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Forum Editorial

Redox Control of Zinc Finger Proteins: Mechanisms and Role in Gene Regulation

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REDUCTION AND OXIDATION (REDOX) are among the major cellular environmental processes that significantly affect the functions of proteins involved in gene expression and DNA metabolism (9). Unless redox-active metals or cofactors are involved, most redox reactions in proteins occur at the thiol group of cysteine, the prominent redox-active amino acid. It is now becoming clear that this redox activity is maintained when cysteine is bound to zinc. This property renders zinc/cysteine coordination sites susceptible to damage by oxidants and, moreover, raises the distinct possibility that such sites control biological events as redox switches. Hence, a major part of this forum is dedicated to zinc finger proteins (ZFPs), which have characteristic zinc/cysteine coordination environments, and the functional consequences of their redox modulation.

About 2% of all human genes are ZFPs, making them one of the largest protein families in eukaryotes. In ZFPs, zinc is tetrahedrally coordinated with either two, three, or four sulfur ligands of cysteines and a corresponding complement of histidine ligands, thus forming essentially three types of motifs: Cys_2His_2, Cys_3His, and Cys_4. Although operationally defined in terms of such coordination, the definition zinc finger (ZF) does not express a single function, although it has become almost synonymous with the nucleic acid binding potential of these proteins. In fact, ZFPs exhibit functional diversity in organizing protein domains for protein–nucleic acid as well as protein–protein interactions. Even if zinc is surrounded by the same set of ligands, different protein environments endow ZF sites with a whole spectrum of reactivities, ranging from chemically inert (structural zinc) to chemically active (catalytic zinc) (5). In contrast to simple thiol/disulfide equilibria, which may also affect or regulate some ZFPs, redox reactions at ZF sites are linked to the binding and release of zinc. Thus, zinc, which is not freely available in the cell, is not tangential to this process, but rather an active participant, and possibly a signal itself. With regard to its effect on protein structure, zinc in ZF structures has been considered an intracellular equivalent of a disulfide bridge. Bringing ZF cysteines close together has also functional consequences: proximity is a critical factor in determining the thiol/disulfide redox potential (3).

The zinc/sulfur bond is the molecular device that links zinc and redox metabolism (4). This link has two facets, which the chapters in this forum address from different viewpoints: (i) the redox state affects zinc metabolism, and (ii) zinc modulates the redox behavior of cysteines. In other words, on one hand, the *redox-active* sulfur ligand confers redox activity on the zinc/sulfur bond for the purpose of mobilizing zinc and controlling its availability from its otherwise thermodynamically strong binding

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sites; on the other hand, the *redox-inert* zinc ion regulates at least some aspects of thiol redox metabolism. Articles in this forum focus only on ZFPs that are involved in gene expression and DNA metabolism, notwithstanding ample evidence of ZFPs participating in other, biologically diverse functions in a redox-dependent manner.

In the introductory chapter, **Webster *et al.*** give a general account of redox-regulated gene expression, then discuss the evidence that implicates zinc sites of transcription factors in redox reactions, and finally present two cases of aging and cancer where an alterered cellular redox state, *i.e.*, oxidative stress, leads to accumulation of oxidized ZFs. The next three chapters enlarge on this general theme by discussing different agents that oxidize ZFs. Thus, **Wilcox and his colleagues** examine the reactivity of zinc/sulfur coordination sites with reactive oxygen species (ROS) and transition metals from a chemical perspective. Zinc/sulfur bonds are generally less reactive than other metal/sulfur bonds. Therefore, redox-inert zinc in ZFPs protects DNA, whereas other metal ions, if they replace zinc in ZFPs, activate the cysteine ligand for oxidative attack. The authors discuss how sulfur accessibility and different coordination environments determine the reactivity of ZF sites, and summarize work where oxidation of the ligand results in oxidation states higher than that of a disulfide. These modifications are toxicologically significant, because they are irreversible. Mounting evidence points to ZFPs as potential targets for the nitric oxide molecule. **Kröncke's** work was the first to show that zinc/sulfur centers are targets of nitric oxide signaling *in vitro* and *in vivo*. He concludes that nitrosative stress may not be specific, but rather selective in repressing certain ZFP-controlled genes while activating others. **Chen and Maret** focus on selenium compounds as oxidants of zinc/sulfur coordination sites in metallothionein, a small zinc-binding protein that is thought to participate in cellular zinc distribution and delivery of zinc to ZFPs. The outcome of this work suggests that selenoproteins and selenodrugs control the zinc content of ZFPs through redox catalysis. The subject of the chapter by **Giedroc and coworkers** is the structure and function of metal-regulated transcription factor 1 (MTF-1), a prototype of a zinc-regulated transcription factor, and a paradigm for metal-regulated gene transcription. Among other genes, MTF-1 controls the expression of thionein, the apoprotein of metallothionein, and the synthesis of glutathione. Thus, MTF-1 is an important zinc-dependent element in controlling the cellular redox state. Coupling between zinc homeostasis and the oxidative stress response is achieved by this protein, which contains six Cys_2His_2 ZFs and is a sensor for both zinc and oxidants.

