Redox Biology 13 (2017) 452–458

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Redox regulation of proteasome function

Maria Lefaki, Nikoletta Papaevgeniou, Niki Chondrogianni*

Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou Avenue, 116 35 Athens, Greece

ARTICLE INFO

Keywords:
Oxidative stress
Proteasome
Free radicals
Redox status

ABSTRACT

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) were initially regarded mainly as metabolic by-products with damaging properties. Over the last decade, our understanding of their role in metabolism was drastically changed and they were recognized as essential mediators in cellular signaling cascades, as well as modulators of biochemical pathways. Proteostasis is highly affected by the various levels of intracellular and extracellular free radicals with either mild or severe outcomes. As part of the proteostatic network, the proteasome system is equally affected by redox alterations. This short review summarizes the effects of oxidative stress on proteasome status while it also recapitulates conditions and processes where redox alterations signal changes to proteasome expression, assembly and function.

1. Oxidative stress

Organisms are obliged to live in an environment that contains 78% nitrogen and 21% oxygen. Therefore, they have adapted to that environment and this is reflected by the mechanisms they possess to maintain a balance between oxidants and antioxidants [1]. Oxidative stress is a term that is used to describe the imbalance between oxidants and antioxidants that occur in favor of oxidants [2].

1.1. Free radicals

Free radicals are in general defined as chemical species that possess unpaired electrons. There are two major species; a) Reactive oxygen species (ROS) that are molecules derived from O_2 and, b) Reactive nitrogen species (RNS) that are molecules derived from nitrogen and oxygen (nitric oxide – NO) [1]. Increase in cellular ROS and RNS levels is caused by both endogenous and exogenous sources. Exogenous sources include smoke, air pollutants, radiation (UV and ionizing) and several drugs. The majority of endogenous ROS are produced through respiration in the mitochondria while the endoplasmic reticulum (ER) and several enzymes are additional ROS sources. In general most of the enzymes that metabolize oxygen may produce ROS i.e. NADPH oxidase (NOX), cytochrome P450 enzymes, lipooxygenases and cyclooxygenase. Superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·) are the most important cellular ROS [1]. Hydroxyl radicals are released by metal storage proteins and heme groups while free copper or iron ions are released from iron-sulphur clusters [3]. Intracellular ROS levels are important. Low levels may lead to cell proliferation, differentiation and in general they can act as signaling messengers, whereas high levels lead to cell death, apoptosis and senescence as they affect all biological macromolecules such as lipids, proteins and nucleic acids. Consequently, the regulation of cellular ROS levels is highly important [2].

1.2. Redox signaling: major pathways involved

ROS are considered as important signaling messengers in the cell, playing a key role in numerous pathways [4]. Nevertheless, when oxidative stress occurs, cellular systems become dysfunctional and cells are led to death. Upon mild or severe oxidative stress, many transcription factors and signaling pathways are triggered, indicating that ROS can promote alterations not only in the gene but also in the protein

Abbreviations: Abcd1, ATP binding cassette subfamily D member 1; AD, Alzheimer's disease; ALD, adrenoleukodystrophy; ALS, amyotrophic lateral sclerosis; AP-1, activator protein-1; APP, amyloid precursor protein; CNS, central nervous system; DDI, DNA-damage inducible 1; D-gal, D-galactose; DS, down syndrome; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-ligase; E4, ubiquitin-prolongation enzyme; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; ESCs, embryonic stem cells; γ-GCS, γ-glutamylcysteinesynthetase; GPX1, glutathione peroxidase-1; GST, glutathione s-transferase; HD, Huntington's disease; HFL-1, human fetal lung fibroblasts; HIF-1, hypoxia inducible factor 1; HO-1, heme oxygenase 1; HSF, heat shock factor; IFN-γ, interferon-γ; JNKs, c-Jun N-terminal kinases; Keap1, kelch-like ECH associated protein 1; Maf, musculoaponeurotic fibrosarcoma protein; MAPKs, mitogen- activated protein kinases; MEFs, mouse embryonic fibroblasts; NF-kB, nuclear factor-kappa B; Nrf2, nuclear factor (erythroid-derived-2)-like 2; NOX, NADPH oxidase; NQO-1, NAD(P)H quinoneoxidoreductase- 1; PARP, poly ADP-ribose polymerase; PKC, protein kinase C; ROS, reactive oxygen species; RNS, reactive nitrogen species; SKN-1, skinhead-1; SESN, sestrin; SINGs, stress-induced nuclear granules; SIRT-1, sirtuin-1; SOD1, superoxide dismutase 1; TNF, tumor necrosis factor; UBC1, ubiquitin-conjugating enzyme 1; UCHL1, C-terminal hydrolase L1; UPS, ubiquitin proteasome system; 18α GA, 18α-glycyrrhetinic acid

