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Triage of oxidation-prone proteins by Sqstm1/p62 within the mitochondria

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ABSTRACT

As the mitochondrion is vulnerable to oxidative stress, cells have evolved several strategies to maintain mitochondrial integrity, including mitochondrial protein quality control mechanisms and autophagic removal of damaged mitochondria. Involvement of an autophagy adaptor, Sqstm1/p62, in the latter process has been recently described. In the present study, we provide evidence that a portion of p62 directly localizes within the mitochondria and supports stable electron transport by forming heterogeneous protein complexes. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) of mitochondrial proteins co-purified with p62 revealed that p62 interacts with several oxidation-prone proteins, including a few components of the electron transport chain complexes, as well as multiple chaperone molecules and redox regulatory enzymes. Accordingly, p62-deficient mitochondria exhibited compromised electron transport, and the compromised function was partially restored by *in vitro* delivery of p62. These results suggest that p62 plays an additional role in maintaining mitochondrial integrity at the vicinity of target machineries through its function in relation to protein quality control.

1. Introduction

Mitochondria provide efficient energy generation that is critical for various processes in the cell. Mitochondria manage this by coupling two separate events; the transport of electrons from reducing equivalents (NADH and $FADH_2$) to oxygen molecules via the electron transport system (ETS), which is composed of inner-membrane protein complexes-I to IV, and ATP synthesis by F_0F_1-ATP synthase complex (complex-V) using the proton gradient built up across the inner-membrane during electron transport [1]. However, due to the unstable nature of single electron transport in this process, about 1% of total electron flow leaks from the complexes, and converts molecular oxygen to reactive oxygen species (ROS) [1–3]. As the major ROS generation site in the cell, the mitochondrion itself is the most vulnerable organelle to oxidative stress. For instance, ROS generated from ETS complexes induce oxidative modifications of mitochondrial proteins, lipids and DNA, which lead to functional instability of mitochondria and thus, links to aging and degenerative diseases [1–3].

In order to cope up with the challenge imposed on mitochondrial integrity, cells have evolved elaborate systems at the levels of damaged protein management and removal of damaged mitochondria by an autophagic process, termed mitophagy [4–6]. In contrast, outer-membrane proteins, which are exposed to the cytosol, can be ubiquitin conjugated and thereby, removed by the 26S proteasome or used as a recognition signal for mitophagy [4,7].

Sqstm1/p62, a protein induced by oxidative stress, interacts with ubiquitin-conjugated proteins and microtubule-associated protein 1A/1B-light chain 3 (LC3), thereby mediating autophagic removal of ubiquitin-conjugated proteins and cellular organelles such as mitochondria [7–9]. In addition, p62 also functions in the non-canonical activation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) through facilitating the degradation of Kelch-like ECH-associated protein 1 (Keap1), a cellular Nrf2 inhibitor [10,11]. Recently, we observed that $p62^{-/-}$ mice exhibited rapid aging in association with compromised mitochondrial electron transport, due to attenuated activation of the non-canonical Nrf2 pathway (Kwon et. al., unpublished results). In the present study, we provide evidence that a portion of p62 directly localizes within the mitochondria and interacts with oxidation-prone proteins including a few ETS components as well as with multiple chaperone molecules and redox regulatory enzymes. Furthermore, *in vitro* delivery of p62 into $p62^{-/-}$ mitochondria partially reduced ROS generation. These implicate p62 as a direct participant in maintenance of mitochondrial integrity. Thus, p62 likely contributes to mitochondrial integrity at three different levels; the non-canonical activation of Nrf2, mediation of ubiquitn-dependent mitophagy, and damaged protein triage within the mitochondria.

