

Max-Delbrück-Center for Molecular Medicine, Berlin

Research Group Neurodegeneration

Supervisor: Dr. Christiane Alexander

**Generation and analysis
of *OPA1 (optic atrophy 1)*-deficient mice**

Dissertation

zur Erlangung des akademischen Grades

Doctor rerum naturalium (Dr. rer. nat.)

vorgelegt beim Fachbereich Biologie/Chemie/Pharmazie
der Freien Universität Berlin

von

Maja Fiket

aus Belgrad

Februar 2007

1. Gutachter: Prof. Dr. Fritz G. Rathjen, Max-Delbrück-Center for Molecular
Medicine, Freie Universität, Berlin

2. Gutachter: Prof. Dr. Markus Schülke, Charité University Medical Center, Berlin

Tag der Disputation: 13.07.2007

ACKNOWLEDGEMENTS

First of all, I would like to thank to my mother, who made my education possible. I want to thank her for her support and patience.

Then, I would like to thank to my supervisor, Dr. Christiane Alexander, in whose group I was “working out” the way to become a “Doctor of philosophy”. She let me work on this interesting project, and read my thesis very carefully. She thought me a lot about mitochondria and was always very critical, so that I could move forward.

I would also like to thank to my lab colleagues, Anita and Vasu for standing me and being good friends, and Jana, Christin and Rene for a fantastic technical and moral support.

Of course, I am very grateful to the people who helped me scientifically during all these years, especially, in order of appearance: Dr. Alistair Garratt, for a great help with ES cells; Dr. Glen Jeffrey for teaching me what a retina is; Dr. Bettina Erdmann for EM pictures of embryos and optic nerves, Prof. Dr. David C. Chan and Dr. Hsiuchen Chen for establishing MEFs, Dr. Nicola Strenzke for analysis of the mouse visual and hearing system, PD Dr. Mathias W. Seeliger for ERG and SLO analysis, Dr. Ansgar Santel for help with confocal microscopy, Dr. Ulrike Ziebold for useful tips in mouse embryology, Prof. Dr. Markus Schülke for helpful tips concerning mitochondrial respiration and Prof. Dr. Fritz G. Rathjen for being my “Dr. Vater”.

I am very happy to have two wonderful sisters and many very good friends who were always there for me.

Lastly, I would like to thank to Boris, because he was and is the best support I could possibly have. And he also took a lot of time to very critically read this thesis.

Hvala!

CONTENTS

1. INTRODUCTION	1
1.1. Mitochondrion	1
1.1.1. The structure of mitochondria	1
1.1.2. The role of mitochondria in cells	3
1.1.3. Shape and dynamics of mitochondria	6
1.1.3.4. Mitochondrial dynamics and dynamins	8
1.1.3.4.1. <i>Mitochondrial fission proteins</i>	8
1.1.3.4.2. <i>Mitochondrial fusion proteins</i>	9
1.2. OPA1	11
1.2.1. Structure, processing and localisation of OPA1	11
1.2.3. Function of OPA1	12
1.3. Mutations in OPA1 cause autosomal dominant optic atrophy	13
1.4. Critical dependence of neurons on functional mitochondria	14
1.5. The aim and purpose of this project	16
2. MATERIALS AND METHODS	17
2.1. Materials	17
2.1.1. Chemicals and enzymes	17
2.1.2. Bacterial strains	17
2.1.3. Vectors/plasmids	17
2.1.4. Cell lines	17
2.1.5. Mouse strains	18
2.1.6. Bacterial and cell culture media	18
2.1.7. Antibodies	19
2.2. Methods	19
2.2.1. Bioinformatics	20
2.2.2. Molecular Biology	20
2.2.2.1. Plasmid DNA isolation	20
2.2.2.2. Isolation of genomic DNA from embryonic stem (ES) cells	20
2.2.2.3. Isolation of genomic DNA from mouse tissue	21
2.2.2.4. Isolation of genomic DNA from the embryos	21
2.2.2.5. Isolation of total RNA from mouse tissues	21
2.2.2.6. Measuring of Nucleic Acid Concentration by UV-Spectrophotometry	22

