Sunil C. Kaul
Renu Wadhwa *Editors*

Mortalin Biology: Life, Stress and Death

Springer

Mortalin Biology: Life, Stress and Death

Sunil C. Kaul • Renu Wadhwa
Editors

Mortalin Biology: Life, Stress and Death

Springer

Editors
Sunil C. Kaul
National Institute of Advanced Industrial Science and Technology
Tsukuba, Ibaraki
Japan

Renu Wadhwa
National Institute of Advanced Industrial Science and Technology (AIST)
Tsukuba, Ibaraki
Japan

ISBN 978-94-007-3026-7 e-ISBN 978-94-007-3027-4
DOI 10.1007/978-94-007-3027-4
Springer Dordrecht Heidelberg London New York

Library of Congress Control Number: 2012933850

Foreword

The heat shock response was originally discovered over 40 years ago in the fruit fly and heat shock proteins were subsequently identified in all organisms. It was found that these protect organisms against exposure to suboptimal temperatures and numerous other stresses many of which are also inducers of the response. Research on the mechanisms of action of these stress proteins was aided by development of molecular technologies, identification and cloning of new genes encoding stress proteins in a variety of assay systems both at the cellular and organism levels and it became clear that these proteins have essential constitutive functions in normal unstressed conditions. While *in vivo* animal systems are necessary to make certain firm conclusions, culture cell systems still offer an excellent platform to dissect the molecular mechanisms of action of a protein and to uncover the signal transduction pathways involved in the response. In my view, mortalin is an excellent example of this. Besides its cloning in the normal and cancer cell hybrid screening assay, many of its characteristics such as multiple subcellular residences, impact on p53 protein activity and carcinogenesis, and involvement in neuro-degenerative pathologies have been found since its discovery. The present book offers a single volume reading on the discovery of mortalin biology by experts from different fields and different parts of the globe. It is a unique volume compiling structural, evolutionary and functional aspects of a single stress protein in a variety of model systems ranging from invertebrates to human cells in culture and clinical samples. Besides making an easily understandable reading, it will be very helpful in asking further questions and designing experiments to advance mortalin-based diagnostics and therapeutics.

IBIS, Pavillon C.E. Marchand
1030 Ave de la médecine
Université Laval
Québec, Qc, Canada G1V 0A6
Phone: 418 656–3339
Fax 418 656–7176
E-mail: robert.tanguay@ibis.ulaval.ca

Robert M. Tanguay, D.Sc.
Professor and Associate Head
Dept. Molecular Biology
Medical Biochemistry & Pathology
Lab Cell & Developmental Genetics

Preface

Since the discovery of heat shock response by Ferruccio Ritossa in 1962, the phenomenon has been well characterized in a variety of cells and organisms as induction of a family of proteins called "heat shock proteins" (HSP). Based on their molecular weight, these proteins are classified into, at least, 6 major subfamilies named as HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs. The fact that the heat shock protein synthesis can be triggered by a variety of other stress conditions such as, infection, inflammation, exercise, starvation, oxygen-, nitrogen- or water-deprivation and exposure to chemical and physical toxins, they are also classified as "stress proteins". Then came the surprise that the HSP also exist under non-stressful conditions and perform housekeeping functions, such as folding and assisting in the establishment of correct protein conformation, mediating protein-protein interactions, intra-cellular trafficking of other proteins, preventing unwanted protein aggregation and channelizing their degradation. A new term "chaperones" evolved to express such functionality of this highly conserved class of proteins.

A new member of HSP70 family of proteins was first cloned in 1993 in a cell hybrid protein-screening assay. Since it was identified to be associated with cellular mortal phenotype, it was named 'mortalin'. Endorsing its multiple functionality, mortalin made its manifestation in many independent experimental regimes, such as those aimed to identify molecules involved in antigen processing, stress-survival and mitochondrial functions. With nearly two decades of experimentation, mortalin has been recognized as an essential protein that not only acts as a chaperone and stress-survival factor but also plays a key role in mitochondrial import motor function, energy generation, ROS management, immune response, control of centrosome duplication and activities of tumor suppressor protein p53. Stemming from these multiple functions is its role in human cancers on one-hand and neurodegenerative diseases on the other. With an aim to introduce mortalin at the graduate and advanced undergraduate levels, this book is organized as a chapter-wise description of structure, evolution and functional role of mortalin in normal and diseased physiology. We hope that this sketch of mortalin biology by the team of experts will help in asking new questions, advancing knowledge and developing mortalin-based diagnostic and therapeutic reagents and technologies.

We are very grateful to all the authors for their interest, enthusiasm and devotion to mortalin research that made this book necessary and possible. Without their hard work to contribute chapters, it was not possible to accomplish this volume suitable for general and specialized reading.

Sunil C. Kaul
Renu Wadhwa

Contents

Contributors

Abdussalam Azem Department of Biochemistry and Molecular Biology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Walter A. Baseler West Virginia University School of Medicine, Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences, 1 Medical Center Drive Morgantown, WV 26506, USA

Renata Batistoni Dipartimento di Biologia, Unità di Biologia Cellulare e dello Sviluppo, Università di Pisa. S.S.12 Abetone e Brennero 4, 56127 Pisa, Italy

S. Anne Böttger Department of Biology, West Chester University, West Chester, Pennsylvania 19383, USA

Lena F. Burbulla Laboratory of Functional Neurogenomics, Center of Neurology, Hertie-Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany

Francesco Cappello IEMEST, Istituto Euro-Mediterraneo di Scienza e Tecnologia, Palermo, Italy

Travis J. Cook Department of Environmental & Occupational Health Sciences, University of Washington School of Public Health, Seattle, WA 98195, USA

James A. Cowan Evans Laboratory of Chemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio 43210, USA

Tara L. Croston West Virginia University School of Medicine, Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences, 1 Medical Center Drive Morgantown, WV 26506, USA

Ritesh P. Daya McMaster University, 1200 Main St. West, HSC 4N81, Hamilton, Ontario, L8N 3Z5, Canada

Custer C. Deocaris Department of Food Science and Technology, College of Home Economics, University of the Philippines, Diliman, Quezon City, Philippines

Sinclair R. Dundas Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, AB25 2ZD, Scotland, United Kingdom

Zvi Fishelson Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Kenji Fukasawa Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA

Ran Gao National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Joseph P. Gabriele McMaster University, 1200 Main St. West, HSC 4N81, Hamilton, Ontario, L8N 3Z5, Canada

Rona G. Giffard Dept. of Anesthesia, Stanford University School of Medicine, 300 Pasteur Drive, S272 Grant Building, CA 94305-5117, USA

Sarah E. Groleau McMaster University, 1200 Main St. West, HSC 4N81, Hamilton, Ontario, L8N 3Z5, Canada

Jake G. Hoekstra Department of Pathology, University of Washington School of Medicine, Seattle, WA 98104, USA

John M. Hollander West Virginia University School of Medicine, Division of Exercise Physiology, Center for Cardiovascular and Respiratory Sciences, 1 Medical Center Drive Morgantown, WV 26506, USA

Ohad Iosefson Department of Biochemistry and Molecular Biology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel

Jinghua Jin Department of Neurobiology, Zhejiang University School of Medicine, Hangzhou, Zhejiang 310058, China

Masayuki Kanai Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612, USA

Sunil C. Kaul National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Zeenia Kaul Center for Childhood Cancer, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA

Rejko Krüger Laboratory of Functional Neurogenomics, Center of Neurology, Hertie-Institute for Clinical Brain Research and German Center for Neurodegenerative Diseases (DZNE), University of Tübingen, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany

Nikki P. Lee Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Ben Low The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA

Wen-Jing Lu Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

John M. Luk Department of Oncology, Roche R&D Center (China) Ltd., 720 Cai Lun Road, Shanghai 201203, China

Wen-I Luo Ohio State Biochemistry Program, The Ohio State University, 100 West 18th Avenue, Columbus, OH 43210, USA

