Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1780 (2008) 1273–1290
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbagen

Review

Redox compartmentalization in eukaryotic cells

Young-Mi Go, Dean P. Jones *

Emory Clinical Biomarkers Laboratory and Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, Emory University, Atlanta GA 30322, USA

Received 19 November 2007; received in revised form 11 January 2008; accepted 14 January 2008
Available online 26 January 2008

Abstract

Diverse functions of eukaryotic cells are optimized by organization of compatible chemistries into distinct compartments defined by the structures of lipid-containing membranes, multiprotein complexes and oligomeric structures of saccharides and nucleic acids. This structural and chemical organization is coordinated, in part, through cysteine residues of proteins which undergo reversible oxidation-reduction and serve as chemical/structural transducing elements. The central thiol/disulfide redox couples, thioredoxin-1, thioredoxin-2, GSH/GSSG and cysteine/cystine (Cys/CySS), are not in equilibrium with each other and are maintained at distinct, non-equilibrium potentials in mitochondria, nuclei, the secretory pathway and the extracellular space. Mitochondria contain the most reducing compartment, have the highest rates of electron transfer and are highly sensitive to oxidation. Nuclei also have more reduced redox potentials but are relatively resistant to oxidation. The secretory pathway contains oxidative systems which introduce disulfides into proteins for export. The cytoplasm contains few metabolic oxidases and this maintains an environment for redox signaling dependent upon NADPH oxidases and NO synthases. Extracellular compartments are maintained at stable oxidizing potentials. Controlled changes in cytoplasmic GSH/GSSG redox potential are associated with functional state, varying with proliferation, differentiation and apoptosis. Variation in extracellular Cys/CySS redox potential is also associated with proliferation, cell adhesion and apoptosis. Thus, cellular redox biology is inseparable from redox compartmentalization. Further elucidation of the redox control networks within compartments will improve the mechanistic understanding of cell functions and their disruption in disease.

Keywords: Mitochondria; Nuclei; Plasma membrane; Endoplasmic reticulum; Cytoplasm; Thioredoxin; Glutathione; Peroxiredoxin; Glutaredoxin; Oxidation-reduction potential; Non-equilibrium thermodynamics

1. Introduction

The compartmentalization of peroxide-metabolizing systems within peroxisomes has long been recognized to provide a means to utilize hydrogen peroxide as a metabolic reagent while at the same time protect redox-sensitive cell components in other cell compartments from oxidative damage. Accumulating knowledge of redox characteristics in mitochondria, cytoplasm, nuclei and the secretory pathway indicate that unique redox characteristics have evolved with each of the major compartments in mammalian cells.

Elucidation of details has been advanced by improved sensitivity and spatial resolution of redox indicators in living cells but remains somewhat limited by difficulties in calibration and ability to confirm with fractionation due to auto-oxidation and redistribution artifacts. However, methods are progressively being improved so that an understanding of subcellular redox characteristics is emerging [1,2]. In particular, development of redox western blot techniques for measuring redox states of proteins [3,4] has allowed quantification of steady-state redox potentials of proteins in different subcellular compartments [2,4,5]. By creating constructs to target accumulation of proteins in specific compartments, systematic mapping of redox potentials in different compartments has become possible. Targeting has been achieved using mitochondrial localization sequences (MLS), nuclear localization signals (NLS), nuclear export signals (NES), and endoplasmic reticulum retention signals. The approaches have been complemented by techniques to measure redox potential in living cells, largely based upon partitioning of lipophilic cationic fluorescent chemicals into mitochondria dependent upon the high mitochondrial membrane potential ($\Delta\psi$) [6] and

* Corresponding author. 205 Whitehead Research Center, Emory University, Atlanta, GA 30322. Tel.: +1 404 727 5970; fax: +1 404 712 2974.
E-mail address: dpjones@emory.edu (D.P. Jones).

0304-4165/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagen.2008.01.011

development of redox-sensitive forms of green fluorescent protein [7]. Detection of disulfide reduction in the endosome has also been achieved by the use of FRET [8]. Application of these methods to diverse systems has begun to clarify structural and chemical aspects of cellular functions which depend upon thiol/disulfide redox elements.

2. Mitochondrial redox regulation

Mitochondria are the most redox-active compartment of mammalian cells, accounting for more than 90% of electron transfer to O_2 as the terminal electron acceptor. The predominant electron transfer occurs through a central redox circuit which uses the potential energy available from oxidation of various metabolic substrates (e.g., pyruvate, fatty acids) to generate ATP. The mitochondrial inner membrane contains 5 multiprotein complexes with central functions in oxidative phosphorylation. Three of these complexes are redox-driven proton pumps: complex I (NADH-quinone oxidoreductase), complex III (coenzyme Q: cytochrome reductase), and complex IV (cytochrome oxidase). Complex II (succinate dehydrogenase) transfers electrons into the chain from succinate, and complex V (ATP synthase), is a reversible proton pump which uses the electrochemical proton gradient ($\Delta\mu_H^+$) to drive ATP synthesis.

