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Oxidative stress causes reversible changes in mitochondrial permeability and structure

Nelson B. Cole [a,b,*], Mathew P. Daniels [c], Rodney L. Levine [a,*], Geumsoo Kim [a,*]

[a] Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, Building 50, Room 2351, Bethesda, MD 20892-8012, USA
[b] Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, Building 50, Room 2507, Bethesda, MD 20892-8017, USA
[c] Electron Microscopy Core Facility, National Heart, Lung, and Blood Institute, Building 14E, Room 111B, Bethesda, MD 20892-5570, USA

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ABSTRACT

Mitochondria are a primary source as well a principal target of reactive oxygen species within cells. Using immunofluorescence microscopy, we have found that a number of mitochondrial matrix proteins are normally undetectable in formaldehyde-fixed cells permeabilized with the cholesterol-binding detergent saponin. However, exogenous or endogenous oxidative stress applied prior to fixation altered the permeability of mitochondria, rendering these matrix proteins accessible to antibodies. Electron microscopy revealed a loss of matrix density and disorganization of inner membrane cristae upon oxidative stress. Notably, the changes in permeability and in structure were rapidly reversed when the oxidative stress was relieved. The ability of reactive oxygen species to reversibly alter the permeability of the mitochondrial membrane provides a potential mechanism for communication within the cell such as between nucleus and mitochondria.

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1. Introduction

Translocation of proteins from one cellular site to another is an important mechanism in cellular regulation. Various stimuli can trigger the movement of proteins through a variety of processes, including receptor-mediated endocytosis, nuclear import and export, and covalent modifications such as phosphorylation and myristoylation. Oxidative stress is capable of triggering translocation of proteins to mitochondria (Orsini et al., 2004; Li et al., 2005; Noh et al., 2009). The methionine sulfoxide reductases are antioxidant enzymes which are present in most organisms, from microbes to plants and animals (Weissbach et al., 2005). They can reduce free or protein bound methionine sulfoxide back to methionine, thus functioning both in repair of oxidatively damaged molecules and as scavengers of reactive oxygen and nitrogen species (Stadtman et al., 2002). In mammals, methionine sulfoxide reductase A (MsrA) is encoded by a single gene but is present both in the cytosol and the mitochondria (Hansel et al., 2002; Vougier et al., 2003). In the course of our studies on the subcellular localization of MsrA, we observed that when cells were treated with exogenous oxidants such as hydrogen peroxide, cytosolic MsrA appeared to physically translocate from the cytosol to mitochondria, as assayed by immunofluorescence microscopy. Subsequent analyses revealed that the apparent translocation was in fact due to the exposure of previously hidden epitopes on mitochondrial matrix-localized MsrA. This effect was not limited to MsrA, but could be demonstrated with a number of different matrix proteins. We also found that the effect was fully reversible, indicating that mitochondria have the capacity to reverse the oxidant-induced changes that altered their accessibility and morphology.

2. Materials and methods

2.1. Cell lines and reagents

Mouse embryonic fibroblasts (MEF) from transgenic mice overexpressing MsrA were kindly provided by Hang Zhao (Laboratory of Biochemistry, National Heart, Lung, and Blood Institute). Generation of these mice will be described elsewhere. Early-to-middle passage (6–20) cells were grown in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, and penicillin and streptomycin and maintained in a humidified 5% CO_2 atmosphere. Glucose oxidase (Type X-S from *Aspergillus niger*; # G7141), rotenone, and antimycin A were from Sigma.

* Corresponding authors.
E-mail addresses: ncole@nhlbi.nih.gov (N.B. Cole), rlevine@nih.gov (R.L. Levine), kimg@nhlbi.nih.gov (G. Kim).

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2.2. Antibodies and immunofluorescence labeling

Polyclonal antiserum against MsrA was generated from rabbits immunized with recombinant mouse MsrA purified from *Escherichia coli*. This recombinant MsrA lacked the N-terminal 20 amino acids that encode the mitochondrial leader peptide. A monoclonal antibody against cytochrome *c* was from BD Pharmingen (# 556432) and used at 1:500 dilution. Anti-Mitochondrial Heat Shock Protein 70 monoclonal antibody was from Affinity Bioreagents (now Thermo Scientific; # MA3-028) and diluted 1:250. Alexa Fluor 488- and 594-conjugated species-specific secondary antibodies were from Invitrogen and used at a 1:500 dilution.

Cells were grown on 12 mm glass coverslips until approximately 50–75% confluent and treated as indicated. They were directly fixed for 20–30 min at room temperature with 2% formaldehyde in phosphate buffered saline (PBS). Fixation with 3.7% formaldehyde in PBS or 3.7% formaldehyde in 0.1 M sodium phosphate, pH 7.2, 150 mM NaCl gave similar results. After fixation, cells were rinsed three times with 10% FBS in PBS. Some cells were treated after fixation with 0.1% Triton X-100 for 1 min, followed by three rinses in PBS prior to rinsing in FBS/PBS. The cells were then incubated with primary antibody or serum diluted in 10% FBS/PBS containing 0.2% saponin for 1 h at room temperature. The cells were then washed three times over a 15 min period with 10% FBS/PBS and incubated with fluorochrome-conjugated secondary antibodies (diluted in 10% FBS/PBS plus 0.2% saponin) for 1 h at room temperature. Cells were again rinsed for 10 min with 10% FBS/PBS and one final rinse with PBS before mounting on a glass slide with Fluoromount G (Southern Biotech) containing 2.5 μg/mL DAPI (4′,6-diamidino-2-phenylindole) to stain nuclei. For treatment of cells with hydrogen peroxide, either a single bolus of hydrogen peroxide (250 μM) was added to cells or alternatively, hydrogen peroxide was generated enzymatically by glucose oxidase (25–125 mU/mL), which produces hydrogen peroxide in a more chronic manner via oxidation of glucose in the cell-culture medium. Similar results were observed for both.