Alberto J. L. Macario Department of Microbiology and Immunology, School of Medicine, University of Maryland at Baltimore; and IMET, Columbus Center, 701 East Pratt Street, Baltimore, Maryland 21202, USA

Everly Conway de Macario Department of Microbiology and Immunology, School of Medicine, IMET, University of Maryland at Baltimore, Columbus Center, 701 East Pratt Street, Baltimore, Maryland 21202, USA

Ram K. Mishra McMaster University, 1200 Main St. West, HSC 4N81, Hamilton, Ontario, L8N 3Z5, Canada

Geneviève Morrow Laboratory of Cell and Developmental Genetics, Department of Molecular Biology, Medical Biochemistry and Pathology, Institut de Biologie Intégrative et des Systèmes and PROTEO, Université Laval, Québec, G1V 0A6, Canada

Oren Moskovich Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Graeme I. Murray Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, AB25 2ZD, Scotland, United Kingdom

Masanori Nishi National Institute of Advanced Industrial Science & Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Angheliki Nomikos Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen, AB25 2ZD, Scotland, United Kingdom

Yi-Bing Ouyang Dept. of Anesthesia, Stanford University School of Medicine, 300 Pasteur Drive, S272 Grant Building, CA 94305-5117, USA

Marie Le Pécheur Laboratory of Cell and Developmental Genetics, Department of Molecular Biology, Medical Biochemistry and Pathology, Institut de Biologie Intégrative et des Systèmes and PROTEO, Université Laval, Québec, G1V 0A6, Canada

Zdenek B. Pristupa McMaster University, 1200 Main St. West, HSC 4N81, Hamilton, Ontario, L8N 3Z5, Canada

Moran Saar Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Robert M. Tanguay Laboratory of Cell and Developmental Genetics, Department of Molecular Biology, Medical Biochemistry and Pathology, Institut de Biologie Intégrative et des Systèmes and PROTEO, Université Laval, Québec, G1V 0A6, Canada

Toshimasa Uemura National Institute of Advanced Industrial Science & Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Renu Wadhwa National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Charles W. Walker Department of Molecular, Cellular and Biomedical Sciences, Center For Marine Biology and Marine Biomedical Research Group, The University of New Hampshire, Durham, NH 03824, USA

Robin E. White Dept. of Anesthesia, Stanford University School of Medicine, 300 Pasteur Drive, S272 Grant Building, CA 94305-5117, USA

Tomoko Yaguchi National Institute of Advanced Industrial Science & Technology (AIST), Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan

Chae-Ok Yun Department of Bioengineering, College of Engineering, Hanyang University, 17 Haengdang-Dong, Seongdong-Gu, Seoul 133-791, Korea

Jing Zhang Department of Pathology, University of Washington School of Medicine, Seattle, WA 98104, USA

Part I
Structure and Function of Mortalin

Chapter 1
Birth of Mortalin: Multiple Names, Niches and Functions Connecting Stress, Senescence and Cancer

Renu Wadhwa and Sunil C. Kaul

Abstract The mitochondrion, arising from the historical endosymbiosis during the stressful period of the Great Oxidation Event 2.4 billion years ago, marks the existence of all eukaryotes. Retaining only a handful of genes from its ancestral symbiont and yet performing life-essential tasks, it is heavily dependent on the nucleus and a consortium of stress chaperones that maintain its structural and functional integrity by regulation of transport of the nuclear-encoded proteins, their quality control by chaperoning and proteolysis, and energy-generation as a part of their housekeeping and stress-survival functions. Mortalin, first identified in 1993 from cell fusion studies as a marker of mortal cell phenotype, was characterized as an Hsp70 family stress chaperone based on its sequence homology. Nearly two decades of experimental data have revealed its residence beyond the mitochondrial boundaries, life essential functions in and outside the mitochondria and those that specifically promote carcinogenesis on one hand and neurodegeneration on the other. Aimed to portrait mortalin characteristics, both in structure and function and drive the mortalin biology to drug discovery, this chapter reviews the events leading to its identification and role in old age diseases including cancer along with its possibility of being a therapeutic target.

Keywords Mortalin · Hsp70 family · Stress protein · Identification · Functions

1.1 Mortalin as a Member of Hsp70 Family of Proteins

Origin of heat shock proteins (Hsp-s, often called stress chaperones) preceded the birth of the mitochondria that marked the Great Oxygenation Event and the origin of the first eukaryote (Margulis 1975). Central to the mitochondrial evolution was the transition from individualistic bacteria to host-dependent organelles. Phylogenetic studies suggest that the earliest bacterial symbiont may have probably carried a genome of 630 distinct genes (Gabaldon and Huynen 2003) that got gradually lost

R. Wadhwa (✉) · S. C. Kaul
National Institute of Advanced Industrial Science and Technology (AIST),
Central 4, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8562, Japan
e-mail: renu-wadhwa@aist.go.jp

S. C. Kaul, R. Wadhwa (eds.), *Mortalin Biology: Life, Stress and Death,*
DOI 10.1007/978-94-007-3027-4_1, © Springer Science+Business Media B.V. 2012

and transformed to the present day human mitochondrion that encodes for only 13 polypeptides, 22tRNAs and 2 rRNAs (Anderson et al. 1981), and functions to actively convert free atmospheric oxygen into water. For this gene-depleted present day organelle to function, its proteome will need to be synthesized in the cytosol; traverse the mitochondrial boundaries, the outer (OM) and inner (IM) membranes; refold back into their native forms; and finally, get sorted into various intra-mitochondrial locations (Elstner et al. 2008; Huynen et al. 2009). Intuitively, the development of machineries for import, post-import folding, maturation and segregation must have originated as 'adaptive' evolutionary phenomenon. Mitochondrial chaperones, being the stress proteins that arose from harsh planetary conditions, are likely to be the most competent guardians of the mitochondrial proteome: its import, protein quality control and stress protective functions. They act both in housekeeping and stress responses based on their ability to bind with unfolded (nascent) and misfolded (denatured) proteins (Ecroyd and Carver 2008; Tatsuta 2009).

It was more than half a century ago when Ferruccio Ritossa reported the unusual puffing patterns in the polytene chromosomes of *Drosophila* after 30-min exposure of its larvae to elevated temperatures (37 °C) and their return to ambient temperatures for recovery (Ritossa 1962). The term Hsp-s was later dubbed when follow-up experiments revealed the increased expression of 70- and 26-kDa proteins suggesting that these gene products may be indispensable molecules that assisted protein refolding to overcome heat stress (Ananthan et al. 1986; Tissieres et al. 1974). The concept of Hsp-s as molecular chaperones was built from the earlier ideas of Laskey and colleagues that described chaperonization, an activity associated with nucleoplasmin in *Xenopus* oocytes (Laskey et al. 1978). The term was expanded to include a diverse class of proteins that aid polypeptide folding, transit across cellular and organelle membranes, assist the disassembly of macromolecular complexes or aggregates, regulate their conformation and target them for proteolysis to assure protein quality control that widely affect bio-signaling and functions (Ellis 1987; Hartl 1991).

Hsp family of proteins is composed of at least 40 members in humans. They are grouped into at least 6 major subfamilies named as Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small Hsp-s based on their molecular weights (Powers and Workman 2007). Hsp-s of the same family share similar domain structure, whereas members of each specific family associate with unique pattern of expression and cellular localization (Lindquist and Craig 1988). These are known for their multifunctional ability such as housekeeping functions in maintaining the protein structure, gene transcription, signal transduction and immunity (Helmbrecht et al. 2000), and induction in response to stress (like high temperature, chemical and physical stress resulting in augmentation of the biological functions for sustaining cell survival) (Sherman and Multhoff 2007). The present chapter portraits events on identification, cloning and functional role of mortalin (a member of Hsp70 family of proteins) in normal and abnormal, stressed and diseased, scenarios.

