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Towards a functional definition of the mitochondrial human proteome

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ABSTRACT

The mitochondrial human proteome project (mt-HPP) was initiated by the Italian HPP group as a part of both the chromosome-centric initiative (C-HPP) and the "biology and disease driven" initiative (B/D-HPP). In recent years several reports highlighted how mitochondrial biology and disease are regulated by specific interactions with non-mitochondrial proteins. Thus, it is of great relevance to extend our present view of the mitochondrial proteome not only to those proteins that are encoded by or transported to mitochondria, but also to their interactors that take part in mitochondria functionality. Here, we propose a graphical representation of the functional mitochondrial proteome by retrieving mitochondrial proteins from the NeXtProt database and adding to the network their interactors as annotated in the IntAct database. Notably, the network may represent a reference to map all the proteins that are currently being identified in mitochondrial proteomics studies.
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The Human Proteome Organization (HUPO) has promoted in recent years a concerted action aimed at the full characterization of the human proteome. Two measures have been put forward in parallel. First, the chromosome-centric initiative (C-HPP) aimed at joining the efforts of several groups with a country-based strategy to unravel the human proteome to reduce to a minimum, and possibly to eliminate, the number of proteins that did not show evidence at the protein level in the dedicated database such as NeXtProt [1,2]. Second, the "biology and disease driven" initiative (B/D-HPP) aimed at functional clustering of proteins around a significant biological issue [3]. To address some of these gaps, several consortia were formed, focusing on the human eye, diabetes, pediatric diseases, liver, brain, cancer, infectious diseases, autoimmune disorders, epigenetics, among others. A great contribution to these initiatives was provided by the technological pillars: the Human Proteome Atlas (HPA), the bioinformatics, and the mass spectrometry technological advancement study groups [4].

The two HPP programs are strongly intersected; in fact, the effort to consolidate the current available tools to identify and quantify all the genome-encoded proteins is providing the fundamental framework to highlight novel molecular targets for the B/D efforts. The Italian HPP group did a great effort in bringing laboratories across the globe to take part in the Italian Proteomics Association (ItPA) project to unravel the mitochondrial human proteome project (mt-HPP) as part of both C-HPP and B/D-HPP initiatives [5].

Mitochondria are essential organelles for the life and death of cells, and several human disorders are associated to mitochondrial dysfunction. While some human diseases are strictly linked to mutations in the mitochondrial genome, many others are someway connected to mitochondrial functionality by defects in the sequence of proteins encoded by nuclear chromosomes that are imported into mitochondria through their mitochondrial transfer sequence (MTS) [6]. Mutations of cytosolic proteins that regulate the elimination of impaired mitochondria segregate with severe degenerative disorders, such as Parkinson's disease. For instance, a number of mutations in the PARK2, PINK1 and PARK7 genes have been shown to cause mitochondrial parkinsonism [7], although PARK2 and PARK7 gene products (parkin and DJ-1, respectively) do not belong, strictly speaking, to the mitochondrial proteome, as they are not imported into mitochondria. In fact, several recent reports highlighted the relevance of cytosolic

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molecular machineries and non-mitochondrial subcellular structures in the governance of mitochondrial functions. Although mitochondria are usually associated to their role in cellular energetics, they exert a number of functions that are not confined to ATP production. In particular, calcium ion homeostasis is regulated by a strict functional and spatial connection between mitochondria and the endoplasmic reticulum, and glycolysis has been shown to be coupled to ATP production through the spatial recognition of the glycolytic enzyme hexokinase by the outer membrane porin VDAC1 [8–10].

In recent years, several papers underlined the role of mitophagy, the disposal of dysfunctional mitochondria by the autophagic machinery on a whole-cell level. Parkin and PINK1 assumed a fundamental role in targeting mitochondria to the autophagosome through ubiquitination of outer surface membrane proteins, although this mechanism still seems to be controversial [11,12]. Additionally, the maintenance of a well-organized mitochondrial network was shown to be a peculiar actor in assuring full functionality of the mitochondrion [13]. To this purpose, a thorough assessment of the mitochondrial interactome should fill the gaps in our knowledge of the complex biological processes sustained by the mitochondrial proteome [14,15].

It is of great relevance to extend our present view of the mitochondrial proteome not only to those proteins that are encoded by the mitochondrial or nuclear genomes, but also to their interactors that take part in mitochondrial functionality, maintenance, dynamics and metabolism [16]. Thus, the integration of information obtained within the C-HPP and B/D-HPP actions with interactomics data deposited in the IntAct database [17] and protein annotation in the NeXtProt database is a priority (Fig. 1). To date, 13 proteins are listed in the NeXtProt database (September 2015 release) as mitochondrial-encoded, whereas another 1869 proteins were annotated as mitochondrial proteins. Among them, 1277 entries were indicated to be evident at the protein level, with 592 remaining missing proteins. A search in the IntAct database allowed us to identify a broad panel of interactors that

