1. INTRODUCTION

1.1. Mitochondrion

Most eukaryotic cells contain a large amount mitochondria, which can occupy up to 25 percent of the volume of the cytosol. The first microscopic observations of these organelles happened more than 100 years ago. Since then we have learned a lot about them, but there are still many questions that need to be answered.

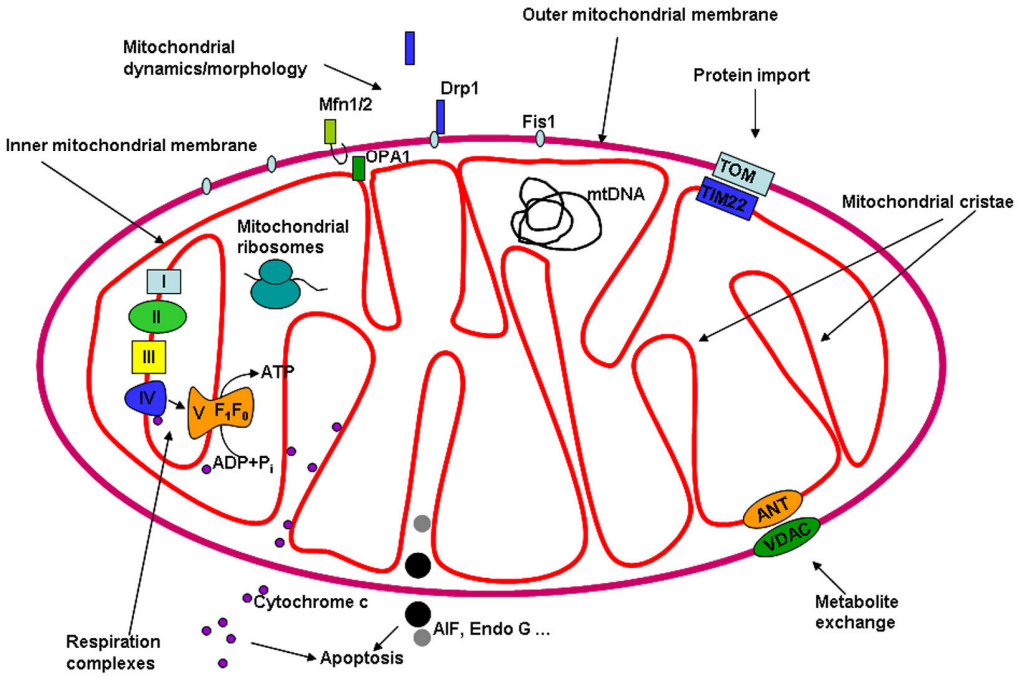

Figure 1. Scheme of a mitochondrion

The mitochondrion is presented with its double membrane, cristae, mitochondrial DNA and many of the proteins important for its structure and function.

1.1.1. The structure of mitochondria

The mitochondrion is a double-membrane organelle and each of the membranes has a different composition and function. The outer membrane, composed of about half lipid and half protein, contains voltage-dependent anion-selective channels (VDAC), also known as porins. They are highly conserved transmembrane channel proteins similar in

structure to bacterial porins. VDAC are the key regulators of metabolite flow across the mitochondrial outer membrane and make the outer membrane permeable to small molecules up to 5 kDa in weight (Bay and Court, 2002; Benz, 1994; Colombini, 1979; Shoshan-Barmatz et al., 2006). As the name suggests, VDAC show both ion selectivity and voltage dependence. In the high conductance, open state, anions are favoured over cations (Colombini, 1983). The mitochondrial inner membrane, on the other hand, has a shape that can extremely vary among different organisms and tissues, but all mitochondria have a similar basic structure: a smooth outer membrane envelops an inner membrane with a considerably larger surface area (Figure 1). The inner membrane is much less permeable than the outer. It consists of about 20 percent lipid and 80 percent protein, making it a membrane with a higher proportion of protein than in any other cellular membrane. Its surface is greatly increased by many invaginations called cristae. Cristae were first thought to be random, wide folds in the inner membrane (Palade, 1952). The next model proposed was that cristae are disc-shaped chambers not connected to the inner membrane (Sjostrand, 1953). A model that created a compromise suggested that cristae are connected to the inner membrane by thin tubules (Daems and Wisse, 1966). This was based on three-dimensional reconstructions of high voltage electron micrographs of mitochondria showing chambers of variable shapes that were connected to the mitochondrial inner membrane by thin tubules, known as cristae junctions (Figure 2) (Mannella et al., 1994; Perkins et al., 1997). Thus, mitochondrial cristae can be defined as involutions of the inner membrane connected via narrow tubular segments, cristae junctions, to the inner boundary membrane, i.e., the region of the inner membrane that parallels the outer membrane on the mitochondrial periphery. Cristae are highly variable. Their shapes can vary depending on the cell type and on the pathophysiological state of the cell (Scheffler, 1999). 75% of cytochrome c and the majority of respiration complexes are located in mitochondrial cristae (Figure 1). The inner mitochondrial membrane envelops the protein-rich mitochondrial matrix. Besides proteins, the matrix contains mitochondrial DNA (mtDNA). The double membrane and its own DNA make the mitochondrion, next to the chloroplast of the plant cell, a ‘unique’ organelle in a cell. Mitochondria contain multiple mtDNA molecules, which are maternally inherited, covalently closed, circular, double stranded DNA molecules. They lack introns and have overlapping genes and a genetic code that differs from the universal one (Clayton, 2000).