1.2 Mortalin-Multiple Births

1.2.1 Cell Fusion Studies for Markers of Mortality and Immortality

In order to track proteins involved in regulation of cellular mortality and immortality, normal and immortal mouse fibroblasts were fused to generate hybrid mortal cells and screened for proteins associated with either the mortal or immortal phenotype. An approximately 66-kDa cytoplasmic protein segregated with the loss of immortality in cybrids and was named 'mortalin' (Wadhwa et al. 1993a). An antibody raised against the protein revealed its pancytoplasmic distribution in normal mortal cells. Surprisingly, it also detected the protein in immortal mouse fibroblasts but was perinuclear in localization. Using the antibody, the cDNA for mortalin was cloned. It is 2850 bp in length, encoding a 74-kDa protein constituting 679 amino acids with a high degree of homology with members of the Hsp70 family, including *Escherichia coli* DnaK (51%), *Saccharomyces cerevisiae* SSC1p (65%), the constitutive cytosolic Hsp70 from rat, Hsc70 (46%) and the rat endoplasmic reticulum isoform, BiP (49%) (Wadhwa et al. 1993a). Although the complete crystal structure of mortalin has not yet been resolved, based on the conserved homology and bioinformatics, its three-dimensional structure was unraveled. Like most Hsp-s, mortalin has 2 principal domains, the amino-terminal ATPase region and carboxyl-terminal region, as illustrated by the kettle pot model (Kaul et al. 2007). A second cloning from mouse immortal cells revealed that the mouse mortalin exists in two isoforms of opposing phenotypes: the mortality-associated pancytoplasmic form (which was renamed as mortalin-1, mot-1) and the immortalization-associated perinuclear mortalin (mot-2) (Wadhwa et al. 1993b, 1996; Kaul et al. 1998). Mouse mot-1 and mot-2 cDNA differed by only two amino acids (V618M and R624G) in the carboxy-terminus and segregated in F1 and F2 progenies suggesting that these were encoded by two alleles on chromosome 18 (Kaul et al. 1995; Wadhwa et al. 1996). The two minutely different proteins were found to have different structural and functional characteristics to the extent that overexpression of mot-1 in immortal NIH3T3 cells induced senescence, while overexpression of mot-2 in the same cells mediated malignant transformation (Kaul et al. 1998; Wadhwa et al. 1993b). Recently, another variant of mouse mortalin, D626G, was identified and awaits functional characterization (Chardonnet et al. 2007). Studies on mortalin in humans revealed only one form of mortalin that possessed malignant transformation activity as mouse mot-2 (Kaul et al. 1998, 2007).

1.2.2 Mortalin as CSA (C3H Strain Specific Antigen)

Nearly at the same time when mortalin took its birth in mortality/immortality screen as described above, in a totally independent scenario, it was identified as a strain-specific antigen, found only in C3H mice and was named CSA (C3H-specific antigen). Mouse mortalin gene was sequenced and shown to contain 17 exons

interrupted by 16 introns and has two dimeric repeats of the consensus sequence of the heat-shock element in the 5′-flanking region (Michikawa et al. 1993); surprising to this fact, the protein is heat un-inducible. Another feature that aligned well with its mitochondrial residence was described as its first intron interrupted within the amino-terminal leader sequence, a pattern found similar to that of cytochrome c1, a well-known mitochondrial protein (Michikawa et al. 1993). Interestingly, the presence of two isoforms of mortalin correlated with the immortalization tendencies of fibroblasts derived from specific mouse strains. Fibroblasts from C3H strain of mouse that contain mot-1 were difficult to immortalize as compared to the mot-2 harboring fibroblasts from Balb/c and C57BL/6 strains. Despite these differences, both types of mortalin are essential for cell survival (Domanico et al. 1993; Michikawa et al. 1993) and one out of these two residues, arginine at residue 578 of C3H mouse, contributed to the immunogenicity of the protein. Using anti-CSA monoclonal antibody, the subcellular localization of CSA was shown to be the mitochondria and the fact that new genetic marker in mice was located on a gene encoding for a mitochondrial protein caught lot of attention.

1.2.3 Mortalin as PBP74 (Peptide-Binding Protein) in Immune Regulation

Yet another lab looking for proteins involved in antigen-processing, identified peptide-binding proteins (PBP72/74) by their ability to bind to a model antigenic peptide from pigeon cytochrome C (Pc). PBP72/74 did not bind to the native Pc and thus were suspected to recognize some feature of peptides not found in the native antigens. Antisera raised against PBP72/74 blocked the presentation of native antigen and of the corresponding fragment. Although the role of PBP72/74 in antigen processing is still a matter of research, it was interesting that the investigators detected the protein on cell surface, endosomes, golgi, ER, plasma membrane and the vesicular cytoplasmic structures. Cloning of PBP74 cDNA revealed that it is identical to mortalin (Domanico et al. 1993). Recently, it was demonstrated that the surface-expressed mortalin plays important role in antigen presentation and in innate immunity (Pilzer and Fishelson 2005; Pilzer et al. 2005). It was shown to bind to complement C8 and C9, shed in vesicles containing C9 and complement membrane attack complex (MAC) and involved in MAC elimination. Anti-mortalin antibodies increased cell sensitivity to MAC-mediated lysis suggesting that mortalin promotes the shedding of membrane vesicles loaded with complement MAC and protects cells from complement-mediated lysis.

1.2.4 Mortalin as Grp75 (Glucose Regulated Protein 75) in Stress Response

To discover novel metabolic stress markers for the central nervous system, Massa et al. screened candidate genes with degenerate RT-PCR primers (Massa et al. 1995)

and reported a rat-brain cDNA that encoded the glucose-regulated protein 75 (Grp75). It turned out to be a mitochondrial member of the Hsp70 family with sequence homology to mortalin and mortalin/PBP74/CSA. *In situ* analysis of normal brain revealed an abundance of Grp75 in neurons of the basal forebrain, reticular and subthalamic nuclei, globus pallidus and amygdala. With focal brain ischemia, Grp75 mRNA was upregulated in a peculiar fashion depending on the degree of injury. If ischemic focus was small, induction occurred only within the affected area, whereas, with a more extensive damage, Grp75 acquired a more global expression pattern. Consistent to this, five isoforms of Grp-s at 74–75 kDa mass were found from proteomic profiling of 2-deoxyglucose-treated murine and human fibroblasts. Cells treated with 2-deoxyglucose provide an *in vitro* model for glucose deprivation (Merrick et al. 1997). Stress from energy deprivation in the tibialis anterior muscle, a type II muscle, after 10 days of chronic contractile activity, stimulated mortalin/Grp75 protein, but not its mRNA (Ornatsky et al. 1995). Orsini et al. showed that a fraction of cytosolic p66Shc (regulates lifespan in mammals and is a critical component of the apoptotic response to oxidative stress) localizes within mitochondria where it forms a complex with mitochondrial Hsp70/mortalin (Orsini et al. 2004). Mortalin was shown to inhibit p66Shc function activated during oxidative stress (ultraviolet radiation) that induced the dissociation of p66Shc-mortalin complexes. Another study identified mortalin as one of the factors responsible for superior stress defense in murine embryonic stem cells (Saretzki et al. 2004) suggesting that it is an important component of the glucose and oxidative stress response of cells.

1.2.5 Mortalin as mtHsp70, a Mitochondrial Chaperone

Combination of immunological, biochemical and functional approaches both *in vitro* and *in vivo* revealed that human mortalin was imported into and stayed in the mitochondrial compartment (Dahlseid et al. 1994). By confocal immunofluorescence microscopy, mortalin was found inside the organelle and co-localized with the mitochondrial Hsp60. Deletion of the N-terminal 46-amino acid pre-sequence resulted in a cytosolic localization of the epitope-tagged protein (Dahlseid et al. 1994). Bhattacharyya et al. (1995) cloned human mtHsp70 gene by screening of an expression library with a monoclonal antibody and demonstrated that the 3A3-reactive protein co-fractionated with mitochondrial proteins. The nucleotide sequence of the respective cDNA clone matched with mortalin.