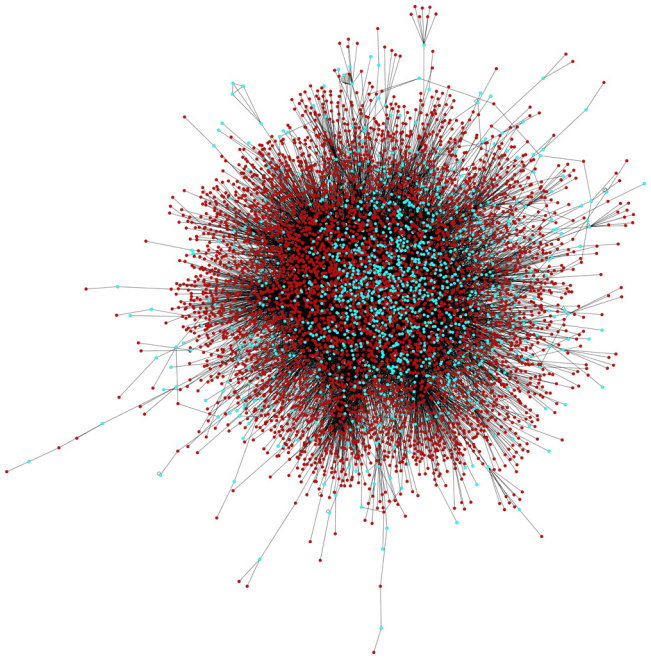

Fig. 2. The mitochondrial human proteome from a functional perspective. Cyan nodes represent proteins encoded by the mitochondrial genome or translocated to the mitochondrion. Protein identities were obtained by querying the NeXtProt database. Red nodes represent interactors of the cyan nodes as obtained by querying the IntAct database using the PSICQUIC query module embedded in Cytoscape.

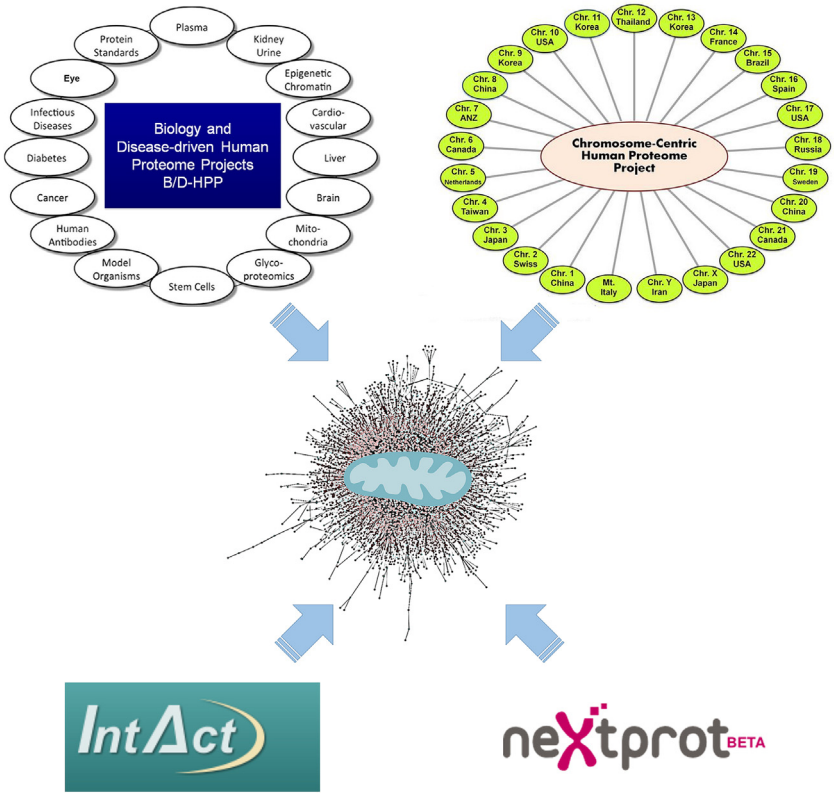

Fig. 1. A functional definition of the human mitochondrial proteome requires the integration of several efforts. From top-left, clockwise: functional studies from the B/D-HPP; a full coverage of the human proteome by C-HPP; protein evidence and functional annotation in the NeXtProt database; interactomics annotation from the IntAct database.

