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Forum Editorial

The Mammalian Cap and Collar Family of Transcription Factors

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THE MATURATION of erythroid cells has been the subject of long-standing and extensive investigations and the mechanisms governing this process serve as paradigms for cell-specific and differentiation-dependent gene regulation. In the late 1980s and early 1990s, analyses of erythroid-specific regulation of the porphobilinogen deaminase (an enzyme of the heme biosynthetic pathway) and the β-globin gene led to the identification of a critical *cis*-acting regulatory element (13, 14) that resembled the binding site for the AP-1 family of transcription factors (e.g., Jun•Jun and Jun•Fos dimers) which had been discovered a few years earlier. DNA-protein interaction assays, however, consistently indicated that the erythroid-specific protein that bound to this element—termed nuclear factor–erythroid 2 (NF–E2)—was distinct from AP-1 factors. This suspicion was eventually confirmed a few years later with the purification of NF–E2 and cloning of the cDNAs encoding the constituent subunits, p45 and p18 (2, 3). Sequence analyses also revealed the nature of the similarity to AP-1 proteins: like Jun and Fos proteins, both p45 and p18 contained a basic-leucine zipper (bZIP) domain for DNA binding and protein dimerization.

Proteins p45 and p18 belong to separate subfamilies of the bZIP superfamily of transcription factors. The bZIP domain of p18 most closely resembles those of the Maf family members, categorized based on their similarity to the Maf oncoprotein of the AS42 avian musculoaponeurotic fibrosarcoma virus (18); p18 is now synonymous with MafK. At the time of isolation, p45 was unique among mammalian bZIP factors in that it contained a structural domain with significant homology to the *Drosophila* homoeotic selector protein encoded by the *cap'n'collar* (*cnc*) gene. Additional members of the CNC subfamily were readily identified by exploitation of the principles of structure and function conservation.

The CNC subfamily of bZIP proteins is comprised of six members. These include proteins with moderate to strong transcription activation domains [p45, NF–E2 related factors (Nrf)1 and 2], with weak domains (Nrf3) and with no apparent activation domains (Bach1 and 2). The regulatory actions of these factors impact on diverse biological activities as evidenced by the phenotypes of the respective gene-targeted mice (Table 1).

Nrf2 and p45 are arguably the best characterized of the CNC regulators: p45 because of its distinction of being the first CNC member identified and also because of its importance in erythrocyte differentiation and globin gene regulation; Nrf2 because of its critical role in the maintenance of redox (reduction-oxidation) homeostasis during cellular oxidative stress and the fact that the latter condition has been implicated in a plethora of human pathologies. Given the scientific focus of *Antioxidants & Redox Signaling*, the majority of the FORUM articles are logically devoted to Nrf2 and address issues such as mechanisms regulating Nrf2 activity in response to oxidants and xenobiotics, Nrf2's mode of action, and the implication of Nrf2 activity to human diseases such as acute respiratory distress syndrome (ARDS), asthma, and cancer. In addition, the pioneering work of Igarashi and colleagues (see Ref. 9) has demonstrated that the regulatory mechanisms employed by NF-E2 and Nrf2 in activation of certain target genes, for instance, those encoding β-globin and the antioxidant enzyme heme oxygenase-1 (HO-1), respectively, are intimately connected to the activity of Bach proteins. This aspect of CNC biology, therefore, is also presented here.

In the lead article, Cho *et al.* (5) provide a brief but broad overview of the antioxidant defense system of cells with particular emphasis on the mechanisms by which various detoxification and antioxidant enzymes maintain redox homeostasis under conditions of stress. Transient activation of the genes encoding these enzymes is critical to the cytoprotective response and many are regulated by Nrf2. In addition to enumeration of the Nrf2 target genes, this report highlights the regulatory mechanisms including subcellular compartmentalization and proteasomal degradation that control Nrf2 activity. The use of *nrf2* germ-line mutant mice, microarray analysis, and genetic linkage analysis to identify novel target genes

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Table 1. CNC Transcription Factors

