NO running on MT: regulation of zinc homeostasis by interaction of nitric oxide with metallothionein

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THE CLASSICAL SIGNALING PATHWAY for nitric oxide (NO) is the binding and subsequent activation of soluble guanylate cyclase. However, the number of pathophysiological pathways mediated by NO has been expanding to include processes that are independent of guanylate cyclase. A number of alternative reaction pathways for NO have been proposed to account for these effects, most notably S-nitrosylation (12). In the study by St. Croix et al., one of this issue’s articles in focus (Ref. 11, see p. L185), the authors propose a novel mechanism for NO-based signaling through the regulation of zinc homeostasis. Central to this proposal is the cysteine-rich protein thionein (T), which, due to the high density of reduced cysteine residues, is an extremely avid binder of divalent cations in general and specifically of zinc. The metal bound form of T, metallothionein (MT), is capable of binding up to seven divalent cations in thiolate clusters. The ability of MT to retain its metal is critically dependent on the redox state of its cysteine residues (9). In their paper, St. Croix et al. (11) demonstrate that within a cell, NO exposure results in MT-based release of zinc.

Using the lipophilic compound zinquin, which increases its fluorescence upon zinc binding, the authors are able to monitor alterations in the intracellular concentration of free zinc upon exposure to NO. By examining fibroblasts from MT knockout and heterozygous mice, they demonstrate that MT is necessary for NO-mediated increases in intracellular free zinc. Furthermore, NO effects on zinc metabolism are restored by transfection of MT−/− fibroblasts with MT expressing adenoviral vector. Having established that MT is essential to this NO-mediated effect, St. Croix et al. further demonstrate that zinc levels are critically dependent on the balance between MT and T. Overexpression of MT in sheep pulmonary artery endothelial cells results in an inhibition of NO-mediated zinc release, which can be restored by growing the cells in media containing high concentrations of zinc. The supposition here is that overexpression of MT in the absence of excess zinc results in a large proportion of MT being in its apo-form T. Therefore, when there is a large intracellular concentration of T, relative to MT, there is no NO-mediated release of zinc. Using fluorescent chimeras of MT, St. Croix et al. showed that MT overexpression inhibited NO-induced conformational changes in MT associated with zinc release and that this inhibition could be alleviated by growth in zinc-rich medium.

These latter observations raise some interesting questions as to the molecular mechanism of NO-mediated release of zinc from MT. Perhaps most critical among them is the chemical nature of the modification(s) of the protein itself, which results in zinc release and conformational change. The nature of the modification of MT induced by NO is critical to understanding how the reported MT/T balance operates to control zinc responses to NO exposure. For instance, does overexpressed T inhibit NO-induced action by rebinding released zinc or by operating as a sink for NO; i.e., could similar inhibition have been achieved with a non-zinc-binding thiol (although such inhibition would clearly not be zinc reversible)? The most obvious suggestion for the mechanism of NO-induced zinc release is the formation of S-nitrosothiol either in the metal binding center of the protein or in some other cysteine residue, which induces a conformational change resulting in zinc release. Significantly, the NO donor used in this study was S-nitrosocysteine, implying that this may be an S-nitrosothiol/nitrosonium-ion mediated pathway. It would be interesting in future studies to investigate how other redox congeners of NO affected this system. Previous experiments have shown that MT can be induced to release zinc upon cysteine oxidation to intramolecular disulfides (9). NO is capable of inducing cysteine oxidation as well as nitrosylation; therefore, it remains to be seen whether NO-mediated release occurs via different molecular mechanisms. This is significant because it may reveal convergent
but alternative mechanisms for oxidative and nitrosative stress within intracellular signaling.

Although zinc has been shown to operate as a signaling molecule (1) and as a mediator of cell death (6), these functions have been mediated via flux of extra- cellular ions. The present study brings to bear a whole new dynamic of the regulation of intracellular zinc homoeostasis. A major key to understanding the relevance of this control mechanism is to identify the cellular targets of MT-released zinc. A number of proteins incorporate zinc as a structural component ranging from enzymes (superoxide dismutase) to transcription factors (TFIIIA) to steroid-thyroid hormone receptors (BRCA1) (3). Interestingly, even NO synthase has been recently shown to contain structurally important zinc (4, 10). Entry of extracellular zinc into GH3 cells has been shown to produce transcriptional activation (2), and zinc removal from inhibitory sites has been shown to activate a number of enzymes, including caspase 3 and glyceraldehyde-3-phosphate dehydrogenase (8). Recently, it was proposed that zinc release from MT inhibits mitochondrial respiration (13), providing a mechanism for redox modulation of mitochondrial bioenergetics. In this way the MT/T balance, which would reflect both zinc concentrations and redox status of the protein, would operate both as a controller and as a sensor of cellular redox status.

Zinc, as opposed to other biological cations such as iron and copper, is a redox-inactive ion. Therefore, the data presented in this paper suggest a novel and interesting paradigm in which the versatile biological signal molecule NO, which can exist in multiple redox states, regulates the intracellular homoeostasis of an entirely redox-insensitive molecule, zinc. This represents a novel mechanism of energy transduction, i.e., the redox chemical energy of NO is transduced to the structural chemical energy of zinc. In this paradigm, MT represents a key transducer of redox energy and provides a novel mechanism for redox regulation in cellular homoeostasis.

There is a strong similarity between NO and zinc function within pathophysiology, namely that they can both be cytoprotective and cytotoxic. Zinc has been implicated in neuronal death (5, 6) and yet it is necessary for normal cellular function and has been shown to inhibit pro-apoptotic proteins, such as caspase 3. Interestingly, NO has also been implicated in cell death following ischemia, is necessary in a number of cellular functions, and has been shown to inhibit caspase 3 (7). Of critical importance to determine the effects of NO exposure is where it is made, how much is made, and how fast it reacts with molecular targets. It may be that the same principles can be applied to the MT/T-mediated regulation of zinc and the role that NO plays in controlling the zinc homeostatic system.

REFERENCES