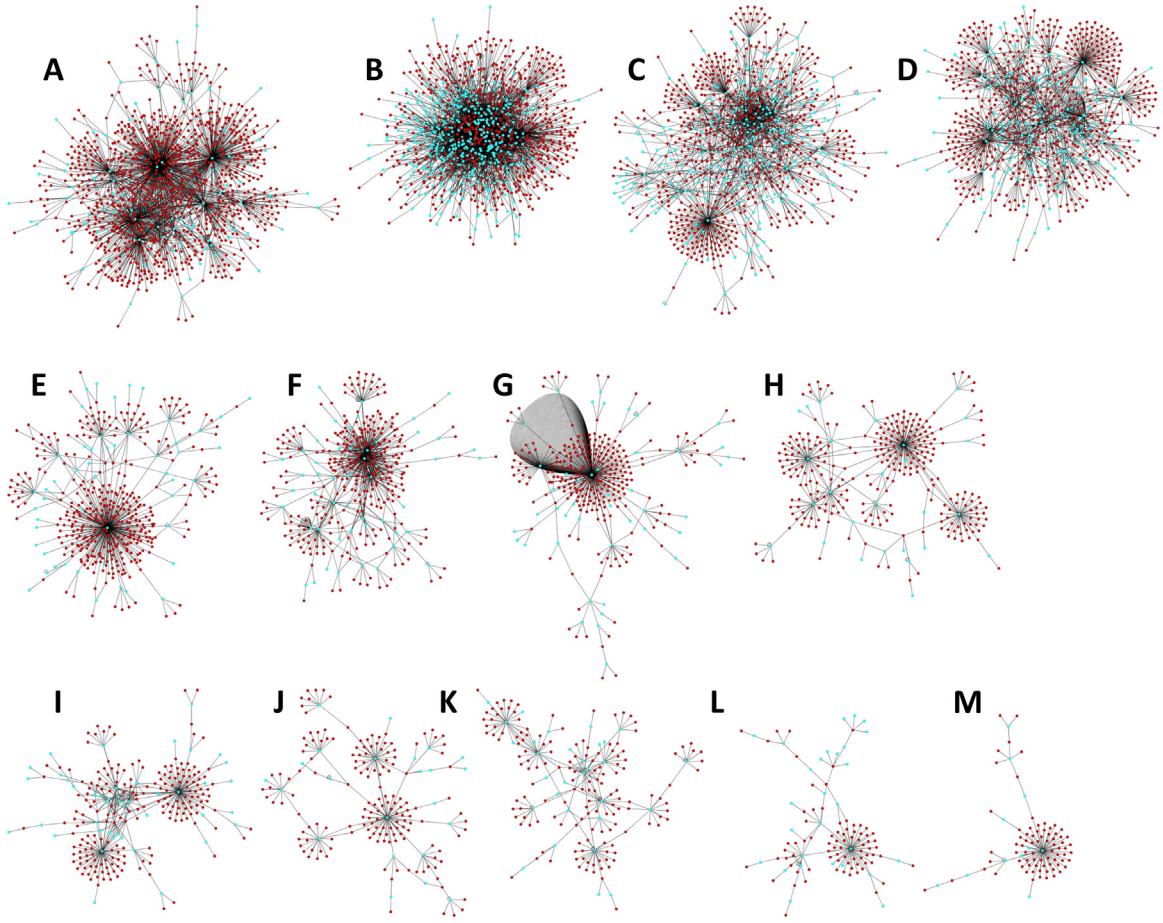

Fig. 3. Segmentation of the mitochondrial proteome network. Subnetworks were obtained by clusterMaker (Cytoscape app) using the GLay algorithm. Over-represented pathways (Reactome) are: A, protein synthesis; B, oxidative phosphorylation; C, energy metabolism; D, apoptosis and mitophagy; E, RNA synthesis and processing; F, signaling by EGFR; G, protein trafficking; H, MAPK signaling pathway; I, WNT signaling; J, Nucleotide excision repair; K, mRNA decay; L, vesicle trafficking; M, Sema3A signaling. Cyan nodes represent proteins encoded by the mitochondrial genome or translocated to the mitochondrion, whereas red nodes represent their interactors.

altogether are likely to constitute an enlarged, functional view of the mitochondrial human proteome. Fig. 2 shows a graph representation of this concept, with proteins encoded by or translocated into the mitochondrion represented as cyan spots and non-mitochondrial interactors as red spots. It appears evident, therefore, that the large majority of the 6592 nodes in the proposed network do not belong to the mitochondrial proteome, although they deeply influence mitochondrial biology.

Due to its complexity, the mitochondrial human proteome in its enlarged, functional view needs to be segmented into subnetworks. Network clustering helps the identification of enriched pathways where mitochondrial complexes play a fundamental role. Fig. 3 shows the more representative clusters obtained by segmentation of the enlarged mitochondrial network. The large network shown in Fig. 2 has been segmented with the clusterMaker Cytoscape app using the GLay algorithm [18,19]. Over-representation analysis of each cluster for Reactome pathways [20] may therefore define the principal biological processes where the functional mitochondrial proteome is involved in. Most notably, each cluster could represent a reference to map all the proteins that are currently being identified within the mt-HPP consortium. Furthermore, reference maps should help to aggregate all proteins identified in interactomics investigations, where several methodologies are employed to connect experimentally identified preys with any mitochondrial bait of interest. Eventually, lists of differentially expressed proteins arising from functional/differential expression proteomics studies might be located on the whole network or on any subnetwork to broaden the viewpoint of a single focused investigation.

In summary, the mitochondrial proteome should be taken into account as a dynamic and possibly chronosteric reality, with significant overlaps with the whole-cell proteome. Several efforts should be made to unravel the contribution of any single protein to biological processes and molecular mechanisms controlled by this organelle and its relationship to human diseases. An audacious and flexible research consolidated by robust analytical data and technological developments has been a fundamental actor for the advance of the human milestones in the hypothesis driven scenario such as the Human Genome Project [21]. Thus, the HPP may well accept to pursue daring goals as long as overall objectives are well grounded in a structured effort in an ardent and humble awareness of the current technological and conceptual limitations.

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