

# The Guardian Recruits Cops: The p53-p21 Axis Delegates Prosurvival Duties to the Keap1-Nrf2 Stress Pathway

Michel B. Toledano<sup>1,\*</sup>

<sup>1</sup>DSV, IBITECS, Laboratoire Stress Oxydants et Cancer, CEA-Saclay, 91191 Gif-sur-Yvette, France

\*Correspondence: [michel.toledano@cea.fr](mailto:michel.toledano@cea.fr)

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In this issue of *Molecular Cell*, Zhang and colleagues (Chen et al., 2009) report their identification of p21 as a positive regulator of Nrf2, which extends p53 prosurvival functions through recruitment of a master stress-response regulator.

Cellular oxidative damage occurs continuously from exposure to many sources of reactive oxygen species (ROS), and reactive nitrogen species (RNS), and electrophilic xenobiotics. Nrf2, a cap'n' collar bZip protein, regulates an adaptive response that increases survival in the face of these stresses (Hayes and McMahon, 2009). Nrf2 is induced by these stresses and functions by activating more than a hundred genes involved in activities such as chemical detoxification, ROS elimination, and restoration of cellular redox homeostasis. Nrf2-induced factors also include chaperones and proteasome subunits. The importance of Nrf2 in stress adaptation is underscored by the increased susceptibility of Nrf2 knockout (KO) mice to the genotoxic, cytotoxic, and inflammatory effects of environmental stressors.

Keap1 is a key regulator of Nrf2, functioning as an adaptor of a Cul3-ubiquitin ligase complex that marks Nrf2 for degradation (Hayes and McMahon, 2009). Stress signals such as ROS and other electrophilic compounds derepress Nrf2 by alkylating and/or oxidizing Keap1 at cysteine residues to inhibit Keap1-mediated Nrf2 ubiquitination. Keap1 specifically binds Nrf2 through a two-site recognition or "hinge-and-latch" model that predicts a Keap1-Nrf2 2:1 stoichiometry; one of the two Kelch domains of dimeric Keap1 contacts Nrf2 through a strong-binding ETGE motif (hinge) and the other through a weak-binding DLG motif (latch) (Hayes and McMahon, 2009) (Figure 1A). The ETGE-DLG intervening sequence carries a stretch of lysine residues supposedly optimally positioned under uninduced

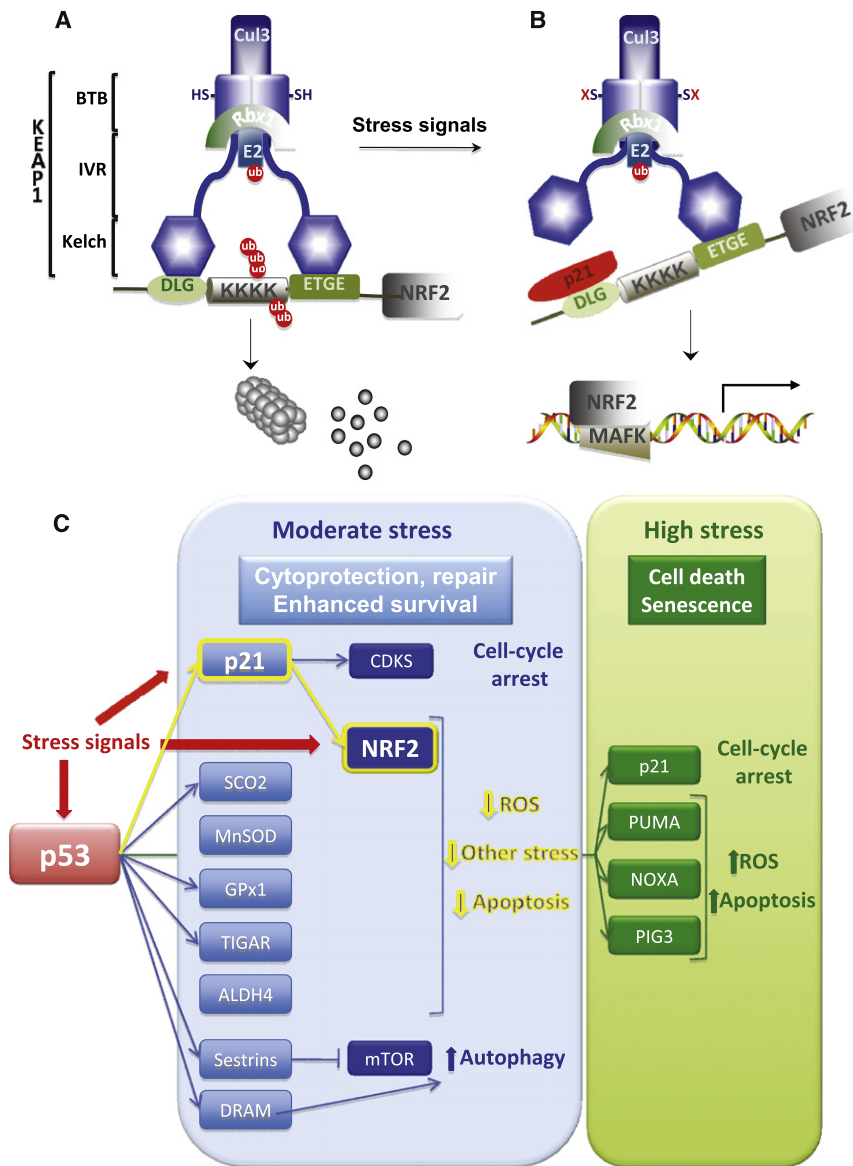
conditions for ubiquitin transfer. Rather than releasing Nrf2 from Keap1, stress signals modify Keap1 conformation in a way that loosens the latch and places lysine residues in a position unfavorable for ubiquitination (Figure 1B).