2. Materials and methods

2.1. Animals and sample preparation

Animal procedures complied with NIH guidelines and were approved by the Sungkyunkwan University Animal Care and Use

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Committee. Mitochondria were isolated from brains of wild-type and $p62^{-/-}$ mice and purified by differential centrifugation and iodixanol density gradient centrifugation [Ref. [12] and Kwon et. al., unpublished results]. Briefly, whole tissues were homogenized in the isolation buffer (10 mM Hepes, 250 mM sucrose, 1 mM EDTA, pH 7.4, supplemented with protease inhibitor cocktail), and tissue debris and nuclei were removed by centrifugation twice at 2000*g* for 5 min at 4 °C. Supernatant was then centrifuged at 17,000*g* for 10 min, and the resultant pellet resuspended in the isolation buffer (P17 fraction). The P17 fraction was adjusted to the 36% iodixanol concentration, by adding 2.57 volume of 50% iodixanol working solution (OptiPrep, Nycomed Pharm). On this, 30% and 10% iodixanol was layered sequentially, and centrifuged at 50,000*g* for 4 h in an SW41 rotor (Beckman). Mitochondria were collected from the 10% and 30% interface, washed with isolation buffer to remove iodixanol, and then suspended in the isolation buffer (Op-M fraction). Mitochondria were always freshly prepared, and lysate was prepared by solubilizing mitochondria in the lysis buffer (20 mM Tris, pH 7.4, 2% CHAPS, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 5% glycerol, supplemented with protease inhibitor cocktail). For preparation of whole tissue lysate, tissues were homogenized in the lysis buffer, centrifuged at 17,000*g* for 30 min at 4 °C, and the resultant supernatant recovered and used.

2.2. Gel filtration and affinity column chromatography

After filtration of total tissue lysate or Op-M lysate through a 0.45 μm polyethersulfone membrane (Millipore), proteins were separated on a Superose-6 10/300 column (Amersham) at the flow rate of 0.5 ml/min and collected 1 ml each. Protein standards (Amersham) were used to determine the molecular weights of proteins. Anti-p62 affinity column, which was prepared by crosslinking antigen-purified rabbit polyclonal anti-p62 antibody to protein A-agarose beads (Upstate), was loaded with total tissue lysate or Op-M lysate after pre-clearing on the normal IgG column, washed with 10 column volume of lysis buffer, eluted with 100 mM Glycine–HCl (pH 2.5) and neutralized using 1 M Tris.

2.2.1. 2D-PAGE and MALDI-TOF

Iso-electric focusing (IEF) was performed with the PROTEAN-IEF Cell (Bio-Rad). Protein was precipitated with the ice-cold acetone, solubilized in rehydration solution (8.0 M urea, 2% NP-40, 20 mM DTT, 0.5% IPG buffer, 0.002% bromophenol blue) and applied to 7 cm pH 3–10 Immobiline dry strip (Amersham). After rehydration for 12 h at 20°C, proteins were focused by a sequential gradient procedure of 100 V for 1 h, 500 V for 1 h, 3000 V for 1 h, and 5000 V for 3 h. After IEF, strips were equilibrated in buffer-I (375 mM Tris–HCl, pH 8.8, 6 M urea, 20% glycerol, 2% SDS and 130 mM DTT) for 15 min, then re-equilibrated in buffer-II containing 135 mM iodoacetamide instead of DTT for 15 min. Strips were then loaded on 12.5% polyacrylamide gels for the second-dimension SDS–PAGE, and gels were stained with silver nitrate. Protein spots were excised, destained with 30 mM potassium ferricyanide and 100 mM sodium thiosulfate (1:1), and reduced and alkylated with 5 mM DTT and 55 mM iodoacetamide in 25 mM ammonium bicarbonate, respectively. Gel pieces were dehydrated with acetonitrile, and rehydrated and digested with 25 mM ammonium bicarbonate containing 50 ng trypsin (Promega). Peptides were then extracted with 2.5% trifluoroacetic acid in 50% acetonitrile, and analyzed by Ultraflex TOF/TOF (Bruker) at the Proteome Lab in Sungkyunkwan Medical School.

2.3. Immunofluorescence staining

Mouse embryonic fibroblasts (MEFs), grown on a coverslip, were incubated with 100 nM Mito-Tracker Red (Molecular Probes) in the culture medium at 37 °C for 30 min, washed briefly with PBS, and then fixed and permearbilized using 4% paraformaldhehyde and 0.1% Triton X-100, respectively. Cells were then washed three times with PBS, blocked with 5% bovine serum albumin in PBS, immunostained with anti-p62 antibody and FITC-conjugated goat anti-rabbit IgG (Zymed), and examined using Zeiss LSM510 confocal microscope.