Regulation of this process is central to cell function because cells must produce ATP while at the same time maintaining an appropriate homeostasis in terms of supply of non-essential amino acids, eliminating excess amino acids, supplying glucose and interconverting energy precursors to allow for long-term energy supply in the face of variable and intermittent food intake. Part of the regulation appears to occur through a continuous low rate of reactive oxygen species (ROS) generation, which regulates the magnitude of $\Delta\mu_H^+$ through control of Fe–S containing dehydrogenases and uncoupling proteins [9]. The molecular sensors and associated redox circuitry for this regulation remain poorly defined, but such regulation requires a specialized redox environment. In addition, the molecular machinery required for oxidative phosphorylation is highly dependent upon critical cysteine residues of proteins for enzymatic and transport activities [10], and the mitochondrial genome is susceptible to oxidative damage [11,12]. These processes are directly relevant to mitochondrial oxidative stress-related diseases such as Parkinson's disease [13], Friedreich's ataxia [14], Huntington disease [15], and diabetes [16], and improved understanding can be expected to aid the development of improved therapies for these diseases.

2.1. Oxidants in mitochondria

ROS, principally superoxide anion radical ($O_2^{\cdot-}$) and its dismutation product H_2O_2, are derived from several sources in mitochondria. Within the electron transport chain, Complexes I and III are the main sites of electron transfer to O_2 to produce $O_2^{\cdot-}$ [17]. Inhibitors of complexes I and III, rotenone and antimycin A, respectively, stimulate $O_2^{\cdot-}$ generation at these sites [18,19]. In addition to the electron transport chain, mitochondrial ROS may also be generated by pyruvate, α-ketoglutarate dehydrogenase, glycerol-3-phosphate dehydrogenase, and monoamine oxidase [20–23]. In apoptotic signaling, release of cytochrome *c* from mitochondria results in stimulated ROS generation [24]. Under excessive oxidative stress, simultaneous collapse of the mitochondrial membrane potential and a transient increase in ROS generation by the electron transfer chain can result in mitochondrial release of ROS to cytosol. This can trigger "ROS-induced ROS release" in neighboring mitochondria [25]. Thus, although a low rate of ROS generation is a normal process in mitochondria, disruption of electron flow with excessive ROS generation can activate cell death.

In addition to ROS, reactive nitrogen species (RNS) including nitric oxide (NO) and peroxynitrite ($ONOO^-$), formed from reaction of NO with $O_2^{\cdot-}$, are present. The interaction of NO with mitochondria is also a potential mechanism for mitochondrial redox regulation. NO at physiological levels in cells does not appear to be directly toxic but can be converted to more reactive species [26]. Similar to ROS reactivity to protein thiols (-SH in cysteine residue), NO is also reactive to protein thiols and glutathione (GSH) to form nitrosothiols [27]. NO-modified proteins or other NO metabolites play a key role in regulating cellular redox signaling [27].

In support of a possible role for NO within mitochondria, mitochondrial nitric oxide synthase (mtNOS) has been identified and its function of NO generation in the mitochondria has been reported [28,29]. In addition to stimulation of mitochondrial NO production by calcium-stimulated mtNOS activation, specific interaction of NO with cytochrome oxidase (complex IV) was shown to affect mitochondrial function [30]. Subsequent research demonstrated that mitochondrial function was affected by interactions of mitochondrial NO and its derivatives with glutathione (GSH) and with protein thiols [31].

NO reacts with $O_2^{\cdot-}$ to produce peroxynitrite at a diffusion controlled rate. Peroxynitrite reacts with tyrosine residues in proteins to form nitrotyrosine residues [32,33]. Similar to NO, $ONOO^-$ has a very short half-life, and its effect on cells is dependent on the concentration and proximity to the target molecule.

The mechanisms of ROS and RNS and their control by free radical scavengers have been extensively reviewed [34–37]. In the present review, we primarily discuss only the thiol/disulfide redox-regulated antioxidant systems. Mitochondria have two major thiol antioxidant systems dependent upon GSH and the small protein thioredoxin-2 (Trx2), and each has several associated proteins, e.g., glutaredoxin-2 (Grx2), glutathione peroxidases 1 and 4 (Gpx1, Gpx4), and thioredoxin reductase-2 (TrxR2) and peroxiredoxins 3 and 5 (Prx3, Prx5), respectively.

2.2. NADPH/$NADP^+$ system as reducing power for Trx and GSH system

In contrast to the respiratory electron transport chain in the mitochondria which is dependent upon NADH/NAD^+, both Trx and GSH systems are dependent on the reducing power of NADPH/$NADP^+$. Most mitochondrial dehydrogenases are linked to NAD^+ rather than $NADP^+$, so the rate of electron flow through