Zhang and colleagues now provide compelling evidence that the cyclin-dependent kinase (CDK) inhibitor p21 positively regulates Nrf2 (Chen et al., 2009). Basal and induced Nrf2 transcriptional activation is significantly less efficient in the absence of p21, and conversely, Nrf2 activity is increased upon p21 overexpression. p21 increases Nrf2 levels by interfering with Keap1-mediated Nrf2 ubiquitination; it binds Nrf2, displaces Keap1 from the DLG motif, and thus loosens the latch (see Figure 1). These data support the two-sites model for Keap1 recognition of Nrf2 and establish a crucial role for p21 in potentiating Nrf2 activation. It will be desirable, though, to fully demonstrate competition between p21 and Keap1 for Nrf2 binding. It will also be interesting to know (1) if p21 potentiates Nrf2 activation by increasing the strength of this activation or by extending its duration, even after Keap1 returns to the uninduced conformation; and (2) whether Nrf2 is constitutive in cells that accumulate p21. The nuclear oncoprotein prothymosin  $\alpha$  specifically binds the Keap1 Kelch to disrupt the Keap1-Nrf2 interaction (Hayes and McMahon, 2009). Once Nrf2 has been stabilized by stress signals and p21, prothymosin  $\alpha$  might allow the full release of Nrf2, which is required for DNA binding and gene activation.

The data from Chen et al. (2009) confirm the suggested p21 ROS protective func-

tion (O'Reilly, 2005). Further, they show that such a p21 ROS protective function is fully dependent on a functional Nrf2 pathway, as it is lost upon Nrf2 depletion. To what extent Nrf2 is dependent on p21 for its cytoprotective functions will have to be evaluated but might already be inferred from phenotypes that are shared by p21 and Nrf2 KO mice. Similar to the Nrf2 KO mice (Kensler et al., 2007), p21 KO mice are hypersensitive to hyperoxic lung injury (O'Reilly, 2005) and to lipopolysaccharide (LPS)-induced endotoxic shock (Lloberas and Celada, 2009), and they develop a lupus-like autoimmune glomerulonephritis (Balomenos et al., 2000). Macrophages from both KO mice display hyper-responsiveness to activation, which at least partly explains their abnormal inflammatory responses to stress and infection. Conspicuously, these KO mice also share a high susceptibility to chemically induced skin carcinoma (Gartel and Tyner, 2002). It will be important to evaluate p21 KO mice for their sensitivity to the environmental stressors affecting Nrf2 KO mice, and the importance of Nrf2 in these responses.