1.2.6 Mortalin as Tumor Necrosis Factor Receptor-Associated Protein 1

Tumor necrosis factor receptor-associated protein 1 (TRAP-1) was originally isolated from yeast two-hybrid system as a protein that interacted with the intracellular domain of the type 1 tumor necrosis factor receptor (TNFR-1). It was also identified

as a mitochondrial heat shock protein in Saos-2 (human osteosarcoma) cells adapted to mild oxidative stress induced by diethylmaleate (DEM). A recent study mentioned mortalin to be TRAP-1/mtHsp70 stimulated by ischemia. To understand the role of TRAP-1 in brain injury, it was overexpressed in astrocytes and was found to drop ROS production with glucose-deprivation. In addition, TRAP-1 preserved mitochondrial membrane potential, maintained ATP levels and cell viability during stress. Such findings endorsed TRAP-1 to be mortalin/mtHsp70/Hsp75/Grp75/PBP74 and an interesting gene that provided protection against ischemia-like *in vitro* injury (Volobouеva et al. 2007). It conferred greater resistance to hydrogen peroxide and cisplatin, and inhibited release of apoptosis-inducing factor upon cisplatin treatment (Montesano et al. 2007). Just like mortalin in other studies, TRAP-1 was detected in the mitochondrial matrix and non-mitochondrial locations, including pancreatic zymogen granules, insulin secretory granules, cardiac sarcomeres, nuclei of pancreatic and heart cells, and on the cell surface of blood vessel endothelial cells (Cechetto and Gupta 2000).

1.3 Mortalin: Inside and Outside the Mitochondria

Representing approximately 1% of the total protein content, mortalin is one of the most abundant proteins in the mitochondrial matrix (Naylor et al. 1996). It fulfills two special needs of the mitochondria: (i) constitutes an essential component of the import machinery and (ii) protein quality control by assisting in functional folding and degradation of unfunctional proteins. It has been identified as the only ATPase component of the pre-protein mitochondrial import complex (Schneider et al. 1994; Brunner et al. 1995) and plays a crucial role in mitochondrial biogenesis: the translocation of cytosolic precursors, their partitioning within the matrix and across the two mitochondrial membranes (Rehling et al. 2004). Along with the second mitochondrial chaperone-Hsp60, mortalin has been shown to maintain mitochondrial homeodynamics by taking care of degradation of the misfolded nonfunctional proteins and ROS by mitochondrial stress response signaling mediated by the transcription factor CHOP (Zhao et al. 2002; Yoneda et al. 2004). Unlike the well-understood ER stress response signaling that is mediated ER-resident Hsp70 BiP/Grp78 and proximal signal transducers IRE1, PERK and ATF6, mechanism of mitochondrial stress response is yet to be resolved.

Despite the fact that mitochondrion was frequently assigned as mortalin's home, it was seen traveling to many other subcellular sites and the idea of mortalin being a mitochondrion's permanent resident, indeed, was ramified to include its 'adventuring' tendencies. Ran et al. (2000) by undertaking subcellular fractionation and immunoelectron microscopy in a variety of human cancer cell lines revealed that mortalin exists in mitochondria of all the tested cells and travels to other subcellular organelles, ER and Golgi, in a cell line specific way. Ma et al. (2006) detected it in the nucleus of dividing cells at the time of chromosome duplication. Most recently, mortalin is also found as a secreted protein and detected in the extra-cellular

space similar to Hsp60 so besides its primary home as mitochondria, mortalin lives in many subcellular sites that is thought to be relevant to its multiple functionality. Some other examples of mitochondrial proteins found at unexpected locations both in the normal and pathogenic states include the mitochondrion proteins aspartate aminotransferase (mAsmAT) and Hsp60. mAsmAT is regarded as a transporter of free fatty acids into the mitochondria (Passarella et al. 1990), albeit, containing an N-terminal mitochondrial targeting sequence, was found on the cell surface and into the culture medium. Hsp60 mitochondrial chaperone was initially discovered in mammalian cells as a protein altered in Chinese Hamster Ovary (CHO) cells that were made resistant to the microtubule (MT)-inhibitor podophyllotoxin (Singh et al. 1997; Soltys and Gupta 2000). Found in various extra-mitochondrial locations, it has been shown to play role as an amino acid transporter, biosynthesis and packaging of insulin, modulating chromatin packing by histone 2B, and the regulation of cell cycle via the plasma membrane resident p21ras protein (Gupta et al. 2008; Gupta and Knowlton 2005; Knowlton and Gupta 2003; Soltys and Gupta 2000). How mortalin could arrive at specific extra-mitochondrial destinations is yet to be understood. Nevertheless, the phenomenon of multiple localizations and multiple functions may reflect a molecular-evolutionary protein economics that argues for a single protein to acquire distinct roles in more than one cellular compartment obviating the need to create a new gene. A comprehensive review of some of the likely mechanisms that could control the export of resident proteins from the mitochondrial matrix to other intra- and extra-cellular sites from evolutionary perspectives can be found elsewhere (Soltys and Gupta 1999, 2000).

Outside the mitochondria, mortalin is expected to collaborate with an expanding list of binding partners, described in several reviews, that endows it an assumption of wider cellular roles ranging from intracellular trafficking, control of centrosome duplication, regulation of p53 activity, calcium and ROS signaling, differentiation among many others (Deocaris et al. 2006, 2007a, b; Kaul et al. 2007; Takano et al. 2001; Wadhwa et al. 2003b, 2005).

1.4 Mortalin: Stress, Aging and Cancer

Sequence homology had placed mortalin in heat shock 70 family of proteins. Although remained unresponsive to the heat shock (Wadhwa et al. 1993a), several other stress conditions such as glucose deprivation, ionizing radiations, hypoxia and increase in the reactive oxygen species (ROS) were shown to induce mortalin that acts as a stress-survival factor (Carette et al. 2002; Hori et al. 2002; Liu et al. 2005; Merrick et al. 1997; Yang et al. 2011b). Suppression of mortalin by antisense mortalin oligonucleotide was found to sensitize cells to ionizing radiation and oxidative stress (Sadekova et al. 1997; Yang et al. 2011a, b). Another study proposed mortalin as a DNA-PK regulated protein that plays a protective role against drug-induced apoptosis and determines drug sensitivity (Um et al. 2003b).

In worms, overexpression of mortalin led to increased longevity (Yokoyama et al. 2002), and its knockdown led to accelerated aging syndrome (Kimura et al. 2007), associated with defects in mitochondrial import, a reduction in the levels of ATP-2, Hsp60 and CLK-1. Mortalin-compromised worms showed progeria like phenotypes including lower motility, defects in oogenesis, earlier accumulation of auto-fluorescent material, and a shorter life span (Kimura et al. 2007). Since a close correlation exists between stress resistance and longevity mechanisms, it may be true for even more complex models, such as mice. In general, when stress and impairment of the chaperone system are combined, the resulting gene-environment interaction may amount to causative impetus to premature aging (Macario and Conway de Macario 2002). Caloric restriction (CR), the only effective experimental manipulation known to retard aging in rodents and primates, restored age-impaired chaperone induction, while reversing the age-induced changes in constitutive level of Hsp (Berner and Stern 2004; Boxenbaum 1991; Kirkwood and Shanley 2000). CR rats were seen to have increased mortalin expression level in the testis (Um et al. 2003a, b). These examples support the hypothesis that mortalin endows a better adaptation capacity to various forms of stresses and increases the longevity in an organism. On the other hand, a sick version of mortalin (oxidized mortalin), in fact, was found in brain tissues of a rodent model for Alzheimer's disease (Berner and Stern 2004), and this may likely be actively involved in etiology of the disease rather than as mere molecular fossil of neurodegeneration. The oxidized form of mortalin was tested to act as an anti-chaperone, promotes protein aggregation, and overrides the chaperone activity of undamaged mortalin protein (Deocaris et al. unpublished data). Several studies have endorsed the involvement of mortalin to age-pathologies including cardiovascular diseases (Massa et al. 1995), diabetes (Matsuoka et al. 2005; Zhang et al. 2006) and neurodegenerative disorders, Alzheimer's and Parkinson's Diseases (PD) (Calabrese et al. 2001; Choi et al. 2004; Jin et al. 2006; Osorio et al. 2007; Seyb et al. 2006; Sirk et al. 2007). It was also shown to be one of the five proteins that form complex with alpha-synuclein and DJ-1 (an oncogene and causative gene for familial form of the PD) and is critically involved in the pathogenesis of PD (Jin et al. 2006, 2007; Shi et al. 2008). Li et al. showed that DJ-1 is associated with HSP70, CHIP and mortalin and the complex is involved in regulation of oxidative stress (Li et al. 2005). An association of wild type DJ-1, but not the mutants found in PD patients, was enhanced by treatment of cells with H_2O_2. Van Laar et al. also showed that the level of mortalin decrease during dopamine oxidation leading to selective dopaminergic terminal degeneration *in vivo* and altered mitochondrial function *in vitro* (Van Laar et al. 2008). In a quantitative proteomic study on comparison of the nigral mitochondrial proteins of Parkinson's disease (PD) patients with those from age-matched controls, mortalin was detected as downregulated in the PD patients (Jin et al. 2006). Indeed, manipulations of mortalin levels in dopaminergic neurons resulted in significant changes in sensitivity to PD phenotypes *via* pathways involving mitochondrial, proteasomal and oxidative stress response functions (Jin et al. 2006) revealing that mortalin is involved in the PD pathogenesis.

