InTech, 2011, pp. 77–98.
26 F. Ciregia, C. Giacomelli, L. Giusti, A. Lucacchini and L. Bazzichi, in *New insights into fibromyalgia*, ed. W. S. Wilke, InTech, 2011, pp. 149–166.
27 M. Schiff, P. Bénit, A. Coulibaly, S. Loublier, R. El-Khoury and P. Rustin, *Nutr. Rev.*, 2011, **69**, 65–75.
28 O. Warburg, K. Posener and F. Negelein, *Biochem. Z.*, 1924, **152**, 319–344.
29 R. A. Cairns, I. S. Harris and T. W. Mak, *Nat. Rev. Cancer*, 2011, **11**, 85–95.
30 C. Frezza and E. Gottlieb, *Semin. Cancer Biol.*, 2009, **19**, 4–11.
31 M. R. Stratton, P. J. Campbell and P. A. Futreal, *Nature*, 2009, **458**, 719–724.
32 F. L. Muller, M. S. Lustgarten, Y. Jang, A. Richardson and H. Van Remmen, *Free Radicals Biol. Med.*, 2007, **43**, 477–503.
33 J. H. Jiang, J. Tong and K. Gabriel, *IUBMB Life*, 2012, **64**, 397–401.
34 D. R. Donohoe, N. Garge, X. Zhang, W. Sun, T. M. O'Connell, M. K. Bunger and S. J. Bultman, *Cell Metab.*, 2011, **13**, 517–526.
35 H. F. Wang, C. Y. Tseng, M. H. Chang, J. A. Lin, F. J. Tsai, C. H. Tsai, Y. C. Lu, C. H. Lai, C. Y. Huang and C. C. Tsai, *Chin. J. Physiol.*, 2012, **55**, 37–46.
36 A. Cossarizza, A. Riva, M. Pinti, S. Ammannato, P. Fedeli, C. Mussini, R. Esposito and M. Galli, *Antiviral Ther.*, 2003, **8**, 315–321.

 