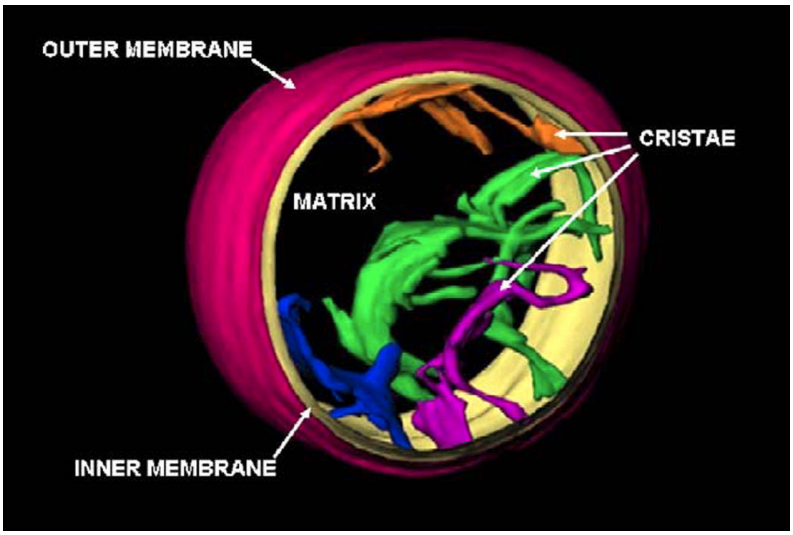

Figure 2. Model of a mitochondrion

Model derived from cryo-electron tomography of the membranes in an intact, frozen-hydrated mitochondrion isolated from rat liver. This mitochondrion has a diameter of 700 nm. Taken from Mannella, (2006).

The entire mitochondrial genome from a number of different organisms has now been cloned and sequenced (see http://megasun.bch.unmontreal.ca/gobase/gobase.html). mtDNA has a small number of genes, usually 37 in animals. They encode rRNAs, tRNAs and essential mitochondrial proteins (reviewed in Garesse and Vallejo, 2001). All proteins encoded by mtDNA are synthesised by mitochondrial ribosomes and never leave mitochondria. The mitochondrial genome encodes only a few proteins (e.g. 8 in *Saccharomyces cerevisiae*, 13 in *Homo sapiens*). These proteins are subunits of multimeric complexes used in electron transport or ATP synthesis. Most other proteins localised in mitochondria are encoded by the nucleus, synthesised on cytosolic ribosomes and imported into the organelle by translocases located in the outer and inner mitochondrial membranes (Figure 1) (Attardi and Schatz, 1988; Herrmann and Neupert, 2000; Neupert and Brunner, 2002).

1.1.2. The role of mitochondria in cells

Mitochondria are organelles important for both life and death of the cell. They can be regarded as “power plants” of the cell because of their production of most of the ATP

by oxidation of glucose and fatty acids (Lodish, 2004). The complete aerobic degradation of glucose to CO_2 and H_2O is coupled to the synthesis of 30 molecules of ATP. Enzymes in the mitochondrial matrix and mitochondrial cristae carry out the terminal stages of this process, creating 28 molecules of ATP. All the ATP formed by oxidation of fatty acids to CO_2 is generated in mitochondria. The synthesis of ATP from ADP and inorganic phosphate (P_i), driven by the transfer of electrons from NADH and $FADH_2$ to O_2, is called oxidative phosphorylation. This process is accomplished by five multiprotein respiratory chain complexes located on the inner mitochondrial membrane, mostly on cristae (Figure 1 and 3). The major components of the electron transport chain are four inner membrane multiprotein complexes: succinate-dehydrogenase (Complex I), NADH-dehydrogenase (complex II), coenzyme Q: cytochrome c-oxidoreductase (Complex III), and cytochrome *c* oxidase (Complex IV). The last complex transfers electrons to O_2 to form H_2O. Each complex in the chain has a greater affinity for electrons than its predecessor, and electrons pass sequentially from one complex to another until they are finally transferred to oxygen, which has the greatest affinity for electrons. During this electron transport from NADH to O_2, protons (H^+) from the mitochondrial matrix are pumped across the inner membrane and this generates a proton concentration gradient. As a consequence, the matrix becomes negative with respect to the intermembrane space resulting in an electric potential across the inner membrane (membrane potential, $\Delta\Psi_m$). The H^+ concentration gradient also generates a pH gradient across the inner mitochondrial membrane, with a higher pH in the matrix than in the cytosol. The large amount of free energy released when H^+ flow back into the matrix provides the basis for ATP synthesis.

One other important role of mitochondria is buffering of intracellular Ca^{2+} (Duchen, 2000; Rizzuto et al., 2000). Mitochondria regulate the local Ca^{2+} concentration in cellular microdomains and shape calcium signals in many cells. Under pathological conditions of cellular calcium overload, mitochondrial Ca^{2+} uptake may trigger processes that lead to cell death.