Within the ROS-bathed cellular environment, mutations stochastically accumulate with time contributing to genomic instability and cancers. Consistent with the major

involvement of ROS-related mutational events, Bert Vogelstein's group found that the majority of mutations in ten human colorectal cancer cell lines were: (a) somatically acquired mtDNA mutations (b) the detected mutations however were not associated with major perturbations of mitochondrial functions, as oxygen consumption (Polyak et al. 1998). Given that cancer cells carry biologically risky and numerous mitochondrial gene mutations that could exacerbate mitochondrial dysfunction, it may be suggested that a strong chaperone buffering system within this organelle could be one plausible strategy how cancer cells are able to tolerate high mutational loads. Several studies have found that the level of mortalin was elevated in many human tumors, the tumor-derived and *in vitro* immortalized cells. Remarkably, overexpression of mortalin matched with the increase in aggressiveness of brain tumors from astrocytoma to glioblastoma (Takano et al. 1997) and was sufficient to increase the malignancy of breast carcinoma cells suggesting that an upregulation of mortalin contributes significantly to tumorigenesis (Wadhwa et al. 2006). Comparative proteomic analysis identified the correlation of mortalin overexpression with poor patient survival in colorectal adenocarcinomas (Dundas et al. 2005) and post-surgery hepatocarcinoma recurrence (Yi et al. 2008). In chronic myeloid leukemia (CML), a hematopoietic stem cell disease containing an aberrant Bcr-Abl protein tyrosine kinase activity, mortalin was identified as a major protein down-regulated during the progression of a benign chronic phase to a rapidly fatal blast crisis. The absence of correlation between mRNA and protein levels pointed at the possible post-translational events that modify protein content (Smith et al. 2002). Another proteomic study on bone marrow cells from CML patients also identified mortalin as one of the 31 proteins that described a chronic phase molecular phenotype (Pizzatti et al. 2006).

In a study on the disease models of hematopoiesis in which Zebrafish mutants with abnormalities at various stages in blood development were used, positional cloning of a developmental blood mutant (crimsonless (*crs*)- anemic) revealed that the mutated gene was mortalin/HSPA9B that shows 84.8% identity and 89.4% similarity with human mortalin (Craven et al. 2005). A single amino acid mutant (G492E) within the substrate-binding domain of HSPA9B was shown to be the cause of the *crs* phenotype. Interestingly, a near-identical mutation in the conserved glycine at position 443 in DnaK (53.5% identity and 63.3% similarity to Zebrafish mortalin) completely abolished pro-peptide binding and chaperone function (Burkholder et al. 1996). To verify that the mutation in HSPA9B is sufficient to cause the *crs* phenotype, investigators used both rescue and antisense morpholino knockdown strategies (Craven et al. 2005). Injection of capped RNA encoding wild-type HSPA9B rescued approximately 95% (53 of 56) injected mutant embryos. Conversely, inactivation of Zebrafish mortalin using antisense morpholino-modified oligonucleotides recapitulated the anemic phenotype demonstrating that a single amino acid change, G492E that abolishes chaperone function of mortalin is the cause of *crs* phenotype in Zebrafish, a model of human MDS (Craven et al. 2005). The role of mortalin in cancer is best explained by its interactions with tumor suppressor protein p53 (Deocaris et al. 2007b; Kaul et al. 2001, 2005; Lu et al. 2011a, b; Ma et al. 2006; Wadhwa et al. 1998, 2010; Walker et al. 2006). Mortalin-p53 interactions were abrogated both in

mammalian and clam cells by a cationic inhibitor (MKT-077) that binds to mortalin and dissociates p53 from the complex resulting in the reactivation of wild type p53 function (Wadhwa et al. 2000; Walker et al. 2006; Deocaris et al. 2007c; Pilzer et al. 2009).

1.5 Mortalin: Therapeutic Target

Considering the wide diversity of mortalin functions, it is likely that mortalin-based therapy would be useful in dampening the impact of some of the chaperone-associated maladies involving both the chaperone-hyperfunction (such as in cancers) and chaperone-deficiency (such as in neurodegenerative disorders) (Macario and Conway de Macario 2007). A variety of reagents hold promises and await further studies on validation. Some worth-mentioning here are mortalin injectibles, mortalin peptides, small molecules and antibodies.

Given the growing interest on developing recombinant stress proteins for chaperonotherapy, the field is expected to expand and validate information on the use of individual stress chaperones as chaperonotherapeutic tools. Some of the initial evidence that an Hsp70 member might serve as a chaperonotherapeutic agent is from the purified bovine brain Hsc70. When administrated exogenously, the chaperone proved useful for repair of peripheral sensory nerve damage. In this particular experiment, axotomy induced death in 33% of dorsal root ganglion neurons and 50% of motoneurons, and damage-control by the recombinant Hsc70 was apparent in virtually all sensory neurons (Houenou et al. 1996). Gifondorwa et al. tested whether the recombinant human Hsp70 could be used for treating amyotrophic lateral sclerosis (ALS), a debilitating neurodegenerative disorder that results in the progressive loss of motor neurons in the central nervous system (Gifondorwa et al. 2007). Using the G93A mutant SOD1 mouse, the group intraperitoneally-injected Hsp70 (3 times/per week) from postnatal day 50 until the end-stage of the disease. Such regimen was observed to lead to increased lifespan, delayed symptom onset, and intact motor functions. Interestingly, it also resulted in a more robust innervations of the neuromuscular junctions compared with control tissues. It was thus suggested that an Hsp70-based chaperonotherapy might be used to delay the disease progression in an ALS model via an unknown peripheral mechanism (Gifondorwa et al. 2007). An alternative approach would involve the use of *protein inducers* (Macario and Conway de Macario 2007). Given the roles played by stress chaperones in the maintenance of proteome integrity during aging and stressed conditions, it would be of considerable benefit to discover new compounds that will induce HSPs without any toxic effects. One of the first Hsp70 inducing agents introduced is Bioclomol, a hydroxylamine derivative developed by the Hungarian biotech company, Biorex Research and Development Co. Originally marketed to prevent microangiopathy in diabetes patients, the drug amplifies induction of Hsp70 when cells are subjected to stressful conditions (Hargitai et al. 2003). Two molecules are found to boost cellular production of mortalin: 2-deoxyglucose (Merrick et al. 1997) and glycerol (Deocaris et al. 2008).