2.4. Biochemical analysis

Proteinase-K protection assay was performed by treating Op-M fraction (100 μg protein) with 50 ng/mL proteinase K (Sigma) in an isotonic medium (10 mM Hepes, 250 mM sucrose, 0.1 mM EGTA, pH 7.4) at 4 °C for 20 min, and the reaction was stopped by addition of Phenylmethylsulfonyl fluoride. Mitochondria were then solubilized in the lysis buffer, and proteins were analyzed by immunoblotting. NADH–Ubiquinone oxidoreductase and Succinate–Ubiquinone oxidoreductase activities were measured by following the decrease in absorbance due to the oxidation of NADH at 340 nm or to oxidation of 2,6-dichlorophenolindophenol (DCPIP) at 600 nm, respectively [13]. For this, Op-M fraction (30 μg protein) were lysed by freeze and thaw three times in 100 μl hypotonic buffer (25 mM potassium phosphate, 5 mM $MgCl_2$, pH 7.2) and used as the activity source. The ATP synthesis rate of mitochondria (100 μg Op-M fraction) in the presence of either complex-I substrate (glutamate/malate) or that of complex-II (succinate) was measured by the luciferin–luciferase method [14], using ATP bioluminescent assay kit (Sigma). The rate of mitochondrial H_2O_2 generation (250 μg/ml Op-M fraction) in the presence of either glutamate/malate or succinate was determined by linear increase in fluorescence of oxidized homovanillic acid, using SPECTRAmax Plus microplate fluorometer [15].

3. Results and discussion

3.1. Proteins associated with the p62-containing mega-dalton complex

When mouse liver total lysate was separated on the Superose-6HR gel-filtration column, p62 was eluted mostly in fractions 7–12 (Fig. 1A). Calibration with standard proteins suggests that p62 migrates in the molecular weight range between 600 kDa and 2 mDa, suggesting that p62 constitutes part of heterogeneous mega-dalton complexes. In order to characterize the mega-dalton sized complexes, proteins specifically bound to p62 in the fraction number 8–11 were purified on an anti-p62 antibody affinity column after passing through a normal IgG pre-affinity column. Anti-p62 antibody used in this study demonstrated high specificity, detecting p62 almost exclusively in the liver lysate, and the anti-p62 antibody column efficiently retained p62 with a number of proteins (Supplementary Fig. S1).

Proteins co-purified from the affinity column were then separated on the two-dimensional gel electrophoresis (2D-PAGE), and some of those characterized by MALDI-TOF analysis (Fig. 1B). These proteins included chaperone molecules (Grp78 and Hsp70), protein redox regulatory enzymes (GST θ and μ), other cytosolic enzymes (PFK-2, 12-LOX, Smok-2) and cytoskeletal proteins (actin and vimentin). Strikingly, the mitochondrial proteins, prohibitin as well as a few ETS components including p24 and p75 subunits of complex-I and α-subunit of F_1-ATP synthase, were also abundantly found in the p62-copurified protein pool. Thus, p62 seems to form a specific type of large protein complex with limited groups of cytosolic and mitochondrial proteins.

As most mitochondrial proteins, including those co-purified with p62, are translated by cytosolic ribosomes, p62 might have interacted with those proteins in the cytosol prior to their mitochondrial