Factor	*Expression Profile*	*Phenotype of Null-Mutant Mice/Cells*	*References*
p45	Hematopoietic progenitor, erythroid, megakaryocytic and mast cells.	Mild anemia; inhibition of platelet production.	16 (and ref. therein)
Nrf1	Multiple tissues (mouse and human).	Embryonic lethality due to fetal liver abnormality and noncell autonomous anemia. Impaired antioxidant defense.	4, 16
Nrf2	Multiple tissues (mouse and human).	Impaired antioxidant defense.	16
Nrf3	Multiple tissues in mouse; highest levels in brain, thymus, testes and placenta.	No gross abnormalities.	6, 16
Bach1	Multiple tissues in mouse; highest levels in intestine and spleen.	Reduced macrophage phagocytic activity and smooth muscle cell proliferation.	16, 19
Bach2	Brain, spleen/B lymphocytes, embryonic neuronal and lens cells (mouse).	Defects in T lymphocyte-dependent and -independent IgG responses.	8, 16, 17, 21

and to elucidate the Nrf2 regulatory network is also discussed, as is the functional implication of this network on lung diseases such as ARDS, idiopathic pulmonary fibrosis, and emphysema.

Asthma is another pulmonary disorder in which oxidative stress is increasingly implicated. Li and Nel (11) review studies supporting a role for airborne particulate matter, in particular diesel exhaust particles and their constituents, in the exacerbation or even development of asthma. These authors propose a three-tiered, hierarchical model of the cellular response to escalating oxidative stress resulting from exposure to increasing levels of particulate matter. In this model, induction of Nrf2 and its target gene products is a quintessential feature of the initial, tier 1 response to mild oxidative stress; failure of the Nrf2-dependent antioxidant and detoxification mechanisms to correct the redox disequilibrium may lead to significant, and possibly irreversible, cellular damage. Li and Nel also suggest that polymorphisms of Nrf2-regulated genes could be potential susceptibility markers for asthma and chemopreventive agents known to induce Nrf2 expression may be used for treating particulate matter-exacerbated asthma.

The latter idea—utilization of chemopreventive agents, particularly phytochemicals derived from foods and edible plants, to induce Nrf2-dependent cytoprotective responses and thus obstruct the toxic and neoplastic effects of xenobiotics and carcinogens—is explored in more depth by Jeong and Kong (10). This article reviews the chemopreventive properties of several classes of natural compounds including isothiocyanates, phenolic antioxidants, and indoles, and examines the various mechanisms by which these agents stimulate Nrf2 activity. Unique among the review articles, this report also provides a comprehensive summation of the numerous protein kinase-dependent signaling pathways implicated in the regulation of Nrf2 and its downstream target genes. These include pathways anchored by mitogen activated protein kinases (MAPKs), the protein kinase C family, phosphoinositide-3-kinase (PI3K) and Akt, and RNA-dependent protein kinase-like endoplasmic reticulum kinase. The mechanisms by which these kinases regulate Nrf2 activity is not well understood but are likely to be complex: some kinases directly phosphorylate Nrf2, whereas others probably target accessory proteins and utilization of a specific pathway is likely to be to be both cell- and inducer-specific. Such complexity is underscored by the study of Papaiahgari *et al.* (20) who demonstrate that activation of Nrf2 and Nrf2-dependent transcription by hyperoxia in pulmonary epithelial cells requires not only signaling via the ERK subfamily of MAPKs and PI3K/Akt but also NADPH oxidase and the epidermal growth factor tyrosine kinase activities.

The bZIP structure of Nrf2 and other CNC proteins precludes formation of stable homodimers and, therefore, such factors bind to target sequences only as heterodimers. Following the paradigm established by NF-E2, Nrf2 dimerizes avidly with "small' Maf proteins including MafF, MafG, and MafK (reviewed in Ref. 16). Functional complexes with other bZIP proteins including ATF4 and Jun proteins, however, have also been reported (1). Whether the multiplicity of dimerization partners simply represents functional redundancy or provides for cell-, inducer-, or development-selective responses is an underexplored question. Thus the study by Massrieh *et al.* (12) examining the nature and regulation of the dimerization partner(s) of activated Nrf2 in placental cells exposed to inorganic arsenic is both timely and informative.