Images were obtained using a LSM 510 confocal microscope (Carl Zeiss, Thornwood, NY) with a 63 × 1.3 numerical aperture

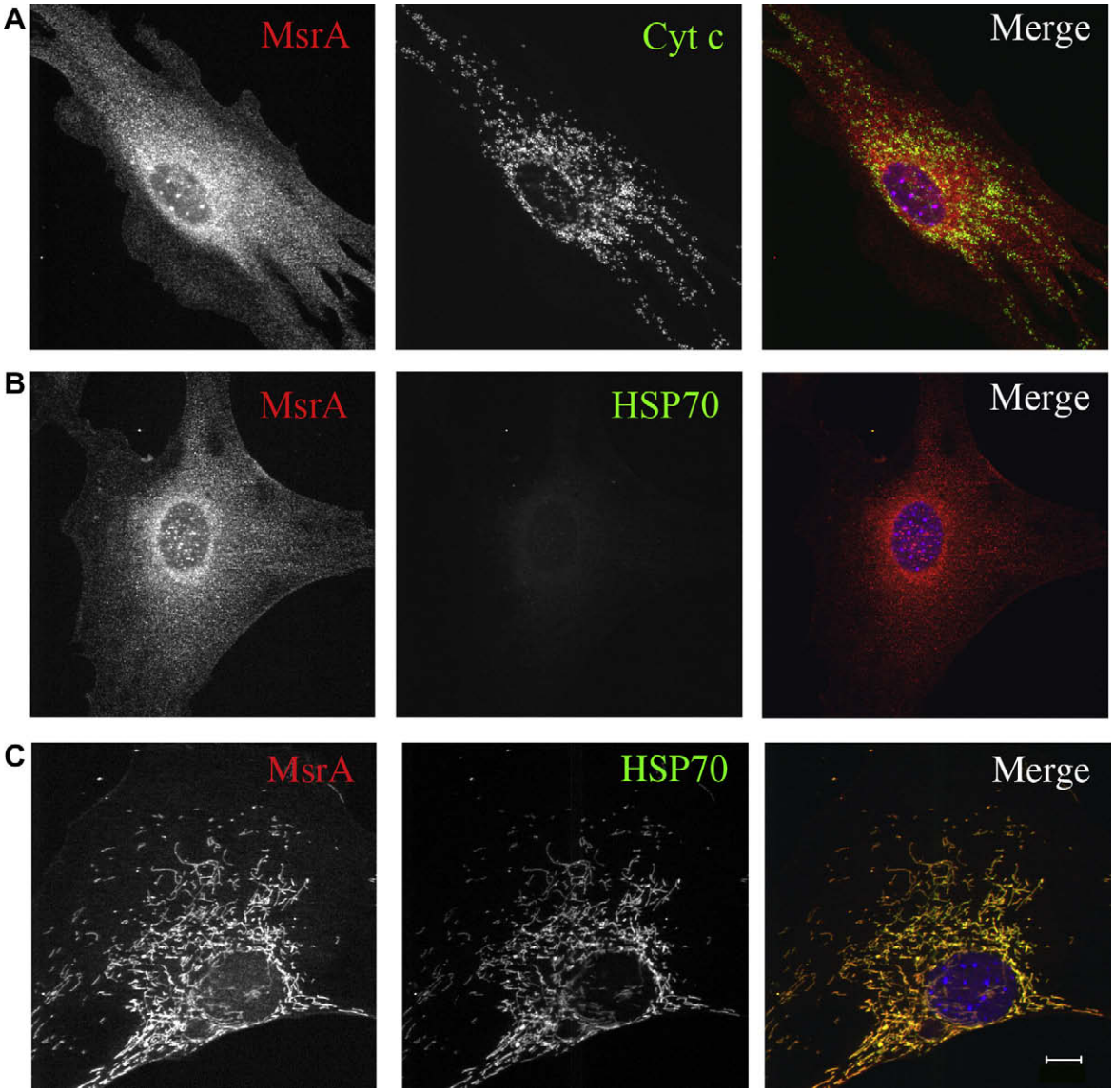

Fig. 1. Immunofluorescence of untreated MEFs expressing mouse MsrA. Cells were fixed in formaldehyde/PBS followed by permeabilization with saponin. Staining was with antibodies to MsrA and cytochrome *c* (A) or MsrA and mtHSP70 (B). Note that MsrA is restricted to the cytosol in saponin permeabilized cells and mtHSP70 is not detectable. In (C) cells were post-fixed with 0.1% Triton X-100 for 1 min prior to antibody staining. Both MsrA and mtHSP70 are visible in mitochondria. Bar = 10 μm.

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