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Editorial

Zinc biochemistry, physiology, and homeostasis – recent insights and current trends

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Reviews in this special issue summarize recent discoveries in the biology of zinc in order to draw further attention to the significance of this metal, which is essential for growth and development (Vallee & Falchuk 1993). Recognition of its full potential was much delayed, because its chemical properties and the way it is utilized in biology posed serious challenges to the investigator. Thus, zinc is colorless and diamagnetic, properties that render it invisible to most spectroscopic methods. Therefore, physicochemical approaches brought comparatively less insight into the biology of zinc than into that of e.g. copper or iron. Moreover, unlike iron, where 80% of a total of about 3 g in a human is in the heme group alone, similar total amounts of zinc are spread among thousands of proteins. This dilution effect makes it much more difficult to establish the presence and role of zinc in low abundance proteins. Yet, from 1950 on, an ever-increasing number of zinc enzymes was discovered (Vallee & Galdes 1984; Vallee & Auld 1990). The number of just those with known 3D structures is now already 200.

TFIIIA was the first transcription factor to be identified as a zinc protein (Hanas *et al.* 1983). When the term DNA-binding *finger* was introduced for the nine repetitive domains in this protein (Miller *et al.* 1985), discovery followed a different path. The close spacing of metal ligands in the primary sequences of zinc finger proteins allowed recognition of recurring zinc binding motifs. Consequently, it became common practice to define any new protein with such a motif as a zinc protein, thus assuming the presence of zinc rather than determining it directly. On this basis, hundreds of zinc finger proteins were identified within about 15 years. The domains that zinc organizes in these functionally and structurally diverse proteins are key elements for the molecular recognition of nucleic acids, proteins, or lipids (Laity *et al.* 2001). With blueprints of entire genomes now in hand, we are beginning to grasp the size of the *zinc proteome*, at least with regard to the number of zinc finger proteins (Clarke & Berg 1998). Over one thousand genes in the human genome encode members of three protein families with zinc finger domains alone, i.e., C2H2 zinc fingers, RING fingers and LIM domains (International Human Genome Sequencing Consortium 2001). In other words, the number of genes containing zinc finger domains exceeds 3% of the about 32,000 identified human genes. The set of signatures for other zinc proteins is less complete or even unknown for sites such as those between protein subunits. Therefore, not all sites can be accounted for by homology searches or data base mining, and a final count is still out.

Having established this impressive number of zinc proteins, the question remains how all these proteins acquire their zinc at the right time. The total zinc concentration of a eukaryotic cell is quite high, i.e., about 200 μM (Palmiter & Findley 1995). The bulk of it is bound very tightly in proteins. One corollary of this strong interaction is that the concentration of 'free' or freely available zinc is very low, with estimates given as picomolar for mammalian cells (Peck & Ray 1971; Simons 1991). This seems to rule out this pool as the source for incorporation into proteins. A key issue, and not a trivial one, therefore, is the chemical form in which zinc is made available. Recent discoveries make it clear that an elaborate homeostatic system of proteins regulates cellular zinc distribution and perhaps controls a hierarchy of zinc-dependent functions. In fact, the coordination dynamics of new types of zinc proteins involved in zinc trafficking now open fascinating and unprecedented aspects of an ingenious bioinorganic chemistry. Discoveries of zinc

transporters gave the first clues about the participation of specific proteins in a homeostatic system (Palmiter & Findley 1995; Eide 1997). Zinc sensors that induce gene transcription as a result of either too much or too little cellular zinc are the second type of molecules belonging to this system, while a third is metallothionein (MT). The function of MT might serve as an example of the uniquely biological chemistry that has evolved in zinc metabolism to deal with the problem of distributing zinc. MT links zinc distribution to the redox state of the cell (Maret & Vallee 1998). The molecular basis for this coupling is that the bonding to sulfur donor atoms of cysteine ligands confers redox activity on the otherwise redox-inert zinc atom. Thus, a change in the cellular redox potential toward more oxidizing conditions can induce kinetic lability in zinc/sulfur coordination sites and thereby provide a driving force for zinc transfer against thermodynamic gradients, e.g. from its tight binding in MT to sites of lower affinity. MT does not only occur in its zinc-loaded form, but also in its apoform thionein (T) at varying ratios with regard to MT (Yang *et al.* 2001). T is an efficient endogenous chelating agent and an effective thermodynamic sink for zinc. Regulation of MT at the protein level by ligand binding (Jiang *et al.* 1998) and at the gene level by multiple inducers provides a means to control the availability of zinc by adjusting the amount of MT and the MT/T ratio. Moreover, eukaryotic cells compartmentalize zinc and MT/T in their organelles. Questions are now which energy, signals, and mechanisms control their subcellular translocations, and what are the characteristics of the pools of available zinc in different compartments of the cell. Finally, on an organizational level higher than the cell, it remains a major challenge to identify the hormones that regulate body zinc homeostasis.