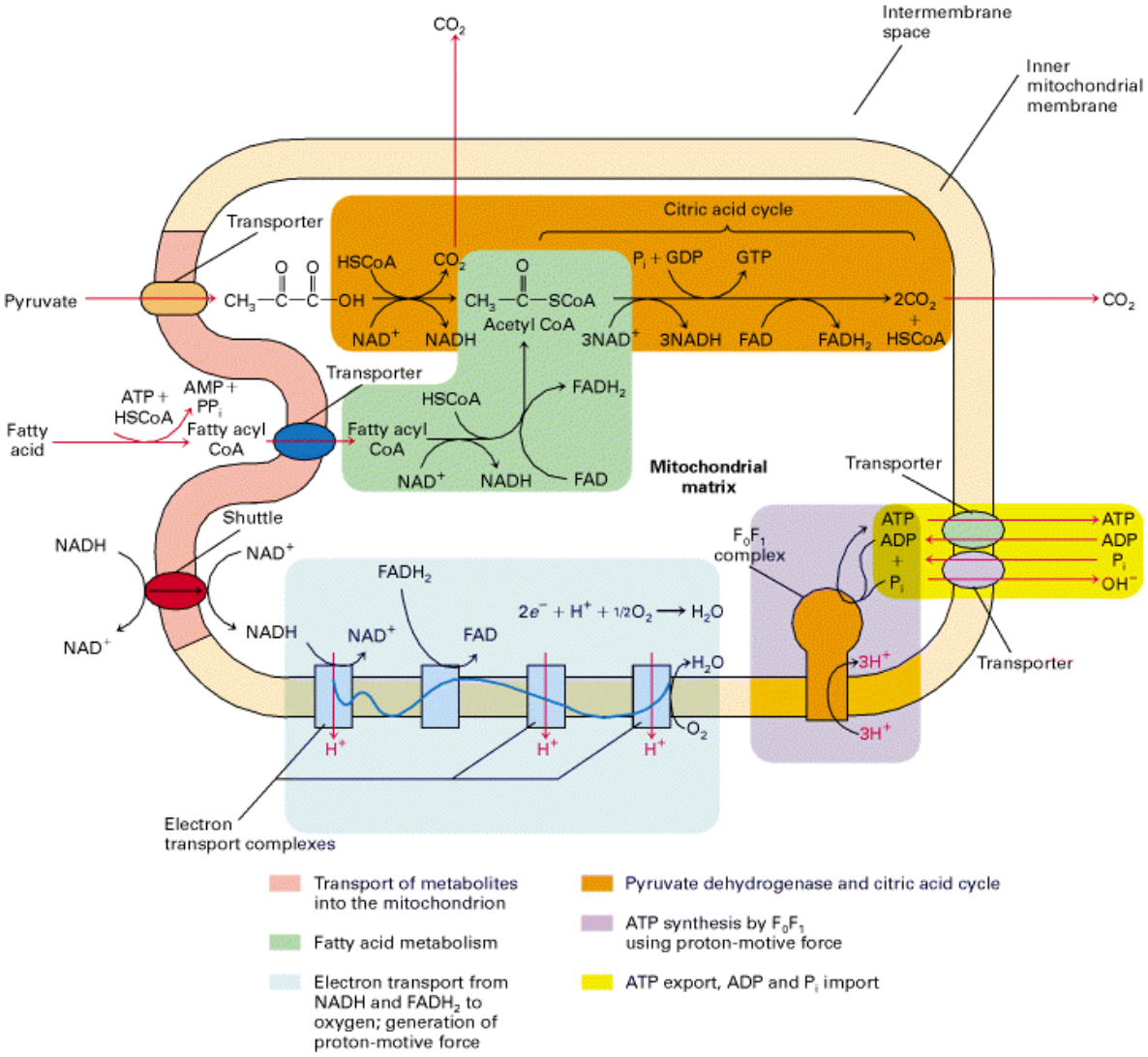

Figure 3. Summary of the aerobic oxidation in mitochondria

The outer membrane is not shown because it is freely permeable to all metabolites. Specific transport proteins (ovals) in the inner membrane import pyruvate (tan), ADP (green), and P_i (purple) into the matrix and export ATP. NADH generated in the cytosol is not transported directly to the matrix because the inner membrane is impermeable to NAD^+ and NADH; instead, a shuttle system (red oval) transports electrons from cytosolic NADH to NAD^+ in the matrix. O_2 diffuses into the matrix and CO_2 diffuses out. HSCoA denotes free coenzyme A (CoA), and SCoA denotes CoA when it is esterified. Fatty acids are linked to CoA on the outer mitochondrial membrane. Subsequently, the fatty acyl group is removed from the CoA, linked to a carnitine carrier that transports it across the inner membrane, and then the fatty acid is reattached to a CoA on the matrix side of the inner membrane (blue oval). Oxidation of pyruvate in the citric acid cycle generates NADH and $FADH_2$. Electrons from these reduced coenzymes are transferred via four electron transport complexes (blue rectangles) to O_2 concomitant with transport of H^+ ions from the matrix to the intermembrane space, generating the proton-motive force. The F_0F_1 complex (orange) then harnesses the proton-motive force to synthesize ATP. Blue arrows indicate electron flow; red arrows indicate transmembrane movement of metabolites. Figure and text taken from Lodish, (2004).

Besides the necessity of mitochondria in processes important for life of the cell, mitochondria are found to be central players in the initiation and execution of apoptosis (Figure 4) (Kroemer and Reed, 2000). Apoptosis is often preceded by mitochondrial dysfunction, like a decline in mitochondrial membrane potential ($\Delta\Psi_m$), respiratory defects, an increase in production of reactive oxygen species (ROS) and changes in ATP levels (Bossy-Wetzel et al., 1998; Danial and Korsmeyer, 2004; Green and Reed, 1998; Petit et al., 1996; Wang, 2001). During apoptosis, mitochondria release apoptogenic factors including cytochrome c, Smac/Diablo, apoptosis inducing factor (AIF) and endonuclease G (Bernardi et al., 1998; Green and Kroemer, 2004; Green and Reed, 1998; Parone et al., 2002). The release of these proteins inevitably leads to apoptosis, indicated by a wide array of morphological hallmarks, ranging from nuclear condensation to the exposure of phosphatidylserine at the surface of the dying cell. Another link between apoptosis and mitochondrial physiology is suggested by the presence of Bcl-2 family proteins in mitochondrial membranes (Krajewski et al., 1993). The Bcl-2 family of proteins can be divided into three groups of proteins: the antiapoptotic, the multidomain proapoptotic and the BH3-only proteins. Many (but not all) Bcl-2 family proteins reside in the mitochondrial outer membrane (Krajewski et al., 1993). Some of them translocate from the cytosol to the mitochondria upon induction of apoptosis (Luo et al., 1998). All in all, a great variety of key events in apoptosis rely on mitochondria.