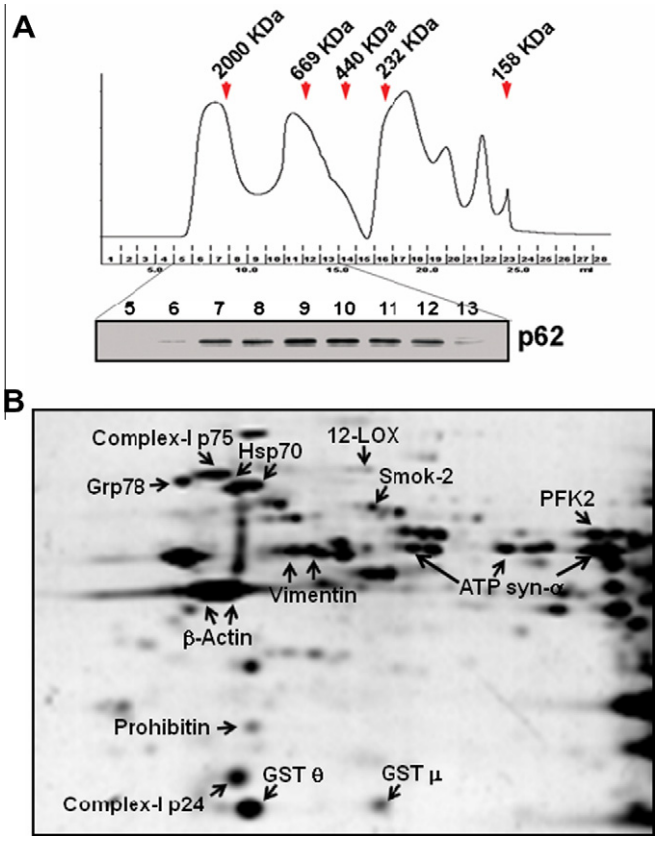

Fig. 1. Proteins associated with the p62-containing mega-dalton complex. (A) Elution profile of p62 in the mouse liver lysate on a Superose-6 10/300 column measured by absorption at 280 nm (upper panel) and immunoblotting (bottom panel). Elution of molecular weight markers including blue dextran (2000 kDa), thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and aldolase (158 kDa) were marked by arrowheads. (B) Resolution of the proteins co-purified with p62 by an anti-p62 antibody column from the fractions 8–11 of Fig. 1A on 2D-electrophoresis. First dimension: Immobilized pH gradient 3–10; second dimension: 12.5% SDS–PAGE. Proteins were stained with silver nitrate and identified by MALDI-TOF.

import. Alternatively, a portion of p62 may interact with proteins within the mitochondria in a yet uncharacterized manner.

3.2. Mitochondrial localization of p62

Interestingly, confocal immuno-fluorescence imaging of a small population of wild-type MEFs exhibited mitochondrial localization of p62 (Fig. 2A). Furthermore, fractionation of wild-type mouse liver homogenate revealed a significant amount of p62 present in the mitochondria-enriched fraction (P17) and iodixanol gradient-purified mitochondrial fraction (Op-M) (Fig. 2B). When estimated, less than 5% of total cellular p62 appeared to associate with the Op-M fraction. Furthermore, both p62 and the mitochondrial matrix protein, ATP synthase α-subunit, in purified mitochondria were similarly protected from exogenous proteinase-K digestion (Fig. 2C). In contrast, the mitochondrial outer-membrane protein, Bcl-2, was degraded by the same protease. Hence, we conclude that a portion of cellular p62 localizes within the mitochondria.

In addition, the $p62^{+/-}$ mitochondria maintained the p62 level comparable to that of the wild-type ($p62^{+/+}$) mitochondria, despite far lower levels of p62 expression in the $p62^{+/-}$ tissues (Fig. 2D). Furthermore, the $p62^{+/-}$ mitochondrial ETS maintained a normal integrity, as evidenced by the rate of ROS production similar to that associated with the wild-type mitochondria (Fig. 2E). These results further support that p62 is primarily targeted to the mitochondria.

3.3. p62-binding proteins in the brain mitochondria

In order to characterize p62-binding proteins within the mitochondria more closely, mouse brain mitochondria were enriched by an iodixanol gradient and used for protein purification (Supplementary Fig. S2A). Gel filtration analysis on proteins of the mitochondrial lysate suggests that p62 also forms protein complexes larger than 1 mDa in the mitochondria (Supplementary Fig. S2B). As expected, p62 in the mitochondrial lysate was retained only in the anti-p62 antibody column but not in the rabbit IgG pre-affinity column (Supplementary Fig. S2C). Proteins co-purified with p62 on the anti-p62 antibody column were then separated on the 2D-PAGE, and 53 protein spots analyzed by MALDI-TOF (Fig. 3A). The analysis identified 46 proteins, including 28 mitochondrial resident proteins and 9 proteins related to protein quality control in the cytoplasm or endoplasmic reticulum (ER) (Fig. 3A and Supplementary Table 1).

The p62-binding mitochondrial proteins belonged to categorized groups of; (i) chaperone molecules (HspA9 and prohibitin), (ii) protein redox regulatory enzyme (thioredoxin reductase-2), (iii) ETS components including p24 and p30 subunits of complex-I, Core-1 and Rieske subunits of complex-III, and α-, β- and δ-subunits of F_1-ATP synthase, and (iv) enzymes for other energy metabolism. Interaction of p62 with a few ETS components and chaperone molecules was further verified by co-immunoprecipitation analysis (Fig. 3B). Interestingly, p70 subunit of complex-II but not Cox4 subunit of complex-VI was clearly co-immunoprecipitated with p62, although both were not detected during the analysis. These results suggest that proteins identified in these experiments were specifically co-purified with p62, but the analysis reveals only an incomplete list of p62-binding proteins.

