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Parallels and contrasts between iron and copper metabolism

Michael D. Garrick[1,*], Marco T. Núñez[2], Manuel Olivares[3] & Edward D. Harris[4]
[1]*Department of Biochemistry, SUNY at Buffalo, NY 14214, USA;* [2]*Department of Biology, Faculty of Sciences, University of Chile, Casilla 653, Santiago, Chile, and Millennium Institute for Advanced Studies in Cell Biology and Biotechnology, Santiago, Chile;* [3]*Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile;* [4]*Department of Biochemistry and Biophysics and the Faculty of Nutrition, Texas A&M University, College Station, TX, USA; *Author for correspondence (Tel: 716-829-3926; Fax: 716-829-225; E-mail: mgarrick@buffalo.edu)*

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Abstract

This paper reviews the Second International Workshop on Iron and Copper Homeostasis, held in Pucón, Chile 10–13 November, 2001. We cover the presentations and papers published (this issue) with the intent to point out parallels, contrasts and cutting edge areas rather than to say something about every paper. Iron and copper metabolism have been intertwined for nearly 150 years and the interrelationship is growing with advances in understanding the role of ceruloplasmin as one example and the probable role of hephaestin as another. The transporter DMT1 (divalent metal transporter 1) clearly plays a major part in iron uptake and trafficking. Emerging evidence suggests that it plays a lesser role in manganese, cadmium and copper transport; but it is still being evaluated there. Yet another interaction may come from the IRE/IRP (Iron Responsive Element/Iron Regulatory Protein) story where a paradigmatic role in iron homeostasis is well established, but interaction with copper is only now emerging. Parallels include the nutrient status of both metals based on their utility for redox reactions as well as their toxicity primarily via reactive oxygen species. The workshop also revealed that alternate splicing of pre-mRNAs for iron and copper related proteins and tissue specific responses are additional similarities. Regulation of gene expression and excretion offered contrasts between the two metals. The workshop also considered a series of continuing and emerging issues.

Introduction

The Second International Workshop on Iron and Copper Homeostasis was held in Pucón, Chile on 10–13 November, 2001. This paper reviews what transpired and is published in this special issue of BioMetals. The intent is to cover the highlights of the workshop, not to summarize each paper nor to comment on every paper. Thus we start by covering the interrelations between iron and copper homeostasis including the rationale for the workshop, next discuss parallels between the two metals, then move to some of the differences, finishing by pointing out issues that continued or emerged at the meeting.

Why hold an iron-copper conference

Iron and copper metabolisms are intimately intertwined; this relationship was the theme of the historical review that initiated the conference (Fox 2002). Systemic copper deficiency generates cellular iron deficiency (Gubler *et al.* 1952). Iron deficiency in humans leads to a constellation of problems including diminished work capacity, reduced intellectual capacity, diminished growth and diminished immune response. Yet the molecular basis for the iron-copper relationship is far from completely known. Copper is a prosthetic group of the ferroxidase involved in high affinity iron transport in yeast (Askwith *et al.* 1994). Deficiency in ceruloplasmin (Cp), a plasma

copper ferroxidase, induces iron deficiency (Harris 1995). Hephaestin, recently identified as the protein altered in the mouse mutant *sla* (sex linked anemia) apparently plays a similar role at the serosal side of the enterocyte (Vulpe *et al.* 1999). The divalent metal transporter DMT1 (also Nramp 2/DCT1) apparently transports both iron and copper (Gunshin *et al.* 1997). Whether this potential is actually realized is a topic for both a paper (Garrick *et al.* 2002) and an abstract (Arredondo *et al.* 2002) from this workshop. Excess iron or copper produces oxidative damage and cell death. Presumably this damage occurs because ferrous iron or cuprous copper catalyze, through the Fenton and Haber-Weiss reactions, the dismutation of hydrogen peroxide to form hydroxyl radicals. Preferential targets of the hydroxyl free radical are membrane lipids and chromatin. The generation of free radicals underlies the free radical-mediated theory of aging. Cells with low replacement rates, such as neurons, accumulate free radical-produced damage until death. Many of the papers from the workshop expand on this theme. Thus, organisms have a love-hate relationship with iron and copper. Both are nearly universally required nutrients whose excess, deficiency and genetic mismanagement cause impaired cellular functions and eventually cell death. Better understanding of iron of copper metabolism, and particularly of their interactions, needs to be developed.

