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Seminars in Cell & Developmental Biology 16 (2005) 462–473

seminars in
CELL & DEVELOPMENTAL
BIOLOGY

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Review

COPing with hypoxia

Peter Mikus [a], Wayne Zundel [a,b,c,*]

[a] *Department of Radiation Oncology, Baxter Research Bldg II, Room 204C (Lab 215), 580 S. Preston Street, University of Louisville School of Medicine, Louisville, KY 40202, USA*
[b] *Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY 40202, USA*
[c] *The J.G. Brown Cancer Center, University of Louisville School of Medicine, Louisville, KY 40202, USA*

Available online 23 May 2005

Abstract

To understand how cells respond to altered oxygenation, a frequent experimental paradigm is to isolate known components of bona fide oxygen responsive proteins. Recent studies have shown that a protein known as CSN5 or JAB1 interacts with both the HIF-1α oxygen-responsive transcription factor and its oxygen-dependent regulator, the Von Hippel-Lindau (pVHL) tumor suppressor. CSN5 is a component of the COP9 Signalosome (CSN) which is a multi-subunit protein that has high homology to the lid of the 19S lid of 26S proteasome. The exact function of the CSN5 interaction with pVHL and HIF-1α remains to be fully elucidated, but it is clear that the interaction is both oxygen dependent and that CSN5 may play different roles under oxic and hypoxic responses. Further, evidence has also been published indicating that pVHL can be potentially post-translationally modified by CSN5 (de-neddylation) and that CSN5 transcription is regulated by hypoxia as are many of the key pVHL/HIF-1α regulatory genes such as the PHDs and OS-9. This review will give a broad overview of known CSN5 and COP9 Signalosome functions and how these functions impact the pVHL/HIF-1α signaling complex and potentially other oxygen-sensitive response networks.

Keywords: COP9 signalosome; CSN; Hypoxia; VHL; CSN5; Jab1

Contents

1. Oxygen sensing and response

Over the last two decades, research from a variety of laboratories has revealed a tremendous amount of information on

* Corresponding author. Tel.: +1 502 852 3445 (O)/
+1 502 852 3446 (Lab)/+1 502 852 3744 (Mobile); fax: +1 502 852 4649.
E-mail address: wz@bcc.louisville.edu (W. Zundel).

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doi:10.1016/j.semcdb.2005.03.002

cellular aspects of oxygen sensing and response (reviewed in [1]). One of the key regulators of this response is the pVHL ubiquitin E3 ligase. This E3 ligase mediates the O_2-dependent destruction of proteins required for cellular and tissue-specific responses to hypoxia and anoxia. It has also become apparent that deregulation of this ubiquitin ligase can occur independently of altered pO_2 in various physiologic and pathophysiologic states [2]. For example, growth factors and oncogenes that activate the PI(3)K pathway can lead to aerobic stabilization of pVHL targets such as the alpha subunits of the hypoxia-inducible (HIF-α) family of bHLH-PAS transcription factors [3–5]. The exact molecular dynamics controlling pVHL ligase activity are very complex however, and novel control mechanisms continue to be revealed [2,6]. Some of these mechanisms have also revealed a great deal about key regulatory events controlling protein degradation in general [2,7]. As many excellent reviews including Peter Ratcliffe's review in this edition of Seminars in Cell & Developmental Biology address pVHL function as an E3 ubiquitin ligase in detail, this review will address other aspects of pVHL control, namely the control of ubiquitin E3 ligase activity by the COP9 signalosome (CSN).

Clearly, proteins other than the pVHL pathway play roles in oxygen sensing and response. There are a wide variety of enzymes that directly utilize dioxygen (i.e. cyclooxygenases, dioxygenases, monooxygenases) or utilize O_2 as a cofactor in enzymatic reactions (i.e. some heme-binding proteins, metalloproteases). The reductive state of the cell also impacts the tertiary structure and function of many if not all proteins and various lipids. Thus, dioxygen availability impacts on the cellular microenvironment directly as well as through glycolytic metabolites and reactive oxygen species generation. Although hypoxia and anoxia generate such pleiomorphic alterations in cellular functions via these mechanisms, many of these proteins/lipids remain relatively unstudied with regard to oxygenation. This review focuses on how one aspect of the protein degradation pathway can impact the pVHL hypoxia-responsive pathway and reciprocally how altered cellular oxygenation could directly impact 26S-mediated protein degradation.

