Chapter 3
Mitochondrial Dysfunction in Huntington's Disease

Catarina Carmo, Luana Naia, Carla Lopes and A. Cristina Rego

Abstract Mitochondrial dysfunction has been described as an early pathological mechanism delineating the selective neurodegeneration that occurs in Huntington's disease (HD), a polyglutamine-expansion disorder that largely affects the striatum and the cerebral cortex. Over the years, mitochondria roles in eukaryotic cells (e.g. in neurons) have largely diverged from the classically attributed cell power source; indeed, mitochondria not only contribute for synthesis of several metabolites, but are also dynamic organelles that fragment and fuse to achieve a maximal bioenergetic performance, are transported along microtubules, regulate intracellular calcium homeostasis through the interaction with the endoplasmic reticulum, produce free radicals and participate in cell death processes. Indeed, most of these activities have been demonstrated to be affected in HD, potentially contributing for the neuronal dysfunction in pre-symptomatic stages. This chapter resumes some of the evidences that pose mitochondria as a main regulatory organelle in HD-affected neurons, uncovering some potentially therapeutic mitochondrial-based relevant targets.

Keywords Calcium dyshomeostasis · Oxidative stress · Metabolic deficits Mitochondrial dynamics · Cell death

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C. Nóbrega and L. Pereira de Almeida (eds.), *Polyglutamine Disorders*, Advances in Experimental Medicine and Biology 1049,
https://doi.org/10.1007/978-3-319-71779-1_3

Abbreviations

$\Delta\psi_m$	Mitochondrial membrane potential
α-KGDH	α-ketoglutarate dehydrogenase
3-NP	3-nitropropionic acid
AIF	Apoptosis inducing factor
Apaf-1	Apoptotic protease-activating factor 1
ATP	Adenosine triphosphate
Bcl-2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
BH3	Bcl-2 homology 3
Bid	BH3 interacting-domain death agonist
Bim	Bcl-2 interacting mediator of cell death
BNIP3	BCL2/adenovirus E1B 19 kDa protein-interacting protein 3
CBP	CREB-binding protein
CK	Creatine kinase
CoQ	Coenzyme Q
CREB	cAMP response element-binding protein
Drp1	Dynamin-related protein 1
ETC	Electron transport chain
Fis1	Mitochondrial fission 1
FMN	Flavin mononucleotide
GABA	γ-aminobutyric acid
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Gpx	Glutathione peroxidases
GTP	Guanosine triphosphate
H_2O_2	Hydrogen peroxide
HD	Huntington's disease
hESC	Human embryonic stem cells
HTT/*HTT*	Human huntingtin protein/gene
Htt	Rodent huntingtin protein
IAP1	Inhibitor of Apoptosis Protein-1
iPSCs	Induced pluripotent stem cells
K	Lysine
LC3	Light chain 3
MCU	Mitochondrial calcium uniporter
Mff	Mitochondrial fission factor
Mfn	Mitofusin
mHTT	Human mutant HTT
mHtt	Rodent mutant Htt
MIM	Mitochondrial inner membrane
MIS	Mitochondrial intermembrane space
MOM	Mitochondrial outer membrane
mtDNA	Mitochondrial DNA
NAD	β-nicotinamide adenine dinucleotide

ND5	NADH dehydrogenase subunit 5
NRF	Nuclear respiratory factor
Nrf2	Nuclear factor-erythroid 2-related factor-2
OPA1	Optic atrophy 1
OXPHOS	Oxidative phosphorylation
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
PGC-1α	PPARγ—coactivator-1α
PINK1	PTEN-induced putative kinase 1
PolyQ	Polyglutamine
PPAR	Peroxisome proliferator-activated receptor
Prx	Peroxiredoxins
PTEN	Phosphatase and tensin homolog
PTP	Permeability transition pore
PUMA	p53 upregulated modulator of apoptosis
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
Smac/DIABLO	Second mitochondria derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI
SOD	Superoxide dismutase
TAF	TBP-associated factor 4
TBP	TATA-binding protein
TCA	Tricarboxylic acid
Tfam	Mitochondrial transcription factor A
TIM	Translocase of the inner membrane
TRAK	Trafficking kinesin protein
XIAP	X-linked inhibitor of apoptosis
YAC	Yeast artificial chromosome

3.1 Introduction

Mitochondria are double-membrane organelles that represent the major bioenergetic hub coordinating cell and organism homeostasis. Mitochondria control the production of energy (in the form of adenosine triphosphate, ATP) through oxidative phosphorylation (OXPHOS), supporting the biosynthetic and degradative metabolic requirements of the cells, intracellular calcium (Ca^{2+}) homeostasis, apoptotic and cell signaling pathways, among other cellular processes. The sustained integrity of mitochondria is critical for preserving cell viability; therefore, mitochondrial dysfunction is a common process connecting several neurodegenerative and age-related disorders [1], either due to defects in respiratory function, modified organelle dynamics and degradation, cumulating in DNA and protein damage and/or producing excessive amounts of reactive oxygen species (ROS).

Mitochondrial dysfunction plays a major role in the pathogenesis of Huntington's disease (HD), an autosomal dominant neurodegenerative disorder that initially affects the striatum (mainly the caudate) and later the cortex. HD is characterized by psychiatric disturbances, cognitive deficits, involuntary choreiform movements, dementia and weight loss. All HD cases possess, at least in one of their two copies of the *HTT* gene, a polymorphic CAG repeat tract expansion that encodes for a N-terminal polyglutamine (polyQ) segment of more than 39 residues in the huntingtin protein (HTT) named mutant HTT (mHTT) [2]. Striatal neurodegeneration linked to mitochondrial deregulation has been demonstrated in genetic and toxin-induced animal and cellular models and post-mortem HD human brain tissue [3, 4]. Of relevance, magnetic resonance spectroscopy revealed impaired ATP synthesis and oxidative function in pre-symptomatic HD carriers [5], suggesting that mitochondrial deficits might initiate disease onset.