Interestingly, treatment of cells with a chemical chaperone, glycerol caused heat-shock and oxidative stress responses, induction of mortalin and stimulation of proteasomal system. In *C. elegans,* it resulted in decreased accumulation of old age-associated lipofuscin suggesting that mortalin inducers may serve as anti-aging reagents. Further studies are warranted to resolve the molecular effects and mechanisms of action.

Mortalin was shown to bind to tumor suppressor protein and inactivate its transcriptional activation function by cytoplasmic sequestration (Kaul et al. 2005). Finding that the carboxy-terminal 312–352 residues of tumor suppressor protein p53 bind to mortalin, shorter peptides were used as mortalin binding antagonists. These peptides were seen to activate endogenous p53 function by displacing mortalin from p53-mortalin complexes and relocating p53 to the nucleus. This was sufficient to activate p53 function causing growth arrest in human osteosarcoma and breast carcinoma cells (Kaul et al. 2005). Similar to the peptides, MKT-077, a cationic rhodacyanine dye analogue and withanone, a phytochemical binds to mortalin and abrogates its interactions with p53 resulting in the release of p53 from cytoplasmically sequestered p53-mortalin complexes and reactivation of its transcriptional activation and apoptotic functions (Deocaris et al. 2007c). Thus, MKT-077 and withanone are the anti-mortalin reagents and candidate anticancer drugs. Induction of senescence like growth arrest by bromodeoxyuridine (Michishita et al. 1999) and 5-aza-2′ deoxycytidine (Widodo et al. 2007) caused shift in subcellular distribution of mortalin from perinuclear to pancytoplasmic type. It was shown that mortalin was direct target of these drugs and undergoes structural changes that may affect its function as chaperone, importer or regulator (Widodo et al. 2007; Deocaris et al. 2008). Besides the peptides and the chemicals, mortalin-specific ribozymes and siRNA that caused suppression of mortalin expression resulted in the growth arrest/apoptosis of transformed human cells (Wadhwa et al. 2003a; Yoo et al. 2010). Anti-mortalin antibodies were seen to have antitumor activity in nude mice xenografts. Cell internalizing feature of these antibodies was also exploited as a nanocarrier tool for gene delivery and imaging (Shiota et al. 2007; Ohyabu et al. 2009; Yoshioka et al. 2011).

1.6 Summary

The concomitant, yet independent, discoveries of mortalin have reflected its multiple functionality. Mortalin is indeed a two-billion year old resident of the mitochondria, the living descendant of the first DnaK in endosymbiotic alpha-proteobacter that become a power-generating organelle and acquired multiple roles reflecting an example of molecular-evolutionary protein economics. Furthermore, this promiscuous chaperone is partnered with a larger array of binding partners, from transcription factors, cell receptors, cytoskeleton elements and many others. Like a “molecular megalomaniac”, mortalin has expanded its biological roles. The qualitative and quantitative nature of mortalin responses, as seen from the upregulation in various tumors to the presence of sick (oxidized) forms in neurodegenerative diseases, render future

experimental and clinical studies to unravel its mechanistic aspects. From the understanding that the typical tools used for the basic study of mortalin functions in the laboratory, such as recombinant mortalin, mini-mortalins, ribozymes and siRNA, internalizing-mortalin antibodies, etc., are also expected to evolve into the next generation chaperonotherapeutics—from mortalin peptide-based cytotoxics, vaccines, tumor senescence-modulators and youth rejuvenators. As we come to appreciate the rapidly growing chaperonology of mortalin, this stress protein has undoubtedly emerged as an extremely versatile molecule. The importance of mortalin in cell biology is underscored by its high degree of structural and phylogenetic conservation, and the fact that no cell survives in its absence. As newer genomic technologies, like chaperonomics and systems biology, offer fresher perspectives, it is anticipated that our present knowledge on the physiological roles of this chaperone may still be limited. Nonetheless, the present book portraits the wealth of information on this versatile stress molecule and picture the mechanisms on how it plays essential roles in stress and survival.

References

Ananthan J, Goldberg AL, Voellmy R (1986) Abnormal proteins serve as eukaryotic stress signals and trigger the activation of heat shock genes. Science 232:522–524

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F et al (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Berner YN, Stern F (2004) Energy restriction controls aging through neuroendocrine signal transduction. Ageing Res Rev 3:189–198

Bhattacharyya T, Karnezis AN, Murphy SP, Hoang T, Freeman BC, Phillips B, Morimoto RI (1995) Cloning and subcellular localization of human mitochondrial hsp70. J Biol Chem 270:1705–1710

Boxenbaum H (1991) Gompertz mortality analysis: aging, longevity hormesis and toxicity. Arch Gerontol Geriatr 13:125–137

Brunner M, Schneider HC, Lill R, Neupert W (1995) Dissection of protein translocation across the mitochondrial outer and inner membranes. Cold Spring Harb Symp Quant Biol 60:619–627

Burkholder WF, Zhao X, Zhu X, Hendrickson WA, Gragerov A, Gottesman ME (1996) Mutations in the C-terminal fragment of DnaK affecting peptide binding. Proc Natl Acad Sci U S A 93:10632–10637

Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB (2001) Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. Neurochem Res 26:739–764

Carette J, Lehnert S, Chow TY (2002) Implication of PBP74/mortalin/GRP75 in the radio-adaptive response. Int J Radiat Biol 78:183–190

Cechetto JD, Gupta RS (2000) Immunoelectron microscopy provides evidence that tumor necrosis factor receptor-associated protein 1 (TRAP-1) is a mitochondrial protein which also localizes at specific extramitochondrial sites. Exp Cell Res 260:30–39

Chardonnet S, Decottignies P, Amar L, Le Caer JP, Davis S, Laroche S, Le Marechal P (2007) New mortalin and histidyl tRNA synthetase isoforms point out a pitfall in proteomic analysis of Egr1 genetically modified mice. Proteomics 7:289–298

Choi J, Forster MJ, McDonald SR, Weintraub ST, Carroll CA, Gracy RW (2004) Proteomic identification of specific oxidized proteins in ApoE-knockout mice: relevance to Alzheimer's disease. Free Radic Biol Med 36:1155–1162

Craven SE, French D, Ye W, de Sauvage F, Rosenthal A (2005) Loss of Hspa9b in zebrafish recapitulates the ineffective hematopoiesis of the myelodysplastic syndrome. Blood 105:3528–3534

Dahlseid JN, Lill R, Green JM, Xu X, Qiu Y, Pierce SK (1994) PBP74, a new member of the mammalian 70-kDa heat shock protein family, is a mitochondrial protein. Mol Biol Cell 5:1265–1275

Deocaris CC, Kaul SC, Wadhwa R (2006) On the brotherhood of the mitochondrial chaperones mortalin and heat shock protein 60. Cell Stress Chaperones 11:116–128

Deocaris CC, Kaul SC, Wadhwa R (2007a) Heat shock chaperone mortalin and carcinogenesis. In: Calderwood SK, Sherman MY, Ciocca DR (eds) Heat shock proteins in cancer. Springer, Netherlands, pp 141–158

Deocaris CC, Kaul SC, Wadhwa R (2007b) Mortalin—a driver on the crossroads of stress, aging and carcinogenesis. In: Sreedhar AS, Srinivas UK (eds) Stress response: a molecular biology approach. Research Signpost, pp 135–158

Deocaris CC, Widodo N, Shrestha BG, Kaur K, Ohtaka M, Yamasaki K, Kaul SC, Wadhwa R (2007c) Mortalin sensitizes human cancer cells to MKT-077-induced senescence. Cancer Lett 252:259–269

Deocaris CC, Takano S, Priyandoko D, Kaul Z, Yaguchi T, Kraft DC, Yamasaki K, Kaul SC, Wadhwa R (2008) Glycerol stimulates innate chaperoning, proteasomal and stress-resistance functions: implications for geronto-manipulation. Biogerontology 9:269–282

Domanico SZ, DeNagel DC, Dahlseid JN, Green JM, Pierce SK (1993) Cloning of the gene encoding peptide-binding protein 74 shows that it is a new member of the heat shock protein 70 family. Mol Cell Biol 13:3598–3610