p21 is a p53-regulated gene mediating cell-cycle arrest at the G1-to-S transition by binding to and inhibiting G1 CDKs. It also has prosurvival properties that will be best considered in the context of its regulation by p53 (Figure 1C). The tumor-suppressor function of p53 relies on its ability to induce apoptosis through induction of pro-oxidant genes such as PUMA and PIG3, or to trigger senescence by p21-mediated cell-cycle arrest (Vouden and Prives, 2009). The p53 pathway also has prosurvival and damage-repair



**Figure 1. The Cyclin-Dependent Kinase Inhibitor p21 Potentiates NRF2 Activation**  
 (A and B) Model depicting (A) the two-site recognition of Nrf2 by Keap1 and (B) how the p21 CDK inhibitor alters this interaction and activates Nrf2. Keap1 assembles into an E3 ubiquitin ligase complex with Cul3, Rbx1, and an ubiquitin-charged E2 enzyme and recruits Nrf2 for ubiquitination and proteosomal degradation. Binding affinity of Keap1 is much higher at the ETGE than at the DLG motif. Stress signals that induce Nrf2, by modifying Keap1 cysteine residues, trigger a change of protein conformation that loosens binding at the DLG and inhibits ubiquitination of lysine residues. By binding Nrf2, p21 displaces Keap1 from the DLG and potentiates Nrf2 activation. BTB is the broad complex, tramtrack, bric-a-brac Keap1 dimerization domain; IVR is the intervening region between BTB and the Kelch domain. Regulatory cysteine residues have been mapped to the BTB and IVR.  
 (C) The guardian recruits cops. By recruiting and potentiating the activation of the Nrf2 stress-response pathway, p21 widens the prosurvival, ROS protective, and antiapoptotic functions of the p53 tumor-suppressor pathway. Nrf2 target genes comprise an important set of antioxidants, activities of the glutathione, thioredoxin, and NADPH regeneration systems that are essential for thiol redox control and ROS elimination, drug metabolism/efflux pumps, chaperones, and proteasome subunits. TIGAR is an antiapoptotic factor and a fructose biphosphatase that increases NADPH levels by shifting carbohydrate fluxes from glycolysis to the pentose phosphate shunt. SCO2 helps assemble cytochrome oxidase 2 and is required for respiration; ALDH4 is an aldehyde dehydrogenase homolog with ROS protective functions; PIG3 is a quinone oxidoreductase homolog; DRAM is damage-regulated autophagy modulator. Note that autophagy promotes both survival and apoptosis.

functions. These functions rely on p21-mediated cell-cycle arrest that favors damage repair, p21-dependent inhibition of apoptosis (Gartel and Tyner, 2002), DNA repair, and ROS protection by antioxidants and TIGAR (see Figure 1C). The role of p21 in potentiating Nrf2 activation not only provides a simple explanation for its ROS protective effect (O'Reilly, 2005) but more importantly widens p53 prosurvival functions by recruitment of a dedicated, fully equipped cytoprotective pathway specialized in cellular ROS and redox homeostatic control. It is as if, in order to protect the genome, the guardian has to recruit cops that fight bad guys. If indeed ROS levels influence p53-mediated cell-fate decisions, Nrf2 should be critical in this decision. By the same logic, p21-induced Nrf2 upregulation would explain the apoptosis-inhibitory function of p21. Of note, Nrf2 can inhibit apoptosis, a function that has not yet been fully appreciated. Hence it might not only be the severity of stress (Sablina et al., 2005) but also the efficiency by which it is lowered by Nrf2 that determines survival versus death. The contradictory observation that p53 inhibits Nrf2 transcription (Faraonio et al., 2006) might be accommodated to the data of Chen et al. (2009) by suggesting that such inhibition only occurs under high stress and/or irreparable damage and contributes to shifting the ROS balance toward death.