In this chapter we discuss the role of mitochondrial deregulation in HD-related neuronal dysfunction and degeneration, particularly focusing on changes in energy metabolism, dynamics and movement and the regulation of apoptosis in both central and peripheral HD humanized cell and animal models, in HD human peripheral cells and post-mortem brain samples, as well as in HD human induced pluripotent stem cells (iPSCs), a powerful model to study HD cellular pathogenesis in a pre-differentiation neural stage of the disease and after differentiation into a striatal-like neuronal fate.

3.2 Mitochondrial Dysfunction in HD

Early evidence of mitochondrial defects in HD came from a study demonstrating ultrastructural abnormalities in mitochondria isolated from post-mortem HD cortical tissue [6]. Additionally, the observation that systemic administration of 3-nitropropionic acid (3-NP, an irreversible mitochondrial complex II inhibitor) in rodents or non-human primates produced preferential degeneration in the caudate–putamen that resembled many behavioral and anatomical features of HD, supported the previous evidence [4, 7, 8]. Defects in complexes II, III and IV activities from HD patients' striatum were demonstrated simultaneously [9]. A few years later, mHTT was shown to directly interact with the mitochondrial outer membrane (MOM) [10–12], triggering Ca^{2+} release and abnormal mitochondrial morphology and trafficking, as shown in *postmortem* HD patient's brain specimens, in human HD lymphoblasts or mice neurons expressing the expanded exon 1 of *HTT* [12–14]. Furthermore, interaction of N-terminal fragments of mHTT with the translocase of the inner membrane TIM23 was shown recently, culminating in the inhibition of protein import machinery and neuronal death. Mitochondria from brain synaptosomes of presymptomatic HD mice also exhibited a protein import defect, but not liver mitochondria, suggesting an early and tissue-specific event of the disease [15]. Considering these observations, this section will focus on reports describing how mHTT may cause mitochondrial dysfunction by either a direct interaction with the

organelle and modulation of respiration, mitochondrial membrane potential and Ca^{2+} buffering and mitochondrial bioenergetics, which further impact on ROS production and oxidative damage.

3.2.1 *Altered Mitochondrial Membrane Potential and Impaired Mitochondrial Respiratory Chain Complex Activity*

Electron flow along the respiratory complexes I–IV, localized at the mitochondrial inner membrane (MIM), is coupled to proton translocation into the mitochondrial intermembrane space (MIS), creating an electrochemical proton gradient (proton motive force) and thus a mitochondrial transmembrane potential ($\Delta\psi_m$) of −150 to −180 mV that drives ATP synthesis. This energy production requires $\Delta\psi_m$ to be maintained at 80–90% of its maximum value ([16], for review). However, in HD mitochondria this percentage is not preserved. Brain mitochondria isolated from two lines of YAC72 mice expressing "low" and "high" levels of full lenght-mHTT displayed depolarized membrane, with mitochondria from YAC72 high expressor depolarizing faster after Ca^{2+} stimulation [11]. Similar defect in $\Delta\psi_m$ was found in mitochondria from chimeric human-mouse mHTT-expressing cells in response to increasing Ca^{2+} concentrations [17]. Remarkably, a large amount of evidence has shown that HD mitochondria from human lymphoblasts are highly susceptible to decreased $\Delta\psi_m$ [11, 18], which was correlated with increased glutamine repeats [19], suggesting that the adverse effect of mHTT is not limited to neurons. Indeed, results obtained in our laboratory in symptomatic HD cybrids (an ex vivo peripheral model obtained from the fusion of HD human platelets with mtDNA-depleted rho0 cells) *versus* control cybrids and in HD human B-lymphocytes evidenced significant changes in $\Delta\psi_m$ linked to apoptotic events [20, 21]. Interestingly, constitutive HTT phosphorylation at serine 421 completely abrogated the deregulation of $\Delta\psi_m$ in HD human lymphoblasts [18], linking the neuroprotective effects of HTT phosphorylation in this residue (e.g. [22]) to improved mitochondrial function.

A dramatically decrease in the activity of complex II (succinate dehydrogenase, SDH)/III (cytochrome *c* reductase) and mildly complex IV (cytochrome *c* oxidase) in the caudate or putamen were observed in *postmortem* studies of symptomatic HD patients or HD models, namely immortalized striatal cell lines, animal model brains or human peripheral cells [9, 23, 24, 25, 26, 27], and may contribute for decreased $\Delta\psi_m$. Additionally, the decrease in complex II in humans, rodents or primates following administration of 3-NP or malonate (complex II reversible inhibitor) causes HD-like symptoms in animal models and striatal cytotoxicity ([28], for review). Although the selective inhibition of the striatum by a chemical compound such as 3-NP has posed several questions, reduced activity of mitochondrial respiratory chain complexes can largely contribute to accelerate mitochondrial dysfuncton in HD.

