BBA - Molecular Cell Research 1865 (2018) 721–733

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

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

Review

Nrf2-Keap1 signaling in oxidative and reductive stress

Ilaria Bellezza[a], Ileana Giambanco[a], Alba Minelli[a], Rosario Donato[a,b,c,*]

[a] Department of Experimental Medicine, University of Perugia, Piazza Lucio Severi 1, 06132 Perugia, Italy
[b] Centro Universitario per la Ricerca sulla Genomica Funzionale, University of Perugia, Piazza Lucio Severi 1, 06132 Perugia, Italy
[c] Istituto Interuniversitario di Miologia (Interuniversity Institute for Miology), Italy

ARTICLE INFO

Keywords:
Nrf2/Keap1
Antioxidant system
Striated muscle
Oxidative stress
Reductive stress
Nrf2/NF-κB cross-talk
Autophagy

ABSTRACT

Nrf2 and its endogenous inhibitor, Keap1, function as a ubiquitous, evolutionarily conserved intracellular defense mechanism to counteract oxidative stress. Sequestered by cytoplasmic Keap1 and targeted to proteasomal degradation in basal conditions, in case of oxidative stress Nrf2 detaches from Keap1 and translocates to the nucleus, where it heterodimerizes with one of the small Maf proteins. The heterodimers recognize the AREs, that are enhancer sequences present in the regulatory regions of Nrf2 target genes, essential for the recruitment of key factors for transcription. In the present review we briefly introduce the Nrf2-Keap1 system and describe Nrf2 functions, illustrate the Nrf2-NF-κB cross-talk, and highlight the effects of the Nrf2-Keap1 system in the physiology and pathophysiology of striated muscle tissue taking into account its role(s) in oxidative stress and reductive stress.

1. Introduction

In mammals, the NF-E2-related factor 2 (Nrf2)-Kelch-like ECH-associated protein 1 (Keap1) system, inherited from ancestors as anti-stress mechanism, is a defense system aimed to preserve cellular homeostasis. During evolution, all the living organisms had to deal with a variety of stressors and only the organisms provided with functional defense systems could survive and evolve. The system is regulated by interactions between Nrf2 and the cytosolic repressor protein Keap1. Nrf2, a member of the Cap-n-Collar family of basic leucine zipper proteins, was first described by Moi et al. [1] as an activator of β-globin gene expression, and later described as a major sensor of oxidative stress in the cell [2,3].

Domain analysis by nuclear magnetic resonance spectroscopy and high-resolution crystal structure showed that Nrf2 has seven functional domains (Neh1–7) that are involved in the regulation of its stability or its transcriptional activity (transactivation) (Fig. 1A). The N-terminal domain is responsible for the interaction at low nanomolar concentration (K_D ~5 nM) between Nrf2 and Keap1, stability of Nrf2, and ubiquitination, while the Neh5 domain regulates the cellular localization of Nrf2 [4,5]. The Neh6 domain controls Keap1-independent degradation of Nrf2 and represents a binding platform for the β-transducin repeat-containing protein. The Neh1 domain, with its basic leucine zipper motif, allows the binding of Nrf2 to the antioxidant response element (ARE) sequence. Moreover, this domain can interact with UbcM2, the E2 ubiquitin-conjugating enzyme, to regulate Nrf2 protein stability [6]. Neh1 domain, after the release from Keap1, uncovers a nuclear localization signal essential to Nrf2 nuclear translocation. The C-terminal of the Neh3 domain interacts with the transcription co-activator CHD6 (a chromo-ATPase/helicase DNA-binding protein), responsible for the transactivation of ARE-dependent genes after chromatin remodeling [4,5,7]. The Neh4 and Neh5 represent domains of transcription activation that bind to the co-activator cyclic adenosine monophosphate-responsive element-binding protein and facilitate Nrf2 transcription [7]. Neh4 and Neh5 can also interact with the nuclear cofactor RAC3/AIB1/SRC-3 and enhance Nrf2-targeted ARE gene expression [4,5,7]. Neh7 domain interacts with retinoic X receptor α thus repressing Nrf2 [8].

Keap1, the main intracellular regulator of Nrf2, is characterized by five domains (Fig. 1B), that is three broad complex-tramtrack-bric a brac (BTB), one intervening region (IVR) and two glycine repeat domains (DGR), each one being important for inhibiting Nrf2 activity. The DGR domains of the Keap1 homodimer bind with different affinity to the DLG (latch) ($Ka = 0.1 \times 10^7\ M^{-1}$) and the ETGE (hinge) ($Ka = 20 \times 10^7\ M^{-1}$) domains in a single Nrf2 molecule (hinge and latch hypothesis). In response to oxidants, the DLG motif in Nrf2 is released from the DGR domain in Keap1 thus blocking Nrf2 ubiquitination and degradation. The binding of Nrf2 to the DGR domain is competitively inhibited by proteins with specific motifs, such as p62 and partner and localizer of BRCA2 [6,9–12], therefore acting as a sensor of cellular stress, such as autophagy deficiency and DNA damage. The IVR domain, in addition to interacting with Cul3 protein

* Corresponding author at: Department of Experimental Medicine, Perugia Medical School, Piazza Lucio Severi 1, 06132 Perugia, Italy.
E-mail address: rosario.donato@unipg.it (R. Donato).

https://doi.org/10.1016/j.bbamcr.2018.02.010
Received 19 September 2017; Received in revised form 25 January 2018; Accepted 22 February 2018
Available online 27 February 2018
0167-4889/