1.1.3. Shape and dynamics of mitochondria

The name mitochondrion originates from two Greek words that describe diverse shapes of these organelles. “Mitos” means thread and describes the tubular shape of mitochondria and “chondros” means grain describing their vesicular shape. Mitochondria are traditionally described as sausage-shaped organelles floating freely in the cytoplasm. While some of them are indeed shaped like sausages, most of them have much more complex morphologies ranging from long, interconnected tubules to individual, small spheres (Bereiter-Hahn and Voth, 1994). Mitochondria can also move rapidly along microtubules or actin in a seemingly deliberate fashion (reviewed in Griparic and van der Bliek, 2001; Rube and van der Bliek, 2004). For example, mitochondria are transported along microtubules to synapses in growth cones in neurons (Hollenbeck, 1996).

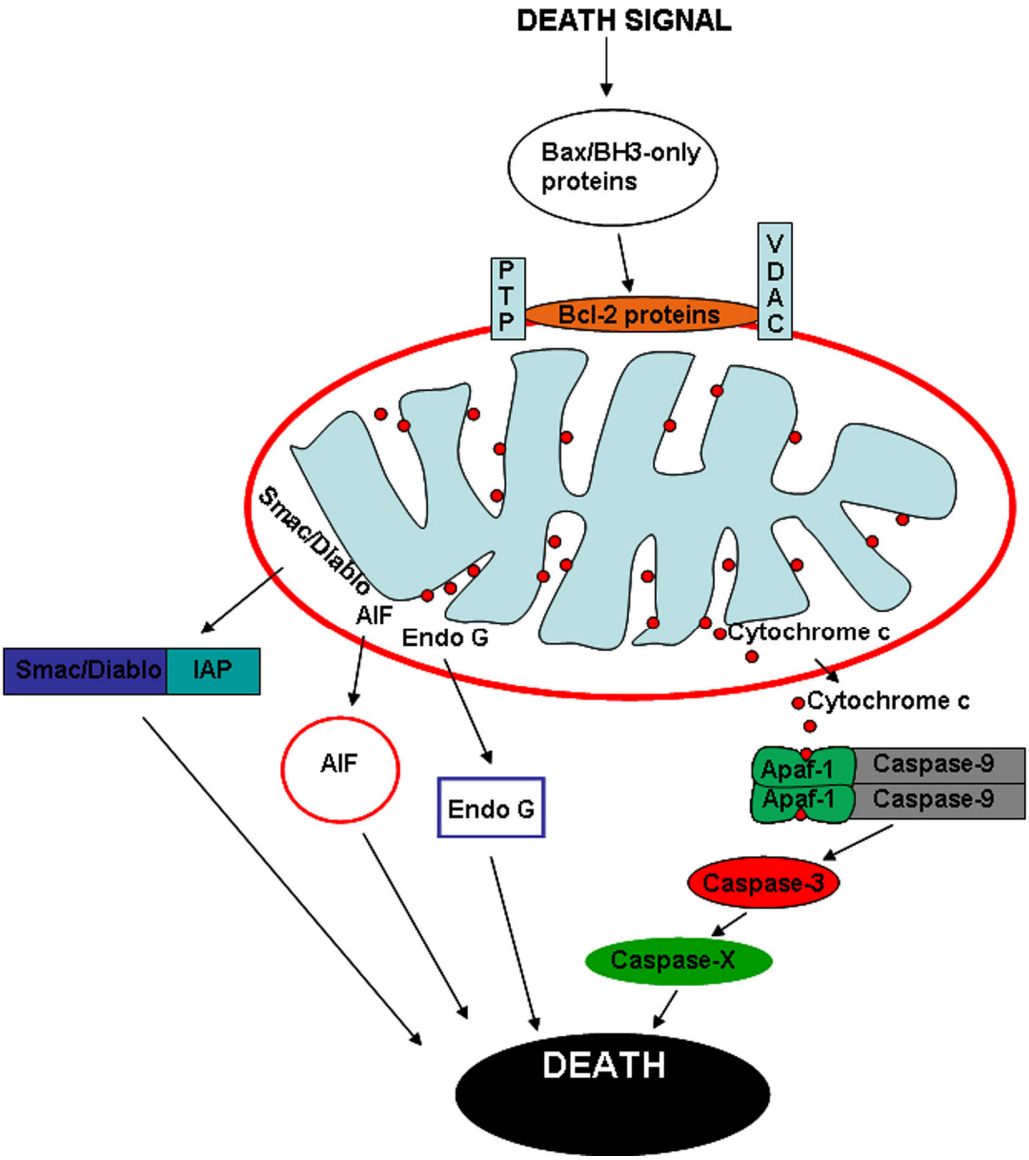

Figure 4. The role of mitochondria in cell death

Upon different death stimuli proapoptotic members of Bcl-2 protein family are activated (Bax, BH3-only). They are involved in mitochondrial outer membrane permeabilisation through permeability transition pore (PTP) or voltage-dependent anion channel (VDAC). Proapoptotic factors, like cytochrome c, apoptosis inducing factor (AIF), Endo G and many others are then released from the intramembrane space into the cytosol. Cytochrome c, for example, binds and activates apoptosis-protease activating factor (Apaf-1) and together they recruit and activate caspase-9. This finally leads to the catalytic maturation of caspase-3 and to apoptosis. On the other hand, AIF and Endo G translocate to the nucleus where they trigger DNA fragmentation and chromatin condensation in a caspase-independent fashion.

The long and short forms of mitochondria are in dynamic equilibrium, making mitochondria not static, but dynamic organelles. They accomplish this dynamics by balancing the two opposing processes: mitochondrial fusion and mitochondrial fission (for review see: Shaw and Nunnari, 2002; Rube and van der Bliek, 2004; Chan, 2006).