3.2.2 Defects in Mitochondrial Ca^{2+} Handling

The MIM possesses a Ca^{2+} uniporter (MCU, mitochondrial calcium uniporter), providing cells with a protective high capacity for Ca^{2+} buffering. Moreover, interaction of mHTT with the MOM may induce the opening of a high conductance pathway, the mitochondrial permeability transition pore (PTP), which is triggered by Ca^{2+}, ROS or decreased adenine nucleotide levels, causing mitochondrial swelling, depolarization and, eventually, cell death [10, 11, 17]. Thus, deficits in mitochondrial Ca^{2+} handling likely contribute to HD neurodegeneration. Studies performed by Panov and colleagues demonstrated that mitochondria isolated from lymphoblasts of HD patients and from brains of transgenic yeast artificial chromosome (YAC) mice expressing full-length mHTT with 72 glutamines (YAC72) exhibited pronounced defects in Ca^{2+} handling. Importantly, these defects persisted even in the presence of PTP inhibitors [11]. Impaired mitochondrial Ca^{2+} homeostasis was also confirmed in intact HD human lymphoblasts following exposure to hydrogen peroxide (H_2O_2) [18]. Moreover, mitochondria obtained from liver of homozygous knock-in $Hdh^{150/150}$ mice or treated with recombinant truncated mHTT protein showed augmented predisposition to Ca^{2+}-stimulated PTP induction [10]. Contrariwise, increased Ca^{2+} uptake was observed in isolated brain non-synaptic mitochondria from R6/2 mice (the most commonly used HD model expressing human *HTT* exon 1 with $\sim$150 CAG repeats) and YAC128 HD mice, when compared to mitochondria from wild-type mice [29]. Indeed, two years earlier, Brustovetsky and colleagues had shown that striatal mitochondria from R6/2 exhibited increased resistance to Ca^{2+}, while in striatal mitochondria from littermate controls, lower doses of Ca^{2+} consistently evoked PTP more easily. Mitochondrial from knock-in HD mice also became more resistant to Ca^{2+} with increasing age and retained these levels of sensitivity throughout life [30], suggesting that mitochondria are capable of compensatory changes towards neuroprotection. Meanwhile, recent data obtained with isolated brain synaptic and non-synaptic mitochondria from YAC128 mice suggest that increased Ca^{2+} uptake capacity can be directly correlated with the amount of mHTT associated with the mitochondrial membrane [31]. In addition, HD-iPSC-derived neuronal-like cells expressing both GABA (γ-aminobutyric acid)-A receptor and ionotropic glutamate receptors (obtained from fibroblasts of symptomatic HD patients retaining 60 or 180 CAGs) revealed a clear CAG expansion-dependent decrease in Ca^{2+} uptake following a chronic glutamate stimulus [32].

3.2.3 Energy Metabolic Deficits

Neurons are highly dependent on mitochondrial ATP production to maintain normal synaptic communication. Therefore, they are very sensitive to disturbed energy metabolism. A number of studies, from HD *postmortem* brains [33] to transgenic

HD mouse brain [34, 35], revealed mHTT-related abnormal ATP/ADP and phosphocreatine/inorganic phosphate (PCr/Pi) ratios and energy charges. This reduction in mitochondrial ATP levels might be linked to increased Ca^{2+} influx through *N*-methyl-D-aspartate receptors, since ATP/ADP ratio could be normalized by blocking Ca^{2+} influx in mHTT-expressing striatal cells [36]. Moreover, a poor creatine kinase (CK)/PCr system in HD brain might also contribute for this reduction [37] (Fig. 3.1). As described before for changes in $\Delta\psi_m$, bioenergetic defects in HD are not only confined to the brain, but are also observed in peripheral tissues, such as muscle [5, 38] or lymphoblasts [36]. These findings raise the possibility that the ubiquitous expression of mHTT may place other cell types at risk, particularly those with high metabolic demand. Remarkably, recent studies conducted in our laboratory showed that glucose deprivation did not exacerbate the defects in ATP/ADP ratio in cortical primary cultures derived from YAC128 mice, contrarily to wild-type neurons, pointing to an abnormal glycolytic pathway linked

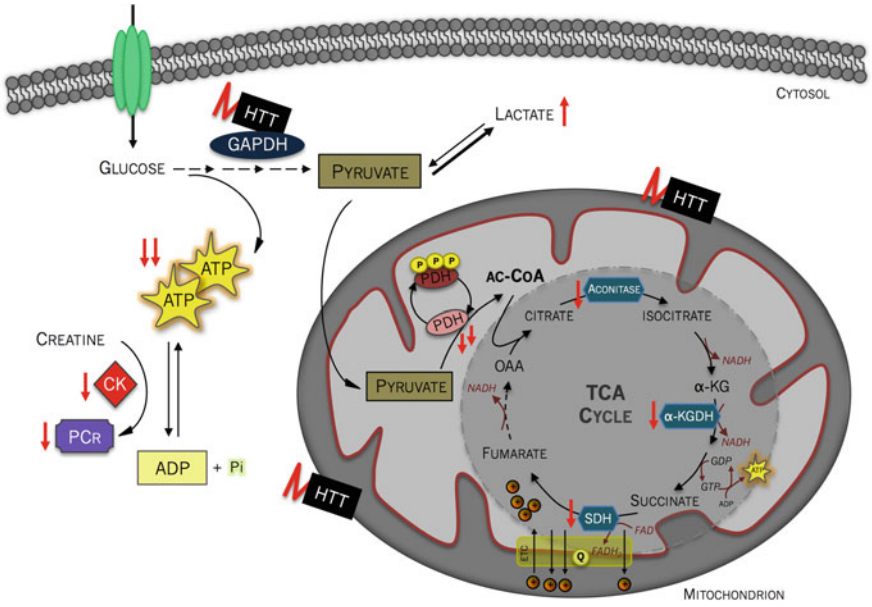

Fig. 3.1 Mutant huntingtin induces energy metabolic failure. Glycolysis is the metabolic pathway that converts glucose into pyruvate, generating ATP and NADH. In the presence of mHTT, ATP production is decreased, along with poor PCr/Pi ratio due to decreased CK activity. Alongside, mHTT interacts with the sixth step glycolysis enzyme GAPDH. Although with compromised glycolysis, pyruvate can still accumulate due to increased phosphorylation/inhibition of PDH, stimulating the conversion to lactate instead of entering in the TCA cycle as acetyl-CoA. Defects in TCA cycle intermediate enzymes such as α-KGDH, aconitase and SDH, as well as the susceptibility of SDH to mHTT interaction severely compromises the generation of reduced equivalents to feed the ETC, which may also explain decreased activities of mitochondrial respiratory chain complexes

to deficient ATP generation [39]. Studies on mitochondrial oxidative metabolism in presymptomatic and symptomatic HD patients previously detected a selective defect in glycolysis in early HD striatum [40–42], suggesting that metabolic deficits in HD may precede neuropathology and clinical symptoms. Indeed, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has an essential function in glycolysis, binding both normal and mHTT, but with a preferential interaction to cleaved polyQ domain, enhancing nuclear translocation of mHTT and cytotoxicity [43]. Interestingly, studies using an allelic series of murine CAG knock-in embryonic stem cell (ESC) lines have shown dominant CAG-length dependent reduction in energy metabolism [44]. In addition, human derived HD specific neural stem cells showed significantly decreased intracellular ATP [32], supporting the hypothesis that bioenergetic dysfunction is an early event in HD.