Whereas dimerization of small Maf proteins with p45 and Nrf2 generates transcription activators, association with Bach proteins should produce transcription repressors as both subunits would lack canonical activation domains but the complex would still bind to target sequences. This assumption has been convincingly validated by a series of studies by Kazuhiko Igarashi and colleagues. These studies, however, have revealed much more including novel mechanisms for regulation of transcription repressors and elaborate mechanisms for inducer-dependent gene activation involving the intricate interplay between the chromatin microenvironment, transcription activators and repressors, and accessory factors. An example of such interplay is provided by Dohi *et al.* (7) who demonstrate that the CNC factor binding sites within the HO-1 gene promoter function as transcription silencers in a *bona fide* chromatin environment but as enhancers in a nonchromatin setting. Interestingly, this silencer activity, a manifestation of the binding of Bach1•MafK heterodimers, can be converted to enhancer activity in response to cadmium treatment by displacement of the Bach1•MafK complex by transcription activators, presumably Nrf2•small Maf dimers. The details of such

a mechanism, along with the structural and regulatory characteristics of Bach1 and its mode of action, are elaborated in the review article by Igarashi and Sun (9).

In reflecting upon the literature on CNC factors, one underlying theme, the cross-talk between these transcription regulators and biological systems that metabolize or are otherwise dependent on heme (or hemoproteins) such as oxygen transport (hemoglobin), xenobiotic transformation (cytochrome p450), and oxidant detoxification (catalase), is readily apparent. For instance, disruption of the *p45* and *nrf1* alleles produces anemia in mice. Targeted deletion of *bach1* results in reduced phagocytic activity, presumably including phagocytosis of senescent red blood cells. NF–E2 regulates erythroid specific expression of genes encoding heme biosynthetic enzymes, whereas Nrf2 modulates the expression of HO-1, the rate-limiting enzyme in heme degradation. Conversely, heme dissociated from proteins, is a potent oxidant and stimulates Nrf2 activity at multiple levels (1). In addition, Bach1 binds heme with high affinity through sequences known as heme-regulatory motifs (HRMs) or CP motifs. Heme binding significantly impacts Bach1 function as it inhibits DNA-binding activity and promotes nuclear extrusion (9). This observation and the well-established fact that heme induces expression of globin genes and promotes differentiation of erythroleukemia cells in an NF-E2-dependent manner has led Volker Blank and colleagues to hypothesize that heme may similarly bind to, and regulate the activity of, the p45 subunit of NF-E2. Moore *et al.* (15) have identified two potential HRMs near the N-terminus of p45 and tested the above hypothesis by mutation analysis. The results of this investigation are presented in the final Forum article.

In summary, in the 15 or so years since the initial detection of NF–E2, rapid progress has been made in our understanding of the basic biology—structure, function, and regulation—of the CNC subfamily of transcription factors. This progress is readily apparent, at least for some of the family members, from the Forum articles presented below. It is also evident that, at least collectively, these regulators play a critical role in the proper operation of at least two biological systems: the erythroid cell lineage and the cellular antioxidant defense network. Malfunctioning of these processes contributes to the development of multiple human diseases. Future investigations, as implicitly or explicitly endorsed in several of the review articles, should reveal to what extent such disorders result from suboptimal activity of CNC factors or their target gene products and whether manipulation of such activity is a viable strategy for managing or reversing the disease state.

ABBREVIATIONS

AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; ARE, antioxidant response element; ATF4, activating transcription factor 4; Bach1, BTB and CNC homology 1; bZIP, basic region-leucine zipper; CNC, Cap'n'Collar; CP, cysteine-proline; ERK, extracellular signal-regulated kinase; HO, heme oxygenase; HRMs, heme-regulatory motifs; Maf, musculoaponeurotic fibrosarcoma; MAPK, mitogen activated protein kinase; MARE, Maf recognition element; NF-E2, nuclear factor-erythroid 2; Nrf, NF-E2-related factor; PI3K, phosphoinositide-3-kinase; redox, reduction-oxidation.

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