1.1.3.4. Mitochondrial dynamics and dynamins

Mitochondrial dynamics not only regulate the morphology of these organelles, but also their distribution and activity. Recent studies have revealed the importance of mitochondrial fusion and fission in normal cell function, mammalian development and human disease. Many proteins are described for their role in these processes. In the past several years, the understanding of mitochondrial dynamics and its molecular basis has extremely increased. Now it is broadly accepted that mitochondria are highly dynamic organelles due to their ability to fuse and divide. Some of the proteins identified so far involved in these processes belong to the superfamily of dynamins, proteins known as large GTPases (Figure 5) (for reviews see: Danino and Hinshaw, 2001; Praefcke and McMahon, 2004).

The importance of dynamin was first shown when the *Drosophila* homologue *shibire* was discovered to be a major component of endocytosis (Kosaka and Ikeda, 1983). Dynamins are distinct from other GTPases by the structure of the large GTPase domain (~300 amino acids) and the presence of two additional domains: the middle domain and the GTPase effector domain (GED). Both domains are involved in oligomerisation and regulation of the GTPase activity (Figure 5). Dynamin family members involved in mitochondrial dynamics can be divided into two groups: mitochondrial fission and fusion proteins.

1.1.3.4.1. Mitochondrial fission proteins

Saccharomyces cerevisiae Dnm1 (Dynamin 1), *Ceanorhabditis elegans* DRP-1 (Dynamin-related protein 1) and mammalian Drp1 (Dynamin-related protein 1) are homologues involved in outer mitochondrial membrane fission. They are cytoplasmic proteins that assemble on the outer mitochondrial membrane at sites of membrane constriction and fission (Bleazard et al., 1999; Labrousse et al., 1998; Otsuga et al., 1998; Sesaki and Jensen, 1999; Smirnova et al., 1998, 2001). Inhibition of Drp1 by expression

of a dominant-negative mutant or by siRNA leads to elongation of mitochondrial tubules due to fission inhibition (Lee et al., 2004; Smirnova et al., 2001).

Fis1 seems to recruit Drp1 to the mitochondrial outer membrane (MOM) (Mozdy et al., 2000), but it is not a member of the dynamin-family. It is uniformly localised to the MOM through a single C terminal transmembrane domain, while most of the protein faces the cytosol (Mozdy et al., 2000). Overexpression of Fis1 leads to mitochondrial fragmentation dependent on Drp1 (James et al., 2003; Yoon et al., 2003). Knockdown of Fis1 causes elongation of mitochondria confirming its function in mitochondrial fission (Lee et al., 2004).

1.1.3.4.2. Mitochondrial fusion proteins

Only nine years ago the first molecule necessary for mitochondrial fusion was described, namely Fzo in *Drosophila melanogaster* (Hales and Fuller, 1997). During spermatid development of *Drosophila*, mitochondria fuse into two giant organelles that wrap around each other to build a large spherical structure, termed Nebenkern, which resembles an onion slice when viewed by electron microscopy (EM). *Fzo* was found to be mutated in flies carrying defective Nebenkern, the *fuzzy onion* (*fzo*) mutants. These flies had misshapen Nebenkerns consisting of many small mitochondria wrapped around each other forming a structure that resembled ‘fuzzy onions’. Without the functional Fzo protein mitochondrial fusion was blocked during Nebenkern formation leading to male sterility. Fzo was later found to be conserved during evolution throughout the fungal and animal kingdoms. The yeast homologue, Fzo1, was also described for its function in mitochondrial fusion (Hermann et al., 1998; Rapaport et al., 1998).

The mammalian homologue of Fzo is mitofusin (Mfn), with two closely related homologues, Mfn1 and Mfn2 (Santel and Fuller, 2001; for review see: Westermann, 2003). Mice lacking either of the mitofusins are committed to die early during embryonic development (Chen et al., 2003), in the case of Mfn2, due to a placental defect. Cells lacking Mfn1 or Mfn2 show greatly reduced levels of mitochondrial fusion (Chen et al., 2003, 2005). In the absence of both Mfn1 and Mfn2, mitochondrial fusion is completely abolished, resulting in total loss of mitochondrial tubules and defective mitochondrial function (Chen et al., 2005). Mitofusins are large, dynamin-related GTPases localised to the mitochondrial outer membrane and contain two predicted transmembrane domains

(Figure 5) (Rojo et al., 2002; Santel and Fuller, 2001). Both N- and C-terminal regions protrude from the mitochondrial outer membrane into the cytosol and each contains one coiled-coil domain (in the middle domain and GED) that are thought to mediate mitochondrial outer membrane fusion. Mitofusins were shown to be required on adjacent mitochondria during the membrane fusion event (Koshiba et al., 2004), likely forming complexes involving mitofusins sitting on adjacent mitochondria. *In vitro* assays show a role of Mfn1 in mitochondrial tethering (Ishihara et al., 2004). Besides, Mfn1 was shown to be necessary for the function of another dynamin-related GTPase involved in mitochondrial fusion, namely OPA1 (optic atrophy 1) (Cipolat et al., 2004). The *OPA1* gene in the mouse is the subject of interest of this thesis, and the next chapter will summarise facts on OPA1 known until today.