Elevated levels of lactate and increased lactate/pyruvate ratio have been also described in striatum, cortex and cerebrospinal fluid from HD patients [45–47], as well as in brains from YAC128 and R6/2 transgenic mouse models [34, 48]. Concordantly, we observed that HD cybrid lines exhibited increased lactate/pyruvate levels, which were correlated with a large decrease in the activity and protein levels of pyruvate dehydrogenase (PDH) [49], a protein complex located in the mitochondrial matrix, responsible for catalyzing the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA (Fig. 3.1). Decreased mitochondrial metabolism in caudate (in particular) and putamen of HD patients was previously assessed through a progressive decrement in PDH complex [50, 51], whereas dichloroacetate, a PDH kinase inhibitor previously shown to increase PDH activity, prevented the increase of cerebral lactate concentrations and attenuated the development of striatal neuron atrophy in R6/2 and N171-82Q transgenic HD models [52]. Besides the PDH complex, tricarboxylic acid (TCA) cycle enzymes that provide the main pathway for generating reducing equivalents, such as aconitase, α-ketoglutarate dehydrogenase (α-KGDH) or SDH are also compromised in central HD tissues [53, 54] (Fig. 3.1). Cultured striatal neurons expressing N-terminal HTT fragment showed decreased SDH activity, the only enzyme that participates in both TCA cycle and electron transport chain (ETC) [23]. Contrariwise, in peripheral HD cybrids α-KGDH enzymatic activity was increased [49, 55, 56], suggesting a compensatory mechanism to re-establish NADH levels.

3.3 Oxidative Dysregulation

Under physiological conditions about 1% of O_2 consumed generates superoxide anion ($O_2^{\bullet -}$) at the mitochondrial respiratory chain due to the leakage of electrons at the level of complexes I and III. Indeed, mitochondria are both sources (at the level of complexes I and III) and targets of ROS, contributing to mitochondrial damage in several pathologies. In complex I (reduced β-nicotinamide adenine dinucleotide (NADH)-ubiquinone:oxidoreductase), flavin mononucleotide (FMN) accepts electrons from NADH passing them through a chain of iron–sulfur centers

to the ubiquinone (CoQ, coenzyme Q) reduction site. Complex I-dependent ROS production is influenced by the matrix redox potential ($NADH/NAD^+$ ratio) and is enhanced by its selective inhibitor, rotenone, which prevents the transfer of electrons to the CoQ-binding site [57]. Complex III catalyzes the reduction of cytochrome *c* by oxidation of CoQ, which has two reaction centers, the ubiquinol-oxidation center (Qo site) and the ubiquinone-reduction center (Qi site). Inhibition of Qi site by antimycin A prompts the production of large amounts of $O_2^{\bullet-}$ on both sides of the MIM [58]. $O_2^{\bullet-}$ is highly membrane impermeable, however it can be readily dismutated to H_2O_2 by superoxide dismutases (SOD); in contrast, H_2O_2 diffuses across membranes [59]. Mitochondria have their own antioxidant defense system, thus minimizing the deleterious effects exerted by ROS. The first line of defense involves the dismutation of $O_2^{\bullet-}$ to H_2O_2 by metal-containing enzymes, comprising Mn-SOD (SOD2), located in the mitochondrial matrix, and Cu,Zn-SOD (SOD1) at the MIS and cytosol. H_2O_2 can also be effectively detoxified by mitochondrial antioxidant enzymes like glutathione peroxidases (Gpx), which are also present in the cytosol and by peroxiredoxins (Prx) and catalase, the latter located in the peroxisomes, which convert H_2O_2 into H_2O and O_2 [60, 61].

The major cause for energetic deficits in HD involves mitochondrial abnormalities and ROS production triggered by mHTT-mitochondria interactions (in a polyQ length-dependent manner), which impair oxidative phosphorylation and ATP production [62]. Aconitase is an iron-sulphur enzyme of the TCA cycle that is particularly susceptible to ROS. Several studies found that it is strongly affected in *postmortem* human HD caudate, putamen and cortex [55, 63, 64] and in striatum from R6/2 mice [65].

A relationship between inhibition of complexes I and III and enhanced levels of mitochondrial-generated $O_2^{\bullet-}$ was previously confirmed by us in HD striatal cells [66]. Enhanced ROS formation occurring mainly via mitochondria, observed in knock-in striatal cells expressing mHtt ($ST\mathit{Hdh}^{Q111/Q111}$), was related with altered activities and levels of antioxidant defense systems, and decreased antioxidant response to exogenous stressors associated to impaired nuclear factor-erythroid 2-related factor-2 (Nrf2) transcriptional activity [66, 67]. An exhaustive proteomic analysis of human HD samples from striatum and cortex showed increased oxidative stress response, defined by the induction of several antioxidant enzymes, namely Prx 1, 2, and 6, and Gpx 1 and 6. Concomitantly, catalase activity was enhanced and SOD2 showed a significant increase of both protein and activity levels in cortex and striatum [63]. Moreover, protein carbonyl formation (a marker of protein oxidation) of glial fibrillary acidic protein, γ-enolase, and CK B was increased in human HD samples from striatum and cortex [63].

Preceding studies in iPSC lines generated from HD patients fibroblasts, ranging from 33 to 180 CAGs, and control fibroblasts, also described increased cell death in HD-iPSC lines in response to toxic stressors, suggesting that HD-iPSCs are increasingly susceptible to ROS [32]. In HD-iPSCs reprogrammed from a juvenile HD patient carrying 72 CAG repeats, Chae and colleagues (2012) shed some light

on protein expression profiles that are key regulators of oxidative stress, DNA damage and expression of cytoskeleton associated proteins [68]. These authors found lower levels of SOD1, GST and Gpx1 in HD-iPSC lines, when compared to human embryonic stem cells (hESCs), in contrast with the upregulation of Prx family members, including Prx1, 2 and 6, which have been implicated as important indicators for cellular ROS signals [68].