Another important aspect is that zinc has regulatory functions. This is best illustrated by its role in neurotransmission (Frederickson *et al.* 2000). So-called zinc-containing neurons innervate the forebrain and contain zinc in synaptic vesicles. Nerve stimulation releases this zinc into the synaptic cleft where it has a neuromodulatory function, either outside the postsynaptic cell or inside it, or at both locales. It is intriguing that regulatory functions may not be limited to neurotransmission and may involve control of biological function by transient zinc binding in general.

The chapters in this special issue have been arranged such that there is a progression from single molecules to cells and then to whole organisms. The opening chapters start with the perennial task of how to make the spectroscopically silent zinc visible for investigations. In proteins, metal substitution techniques are the classical approach to achieve this. Thus, cobalt is frequently used to study the function and coordination environment of zinc in proteins *in vitro*. When analyzing zinc or imaging zinc fluxes *in vivo*, substitution, of course, is not an option. Instead, visualization is approached in very much the same manner as for calcium, i.e. with specific fluorophore sensors. **Kimura and Aoki** summarize the structural types of presently available zinc fluorophores and their chemical and physical properties, define the limits of their use for *in vitro* and *in vivo* studies, and examine questions that stipulate development of new fluorophores. **Fierke and Thompson** have developed protein fluorescence sensors based on the very high sensitivity and selectivity of carbonic anhydrase for zinc. They have used the understanding of the coordination chemistry of this protein in combination with protein engineering to optimize affinity for zinc, selectivity and binding kinetics as well as the physical properties of the fluorophore. **Andrews** then describes how cells sense zinc by metal-response element-binding transcription factor-1 (MTF-1), a six zinc-finger protein that is essential for embryonic development. MTF-1 has been a paradigm for metal-dependent gene expression of metallothionein and has now been shown to coordinate the expression of other proteins involved in zinc homeostasis, e.g., zinc-transporter-1. This theme is enlarged in the chapters of **Hantke, Gaither and Eide** who discuss transporters and their regulators. Prokaryotes have at least three types of zinc export systems as well as high- and low-affinity uptake systems, all under control of individual repressors and activators. Gaither and Eide point out the major advances that have been made in the last decade through the discovery of two families of zinc transporters and their regulators in eukaryotes. Many of those belonging to the ZIP (Zrt, Irt-like Protein) family are involved in uptake, including one member that transports zinc out of a cellular compartment. Those of the CDF (cation diffusion facilitator) family mediate zinc efflux or zinc transport into cellular compartments. Neither zinc binding sites nor mechanisms of actions of these proteins are known. **Auld** gives a unique, comprehensive, and up-to-date account of those zinc proteins for which this information is available. He summarizes the number of zinc sites in proteins as characterized by high resolution structural methods, their characteristics and their classification in structural, catalytic, co-catalytic,

and protein interface sites. Though zinc finger proteins are not even included, the sheer number of zinc sites in proteins illustrates to what extent zinc proteins impact cell functions. The following chapters then focus on cellular aspects. Zinc deficiency increases apoptosis and halts differentiation and proliferation of cells. **Truong-Tran, Carter, Ruffin and Zalewski** review the evidence suggesting a role of zinc in apoptosis. Zinc is cytoprotective and suppresses apoptotic pathways. Among the multiple targets and mechanisms, regulation of caspase activity is perhaps the critical zinc-dependent event. The chapter by **Beyersmann and Haase** covers the essential role of zinc in cellular proliferation and differentiation. They propose a new role of zinc in cGMP signaling. Again zinc is involved at multiple levels of signal transduction, and the most sensitive zinc-dependent targets in growth and development have not yet been identified. Two chapters then address the role of zinc in brain, where it has a specific function as a neuromodulator in addition to its other typical cellular functions. **Frederickson and Bush** focus on the role of zinc in zinc-containing synaptic terminals in brain. Zinc acts as a signaling substance akin to conventional neurotransmitters in normal physiology. It also exerts pathophysiological effects by participating in plaque deposition in Alzheimer disease and by causing injury to neurons in excitotoxicity. **Takeda** outlines the role of the brain barrier systems for brain zinc homeostasis and discusses learning impairment and olfactory dysfunctions as a result of diet-induced zinc deficiency. **Rink and Gabriel** provide an overview of the extracellular and immunological actions of zinc. The responsiveness of leukocytes and the action of immunostimulants critically depend on the concentration of zinc. Both zinc deficiency and supraphysiological levels of zinc modulate the immune response. Evidence provided in these chapters suggests intracellular, extracellular, and intercellular signaling functions of zinc. **Falchuk and Montorzi** give an account of a complex zinc storage, transport, and distribution system in *Xenopus laevis* oocyte and embryo development. In the frog, the liver protein vitellogenin transports zinc to the egg. A cytosolic pool amounts to about 10% of the total zinc and is responsible for embryogenesis and organogenesis in this closed system. The second pool contains 90% of the total zinc bound to lipovitellin in the yolk sac and is mobilized for later stages of development. **Krebs and Hambidge** discuss tracer kinetic techniques with stable zinc isotopes in humans, which clearly demonstrate the role of the gastrointestinal tract in maintaining whole body zinc homeostasis. Excessive losses, increased requirements, and redistribution are perturbations of homeostasis in disease processes. Such zinc metabolic studies define the regulation of zinc balance to the point where specific questions can be addressed regarding the molecules involved in homeostasis.