Dundas SR, Lawrie LC, Rooney PH, Murray GI (2005) Mortalin is over-expressed by colorectal adenocarcinomas and correlates with poor survival. J Pathol 205:74–81

Ecroyd H, Carver JA (2008) Unraveling the mysteries of protein folding and misfolding. IUBMB Life 60:769–774

Ellis J (1987) Proteins as molecular chaperones. Nature 328:378–379

Elstner M, Andreoli C, Ahting U, Tetko I, Klopstock T, Meitinger T, Prokisch H (2008) MitoP2: an integrative tool for the analysis of the mitochondrial proteome. Mol Biotechnol 40:306–315

Gabaldon T, Huynen MA (2003) Reconstruction of the proto-mitochondrial metabolism. Science 301:609

Gifondorwa DJ, Robinson MB, Hayes CD, Taylor AR, Prevette DM, Oppenheim RW, Caress J, Milligan CE (2007) Exogenous delivery of heat shock protein 70 increases lifespan in a mouse model of amyotrophic lateral sclerosis. J Neurosci 27:13173–13180

Gupta S, Knowlton AA (2005) HSP60, Bax, apoptosis and the heart. J Cell Mol Med 9:51–58

Gupta RS, Ramachandra NB, Bowes T, Singh B (2008) Unusual cellular disposition of the mitochondrial molecular chaperones Hsp60, Hsp70 and Hsp10. Novartis Found Symp 291:59–68; discussion 69–73, 137–140

Hargitai J, Lewis H, Boros I, Racz T, Fiser A, Kurucz I, Benjamin I, Vigh L, Penzes Z, Csermely P et al (2003) Bimoclomol, a heat shock protein co-inducer, acts by the prolonged activation of heat shock factor-1. Biochem Biophys Res Commun 307:689–695

Hartl FU (1991) Heat shock proteins in protein folding and membrane translocation. Semin Immunol 3:5–16

Helmbrecht K, Zeise E, Rensing L (2000) Chaperones in cell cycle regulation and mitogenic signal transduction: a review. Cell Prolif 33:341–365

Hori O, Ichinoda F, Tamatani T, Yamaguchi A, Sato N, Ozawa K, Kitao Y, Miyazaki M, Harding HP, Ron D et al (2002) Transmission of cell stress from endoplasmic reticulum to mitochondria: enhanced expression of Lon protease. J Cell Biol 157:1151–1160

Houenou LJ, Li L, Lei M, Kent CR, Tytell M (1996) Exogenous heat shock cognate protein Hsc 70 prevents axotomy-induced death of spinal sensory neurons. Cell Stress Chaperones 1:161–166

Huynen MA, de Hollander M, Szklarczyk R (2009) Mitochondrial proteome evolution and genetic disease. Biochim Biophys Acta 1792:1122–1129

Jin J, Hulette C, Wang Y, Zhang T, Pan C, Wadhwa R, Zhang J (2006) Proteomic identification of a stress protein, mortalin/mthsp70/GRP75: relevance to Parkinson disease. Mol Cell Proteomics 5:1193–1204

Jin J, Li GJ, Davis J, Zhu D, Wang Y, Pan C, Zhang J (2007) Identification of novel proteins interacting with both alpha-synuclein and DJ-1. Mol Cell Proteomics 6:845–859

Kaul SC, Wadhwa R, Matsuda Y, Hensler PJ, Pereira-Smith OM, Komatsu Y, Mitsui Y (1995) Mouse and human chromosomal assignments of mortalin, a novel member of the murine hsp70 family of proteins. FEBS Lett 361:269–272

Kaul SC, Duncan EL, Englezou A, Takano S, Reddel RR, Mitsui Y, Wadhwa R (1998) Malignant transformation of NIH3T3 cells by overexpression of mot-2 protein. Oncogene 17:907–911

Kaul SC, Reddel RR, Mitsui Y, Wadhwa R (2001) An N-terminal region of mot-2 binds to p53 in vitro. Neoplasia 3:110–114

Kaul SC, Aida S, Yaguchi T, Kaur K, Taira K, Wadhwa R (2005) Activation of wild type p53 function by its mortalin-binding cytoplasmically localizing carboxy-terminus peptides. J Biol Chem 280:39373–39379

Kaul SC, Deocaris CC, Wadhwa R (2007) Three faces of mortalin: a housekeeper, guardian and killer. Exp Gerontol 42:263–274

Kimura K, Tanaka N, Nakamura N, Takano S, Ohkuma S (2007) Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in caenorhabditis elegans. J Biol Chem 282:5910–5918

Kirkwood TB, Shanley DP (2000) Caloric restriction, hormesis and life history plasticity. Hum Exp Toxicol 19:338–339

Knowlton AA, Gupta S (2003) HSP60, Bax, and cardiac apoptosis. Cardiovasc Toxicol 3:263–268

Laskey RA, Honda BM, Mills AD, Finch JT (1978) Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA. Nature 275:416–420

Li HM, Niki T, Taira T, Iguchi-Ariga SM, Ariga H (2005) Association of DJ-1 with chaperones and enhanced association and colocalization with mitochondrial Hsp70 by oxidative stress. Free Radic Res 39:1091–1099

Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Genet 22:631–677

Liu Y, Liu W, Song XD, Zuo J (2005) Effect of GRP75/mthsp70/PBP74/mortalin overexpression on intracellular ATP level, mitochondrial membrane potential and ROS accumulation following glucose deprivation in PC12 cells. Mol Cell Biochem 268:45–51

Lu WJ, Lee NP, Kaul SC, Lan F, Poon RT, Wadhwa R, Luk JM (2011a) Induction of mutant p53-dependent apoptosis in human hepatocellular carcinoma by targeting stress protein mortalin. Int J Cancer 129(8):1806–1814

Lu WJ, Lee NP, Kaul SC, Lan F, Poon RT, Wadhwa R, Luk JM (2011b) Mortalin-p53 interaction in cancer cells is stress dependent and constitutes a selective target for cancer therapy. Cell Death Differ 18:1046–1056

Ma Z, Izumi H, Kanai M, Kabuyama Y, Ahn NG, Fukasawa K (2006) Mortalin controls centrosome duplication via modulating centrosomal localization of p53. Oncogene 25:5377–5390

Macario AJ, Conway de Macario E (2002) Sick chaperones and ageing: a perspective. Ageing Res Rev 1:295–311

Macario AJ, Conway de Macario E (2007) Chaperonopathies and chaperonotherapy. FEBS Lett 581:3681–3688

Margulis L (1975) Symbiotic theory of the origin of eukaryotic organelles; criteria for proof. Symp Soc Exp Biol 1975(29):21–38

Massa SM, Longo FM, Zuo J, Wang S, Chen J, Sharp FR (1995) Cloning of rat grp75, an hsp70-family member, and its expression in normal and ischemic brain. J Neurosci Res 40:807–819

Matsuoka T, Wada J, Hashimoto I, Zhang Y, Eguchi J, Ogawa N, Shikata K, Kanwar YS, Makino H (2005) Gene delivery of tim44 reduces mitochondrial superoxide production and ameliorates neointimal proliferation of injured carotid artery in diabetic rats. Diabetes 54:2882–2890

Merrick BA, Walker VR, He C, Patterson RM, Selkirk JK (1997) Induction of novel Grp75 isoforms by 2-deoxyglucose in human and murine fibroblasts. Cancer Lett 119:185–190

Michikawa Y, Baba T, Arai Y, Sakakura T, Kusakabe M (1993) Structure and organization of the gene encoding a mouse mitochondrial stress-70 protein. FEBS Lett 336:27–33

Michishita E, Nakabayashi K, Suzuki T, Kaul SC, Ogino H, Fujii M, Mitsui Y, Ayusawa D (1999) 5-Bromodeoxyuridine induces senescence-like phenomena in mammalian cells regardless of cell type or species. J Biochem 126:1052–1059