Sun and his colleagues review a unique group of ZF proteins with a so-called RING (really interesting new gene) finger motif. This motif ($Cys_3HisCys_4$ or $Cys_3His_2Cys_3$) also represents a large protein family of 210 genes in the human and differs from other ZFs in that it organizes a protein domain with two bound zinc ions, a property that it shares with other 2-zinc motifs such as the Lim domain, protein kinase C cysteine-rich domain, or FIVE domain. The RING finger protein SAG/ROC/Rbx/Hrt is a redox-sensitive essential component of E3 ubiquitin ligase and as such appears to play an important role in the cell cycle and tumorigenesis.

Another emphasis in this forum is the redox regulation of several key DNA repair factors. Some DNA repair proteins are ZFPs, *e.g.*, xeroderma pigmentosum group A protein (XPA), replication protein A (RPA; also known as human single-stranded DNA-binding protein), OGG1 (8-oxoguanine glycosylase), and poly (ADP ribose) polymerase. Nonetheless, the precise role of ZFs in DNA repair has yet to be established. **Lee's** laboratory provided the first example of redox regulation involving the Cys_4 site of a DNA-binding protein, RPA (6). RPA's ZF is not essential for DNA-binding activity, but is involved in redox regulation of its single-stranded DNA-binding activity. Importantly, a mutation at the ZF site has very little effect on its DNA-binding activity, which makes it an excellent tool to study the role of ZFs in redox regulation. RPA's role in recognition of damaged DNA is also redox-regulated, although its role in stabilizing the XPA-damaged DNA complex is not, suggesting that the ZF motif may mediate the transition of the RPA–XPA interaction to a stable RPA–XPA-

damaged DNA complex in a redox-dependent manner.

In the base excision repair (BER) pathway, OGG1 is a repair factor that removes the oxidized, damaged base prior to the apurinic/apyrimidinic endonuclease Ape-1, also known as the redox factor 1, ref-1. **Shinmura and Yokota** describe a vital role of human OGG1, which has a potential Cys_2His_2 ZF motif, in DNA repair. OGG1 suppresses mutations that could otherwise lead to cancer. **Kelley and Parsons** show that the activity of Ape-1/ref-1 is regulated by redox. This protein is not only an essential BER factor that hydrolyzes the 5′-phosphodiester backbone of an abasic residue, but also a key redox factor that controls the functions of transcription factors such as Fos, Jun, and p53, and the action of redox drugs (2). Recent findings demonstrated that Ape-1/ref-1 physically associates with strand break repair protein (Ku70/Ku80), as well as mismatch repair factor (human mutY) (1, 7), indicating its involvement in other DNA repair pathways. In this regard, the findings of Kelley and Parsons provide novel insight into the redox regulation of Ape-1/ref-1 in DNA repair.

Hainaut and Mann provide a comprehensive overview of the redox regulation of the tumor suppressor p53, a critical nodal point in cellular signaling networks and a link between DNA repair and cell-cycle progression/arrest. They cover an important body of work: p53 is part of the cellular redox network, interacts with redox signaling molecules such as thioredoxin and ref-1, controls the production of ROS, and may act as a sensor for redox stress. They also present findings that metals and redox affect the Cys_3His ZF site in p53. **Hartwig** considers deleterious effects of transition metals on the structure and function of ZFPs. Some metals such as cadmium exhibit higher affinity to sulfur than zinc and therefore displace zinc, in the Cys_4 motif in particular. Other metals may form mixed ligand complexes. Both toxic metals and oxidants target ZFPs and affect gene regulation and the DNA repair activity.

ZF motifs in transcription factors and DNA-binding proteins, so far believed to be the structural element for the recognition of a specific DNA sequence, now turn out to be one of the key redox-sensitive elements in protein–DNA and protein–protein interactions. In this rapidly evolving field, new functions of ZFPs in DNA metabolism have emerged and now define a new general area for ZFPs/ZF motifs as targets in redox signaling.

Although most of the oxidative reactions abrogate the DNA-binding activity of ZFPs, oxidative stress also activates ZFPs through a different mechanism, possibly by releasing zinc from one site and activating the apoform of a ZFP. This suggests that redox signaling pathways actually operate through more than one mechanism, resulting in either activation or inactivation of ZFPs, and raises a series of questions as to how the oxidants are generated and sensed in various compartments of the cell. *In vitro* studies identified many different oxidants, *e.g.*, ROS, nitric oxide, metal ions, selenium compounds. The challenge remains to identify the oxidants *in vivo*. Which redox pathway controls which ZFP, and how are the redox pathways integrated into the cellular signaling network?

The link between reactive ZF coordination environments and the redox state is a new molecular aspect that suggests a central role of zinc in degenerative diseases, including aging, and in toxicological mechanisms of oxidants and metals. The investigations summarized in this forum reveal specific targets for oxidant injury with multiple consequences for the involvement of zinc in pathogenesis and hence open fertile areas of research for future intervention by antioxidant therapy. Moreover, specific redox drugs can be designed and directed against highly specific ZFPs. One example that illustrates the feasibility of this approach is the disulfide benzamide drugs. These compounds target and inactivate the HIV nucleocapsid protein NCp7 by releasing zinc from its Cys_3His ZF site (8, 10).

ABBREVIATIONS

Ape-1, apurinic/apyrimidinic endonuclease; BER, base excision repair; MTF-1, metal-regulated transcription factor 1; OGG1, 8-oxoguanine glycosylase; ref-1, redox factor 1: RING, really interesting new gene; ROS, reactive oxygen species; RPA, replication protein A; XPA,

xeroderma pigmentosum group A protein; ZF, zinc finger; ZFP, zinc finger protein.

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