In summary, mitochondrial dysfunction leads to increased production of ROS, which has a major role in both necrotic and apoptotic mechanisms of cell death (detailed in Sect. 3.6).

3.4 Mitochondrial Biogenesis

Mitochondrial network is constantly being renewed through an equilibrium between biogenesis and degradation of damaged mitochondria (mitophagy) [69]. Mitochondria biogenesis comprises a multistep process, where mtDNA transcription and translation, along with translation of nuclear-encoded mitochondrial-related transcripts, mitochondrial protein import and overall assembly into a mitochondrial network must proceed correctly [70].

cAMP response element-binding protein (CREB)/TATA-binding protein (TBP)-associated factor (TAF)4 signaling pathway regulates various mitochondrial genes, such as NADH dehydrogenase subunit 5 (ND5) that codes for a subunit of complex I, and is severely disrupted in HD. In fact, mHTT can interact with several transcription factors involved in this pathway, such as CREB-binding protein (CBP) or TAF4/TAFII130 [71, 72]. Ultimately, CREB/TAF4 signaling pathway regulates the expression of peroxisome proliferator-activated receptor γ—PPARγ—coactivator-1α (PGC-1α), one of the major transcriptional regulators of organelle biogenesis [73]. PGC-1α is a major regulator of mitochondrial function, mediating mitochondrial biogenesis and respiration. Being a transcriptional co-activator, it regulates the expression of nuclear-encoded subunits of each of the electron transport-chain complexes, along with genes involved in antioxidant response such as ATP synthase or SOD2 [74]. PGC-1α also regulates nuclear respiratory factor (NRF)1 and 2 and PPAR α, δ and γ by forming heteromeric complexes, sharing a role in the expression of genes such as cytochrome *c*, complexes I-V and the mitochondrial transcription factor A (Tfam), the major transcriptional regulator of mtDNA [75]. In HD in vitro and in vivo models PGC-1α was found to be repressed, partially due to the direct interaction of mHTT with the signaling pathway mentioned above that regulates its expression, but also by a direct binding to its promoter [73]. HD patients also showed reduced levels of Tfam and PGC-1α as disease severity increases, along with evidences of mitochondrial loss [3, 73, 76, 77] (Fig. 3.2).

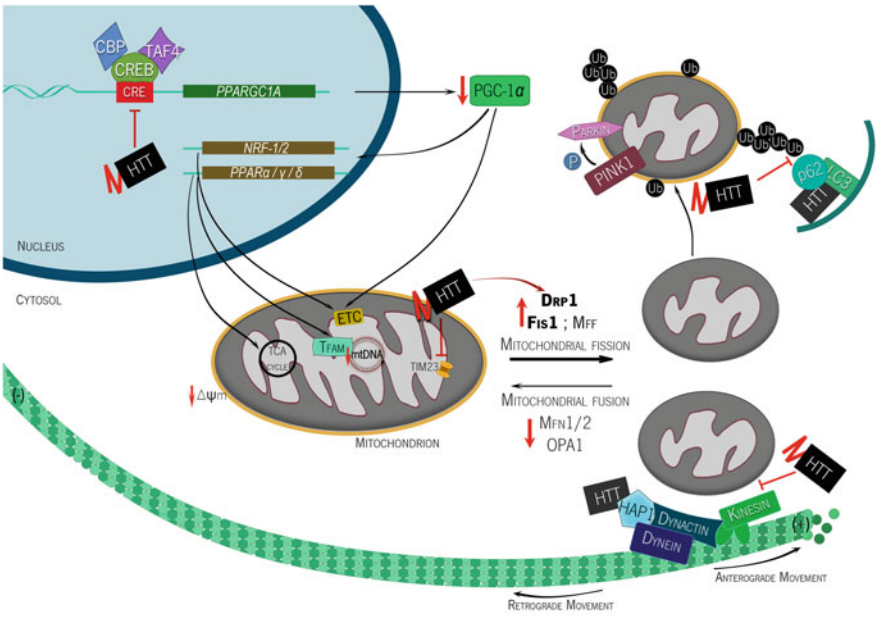

Fig. 3.2 Mutant huntingtin severely impacts mitochondrial turnover. mHTT disrupts CREB/TAF4 signaling culminating in decreased PGC-1α levels and activity. Ultimately, signaling pathways regulated by downstream targets of this transcriptional coactivator may be affected, namely NRF-1, NRF-2, PPARα, PPARδ and PPARγ. Expression of genes involved in TCA cycle, ETC and Tfam are significantly impaired, with compromised mitochondrial biogenesis. By directly interacting with mitochondria, mHTT inhibits mitochondrial protein import, a major factor not only for mitochondrial biogenesis, but also for overall mitochondrial function. Moreover, mHTT disrupts the balance between fission and fusion, with increased mitochondrial fragmentation due to decreased levels of Mfn1, Mfn2 and OPA1 and increased levels of Drp1 and Fis1. Additionally, mHTT interacts with and increases Drp1's GTPase activity. Mitochondrial trafficking is impaired in a retrograde (dynactin/dynein-dependent) and anterograde (kinesin-dependent) manner. mHTT may also interfere with the selective degradation of dysfunctional mitochondria, namely by hampering HTT's function in aiding p62 to associate with LC3

3.5 Mitochondrial Dynamics and Mitophagy

Mitochondria are dynamic organelles with frequent changes in size, shape, number and even cellular distribution that directly relate with their function in response to cellular needs or to diverse stimuli [78]. Increasing evidence suggests that unbalanced mitochondrial dynamics take an important role in neurodegeneration in HD ([79] for review). The presence of mHTT appears to reduce the number of mitochondria and leads to their fragmentation, with defects in anterograde and retrograde transport and velocity, ultimately causing neuronal death [80].

3.5.1 Mitochondrial Fission/Fusion Balance

Mitochondria hold the ability to divide (fission) and unite (fusion) in response to diverse stimuli. Both processes allow the exchange of membranes and intramitochondrial content or mobility of the organelle to specific subcellular locations; moreover, fission facilitates apoptosis by regulating the release of MIS proteins into the cytosol, such as cytochrome *c* [78].

