Available online at www.sciencedirect.com
SCIENCE DIRECT®
Toxicology 208 (2005) 223–233
TOXICOLOGY
www.elsevier.com/locate/toxicol

NO and transcriptional regulation: from signaling to death

Jie Zhou, Bernhard Brüne*

Department of Cell Biology, Faculty of Biology, University of Kaiserslautern, Erwin-Schroedinger-Strasse, 67663 Kaiserslautern, Germany

Available online 19 December 2005

Abstract

It is nearly 20 years that nitric oxide (NO) entered the scene to become an integral component in understanding physiological and pathophysiological processes ranging from fine-tuned signaling to promoting cell demise. Among multiple activities attributed to NO we find regulation of gene expression. Although there is no evidence for direct NO-responsive DNA elements within promotor regions of eukaryotic genes numerous signaling pathways exist to understand NO-regulated gene expression. A characteristic feature of may transcription factors is their redox sensitivity as well as their low protein abundance in unstressed cells due to efficient 26S proteasomal degradation. Examples comprise the hypoxia inducible factor-1α (HIF-1α) and p53 (tumor suppressor p53). It became apparent that NO is able to mimic a hypoxic response by stabilizing HIF-1α and/or to affect viability decisions by accumulating p53. We will review recent molecular understanding how NO affects stability regulation of HIF-1α and p53, considering basic chemical reactions and cellular transducing pathways. Targeting HIF-1α and p53 by reactive nitrogen intermediates (RNI) may help to understand a sphere of NO-evoked transcriptional regulation ranging from cellular adaptation to death, i.e. apoptosis with important implications for medicine.

Keywords: p53; HIF-1α; Ubiquitination; Proteasome; Hypoxia; Mdm2; pVHL; Apoptosis

1. Formation of NO and RNI-signaling

Life demands intra and intercellular signaling and it came with some surprise that a small molecule being a gas powers signal transmission that touches nearly all areas of life (Nathan, 1992; Schmidt and Walter, 1994). NO taught us to revise traditional thinking and to incorporate new concepts in biology/medicine that formation of a radical may initiate efficient pathophysiological signaling. Biological actions of NO often can be attributed to "reactive nitrogen intermediates" (RNI)

Abbreviations: ATM, ataxia telangiectasia-mutated; ATR, ATM- and Rad3-related; AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; cGMP, cyclic guanosine monophosphate; CTAD, C-terminal transactivation domain; GSNO, *S*-nitrosoglutathione; HIF-1, hypoxia inducible factor-1; HRE, hypoxia-response element; iNOS, (inducible) nitric oxide synthase; MAPK, mitogen-activated protein kinase; Mdm2, murine double minute; ODD, oxygen-dependent degradation domain; p53, tumor suppressor p53; PHD, prolyl hydroxylase domain-containing protein; pVHL, von Hippel-Lindau protein; RNI, reactive nitrogen intermediates; ROI, reactive oxygen intermediates; VEGF, vascular endothelial growth factor

* Corresponding author. Tel.: +49 631 205 2406; fax: +49 631 205 2492.
E-mail address: bruene@rhrk.uni-kl.de (B. Brüne).

0300-483X/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.tox.2004.11.021

rather than the radical itself. The term RNI refers to oxidation states and adducts of the products of NOS, including NO-radical ($^{\bullet}NO$), NO^- and NO^+, as well as for the subsequent adducts of these species such as NO_2, NO_2^-, NO_3^-, N_2O_3, N_2O_4, *S*-nitrosothiols, peroxynitrite and nitrosyl-metal complexes (Stamler, 1994; Grisham et al., 1999). Despite the complexity of RNI metabolism determination of RNI involvement in biology is largely based on the use of compounds that mimic an endogenous response by administration of chemical diverse NO donors, by blocking RNI formation with NOS-inhibitors or by using knockout mice that lack isotype specific NOS (Moncada and Higgs, 1995). NO is generated by nitric oxide synthase (NOS) that convert L-arginine to citrulline and NO (Alderton et al., 2001). Isoenzymes known as neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) are named after the cell type from which they were first isolated and cloned. They differ with respect to NO output and basic regulation. A major distinction is regulation of nNOS as well as eNOS by a cytosolic calcium increase versus a cytokine-inducible form, known as iNOS.

Biological activities attributed to RNI, for reasons of simplicity, are distinguished as either being cGMP-dependent or cGMP-independent (Schmidt and Walter, 1994).

Activation of soluble guanylyl cyclase can be considered as the best characterized NO target with broad implications for vascular biology, at the same time referring to the landmark discovery of the endothelium derived relaxing factor (EDRF) (Furchgott and Zawadzki, 1980). We now appreciate that alternative signaling pathways are achieved through RNI via redox and additive chemistry, that may promote covalent modification of proteins as well as oxidation events that do not require attachment of the NO group (Stamler, 1994; Butler et al., 1995). These signaling properties gain steadily growing interest in explaining effects that are not mimicked by lipophilic cGMP analogs. Among those, *S*-nitrosylation/*S*-nitrosation (Koppenol, 2002), protein nitration, oxidation and phosphorylation gained considerable attention as (ir)reversible post-translational protein modification mechanisms. Protein modification by these cGMP-independent mechanisms often contributes to intracellular signal transduction cascades provoking activation or suppression of certain genes (Marshall et al., 2000; Bogdan, 2001). Most, if not all, signaling qualities attributed to RNI in modulating gene expression are indirect. So far there is no evidence for the existence of DNA elements within promotor regions of eukaryotic genes that respond directly to RNI. Therefore, RNI-evoked gene regulation comprises modulation of transcription factors, translation and/or stability of mRNA, protein stability regulation as well as compartmentalization.

Often, the primary target of RNI as well as specific molecular modification(s) remained unknown and sometimes contradictory reports emerged on the role of RNI in modulating eukaryotic transcription regulation. This may reflect the use of RNI with different signaling properties, different concentrations of RNI, cell-free versus intact cell systems, different type of cells, as well as the fact that activation of transcription factors is a result of complex upstream signaling cascades that themselves are targeted by RNI. Often, signaling qualities of RNI depend on the biological milieu, i.e. the presence or absence of stimulatory or inhibitory co-signals (Nathan and Shiloh, 2000). Thus, regulation by RNI in cells may occur at multiple levels in signal transduction pathways and different incoming or intercepting signals may be subjected to different redox control mechanisms. At the end integration of all these variables will dictate cell fate.

Conditions of stress are potentially harmful to cells and require appropriate defense systems. The hypoxia inducible factor-1α (HIF-1α) (Semenza, 2002; Brüne and Zhou, 2003; Huang and Bunn, 2003; Pugh and Ratcliffe, 2003) and the tumor suppressor protein p53 (Haupt et al., 2002; Vousden, 2002; Oren, 2003) are important regulators that transmit stress signals either by inducing growth arrest, apoptosis or adaptation, to a great extend by altering gene expression (Brüne et al., 2001). The nature and intensity of the stress signal, the cell type, and the cellular context dictate the final outcome. Multiple levels of regulation ensure that p53 and HIF-1α are efficiently activated in space and time in response to stress. Upon activation, p53 and HIF-1α function largely through transcriptional regulation of target genes to cope with stress situation. Activation and accumulation of p53 is triggered by a variety of stress signals including DNA damage, oncogenic deregulation, hypoxia and RNI while accumulation of HIF-1α is achieved among others signals by hypoxia, oncogenes, growth factors and RNI. Interestingly, RNI cause activation of both, HIF-1α and p53.