* Corresponding author.
E-mail address: nikichon@eie.gr (N. Chondrogianni).

http://dx.doi.org/10.1016/j.redox.2017.07.005
Received 31 May 2017; Received in revised form 1 July 2017; Accepted 3 July 2017
Available online 06 July 2017

expression [5].

ROS are involved in the activation of several serine/threonine kinases. There are two major examples; mitogen- activated protein kinases (MAPKs) and protein kinase C (PKC). MAPKs include the extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNKs) and p38 kinases that are involved in cell proliferation, differentiation and apoptosis. It has been shown that mild oxidative stress has a mitogenic effect through the activation of the growth factor receptor-related ERK pathways. Under mild oxidative stress, p38 promotes mitotic arrest while upon severe oxidative stress JNK and p38 regulate cellular apoptotis [6]. PKC is the other important serine/threonine kinase that alters the expression of various genes during oxidative stress. PKC is highly responsive to oxidative stress due to its numerous cysteines that can be modified by oxidants. It is involved in various pathways such as cell death, growth and stress response [7].

Several transcription factors such as Nrf2 (Nuclear factor (erythroid-derived-2) like-2), NF-kB (Nuclear factor-kappa B), tumor protein p53, HIF-1 (Hypoxia inducible factor 1), FOXO (Forkhead box class O) and AP-1 (Activator protein-1) are also affected by ROS. There are three nuclear factor (erythroid-derived-2) related factors (Nrfs) namely Nrf1, Nrf2 and Nrf3. Nrf1 and Nrf2 have been shown to play a key role in oxidative response. Nrf2 is a transcription factor that under normal conditions is bound to Keap1 (Kelch-like ECH Associated Protein 1) in the cytoplasm, it is ubiquitinated by the E3 ligase complex, Keap1-Cul3-Rbx-1 and degraded by the proteasome [8]. Under oxidative stress, Keap1 is modified and the E3 ligase complex is inactivated allowing Nrf2 to accumulate and translocate to the nucleus where it heterodimerizes with small Maf proteins (Musculoaponeuroticfibrosarcoma proteins) to bind antioxidant-response elements (ARE), thus driving the transcription of several protective genes including NQO-1 (NAD(P)H quinoneoxidoreductase- 1), HO-1 (Heme Oxygenase 1), GSTs (Glutathione S-Transferase), γ-GCS (γ-Glutamylcysteinesynthetase) and several proteasome subunits. In that sense, Nrf2 and its inhibitor Keap1 are sensors of oxidative stress and Nrf2 promotes cell survival. Impairment of Keap1 and Nrf2 leads cells to reduced response or to Nrf2overactivation [6]. Upon proteasome inhibition, Nrf1 forms heterodimers with small Mafs on the ARE of proteasome subunits to promote their gene expression [9]. Under normal conditions, Nrf1 is located in the ER and degraded by the proteasome. In response to proteasome inhibition, Nrf1 is cleaved and thus gets activated and translocated to the nucleus promoting the expression of proteasome subunits [10]. In support, Nrf1 knock out MEFs (Mouse embryonic fibroblasts) fail to produce proteasomes upon treatment with the proteasome inhibitor YU101 [11]. DDI2 (DNA-damage inducible 1 homolog 2) has been found to be necessary for the activation of Nrf1. Upon deletion of DDI2, an increase in the cytosolic form of Nrf1 occurs and production of proteasomes is reduced [12]. Surprisingly, proteasomes are needed for Nrf1 activation since Nrf1 produces proteasomes less effectively upon exposure to high concentrations of proteasome inhibitors [13]. In *Caenorhabditis elegans*, SKN-1 (Skinhead-1; the ortholog of Nrf1/2/3) has a crucial role in response to oxidative stress by promoting the expression of detoxifying genes and the expression of proteasome genes upon proteasome inhibition [14]. It has been shown that for SKN-1 activation following proteasome impairment a peptide-N- glycanase and DDI1, a conserved aspartic protease, are required [15]. Similar observations have been made in *Saccharomyces cerevisiae* with the stress-regulated transcription factor Rpn4 governing the expression of proteasome genes in response to stress and proteasome inhibition [16].