Fission/fusion balance has been reported to be altered in HD, with altered expression of genes involved in these processes, culminating in abnormal mitochondrial morphology and consequently in neuronal dysfunction [81]. Dynamin-related protein 1 (Drp1) takes control of mitochondrial fission. This is a cytosolic protein that can transit towards the MOM upon a fission stimulus, having an effector guanosine triphosphate (GTP)ase domain [82]. Mitochondrial fission 1 (Fis1) and mitochondrial fission factor (Mff), two integral proteins of the MOM, serve as adaptors for Drp1, allowing the recruitment of Drp1 to the effector sites [83]. Meanwhile, fusion counts with machinery of both the MIM and MOM. Mitofusins (Mfn) 1 and 2 are also GTPases, located at the MOM, which are responsible for the fusion of MOMs of the juxtaposing mitochondria. Optic atrophy 1 (OPA1) is the regulator for the MIM fusion process; OPA1 is found at the MIS and shows association with the MIM. Maintenance of $\Delta\psi_m$ is required for mitochondrial fusion. As such, after dissipation of $\Delta\psi_m$, fusion is inhibited, but fission can still occur and mitochondrial fragmentation becomes a dominant morphology [84].

A visible decrease in the number of mitochondria in striatal spiny neurons derived from neostriatal tissue of HD patients appears to directly correlate with disease severity [3]. Increased levels of Drp1 and Fis1, along with decreased levels of Mfn1, Mfn2 and OPA1 were found in striatum and cortex of several HD animal models and patients, resulting in excessive mitochondrial fragmentation [80, 85]. In addition to increased expression, interaction of Drp1 with mHTT results in increased GTPase activity [12] (Fig. 3.2), leading to fragmented and less efficient mitochondria that culminates in loss of energy required for neuronal function.

3.5.2 Mitochondrial Trafficking

Impairment in mitochondrial transport along neuronal processes, with slower translocation of the organelle has been associated to HD. Mitochondria trafficking along the cells allows the organelle to be present in subcellular compartments that are in need of a high energy demand. This process is critically important when considering neurons that need energy outside the regular bioenergetic requirements, such as for synaptic transmission. In fact, mHTT-induced fragmented mitochondria are mainly localized in the cell body, not being able to be transported to dendrites, axons or synapses (anterograde movement), which consequently results in low ATP levels at these sites and in overall synaptic degeneration [76].

The processes of mitochondrial fusion and fission can be directly related to their motility. Fission allows for smaller mitochondria to be separated from the rest of the network and to be transported along the microtubules with the aid of dynein, dynactin (retrograde transport) and kinesins motors (anterograde transport) [86]. Mitochondria enlist motor adaptors such as trafficking kinesin protein (TRAK)1 and TRAK2 that bind Miro (MOM protein) to kynesin motors and ensures targeted and precise trafficking in response to neuronal activity [87]. Both N-terminal fragments and full-length mHTT can directly affect mitochondria motility in either anterograde or retrograde movement, resulting in accumulation of the organelle in the soma [76, 88, 89] (Fig. 3.2). Sequestration of mitochondrial transport machinery and blockage by mHTT aggregates may take place, being impossible for mitochondria to move through narrow neuronal projections, as seen in cortical neurons overexpressing mHTT and in HD striatal neurons [89, 90]. Moreover, Orr and colleagues reported altered mitochondrial trafficking in the absence of mHTT aggregates, suggesting an early impairment due to reduced association of mitochondria with motor proteins [76, 88, 89].

3.5.3 Mitophagy

Accumulation of damaged mitochondria occurs in HD cells and can be due to the loss in $\Delta\psi_m$, oxidative stress, impaired OXPHOS, excessive fragmentation and/or decreased biogenesis. Selective mitochondrial degradation by macroautophagy (hereafter termed mitophagy) ensures mitochondrial quality control and recycling, but must be balanced with biogenesis [91]. Phosphatase and tensin homolog (PTEN)-induced putative kinase 1 (PINK1)/Parkin-dependent mitophagy pathway is the most well characterized mitophagy pathway, although PINK1/Parkin-independent mitophagy can also occur [92, 93]. One example comes from the redistribution of cardiolipin, naturally in the MIM, to the MOM, signaling damaged mitochondria from neuronal cells to undergo degradation. This allows cardiolipin to interact with autophagosome-associated light chain 3 (LC3) [94].

PINK1 is a serine/threonine kinase that localizes in the cytosol and is normally imported into MIM where it is degraded by mitochondrial proteases [95–97]. In the presence of damaged mitochondria exhibiting decreased $\Delta\psi_m$, PINK1 is stabilized at the MOM, inducing Parkin translocation to mitochondria [98]. Since Parkin is an E3 ubiquitin ligase, it ligates ubiquitin chains on MOM proteins that are recognized by autophagy adaptors such as p62 [99]. Khalil and colleagues reported a protective role for PINK1 overexpression in HD flies and ST*Hdh*$^{Q111/Q111}$ cells [100]. Nevertheless, the significance of alterations in PINK1/Parkin-dependent mitophagy has remained elusive in HD.

HTT was proposed to have a role in the control of autophagosome dynamics, along with huntingtin-associated protein 1 (HAP1), through the regulation of dynein and kinesin. Moreover, axonal transport of autophagosomes was found to be impaired in the presence of mHTT. It ultimately ended in inefficient degradation of

internalized mitochondria, probably due to inhibition of autophagosome/lysosome fusion [101]. Additionally, wild-type HTT may serve as a scaffold protein in selective autophagy (not just mitophagy), aiding autophagic adaptor p62 to associate with LC3 and lysine 63 (K63)-linked ubiquitinated substrates [102]. PolyQ-expanded HTT may thus impede this scaffold function (Fig. 3.2). Indeed, expression of a mHTT transgene in a mice expressing mitochondria-targeted Keima, a protein exhibiting pH-dependent excitation, reduced the levels of mitophagy [103].