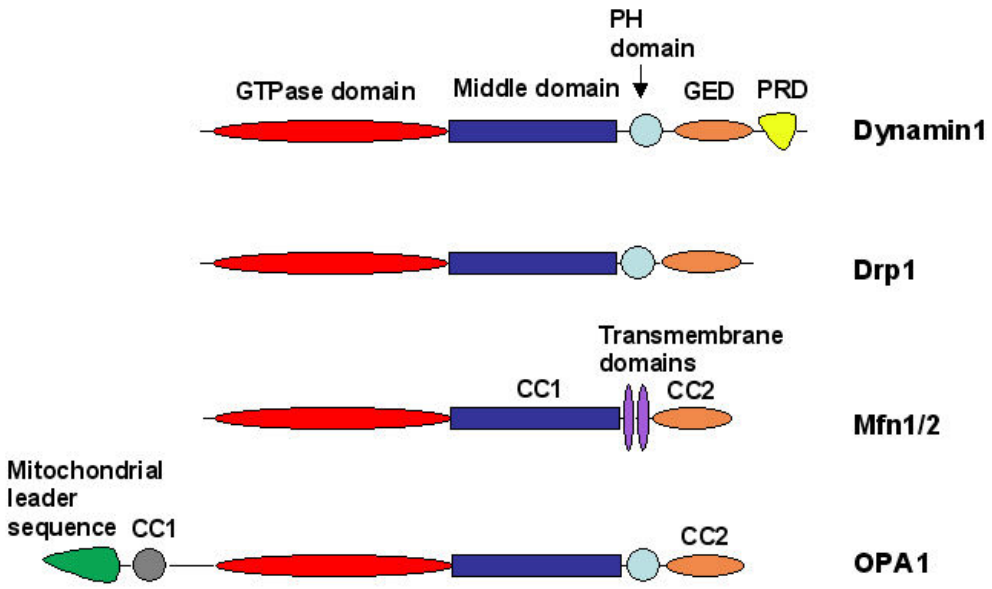

Figure 5. Scheme of dynamin and dynamin related GTPases

Dynamin 1 and dynamin family members involved in mitochondrial fission (Drp1) and fusion (Mfn1, Mfn2 and OPA1) are presented with their protein domains. They all possess large GTPase domain (red), middle domain (dark blue) and GTPase effector domain (GED; orange). Mfn1 and 2 posses long transmembrane domains that make a U-turn in the mitochondrial outer membrane. Both mitofusins and OPA1 contain coiled-coil domains (CC1 and 2), most-likely involved in protein-protein interactions. OPA1 is the only dynamin that is inserted into mitochondria through its unique mitochondrial leader sequence. PRD stands for proline rich domain and PH for plextrin homology domain.

1.2. OPA1

1.2.1. Structure, processing and localisation of OPA1

OPA1 (accession numbers: NM 012062, OMIM 605290, GenBank AB011139, SwissProt 060313) maps to the human chromosome 3q28-q29 (Alexander et al., 2000; Delettre et al., 2000). It is a large gene spanning more than 100 kb of genomic DNA and it comprises 31 exons, of which 29 are coding exons. Three exons, 4, 4b and 5b, are found to be alternatively spliced, resulting in eight transcript variants in human and mouse (Delettre et al., 2001; 2003).

Yeast homologues of OPA1 are Mgm1 (*Saccharomyces cerevisiae*) and Msp1 (*Schizosaccharomyces pombe*). Besides the "basic" dynamin domains, they possess two coiled-coil (CC) domains and an N-terminal mitochondrial import sequence (MIS) (Figure 5). The first coiled-coil domain is located downstream of the MIS and short hydrophobic stretches. The second CC domain forms the C-terminus, known as the GTPase effector domain (GED) (Figure 5) (for review see: Olichon et al., 2006; Praefcke and McMahon, 2004). Coiled-coil domains are known to be involved in protein-protein interactions and oligomerisation of proteins, but no binding partners for these domains of OPA1 have been described so far. The MIS targets the OPA1 protein to the mitochondria and is cleaved by the mitochondrial processing peptidase (MPP) upon import (Herlan et al., 2003; Misaka et al., 2002; Olichon et al., 2002; Pelloquin et al., 1999; Satoh et al., 2003). OPA1 in mammals and Mgm1 in yeast are located within the mitochondrial intermembrane space (Griparic et al., 2004; Herlan et al., 2003; Olichon et al., 2002; Satoh et al., 2003; Wong et al., 2000). Different studies have described Mgm1 to be associated with the inner or outer membrane, or to be integrated into those membranes (Herlan et al., 2003; Sesaki et al., 2003; Shepard and Yaffe, 1999; Wong et al., 2000). OPA1 was cosedimented with both mitochondrial membranes but it was shown to interact more tightly with the inner mitochondrial membrane (IMM) (Griparic et al., 2004; Olichon et al., 2002; Satoh et al., 2003). These studies in yeast and human cells might be controversial, but knowing that OPA1 has several different isoforms and processed forms could be an explanation (Delettre et al., 2001).

Protein isoforms are in the case of Mgm1 formed by posttranslational processing of the protein. After the cleavage of the MIS of Mgm1 by MPP, the protein exits the inner membrane translocation machinery as the long form, l-Mgm1p. If this exit is delayed, the hydrophobic region of Mgm1p is pulled into the inner membrane, where it is further processed by the mitochondrial rhomboid protease (Rbd1p/Pcp1p) into a short form, s-Mgm1p (Herlan et al., 2003; McQuibban et al., 2003; Sesaki et al., 2003). It was shown that the ratio of long to short Mgm1p isoforms affects mitochondrial dynamics and neither isoform by itself is sufficient for normal mitochondrial morphology. Only recently it was shown that the mammalian orthologue of Rbd1p/Pcp1p, PARL, is involved in OPA1 processing (Cipolat et al., 2006). PARL was shown to support the production of a short, soluble, intermembrane space (IMS) isoform of OPA1. Cells lacking PARL contained predominantly the long, inner membrane forms of OPA1, though traces of the IMS isoform were present. This suggests the existence of additional proteases that contribute to the cleavage of OPA1, what remains to be confirmed experimentally.