2. Cullin-based ubiquitylation

Ubiquitylation is a mechanism for targeting specific proteins for degradation by the 26S proteosome (reviewed in [8,9]). This process is mediated by three types of enzymes termed E1, E2 and E3 that facilitate the transfer of ubiquitin (Ub) moieties to target proteins. While this review focuses primarily on the regulation of the E2 and E3 enzymes utilized in the pVHL degradation pathway, other E2–E3 pathways are likely impacted upon by oxygen availability in a similar fashion. E3 enzymes recognize their substrate protein(s) and tether an E2 Ub conjugating enzyme, thereby catalyzing/promoting the transfer of Ub from the E2 to the target. There are two main classes of Ub E3 ligases. The first class contains a HECT domain and the second class possesses a RING finger motif. While HECT E3s utilize a conserved catalytic cysteine residue forming a thiol–ester intermediate with Ub, RING E3s recruit the E2 via the RING domain and promote the transfer of Ub from the E2 to a bound substrate.

Cullins, together with their RING finger partner ROC1/Rbx1 (also named Hrt1) [10–14], are the largest subfamily of RING-based E3s. The interaction between a cullin and ROC1/Rbx1 assembles a core complex, which facilitates the synthesis of polyubiquitin chains [14]. The pVHL complex composed of the SOCS-box protein pVHL, elongins C and B and cullin 2 (CUL2) will be the major subject of this review, but as the cullin-based E3s are thought to be regulated similarly, the mechanisms discussed in the following pages are broadly applicable to other Cullin-based E3s. In the pVHL complex, elongins C and B are referred to as the recognition complex that stabilize and assist in the correct folding of pVHL [15]. CUL2 can then bind to the pVHL–elongin complex but the mechanism facilitating this interaction is currently largely unknown. It is thought that the intact pVHL E3 complex (pVHL/elongin C and B/CUL2) can then recruit the ROC1/Rbx1 RING finger protein via the C-terminal cullin domain. In this manner, CUL2 places pVHL and its target within close proximity to ROC1/Rbx1, which then can recruit the E2 conjugating enzyme Ubc12 [12,13,16]. Consequently, a substrate such as HIF-1α when bound by pVHL via its prolyl-hydroxylated domains is positioned optimally for covalently accepting a Ub moiety in a Ubc12-catalysed transfer reaction.

3. The COP9 signalosome (CSN)

Within the last five years, an evolutionarily conserved protein complex that plays a role in protein degradation in eukaryotes has been identified. This complex has been termed the COnstitutive Photomorphogenesis mutant 9 (COP9) signalosome and is a protein complex composed of eight subunits (CSN1–CSN8 based on mass) and fractionates as a 450–550 kDa complex in gel filtration columns. The CSN was originally identified in *Arabidopsis thaliana* as a regulator of photomorphogenesis (COP9) [17] and the mammalian complex was isolated as a co-purifying byproduct of the 26S proteasome [18]. The CSN has subsequently been reported to control pleiotropic functions in various eukaryotes from yeast to mammals. For example, the CSN has been implicated in various functions including cytokine and growth factor signaling, the control of invertebrate development, nuclear transport, mating in budding yeast, aspects of DNA repair, and oxygen homeostasis [2,19–30].

Currently, the exact function(s) of the CSN complex in these various phenotypes remains undefined although it clearly plays a role in protein stability within the 26S proteosomal pathway. Indeed, the CSN has high homology to the 19S lid of the 26S proteosome, and the CSN subunits are considered paralogs of the eight lid subunits of the proteasome