3.4. ETS as a direct target of p62 within the mitochondria

The p24, p30, and p75 subunits of complex-I and the p70 subunit of complex-II are core components for electron transport to ubiquinone from NADH and $FADH_2$, respectively [16,17]. Thus, we examined if p62 affects the electron transport of complex-I and -II. As anticipated, $p62^{-/-}$ mitochondria exhibited reduced activities of both complex-I (NADH–Ubiquinone oxidoreductase) and complex-II (Succinate–Ubiquinone oxidoreductase) by 15–20%, compared to wild-type counterparts (Fig. 4A and B). Furthermore, the ATP synthesis rate of $p62^{-/-}$ mitochondria retained only about 50% that of wild-type mitochondria in the presence of glutamate and malate (complex-I substrate) or succinate (complex-II substrate) (Fig. 4C). Reflecting this, $p62^{-/-}$ mitochondria always generated more H_2O_2 than wild-type controls in the presence of either substrate (Fig. 4D). Remarkably, pre-incubation of $p62^{-/-}$ mitochondria for 1 h with the wild-type cytosolic fraction resulted in mitochondrial localization of p62 (Fig. 4E, bottom panel) as well as reduction in the rate of H_2O_2 generation in the presence of either glutamate/malate or succinate (Fig. 4E, top panel). Together, our data strongly imply that a portion of p62 localizes within the mitochondria and directly supports the integrity of mitochondrial functions at the vicinity of target components.

3.5. Implicated roles of p62 within the mitochondria

p62 has been postulated to support mitochondrial integrity in the cell, either through recruiting damaged mitochondria for mitophagy [7] or through securing persistent expression of Nqo1 by non-canonical activation of Nrf2 (Kwon et. al., unpublished results). In the present study, we provide additional insight into the role of p62 as a direct promoter of mitochondrial integrity.

Mitochondrial proteins co-purified with p62 can be grouped into four different classes: chaperones, protein redox regulatory enzyme,