1.2.3. Function of OPA1

In the last couple of years, many efforts were focused on the elucidation of the exact role of OPA1 in the cell. It has been shown that loss of *Mgm1* and *Msp1* by gene deletion, or *OPA1* by RNAi experiments leads to fragmentation of the mitochondrial tubules (Chen et al., 2005; Griparic et al., 2004; Guillou et al., 2005; Olichon et al., 2003; Wong et al., 2000). Fragmentation occurred due to decrease in mitochondrial fusion (Chen et al., 2005; Cipolat et al., 2004; Lee et al., 2004; Sesaki et al., 2003; Wong et al., 2003). Thus, OPA1, Mgm1 and Msp1 are believed to promote fusion of mitochondrial membranes. Another feature noticed in cells lacking OPA1/Mgm1 is altered cristae structure (Griparic et al., 2004; Olichon et al., 2003; Sesaki et al., 2003). Cristae were disorganised with irregular shapes and volumes and with enlarged cristae junctions. In Mgm1-deleted yeast, *Dnm1* inactivation rescued the disorganised cristae (Sesaki et al., 2003). This indicates that the role of Mgm1 in cristae organisation might be an indirect effect of its function in mitochondrial fusion. However, it was recently shown that OPA1 overexpression is able to rescue altered cristae structure in Mfn1 knockout cells, but not the lack of fusion (Frezza et al., 2006). In addition, OPA1-RNAi cells were also shown to be respiration deficient (Chen et al., 2005), but this is most likely a secondary effect of the

loss of fusion and cristae disorganisation. Further, downregulation of OPA1 in cells led to an increase in spontaneous apoptosis (Arnoult et al., 2005) and also to an extreme sensitivity to exogenous proapoptotic stimuli (Lee et al., 2004; Olichon et al., 2003). Thus, OPA1 was also implicated in having an antiapoptotic role in the cell.

1.3. Mutations in OPA1 cause autosomal dominant optic atrophy

The *OPA1* gene was first found by mapping of the locus common to families with autosomal dominant optic atrophy (adOA) (Alexander et al., 2000; Delettre et al., 2000). AdOA (MIM #165500) is the most common form of inherited optic neuropathy, with a frequency of 1:12000 (Kivlin et al., 1983; Kjer et al., 1996) to 1:50000 (Lyle, 1990). The disease is highly variable in expression and shows incomplete penetrance in some families (Hoyt, 1980; Johnston et al., 1999; Votruba et al., 1998). Clinical symptoms are progressive loss of visual acuity, colour vision defects, central visual field defects and temporal optic disc pallor with an onset within the first two decades of life (Hoyt, 1980; Votruba et al., 1998). The fundamental pathology was suggested to be a primary degeneration of retinal ganglion cells followed by an ascending atrophy of the optic nerve (Johnston et al., 1979; Kjer et al., 1983). It was reported that the mtDNA content was decreased in leukocytes of adOA patients (Kim et al., 2005). So far, no ultrastructural studies of mitochondria in retinal ganglion cells from patient samples have been reported. As expected, OPA1 is highly expressed in the retina, but also in other parts of organism (Alexander et al., 2000; Delettre et al., 2000). It still remains unclear whether OPA1 is exclusively located in retinal ganglion cells (RGC) and the optic nerve, or it is broadly expressed in the entire retina, since several different groups reported inconsistent results (Aijaz et al., 2004; Ju et al., 2005; Kamei et al., 2005; Pesch et al., 2004).

More than 100 mutations have been identified to date to cause adOA (Figure 6) (Ferre et al., 2005; URL: http://lbbma.univ-angers.fr/eOPA1/). The majority of these mutations cause premature truncations of OPA1 (50%), possibly leading to loss of function of OPA1 (Delettre et al., 2001). Besides, two deletions of one complete allele have been reported; one is a deletion of the entire gene, and the other of the entire open reading frame (ORF) (Marchbank et al., 2002; Pesch et al., 2001). These cases and the autosomal dominant nature of adOA speak in favour of haploinsufficiency of OPA1 as the major mechanism of the disease.