3.6 Mitochondrial-Dependent Apoptosis

Intrinsic apoptosis is triggered following the loss of integrity of the MOM, which allows the release of pro-apoptotic factors to the cytosol. The release of proteins such as cytochrome *c* and Smac/DIABLO (second mitochondria derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI) induces the formation of the apoptosome. After release from the MIS, cytochrome *c* forms a complex with deoxy-ATP, Apaf-1 (apoptotic protease-activating factor 1) and the initiator caspase 9, which when activated further activates the executioner caspases (3, 6 and 7) and induces the well-recognized nuclear apoptotic features, such as DNA fragmentation and chromatin condensation [104–106]. In neurons, p53 has also been implicated in upregulating the pro-apoptotic proteins Bax and the BH3-only proteins PUMA (p53 upregulated modulator of apoptosis) and Noxa [107].

Different evidences reinforce the deleterious influence of mHTT in the apoptotic pathway, particularly by activating several BH3-only proteins. Changes in Bcl-2 (B-cell lymphoma 2), an anti-apoptotic protein, in HD are controversial. In several cell lines with endogenous expression of mHTT or induced to overexpress mHTT, Bcl-2 family protein dysregulation was common; several studies showed decreased Bcl-2 protein and mRNA levels, Bax activation and translocation from the cytosol to mitochondria, followed by Bim (Bcl-2 interacting mediator of cell death) activation [108–112] (Fig. 3.3). However, in animal models of HD, the results are largely controversial, particularly when considering the anti-apoptotic proteins. Initial studies showed that Bcl-2 protein levels were unchanged in total brain or in mitochondrial fractions from R6/1 and R6/2 mice [113–115]. Conversely, other studies reported down-regulation of Bcl-2 levels in R6/2 mouse brain [109, 116] and in striatal tissue from N171-82Q mice [108]. Importantly, overexpression of Bcl-2 was shown to slow the disease progression [117]. For other pro-survival proteins, such as Bcl-XL, no major differences were found in total brain and striatum lysates or mitochondrial fractions of R6/1 (control and with low levels of the neurotrophin brain derived neurotrophic factor, BDNF), HD94 (tetracycline inducible expression of mHtt) and R6/2 mouse models [113, 117]. Moreover, higher Smac/DIABLO levels were found in the cytosol of cells overexpressing mHTT and this release was responsible for the degradation of anti-apoptotic IAP1

(Inhibitor of apoptosis protein-1) and XIAP (X-linked inhibitor of apoptosis) proteins in the cytosol of HD striatal cells [118]. The mitochondrial levels of AIF (apoptosis inducing factor), which induces apoptotic cell death through a caspase-independent pathway, were also found to be reduced in HD mouse striatal cells [119].

More consistent data has been observed in pro-apoptotic proteins examined in HD animal models and patient samples. In mitochondrial brain fractions of R6/1 and R6/2 mice the pro-apoptotic protein Bax was increased; moreover, increased Bax mRNA was described in the cortex and cerebellum of R6/1 mice [113, 115, 117]. A decrease in phosphorylated Bad and activation of caspases-1 and -3 was also reported in R6/2 mice models [117]. In caudate samples from HD patients neostriatal neurodegeneration was associated with enhanced Bax levels, weak caspase-3 immunostaining and cells exhibiting apoptotic morphology, in comparison with controls without neurological disease [120]. These changes seem not to be specific for HD human brain, since increased Bax levels were also described in peripheral B and T lymphocytes and monocytes derived from symptomatic HD patients [20] (Fig. 3.3). Moreover, untreated HD human cybrid lines showed increased mitochondrial Bim and Bak levels, and a slight release in cytochrome c, evidencing their increased susceptibility to intrinsic apoptosis [21]. Another mitochondrial localized BH3-only protein implicated in HD is BNIP3; when activated, BNIP3 causes loss in $\Delta\psi_m$ and induces mitochondrial fragmentation prior to mitophagy [112]. Although the molecular mechanisms for BNIP3 activation are not well understood, BNIP3 levels were described to be increased in total lysates of HD myoblasts [121].

Numerous studies in vitro and in vivo support the interaction between caspases and HTT in vulnerable cell types in the striatum and cortex leading to increase cell death [122–125]. Increased expression of caspase-2 was correlated with decreased BDNF levels in the cortex and striatum of YAC72 mice. Htt can be recruited and cleaved by initiator caspase-2, which induces neuritic degeneration. The generated N-terminal toxic fragment was shown to associate with synaptic vesicles and inhibit glutamate uptake. Caspase-7, which is specifically expressed in medium-sized neurons and enriched in the striatum, may also associate with Htt, triggering the activity of other caspases, namely caspase-6, and accelerating the production of Htt fragments and the induction of apoptosis [122, 124]. Two active caspase-3 sites and one caspase-6 site have been largely studied in HTT. Caspase 6 cleavage at the 586 cysteine residue of Htt, but not caspase 3 cleavage, was shown to be required for the development of the characteristic behavioral and neuropathological features of HD [123, 126, 127]. However, recent studies showed that the cleavage pattern was unaltered when mHtt expressing mice were crossed onto caspase knockout background [128, 129], raising the question of whether other caspases might be involved in the generation of cytotoxic mHTT fragments.