2.2.2.7. PCR	22
2.2.2.8. RT-PCR reaction	27
2.2.2.9. Real-time PCR	28
2.2.2.10. Southern blotting	28
2.2.2.11. Northern blotting	28
2.2.2.12. Hybridisation with radioactively labelled DNA probes to Southern and Northern blots	29
2.2.2.13. Cloning	29
2.2.2.13.1. <i>Restriction digestion</i>	29
2.2.2.13.2. <i>Ligation</i>	30
2.2.2.13.3. <i>Transformation of chemi-competent bacteria</i>	30
2.2.2.14. Preparation of total protein extracts from cells and tissues	30
2.2.2.15. SDS-PAGE	31
2.2.2.16. Western blot	31
2.2.3. Cell culture	32
2.2.3.1. Embryonic Feeder cells	32
2.2.3.2. Growth arrest of Feeder cells by Mitomycin C	32
2.2.3.3. Cell culture of ES cells	33
2.2.3.4. Electroporation of ES cells and selection for G418-resistant clones	33
2.2.3.5. Manipulation of blastocyst and transfer into pseudopregnant mice	34
2.2.3.6. Mouse embryonic fibroblasts (MEFs)	34
2.2.3.7. Apoptosis assay	34
2.2.3.8. Membrane potential measurement	35
2.2.4. Histology and cell staining	35
2.2.4.1. Histological analysis of mouse retinae, optic nerves and embryos	36
2.2.4.2. Electron microscopy (EM) of mouse tissues and cells	36
2.2.4.3. Whole-mount <i>in situ</i> TUNEL assay in embryos	37
2.2.4.4. Staining of mitochondria	37
2.2.4.5. Immunocytochemistry	37
2.2.4.6. Staining for activity of respiratory chain complexes	38

3. RESULTS **39**

3.1. Human and mouse OPA1 expression	39
3.1.1. Human and mouse tissues contain <i>OPA1</i> transcripts of several lengths	39
3.1.2. Steady-state levels of different <i>OPA1</i> transcripts vary in different tissues in both organisms	39
3.1.3. Analysis of polyadenylation signals in the human and mouse <i>OPA1</i> 3' untranslated region	41

3.2. Generation of <i>OPA1</i> -deficient mice	45
3.2.1. Intron-Exon structure of the mouse <i>OPA1</i> gene	45
3.2.2. Generation of the targeting vector	48
3.2.3. Generation of mice lacking one <i>OPA1</i> allele	48
3.2.4. Confirmation of the knockout event	49
3.3. Phenotypic analysis of heterozygous <i>OPA1</i> knockout mice	49
3.3.1. <i>OPA1</i> ^{+/-} mice contain reduced levels of mitochondrial DNA	51
3.3.2. Histological examination of the retina and the optic nerve	52
3.4. Phenotypic analysis of homozygous <i>OPA1</i> knockout mice	52
3.4.1. Loss of <i>OPA1</i> causes early embryonic lethality	54
3.4.2. <i>OPA1</i> ^{-/-} embryos have fragmented mitochondria	54
3.4.3. <i>OPA1</i> ^{-/-} embryos contain reduced level of mitochondrial DNA	56
3.4.4. <i>OPA1</i> ^{-/-} embryos show massive cell death at embryonic day 7.5	56
3.4.5. Fragmented mitochondria in <i>OPA1</i> ^{-/-} cells	57
3.4.6. Ultrastructural changes of mitochondria in <i>OPA1</i> ^{-/-} cells	59
3.4.7. <i>OPA1</i> ^{-/-} cells are respiration deficient	60
3.4.8. Cells deprived of <i>OPA1</i> are less sensitive to staurosporine-induced apoptosis	63
3.4.9. <i>OPA1</i> isoform 1 rescued the severe mitochondrial phenotype seen in <i>OPA1</i> knockout cells	66
4. DISCUSSION	68

4.1. Human and mouse <i>OPA1</i> transcripts differ mainly in their 3'untranslated region	68
4.2. Reduced levels of <i>OPA1</i> protein do not lead to retinal ganglion cell death in mice	70
4.3. Speculations on how <i>OPA1</i> mutations may lead to adOA	73
4.4. Why do <i>OPA1</i> heterozygous knockout mice not show the same phenotype as human carriers of <i>OPA1</i> mutations?	74
4.5. Loss of <i>OPA1</i> leads to early mammalian embryonic death	75
4.6. Decrease of mtDNA levels in <i>OPA1</i> ^{-/-} embryos	76
4.7. Cells devoid of <i>OPA1</i> have lost the ability to fuse their mitochondria	76
4.8. Connection between fragmented mitochondrial phenotype of <i>OPA1</i> knockout embryos and their apoptosis	79

4.9. OPA1 is required for cristae maintenance, oxidative phosphorylation and mitochondrial membrane potential	82
4.10. OPA1 is needed for the activation of apoptosis via mitochondria	83
4.11. OPA1 isoform 1 was able to rescue respiration and fusion but not apoptosis phenotype in OPA1 ^{-/-} cells	85
4.12. Importance and future prospects	87
5. SUMMARY	88
6. ZUSAMMENFASSUNG	90
7. BIBLIOGRAPHY	92
8. LIST OF PUBLICATIONS	111
9. CURRICULUM VITAE	112
10. APPENDIX	115
10.1. List of figures	115
10.2. List of tables	116
10.3. Abbreviations	116