

## 10. APPENDIX

### 10.1. List of figures

Figure 1. Scheme of a mitochondrion .....	1
Figure 2. Model of a mitochondrion .....	3
Figure 3. Summary of the aerobic oxidation in mitochondria .....	5
Figure 4. The role of mitochondria in cell death .....	7
Figure 5. Scheme of dynamin and dynamin related GTPases .....	10
Figure 6. Mutations in the <i>OPA1</i> gene .....	14
Figure 7. Northern blot analysis of human anatomical brain areas .....	40
Figure 8. Tissue specific differential polyadenylation in human tissues .....	42
Figure 9. Tissue specific differential polyadenylation in mouse tissues .....	42
Figure 10. Verification of the mOPA1 3'untranslated region by RT-PCR .....	44
Figure 11. Northern blot analysis of human brain samples with probes of the 3'untranslated region of <i>hOPA1</i> .....	46
Figure 12. Construction of knockout mice and confirmation of the targeting event .....	50
Figure 13. Analysis of <i>OPA1</i> <sup>+/-</sup> versus <i>OPA1</i> <sup>+/+</sup> mice .....	51
Figure 14. Histological analysis of <i>OPA1</i> <sup>+/-</sup> versus <i>OPA1</i> <sup>+/+</sup> mice .....	53
Figure 15. Analysis of <i>OPA1</i> deficient embryos .....	54
Figure 16. Morphological alterations in mitochondrial networks of mouse embryos .....	55
Figure 17. Decreased mtDNA levels in <i>OPA1</i> -deficient embryos .....	56
Figure 18. Analysis of apoptosis in <i>OPA1</i> -deficient embryos .....	58
Figure 19. Mitochondrial fragmentation <i>OPA1</i> -null mouse embryonic fibroblasts (MEFs) .....	59
Figure 20. Electron micrographs of mitochondria of mouse embryonic fibroblasts .....	60
Figure 21. <i>OPA1</i> -deficient mouse embryonic fibroblasts show reduced respiration .....	62
Figure 22. <i>OPA1</i> -deficient mouse embryonic fibroblasts show reduced membrane potential .....	63
Figure 23. <i>OPA1</i> -deficient cells display reduced sensitivity to staurosporine-induced apoptosis .....	65
Figure 24. Analysis of rescued <i>OPA1</i> -null mouse embryonic fibroblasts .....	67
Figure 25. Analysis of visual system of <i>OPA1</i> <sup>+/-</sup> versus <i>OPA1</i> <sup>+/+</sup> mice .....	71
Figure 26. Analysis of acoustic system of <i>OPA1</i> <sup>+/-</sup> versus <i>OPA1</i> <sup>+/+</sup> mice .....	72

Figure 27. Mitochondrial fragmentation and lack of fusion in OPA1-null fibroblasts.....	78
Figure 28. Hypothesis on function of OPA1 deduced form MEF-studies .....	86

## 10.2. List of tables

Table 1. Mitochondrial proteins mutated in neurodegenerative diseases.....	15
Table 2. Human OPA1 polyadenylation sites and their position after the UAA stop codon .....	43
Table 3. Mouse OPA1 polyadenylation sites and their position after UAG stop codon ....	44
Table 4. Intron-Exon structure of the mouse <i>OPA1</i> gene with their lengths in base pairs and intron/exon and exon/intron borders .....	47
Table 5. Genotyping of newborn pups and different staged embryos on hybrid C57BL/6/129/Ola background .....	51
Table 6. Percentage of OPA1 wild-type, heterozygous and homozygous knockout mouse embryonic fibroblasts that have undrgone apoptosis after 4 and 24 hours of different treatments .....	66

## 10.3. Abbreviations

ABR	auditory brainstem responses
AdOA	autosomal dominant optic atrophy
AIF	apoptosis inducing factor
ANT	adenine nucleotide translocator
AT	annealing temperature
ATP	adenosinetriphosphate
bp	base pairs
BSA	bovine serum albumin
CC domain	coiled-coil domain
cDNA	complementary DNA
CMT	Charcot-Marie-Tooth disease
COX	cytochrome c oxidase
CPSF	cleavage/polyadenylation specificity factor
CstF	cleavage stimulation factor
DAB	3,3' diaminobenzidine

---

DEPC	diethylpyrocarbonate
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
Dnm1	Dynamin 1
dNTP	deoxyribonucleotide triphosphate
dpc	days post-coitum
DPOAE	distortion product otoacoustic emissions
DRP-1/Drp1	Dynamin-related protein 1
dsDNA	double-stranded DNA
ECL	enhanced chemiluminescence
EDTA	ethylene-diaminetetraacetic acid
EGTA	ethylene glycol tetraacetic acid
EM	electron microscopy
ER	endoplasmatic reticulum
ERG	electroretinogram
ES cells	embryonic stem cells
EST	expressed short tags
ET	elongation time
et al.	et altera (and others)
FACS	fluorescence activated cell sorting
FCS	fetal calf serum
<i>Fzo</i>	<i>fuzzy onion</i>
G418	geneticin
GED	GTPase effector domain
GTP	guanosinesinetriphosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
<i>hOPA1</i>	human <i>OPA1</i>
HRP	horsereddish peroxidase
IMM	inner mitochondrial membrane
IMS	intermembrane space
JC-1	5,5',6,6'-tetrachloro-1,1',3,3' tetraethylbenzimidazolocarboyanine iodide
kb	kilo base pairs
LB	Luria broth
LHON	Leber's Hereditary Optic Neuropathy

---

LIF	leukaemia inhibitory factor
MEFs	mouse embryonic fibroblasts
Mfn	mitofusin
MIS	mitochondrial import sequence
MOM	mitochondrial outer membrane
<i>mOPA</i>	mouse OPA1
MOPS	3-(N-morpholino)propanesulfonic acid
MPP	mitochondrial processing peptidase
mRNA	messenger RNA
mtDNA	mitochondrial DNA
NADH	nicotinamide adenine dinucleotide (reduced form)
NBT	4-nitrobluetetrazoliumchlorid
<i>neoR</i>	<i>neomycin</i> resistance cassette
OPA1	optic atrophy1
ORF	open reading frame
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PFA	paraformaldehyde
PI	propidium iodide
PTP	permeability transition pore
RGC	retinal ganglion cells
RNA	ribonucleic acid
RNase	ribonuclease
ROS	reactive oxygen species
rpm	rotations per minute
RT	reverse transcriptase
SDH	succinate dehydrogenase
SDS	sodium dodecyl sulphate
ssDNA	single-stranded DNA
SSC	saline sodium citrate
SU	subunit
TAE	Tris/Acetate/EDTA
TBS	Tris-Buffered Saline
TdT	Terminal deoxynucleotidyl transferase

---

TNF	tumor necrosis factor
Tris	Tris-(hydroxymethyl) aminoethane
TUNEL	TdT-mediated dUTP nick end labelling
U	unit (enzymatic activity)
UTR	untranslated region
UV	ultraviolet (light)
VDAC	voltage-dependent anion-selective channels
VEP	visually evoked potentials
$\Delta\Psi_m$	mitochondrial membrane potential