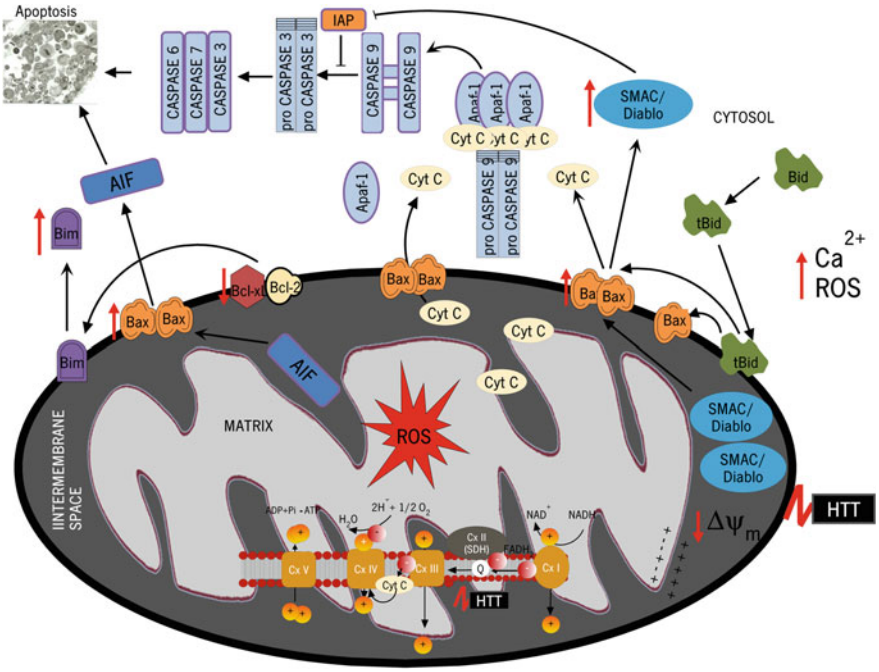

Fig. 3.3 Intrinsic apoptotic pathway is triggered by intracellular stimuli such as Ca^{2+} overload and excessive ROS levels. Following the stress signals, the apoptotic process is controlled by Bcl-2-protein family, classified into anti-apoptotic and pro-apoptotic members. The pro-apoptotic effector proteins, Bak and Bax, can be activated by pro-apoptotic BH3-only proteins —Bim and active Bid (truncated Bid, tBid)—or by a noxious stimulus, upon which these proteins form oligomers that insert into the MOM, being responsible for the loss of membrane integrity. Destabilization of the mitochondrial membrane causes the release of apoptotic factors such as cytochrome *c* and Smac/Diablo, the latter promoting apoptosis by neutralizing the inhibitory effect of IAPs on caspases activity. After being released, cytochrome *c* assembles into a multiprotein caspase activating complex formed by procaspase-9, Apaf-1 and deoxy-ATP, the apoptosome. Subsequently, activated caspase 9 cleaves and activates the downstream executioner caspases 3, 7 and 6 to induce cell death. Other apoptotic factors are released from the MIS into the cytosol, namely AIF, which acts independently of caspases. Stress factors such as increased intracellular Ca^{2+} levels and ROS production can trigger the opening of the PTP (*not shown*)

3.7 Concluding Remarks

In conclusion, numerous studies have shed some light on the role of mitochondria in the regulatory mechanism of biochemical/molecular functions involved in HD pathophysiology. Increasing evidence support the contribution of mitochondria and oxidative phosphorylation defects in HD patients and cellular and animal HD models as a trigger event for the irreversible cascade of events involved in disease phenotype, as demonstrated by some of the most relevant publications that were generated in this field since 1978 until 2015 (Fig. 3.4). However, many questions

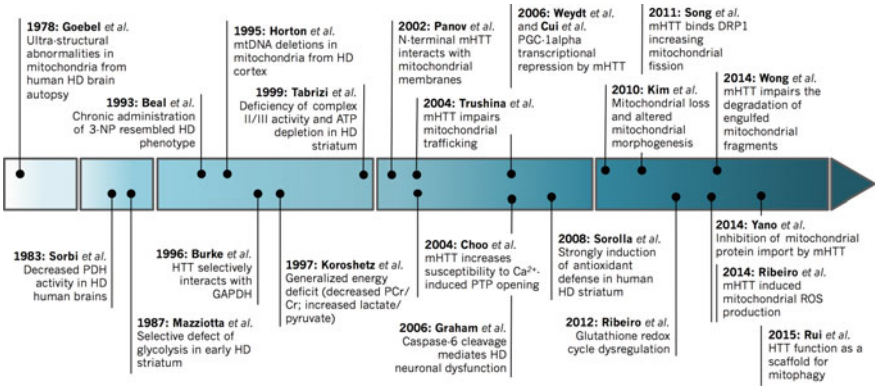

Fig. 3.4 **Key discoveries involving mitochondria in HD pathogenesis**. This timeline resumes some of the major discoveries involving mitochondrial dysfunction in HD and related oxidative stress changes, from dysfunctional glycolysis described in the late 80 s, to deficiency of respiratory chain complexes in the late 90 s, which was later explained by the interaction of mHTT with mitochondria and by transcriptional deregulation. More recently, research has focused on the role of both wild-type and mHTT on mitochondrial dynamics and turnover. All studies are indicated in the reference list

remain to be answered regarding the role of mitochondria dysfunction in HD and its influence on other pathological processes and thus on disease progression. The full understanding of how mHTT targets mitochondria leading to a dysfunctional organelle can be foreseen an important advance to identify new molecular pathways and eventually therapeutic targets. So far, few clinical trials aimed to directly improve mitochondrial function in HD, although the results have not been very promising. A study concerning coenzyme Q10 intake was canceled at phase III due to the lack of results. Other bioenergetic agents tested include creatine and cysteamine bitartrate, the latter the neuroactive metabolite of cystamine. The present chapter clearly points out several molecular changes that can be the targets of more efficient mitochondrial-based therapies and may impact on the organelle physiology. Considering the diversity of changes operated just in a single organelle such as mitochondria, development of combined therapies aiming to improve these alterations may help to delay HD onset and/or disease progression.

Acknowledgements The authors acknowledge financial support from 'Fundação para a Ciência e a Tecnologia' (FCT), Portugal (projects ref. EXPL/BIM-MEC/2220/2013 and Pest-C/SAU/LA0001/2013–2014), co-financed by 'Programa Operacional Temático Factores de Competividade' (COMPETE) and supported by the European community fund (FEDER). ACR also acknowledges financial support from 'Santa Casa da Misericórdia de Lisboa' (SCML)—Mantero Belard Neuroscience Prize 2013, and 'Fundação Luso-Americana para o Desenvolvimento' (FLAD)—Life Science 2020, Portugal. LN holds a Ph.D. fellowship from 'Fundação para a Ciência e a Tecnologia' (FCT), Portugal (Reference SFRH/BD/86655/2012). CL was supported by 'Fundação Luso-Americana para o Desenvolvimento' (FLAD) Life Science 2020 Postdoctoral Fellowship.

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