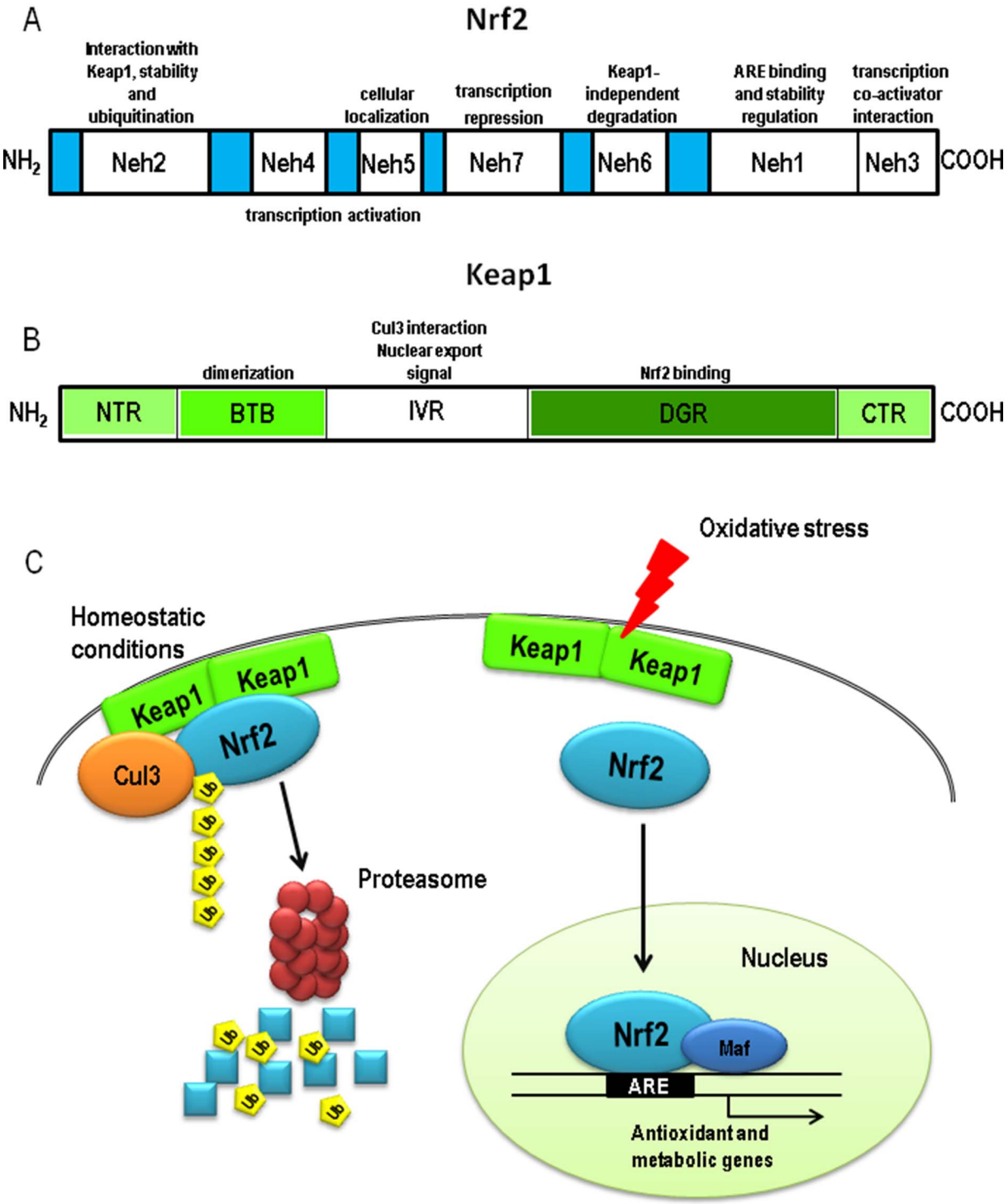

Fig. 1. (A, B) Scheme of Nrf2 (A) and Keap1 (B) primary structure. Relevant functions of Nrf2 and Keap1 domains are indicated. (C) Under homeostatic conditions Nrf2 (light blue) is kept inactive being bound to its endogenous inhibitor, Keap1 (green), associated with the F-actin cytoskeleton [14,48]. In this condition, levels of Nrf2 are principally regulated by the proteasome. Oxidative stress causes Nrf2 to detach from Keap1 and translocate to the nucleus where it heterodimerizes with Maf (blue): the Nrf2-Maf heterodimer binds to ARE (black) to induce the expression of antioxidant and metabolic genes.

which contains the E3 ligase complex together with Roc1 [13] has a consensus sequence of nuclear export signal, important for localization of Keap1 at the cytoplasm [14]. Under basal conditions, Nrf2 is sequestered by cytoplasmic Keap1 and targeted to proteasomal degradation [2,15] (Fig. 1C). Under conditions of oxidative stress, the Nrf2-Keap1 interaction is resolved in a dose-dependent manner [2] and free and newly synthesized Nrf2 translocates to the nucleus and heterodimerizes with one of the small Maf (musculoaponeurotic fibrosarcoma oncogene homolog) proteins (Fig. 1C). The heterodimers recognize the AREs, that are enhancer sequences present in the regulatory regions of Nrf2 target genes, essential for the recruitment of key factors for transcription [16]. Nrf2 affects the expression of nearly 500 genes that encode proteins acting as redox balancing factors, detoxifying enzymes, stress response proteins and metabolic enzymes [17–19].