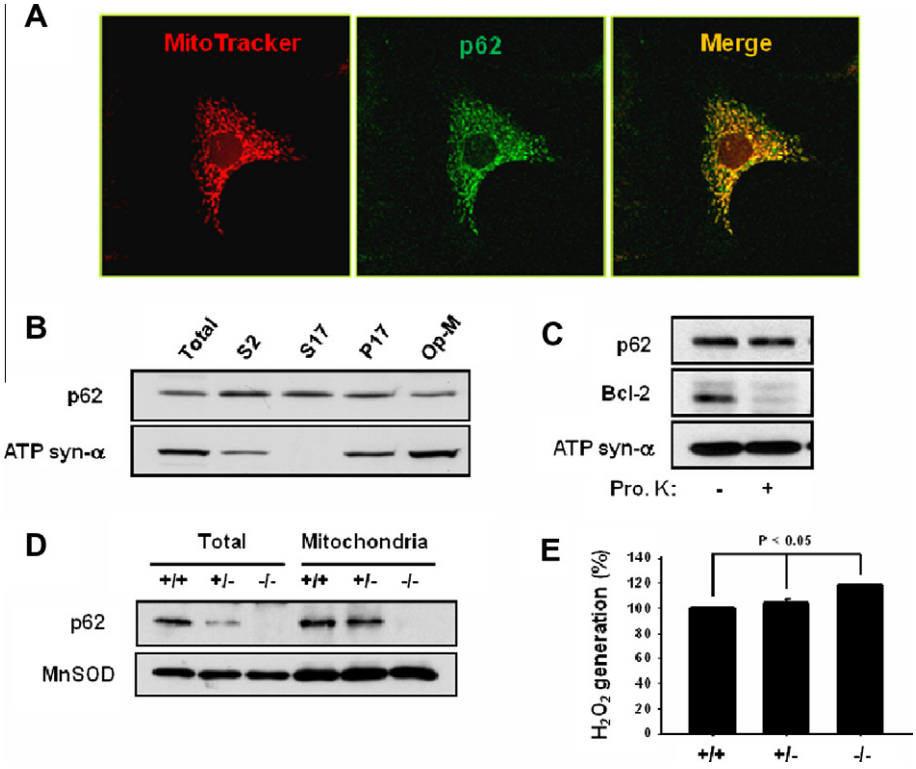

Fig. 2. Mitochondrial localization of p62. (A) Confocal images of MEF stained with an anti-p62 antibody and MitoTracker Red. (B) p62 and ATP synthase α-subunit (ATP syn-α) in the mouse liver subcellular fractions. S2, supernatant of 2000*g* centrifugation; S17 and P17, supernatant and pellet, respectively, of 17,000*g* centrifugation; Op-M, mitochondria purified by Opti-Prep (iodixanol) gradient of P17 fraction. 20 μg protein each for samples except 40 μg for Op-M was immunoblotted. (C) Proteinase-K protection assay; p62, Bcl-2, and ATP synthase α-subunit in Op-M fraction, before and after proteinase-K digestion. (D) p62 levels in mitochondrial lysate and total liver homogenates of wild-type ($p62^{+/+}$), $p62^{+/-}$ and $p62^{-/-}$ mice measured by immunoblot analysis. (E) The rates of H_2O_2 generation in the mitochondria isolated from wild-type, $p62^{+/-}$ and $p62^{-/-}$ mice were presented as the per cent that of wild-type mitochondria ($n = 5$ per group).