Faced with the rich zinc biochemistry outlined in this special issue one wonders why so far zinc has had so little impact on medicine. Perhaps the zinc homeostatic system is so critical and efficient that organisms cannot afford to compromise the supply and distribution of zinc. There is only one known genetic disease of zinc metabolism, acrodermatitis enteropathica, and its pathogenesis is unknown. No other disease has been linked directly to zinc, clearly marginalizing its clinical significance. Yet, imbalances are known. Even mild zinc deficiency, which is quite common, impairs immunological function and defense mechanisms, and increases infectivity. On the other hand, accumulation of zinc in neurons leads to neurodegeneration. Therefore, zinc may be a major etiological factor, and nutritionally a most important metal for human health. Insights into the factors that control, interact with, and perturb cellular zinc and lead to reversible or irreversible changes will fill the large gap between the now known functions of zinc at the molecular level and gross biological observations of stunted growth, developmental abnormalities, and other characteristic signs of zinc deficiency. Therapeutic intervention will eventually follow, not necessarily only with zinc itself, but by characterizing substances that participate in the efficient regulation of zinc availability in the gastrointestinal tract and by targeting specific proteins in the zinc homeostatic system.

References

Clarke ND, Berg JM. 1998 Zinc Fingers in *Caenorhabditis elegans*: Finding Families and Probing Pathways. *Science* **282**, 2018–2022.

Eide D. 1997 Molecular biology of iron and zinc uptake in eukaryotes. *Curr Opin Cell Biol* **9**, 573–577.

Frederickson CJ, Su SW, Silva D, Frederickson CJ, Thompson RB. 2000 Importance of Zinc in the Central Nervous System: The Zinc-Containing Neuron. *J Nutr* **130**, 1471S–1483S.

Hanas JS, Hazuda D, Bogenhagen DF, Wu FY-H, Wu C-W. 1983 *Xenopus* trancription factor A requires zinc for binding to the 5S gene. *J Biol Chem* **258**, 14120–14125.

International Human Genome Sequencing Consortium. 2001 Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921.

Jiang L-J, Maret W, Vallee BL. 1998 The ATP-metallothionein complex. *Proc Natl Acad Sci USA* **95**, 9146–9149.

Laity JH, Lee BM, Wright PE. 2001 Zinc finger proteins: new insights into structural and functional diversity. *Curr Opin Struct Biol* **11**, 39–46.

Maret W, Vallee BL. 1998 Thiolate ligands in metallothionein confer redox activity on zinc clusters. *Proc Natl Acad Sci USA* **95**, 3478–3482.

Miller J, McLachlan AD, Klug A. 1985 Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. *EMBO J* **4**, 1609–1614.

Palmiter RD, Findley SD. 1995 Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *EMBO J* **14**, 639–649.

Peck Jr. EJ, Ray Jr. WJ. 1971 Metal Complexes of Phosphoglucomutase *in vivo*. *J Biol Chem* **246**, 1160–1167.

Simons TJB. 1991 Intracellular Free Zinc and Zinc Buffering in Human Red Blood Cells. *J Membr Biol* **123**, 63–71.

Vallee BL, Galdes A. 1984 The Metallobiochemistry of Zinc Enzymes. *Adv Enzymol Relat Areas Mol Biol* **56**, 283–430.

Vallee BL, Auld DS. 1990 Zinc Coordination, Function, and Structure of Zinc Enzymes and Other Proteins. *Biochemistry* **29**, 5647–5659.

Vallee BL, Falchuk KH. 1993 The Biochemical Basis of Zinc Physiology. *Physiol Rev* **73**, 79–118.

Yang Y, Maret W, Vallee BL. 2001 Differential fluorescence labeling of cysteinyl clusters uncovers high tissue levels of thionein. *Proc Natl Acad Sci USA* **98**, 5556–5559.