Montesano GN, Chirico G, Pirozzi G, Costantino E, Landriscina M, Esposito F (2007) Tumor necrosis factor-associated protein 1 (TRAP-1) protects cells from oxidative stress and apoptosis. Stress 10:342–350

Naylor DJ, Hoogenraad NJ, Hoj PB (1996) Isolation and characterisation of a cDNA encoding rat mitochondrial GrpE, a stress-inducible nucleotide-exchange factor of ubiquitous appearance in mammalian organs. FEBS Lett 396:181–188

Ohyabu Y, Kaul Z, Yoshioka T, Inoue K, Sakai S, Mishima H, Uemura T, Kaul SC, Wadhwa R (2009) Stable and nondisruptive in vitro/in vivo labeling of mesenchymal stem cells by internalizing quantum dots. Hum Gene Ther 20:217–224

Ornatsky OI, Connor MK, Hood DA (1995) Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle. Biochem J 311:119–123

Orsini F, Migliaccio E, Moroni M, Contursi C, Raker VA, Piccini D, Martin-Padura I, Pelliccia G, Trinei M, Bono M et al (2004) The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. J Biol Chem 279:25689–25695

Osorio C, Sullivan PM, He DN, Mace BE, Ervin JF, Strittmatter WJ, Alzate O (2007) Mortalin is regulated by APOE in hippocampus of AD patients and by human APOE in TR mice. Neurobiol Aging 28:1853–1862

Passarella S, Marra E, Atlante A, Barile M, Doonan S, Quagliariello E (1990) Uptake of aspartate aminotransferase into mitochondria in vitro causes efflux of malate dehydrogenase and vice versa. Biochim Biophys Acta 1022:273–282

Pilzer D, Fishelson Z (2005) Mortalin/GRP75 promotes release of membrane vesicles from immune attacked cells and protection from complement-mediated lysis. Int Immunol 17:1239–1248

Pilzer D, Gasser O, Moskovich O, Schifferli JA, Fishelson Z (2005) Emission of membrane vesicles: roles in complement resistance, immunity and cancer. Springer Semin Immunopathol 27:375–387

Pilzer D, Saar M, Koya K, Fishelson Z (2009) Mortalin inhibitors sensitize K562 leukemia cells to complement-dependent cytotoxicity. Int J Cancer 126:1428–1435

Pizzatti L, Sa LA, de Souza JM, Bisch PM, Abdelhay E (2006) Altered protein profile in chronic myeloid leukemia chronic phase identified by a comparative proteomic study. Biochim Biophys Acta 1764:929–942

Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B (1998) Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20:291–293

Powers MV, Workman P (2007) Inhibitors of the heat shock response: biology and pharmacology. FEBS Lett 581:3758–3769

Ran Q, Wadhwa R, Kawai R, Kaul SC, Sifers RN, Bick RJ, Smith JR, Pereira-Smith OM (2000) Extramitochondrial localization of mortalin/mthsp70/PBP74/GRP75. Biochem Biophys Res Commun 275:174–179

Rehling P, Brandner K, Pfanner N (2004) Mitochondrial import and the twin-pore translocase. Nat Rev Mol Cell Biol 5:519–530

Ritossa F (1962) A new puffing pattern induced by a temperature shock and DNP in Drosophila. Experientia 18:571–573

Sadekova S, Lehnert S, Chow TY (1997) Induction of PBP74/mortalin/Grp75, a member of the hsp70 family, by low doses of ionizing radiation: a possible role in induced radioresistance. Int J Radiat Biol 72:653–660

Saretzki G, Armstrong L, Leake A, Lako M, von Zglinicki T (2004) Stress defense in murine embryonic stem cells is superior to that of various differentiated murine cells. Stem Cells 22:962–971

Schneider HC, Berthold J, Bauer MF, Dietmeier K, Guiard B, Brunner M, Neupert W (1994) Mitochondrial Hsp70/MIM44 complex facilitates protein import. Nature 371:768–774

Seyb KI, Ansar S, Bean J, Michaelis ML (2006) beta-Amyloid and endoplasmic reticulum stress responses in primary neurons: effects of drugs that interact with the cytoskeleton. J Mol Neurosci 28:111–123

Sherman M, Multhoff G (2007) Heat shock proteins in cancer. Ann N Y Acad Sci 1113:192–201

Shi M, Jin J, Wang Y, Beyer RP, Kitsou E, Albin RL, Gearing M, Pan C, Zhang J (2008) Mortalin: a protein associated with progression of Parkinson disease? J Neuropathol Exp Neurol 67:117–124

Shiota M, Ikeda Y, Kaul Z, Itadani J, Kaul SC, Wadhwa R (2007) Internalizing antibody-based targeted gene delivery for human cancer cells. Hum Gene Ther 18:1153–1160

Singh B, Soltys BJ, Wu ZC, Patel HV, Freeman KB, Gupta RS (1997) Cloning and some novel characteristics of mitochondrial Hsp70 from Chinese hamster cells. Exp Cell Res 234:205–216

Sirk D, Zhu Z, Wadia JS, Shulyakova N, Phan N, Fong J, Mills LR (2007) Chronic exposure to sub-lethal beta-amyloid (Abeta) inhibits the import of nuclear-encoded proteins to mitochondria in differentiated PC12 cells. J Neurochem 103:1989–2003

Smith DL, Evans CA, Pierce A, Gaskell SJ, Whetton AD (2002) Changes in the proteome associated with the action of Bcr-Abl tyrosine kinase are not related to transcriptional regulation. Mol Cell Proteomics 1:876–884

Soltys BJ, Gupta RS (1999) Mitochondrial-matrix proteins at unexpected locations: are they exported? Trends Biochem Sci 24:174–177

Soltys BJ, Gupta RS (2000) Mitochondrial proteins at unexpected cellular locations: export of proteins from mitochondria from an evolutionary perspective. Int Rev Cytol 194:133–196

Takano S, Wadhwa R, Yoshii Y, Nose T, Kaul SC, Mitsui Y (1997) Elevated levels of mortalin expression in human brain tumors. Exp Cell Res 237:38–45

Takano S, Wadhwa R, Mitsui Y, Kaul SC (2001) Identification and characterization of molecular interactions between glucose-regulated proteins (GRPs) mortalin/GRP75/peptide-binding protein 74 (PBP74) and GRP94. Biochem J 357:393–398

Tatsuta T (2009) Protein quality control in mitochondria. J Biochem 146:455–461

Tissieres A, Mitchell HK, Tracy UM (1974) Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J Mol Biol 84:389–398

Um JH, Kang CD, Hwang BW, Ha MY, Hur JG, Kim DW, Chung BS, Kim SH (2003a) Involvement of DNA-dependent protein kinase in regulation of the mitochondrial heat shock proteins. Leuk Res 27:509–516

Um JH, Kim SJ, Kim DW, Ha MY, Jang JH, Chung BS, Kang CD, Kim SH (2003b) Tissue-specific changes of DNA repair protein Ku and mtHSP70 in aging rats and their retardation by caloric restriction. Mech Ageing Dev 124:967–975

Van Laar VS, Dukes AA, Cascio M, Hastings TG (2008) Proteomic analysis of rat brain mitochondria following exposure to dopamine quinone: implications for Parkinson disease. Neurobiol Dis 29:477–489

Volobouева LA, Duan M, Ouyang Y, Emery JF, Stoy C, Giffard RG (2007) Overexpression of mitochondrial Hsp70/Hsp75 protects astrocytes against ischemic injury in vitro. J Cereb Blood Flow Metab 28:1009–1016

Wadhwa R, Kaul SC, Ikawa Y, Sugimoto Y (1993a) Identification of a novel member of mouse hsp70 family. Its association with cellular mortal phenotype. J Biol Chem 268:6615–6621

Wadhwa R, Kaul SC, Sugimoto Y, Mitsui Y (1993b) Induction of cellular senescence by transfection of cytosolic mortalin cDNA in NIH 3T3 cells. J Biol Chem 268:22239–22242