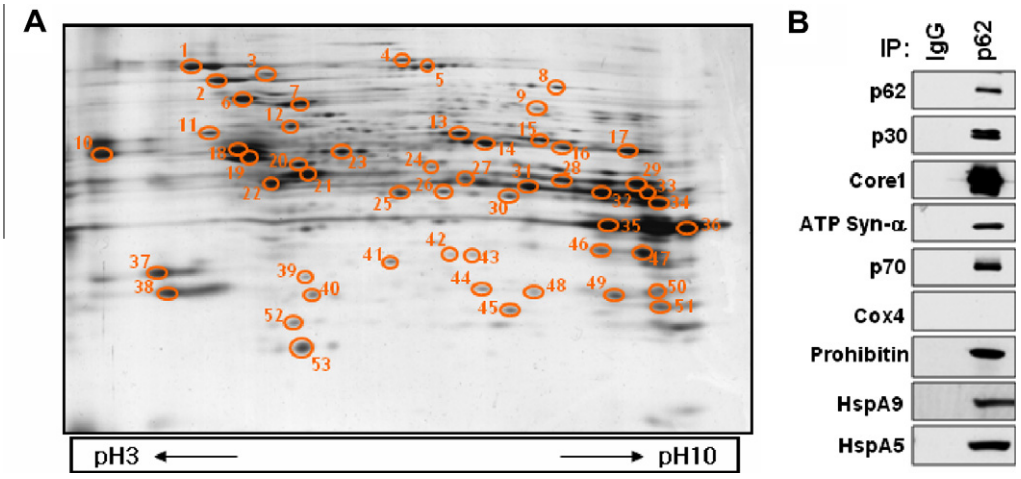

Fig. 3. Interaction of p62 with mitochondrial proteins. (A) Resolution of the proteins co-purified with p62 from mitochondrial lysate on 2D-electrophoresis. Protein spots identified by MALDI-TOF were marked by numbers, and full description is in Supplementary Table 1. (1) Hsp90B1, (2) Hsp90A1, (3) VCP, (4) oxoglutarate dehydrogenase-like, (5) Dynamin, (6) HspA5, (7) HspA9, (8) Aconitase-2, (9) Synapsin-II, (10) Calreticulin, (11) Protein disulfide-isomerase, (12) ST7 protein isoform-a, (13) Dihydrolipoamide dehydrogenase, (14) Glutamate dehydrogenase-1, (15) Thioredoxin reductase-2, (16,17) ATP synthase-α, (18) ATP synthase-β, (19) Enolase-2, (20) Complex-III Core-1, (21) Creatine kinase B-type, (22) Endophilin-A1, (23) E2 component of oxoglutarate dehydrogenase complex, (24) EF-Tu, (25) DnaJB11, (26) Short-chain specific acyl-CoA dehydrogenase, (27) Long-chain specific acyl-CoA dehydrogenase, (28) Mitochondrial creatine kinase, (29) Citrate synthase, (30) Aldolase-C, (31,32,33) Acetyl-CoA acetyltransferase, (34) Aldolase-A, (35,36) Malate dehydrogenase, (37) 14-3-3 ε, (38) 14-3-3 ζ, (39) Prohibitin, (40) Complex-I 30 kDa, (41) Steroid dehydrogenase, (42,43) Arp2/3 complex, (44) Adenylate kinase isozyme-4, (45) Complex-III Rieske subunit, (46,47) Short chain Hydroxyacyl-coenzyme dehydrogenase, (48,49) Enoyl-CoA hydratase, (50) Proline synthase co-transcribed, (51) Hydroxyacyl-Coenzyme A dehydrogenase type II, (52) Complex-I 24 kDa, (53) ATP synthase-δ. (B) Immunoblot analysis of ETS components and chaperones co-immunoprecipitated from the mitochondrial lysate by rabbit normal IgG or anti-p62 antibody.

ETS components, and enzymes for other energy metabolism. Proteins in the first two categories function in proper folding, repair or removal of damaged or newly synthesized proteins. For instance, prohibitin forms a ring-like structure at the inner-membrane and functions as a holdase/unfoldase type of chaperone, controlling the quality of ETS in association with the mitochondrial m-AAA