Nrf2 is a double-edged sword, as it inhibits chemical carcinogenesis but increases cancer cell survival and promotes drug resistance (Hayes and McMahon, 2009). Such beneficial effects are illustrated by cancer somatic mutations that precisely lie within Keap1- and Nrf2-regulatory regions and cause Nrf2 constitutive activation. Thus, in view of the Nrf2-dependent prosurvival role of p21, Keap1 and Nrf2 cancer somatic mutations should be evaluated with regard to the mutational and functional status of the p53-p21 axis.

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## A Histone Code for Regulating V(D)J Recombination

Mark S. Schlissel,<sup>1,\*</sup> Danae Schulz,<sup>1</sup> and Christian Vettermann<sup>1</sup>

<sup>1</sup>Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720, USA

\*Correspondence: [mss@berkeley.edu](mailto:mss@berkeley.edu)

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In a recent issue of *Molecular Cell*, Shimazaki et al. (2009) show that an interaction between RAG2 and a methylated histone might play a critical regulatory role in V(D)J recombination by enhancing DNA binding and enzymatic activity of the V(D)J recombinase.

The lymphocyte-restricted RAG1 and RAG2 proteins are essential for V(D)J recombination. A multimeric complex containing these proteins recognizes and cleaves pairs of recombination signal sequences (RSSs) that flank all rearranging gene segments in the seven immunoglobulin (Ig) or T cell receptor (TCR) loci (Schatz and Spanopoulou, 2005). This recognition depends upon the sequence-specific interaction between a region within RAG1 and the RSS. Despite the conserved nature of these RSSs and the use of a common recombinase, rearrangement is independently regulated at each locus. In 1985, Yancopoulos and Alt presented the prevailing model of how V(D)J recombination is targeted to specific loci at precise stages of lymphoid development (Yancopoulos and Alt, 1985). They reported that unrearranged gene segments are transcribed in a pattern that correlates with their activation as recombination substrates. Their “accessibility hypothesis” proposes that this “germline” transcription alters local chromatin structure, thus rendering specific loci accessible to the V(D)J recombinase and dictating the developmental regulation of antigen receptor gene assembly. Subsequent work showed that purified RAG proteins added to isolated nuclei

could recapitulate the in vivo pattern of locus accessibility, thus providing strong support for the model (Stanhope-Baker et al., 1996).

Exciting new work from Lieber and coworkers, recently reported in *Molecular Cell*, provides two likely biochemical bases for the link between germline transcription and V(D)J recombination (Shimazaki et al., 2009). This series of experiments builds on recent reports showing that a PHD domain in the C-terminal region of RAG2 specifically binds to a region of histone H3 when it is modified at lysine 4 by trimethylation (H3K4me3) (Liu et al., 2007; Matthews et al., 2007). Immunodeficiency disease-associated mutations in this PHD domain disrupt H3K4me3 binding and result in diminished recombination activity in vivo. H3K4me3-containing nucleosomes are enriched in the promoter regions of actively transcribed genes, including transcribed gene segments in the Ig and TCR loci. The current report shows that binding between an H3K4me3-containing peptide and RAG2 enhances the recruitment of the RAG complex to an RSS substrate in vitro. This finding leads the authors to suggest that binding of the RAG complex to H3K4me3-modified chromatin is a component of the mechanism that re-

stricts recombination to actively transcribed loci. Given the widespread nature of this modification, however, it is difficult to imagine it playing a decisive role in the specificity of recombinase targeting.

Perhaps more surprising is their discovery that binding to this same H3K4me3-modified peptide increases the catalytic activity of the RAG complex in vitro. The implication of this result is that recombinase binding to RSSs might not result in efficient dsDNA cleavage and recombination unless the RSSs are within a domain of H3K4me3-containing chromatin (Figure 1). This mode of regulation might play an important role in suppressing recombinase activity at the large number of “cryptic” RSSs scattered throughout the genome, as these sequences are generally found in nontranscribed regions that are poor in H3K4me3-modified nucleosomes.

These new data must be considered in light of several reports detailing the activity of a RAG2 mutant that lacks the entire C terminus, including the PHD domain, termed core RAG2. The core domain of RAG2 (amino acids 1–383 of this 527 amino acid protein) is the minimal region required to cooperate with RAG1 and catalyze V(D)J recombination in vivo. Two independent groups generated targeted homozygous mutant mice that express