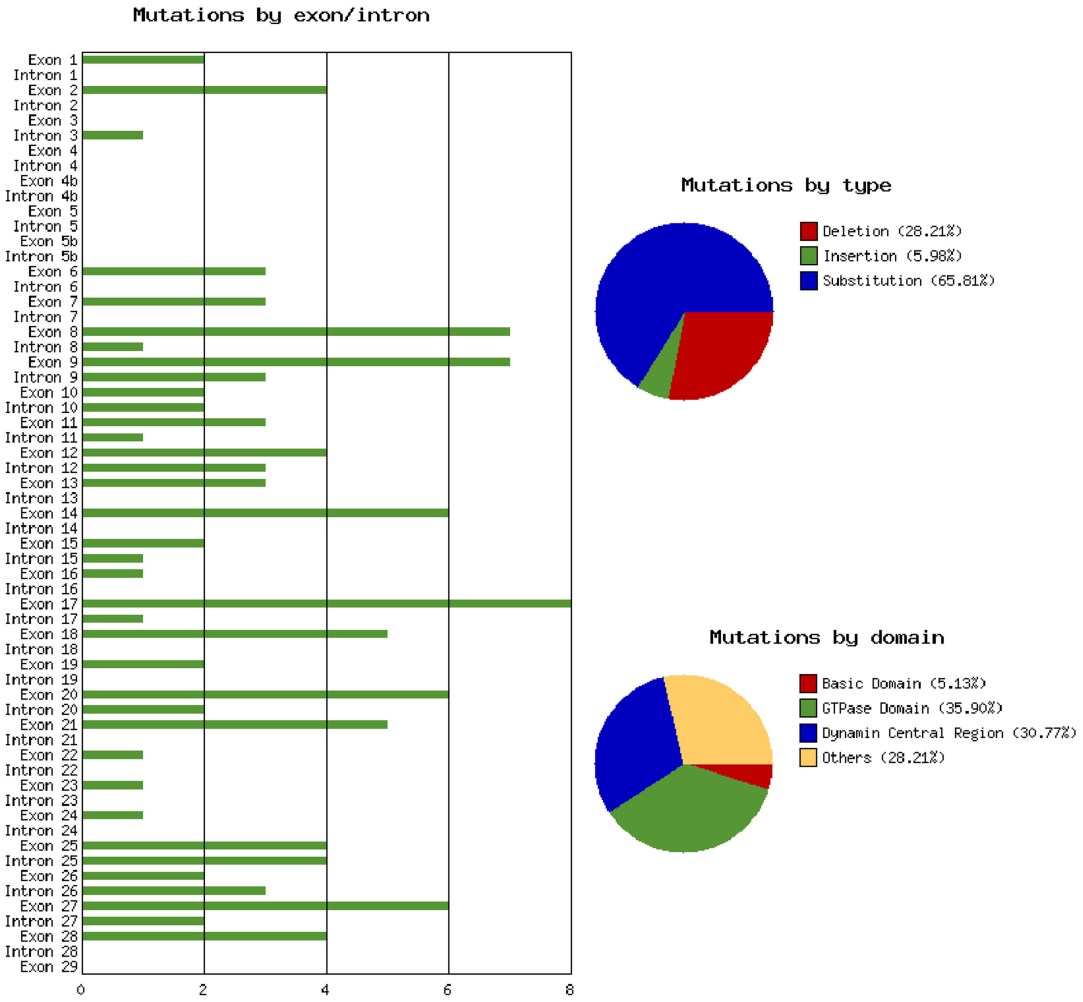

Figure 6. Mutations in the *OPA1* gene

Mutations are shown by introns/exons, different domains of OPA1 and by different type. Taken from Ferre et al., (2005), URL: **http://lbbma.univ-angers.fr/eOPA1/**.

1.4. Critical dependence of neurons on functional mitochondria

AdOA is far from being the only neuronal disease with mutations in mitochondrial genes (Table 1), indicating a crucial function of mitochondria in neurons.

Table 1. Mitochondrial proteins mutated in neurodegenerative diseases

Disease	Gene	Function
Dominant Optic Atrophy	OPA1	Mitochondrial fusion and cristae structure?
Recessive Optic Atrophy	OPA3	Unknown
Leber's Hereditary Optic Neuropathy	ND genes in mtDNA	Oxidative phosphorylation
Charcot-Marie-Toothe Type 2A	Mfn2	Mitochondrial fusion
Charcot-Marie-Toothe Type 4A	GDAP1	Mitochondrial dynamics/Fission?
Hereditary Spastic Paraplegia	Paraplegin	Mitochondrial ATPase/Protease
Hereditary Spastic Paraplegia	HSP60	Mitochondrial heat shock protein
Familial Parkinson's	PINK1	Mitochondrial serine/threonine kinase
Familial Amyotrophic Lateral Sclerosis	SOD1	Superoxide dismutase
Friedrich's Ataxia	Frataxin	Formation of Fe-S clusters in mitochondria

For example, there are parallels that link adOA and Leber's Hereditary Optic Neuropathy (LHON), another mitochondrial disease with an early loss of vision affecting RGCs (for a review see: Carelli et al., 2004a). LHON, caused by mutations in mtDNA (Carelli et al., 2002b), leads to changes in the function of the respiration complex I (Brown, 1999). Another interesting parallel to adOA is found in Charcot-Marie-Tooth (CMT) disease type 2A. This disease is found to be caused by mutations in the *Mfn2* gene (Lawson et al., 2005; Zuchner et al., 2004), discussed above as necessary for mitochondrial fusion. CMT is a hereditary peripheral neuropathy that affects both motor and sensory neurons (Shy, 2004; Young and Suter, 2003). CMT type 2A is the autosomal dominant, axonal form of CMT, in which mutations are found throughout the gene (Kijima et al., 2005; Lawson et al., 2005; Zuchner et al., 2004), but most of them cluster to the GTPase region of Mfn2.

From the results presented above and from table 1, we can see that mutations in genes that encode mitochondrial proteins lead to various neurodegenereative diseases. This shows the extreme importance of functional mitochondria for the neurons.

1. 5. The aim and purpose of this project

In the last five years many data were collected from *in vitro* experiments exploring OPA1, but the function of this protein is not yet clear. These studies suggested that OPA1, as mitochondrial protein, has a role in mitochondrial inner membrane fusion, cristae maintenance and apoptosis. In order to verify these results an *in vivo* model to study OPA1 was necessary.

An effective targeting strategy for the successful generation of an OPA1-deficient mouse can be developed only if the regulation of expression of the target-gene is known. The OPA1 gene in the mouse consists of many exons and undergoes alternative splicing. In order to identify the most promising site of gene disruption, expression studies on the mouse OPA1 transcript are necessary.

In adOA, *OPA1* is found to be mutated in one allele only. As described in Chapter 1.3. , the majority of these mutations lead to either loss of one allele or to the loss of function of one allele. This is the reason why, in most of the cases, haploinsufficiency is thought to be the cause of the disease. Heterozygous OPA1-defficient mice should genetically mimic the disease observed in humans and represent a good model to study the role of OPA1 in adOA.

Second, OPA1-deficient mice should allow to study the role of this protein in mammalian development. Until today, it was not known whether OPA1 is important only for the development and/or maintenance of the visual system or it also has an influence on the development of the whole organism.

Finally, OPA1-deficient mice would be the first model with complete abolishment of OPA1 expression. Experiments exploring the loss of function of OPA1 were so far using siRNA technology. This method does not eliminate all endogenous OPA1 transcripts. In contrast, cells derived from animals carrying null alleles of OPA1 represent a perfect tool for many further studies concerning OPA1.