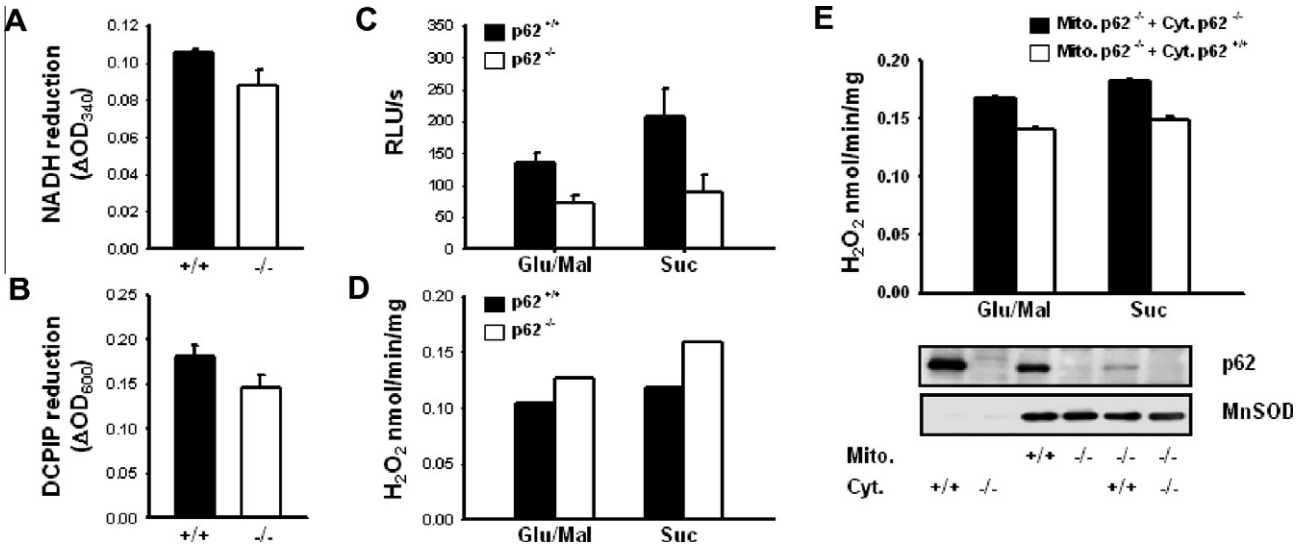

Fig. 4. Effect of mitochondrial localization of p62 on electron transport. (A) Complex-I (NADH–Ubiquinone oxidoreductase) activity of wild-type ($p62^{+/+}$) and $p62^{-/-}$ mitochondria as the rate of decrease in absorbance of NADH (ΔOD_{340}/min), (B) Complex-II (Succinate–Ubiquinone oxidoreductase) activity of wild-type and $p62^{-/-}$ mitochondria as the rate of decrease in absorbance of DCPIP (ΔOD_{600}/min). (C) The rates of ATP synthesis of wild-type and $p62^{-/-}$ mitochondria in the presence of glutamate/malate or succinate. (D) The rates of H_2O_2 production (H_2O_2 nmol/min/mg) in wild-type and $p62^{-/-}$ mitochondria. (E) The rates of H_2O_2 production of $p62^{-/-}$ mitochondria after reconstitution with either wild-type or $p62^{-/-}$ cytosolic fraction in the presence of glutamate/malate or succinate (upper panel). Immunoblots (bottom panel, 40 μg protein/lane) show the p62 levels in the $p62^{-/-}$ mitochondria after reconstitution. Data are presented as mean ± SD (n = 3).

proteases [18]. HspA9, also called mortalin or mtHsp70, unfolds mitochondrial targeting polypeptides for unidirectional translocation across mitochondrial membranes or disaggregates misfolded proteins of the inner-membrane or matrix for degradation by m-AAA and LON proteases, respectively [19]. Tu translation elongation factor also functions as a chaperone at the inner-membrane where mitochondrial ribosomes are found [20]. In addition, thioredoxin reductase-2 donates electrons to thioredoxin- or glutaredoxin-mediated regeneration of thiol-oxidized proteins in the mitochondria [21].

Interestingly, 9 of 16 non-mitochondrial proteins co-purified with p62 also belong to the same categories. These include cytoplasmic or ER chaperones (Hsp90A1, Hsp90B1, HspA5, DnaJB11, calreticulin and 14-3-3 ε and ζ), thiol-redox regulatory enzyme (protein disulfide isomerase) and vasolin-containing protein (VCP), an AAA+ ATPase that dislodges ubiquitin-conjugated proteins from the ER for proteasomal degradation [22]. Interaction of p62 with these groups of proteins implicates the protein triage role of p62 for repair or removal of damaged proteins in the context of the mega-dalton complex in the mitochondria and other intracellular compartments.

Many proteins classified in the latter two categories are well-known oxidation-prone enzymes, particularly through oxidation of cysteinyl thiol groups. Generally, proteins containing Fe/S cluster are more susceptible to oxidative damages [6]. Of the p62-binding proteins in these categories, all three subunits of complex-I (p24, p30 and p75) and the Rieske subunit of complex-III contain 2–4 Fe/S centers [17,23]. Aconitase of the TCA cycle, another Fe/S-containing enzyme, is a well-known oxidation-prone protein [24]. In addition, thiols of lipoate prosthetic group attached to the E2 component of α-ketoglutarate dehydrogenase (α-KGDH) complex has been shown to be sensitive to ROS [25]. Importantly, although the ETS complex-I and -III are the major ROS generation sites in the mitochondria, the α-KGDH complex and the ETS complex-II are also involved in mitochondrial ROS generation [25–27]. Thus, as all of these oxidation-prone proteins are part of multimeric protein complexes generating oxidants themselves, they need to be monitored more thoroughly by protein quality control system for repair or removal. In addition, Creatine kinase and Adenylate kinase, which constitute the enzymatic phosphotransfer network for energy homeostasis, as well as other p62-binding proteins such as Core-1 subunit of complex-III, ATP synthase α-subunit, Aldolase A and C, Enoyl-CoA hydratase, Malate dehydrogenase and Synapsin-2 are also sensitively oxidized by ROS [28–31]. Given that the p62 gene is activated upon oxidative stress [10], p62-binding proteins of these categories are seemingly direct substrates of the p62 function.

Considered in the context of compromised mitochondrial function by the loss of p62, these observations strongly implicate p62 in the triage of oxidatively-damaged proteins. This is done in cooperation with chaperone molecules, thereby creating an environment suitable for the efficient regeneration or removal of oxidatively modified proteins that reflecting the role of cytosolic p62 in sequestration of ubiquitin-conjugated proteins [8,9]. Further analysis on the biochemical role of p62 in the context of a mega-dalton complex associated with other protein quality control mechanisms will elucidate how cells maintain intact mitochondrial function.

Conflict of interest

The funding agency (STC Life, Inc.) was not involved in any process of the present study including research design, data collection, analysis and interpretation, and preparation and submission of the manuscript.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.08.067.

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