NF-kB is a transcription factor that activates genes responsible for cell growth, differentiation, inflammation and cell death [17]. NF-kB can be activated by a variety of signals including ROS that are considered as second messengers involved in its activation via tumor necrosis factor (TNF) and interleukin-1 [18].

P53 has an important impact on the cellular redox state. Under normal conditions, p53 activates the expression of several antioxidant proteins, such as *SESN1*(mammalian sestrin homolog), *SESN2*, and *GPX1*(glutathione peroxidase-1) while reduced p53 levels enhance ROS production [19].

AP-1 is another transcription factor that is responsive to oxidative stress driving the expression of genes involved in cell proliferation, differentiation and apoptosis [20].

HIF-1 consists of HIF-1α and HIF-1β subunits. HIF-1β expression is independent of O_2whereas HIF-1α subunit is modulated by the relative O_2levels. During hypoxia, HIF-1α translocates into the nucleus and dimerizes with HIF-1β subunit and p300/CBP or other coactivators inducing the expression of genes that assist the cells to overcome the hypoxic stress [21].

FOXO transcription factor can be activated by oxidative signals through various mechanisms. For example, upon oxidative stress FOXO is phosphorylated by JNK and Mst1 thus being translocated in the nucleus and activated. FOXO may also get deacetylated by SIRT-1 (sirtuin-1) upon oxidative stress thus acquiring enhanced activity and exhibiting increased DNA binding activity [22]. Monoubiquitination enhances the activity of FOXO proteins whereas poly-ubiquitination drives them to proteasome degradation [5].

The intensity of oxidative stress is crucial not only for the final outcome and the cellular fate but also for the signaling pathway that will be eventually modulated. For example during mild stress, Nrf2 is activated transcribing antioxidant enzymes but when the intensity of the stress is high, NF-kB, AP-1, MAPKs and HSF (heat shock factor) are activated in order to transcribe antioxidant enzymes, inflammatory proteins and heat shock proteins. Finally, under high oxidative stress the cell is driven to death by necrosis and apoptosis; there is not specific evidence regarding the pathway that mediates this response [2].

2. Proteasome system

Oxidative stress affects the correct function of cellular and molecular mechanisms that assures cellular integrity, leading to homeostasis deregulation. Proteostasis is highly disturbed upon oxidative stress with the proteasome system playing a pivotal role.

2.1. Structure

Proteasomes are large protein complexes responsible for the proper regulation of the cellular protein load. They constitute one of the main cellular proteolytic mechanisms. The proteasome consists of catalytic and regulatory subunits. The basic particle of the proteasome is the 20S core, a barrel-shaped complex formed through the assembly of four protein rings. The outer rings are identical and consist of seven different α (alpha) regulatory subunits (α_{1-7}). The α-rings embrace two identical inner rings that consist of seven different β (beta) catalytic subunits (β_{1-7}) [23]. Three β subunits possess catalytic properties; β1 with caspase-like activity (peptidyl-glutamyl-peptide-hydrolase), β2 with trypsin-like activity and β5 with chymotrypsin-like activity [24].

The barrel-shaped 20S proteasome forms a gated channel through which a limited number of peptides and proteins enter [25]. To alter the gate conformation and allow the degradation of a wider range of proteins (ubiquitinated, damaged and misfolded proteins), 19S regulatory complexes bind onto the 20S proteasome. The assembly of one or two 19S regulatory complexes on either end of the core proteasome leads to the formation of 26S or 30S complexes, respectively. The 19S regulatory complex consists of a “base” and a “lid” [26]. The base is the component that is adjusted to the core and through its hexameric ring of six AAA-ATPases (Rpt1-6) can modify the conformation of the α-gated channel. Four non-ATPases are also part of the 19S “base”. The horseshoe-shaped “lid” is attached to the base and consists of nine non-ATPases.