Table 1. Iron/Copper interactions.

Fe & Cu intertwined for nearly 150 years
Both are required to prevent anemia
Hart and Elvejhem
Cu deficient pigs
Iron status affects Cu status
Ceruloplasmin & Hephaestin
DMT1
Ceruloplasmin
Aids iron exit, but not necessarily for all cells
Mutations point up role in Fe metabolism
Possible biomarker for Cu status
References to GPI-linked form
Hephaestin
Aids Fe exit; *sla* mouse
DMT1
Potential competition between Fe and Cu as transports both
Competition must be appropriately tested
Antisense confirms that DMT1 transports Cu in Caco-2 cells
IRP/IRE
Fe paradigm
Cu affects IRP1 too!?
How could Cu affect IRP1?

Iron/copper interactions

Fox (2002) reminded trainees attending the workshop that one should examine the foundation of current research frontiers. To quote Santayana: *Those who cannot remember the past are condemned to repeat it*. This fate is undesirable for one's own research. Table 1 highlights some of Fox's points, but the whole paper is also a part of this issue. Cp and Hephaestin are copper oxidases that clearly play a role in iron metabolism (Table 1). Both are involved in iron export from some cell types. Fox (2002) also reviewed work mostly from this laboratory that suggests that Cp may also be involved in a step (or steps) well before exit of iron. Miyajima *et al.* (2002) covered the disorder, Aceruloplasminemia. This genetic disease clearly underlines the critical role of the protein in iron metabolism in the brain and nervous system. Although Cp's copper content could make it a potential biomarker for exposure to toxic levels of copper, Araya *et al.* (2002) demonstrated that the levels of the protein in the serum were not responsive enough to copper exposure to make it a good predictor. In considering the role of Cp as part of the interaction between iron and copper metabolisms, one must keep in mind that Patel *et al.* (2000) identified an isoform of the protein that was GPI-anchored and detected particularly in the brain. This remarkable finding emphasizes potential partial overlap in roles between Cp and Hephaestin because not all of the former protein must be in the serum. Instead some of it could be directly involved in iron exit on the surface of cells. This new form was mentioned multiple times during the workshop and is a candidate for an activity identified by McArdle *et al.* (2002) in the placental model cell line, BeWo cells, that they initially considered was likely to be due to Hephaestin.

Hephaestin, named after the Greek god of the hearth who was a metal worker (Vulpe *et al.* 1999), clearly must be involved in iron exit from the enterocyte based on the phenotype of the *sla* mouse. The same phenotype also suggests that its role becomes redundant with that of another protein as the mouse ages past the weanling stage. Although Linder *et al.* (2002) reported studies premised on its role, it is still early

in the characterization of this probable copper oxidase and one hopes that advances in understanding its role more precisely will be part of a future conference on iron and copper homeostasis.

DMT1 (Table 1) is another potential crossroad for iron and copper homeostasis, given that Gunshin *et al.* (1997) reported that iron and copper elicited similar currents when they expressed the transporter ectopically in *Xenopus* oocytes. Garrick *et al.* (2002) noted that Cu^{2+} competes with $^{59}Fe^{2+}$ during transient expression of DMT1 in HEK293t cells; but that they had not been able to test directly for uptake of copper so could not test whether Fe^{2+} competed with copper uptake. They also point out that examining a range of concentrations of a potentially competitive metal is critical as evidence on the mode of inhibition. Arredondo *et al.* (2002) showed that an antisense oligonucleotide designed against DMT1 inhibited both iron and copper uptake in Caco-2 cells and that copper did compete with iron uptake in these cells. Their work is direct evidence that DMT1 is a copper transporter, but the extent to which its activity is redundant in this role remains to be determined. Linder *et al.* (2002) examined interactions between copper and iron in the same cell culture system. They found that depletion of cellular iron or copper increased apical uptake of both metal ions and that depletion of iron or copper also enhanced transepithelial transport of iron from the apical to the basal chamber. Some of these results probably reflect DMT1 behavior.

The IREIRP (Iron Responsive Element/Iron Regulatory Protein) regulatory paradigm (Table 1) was reviewed by Kim and Ponka (2002) and by Hentze (2002). (The latter unfortunately could not provide a full paper.) This response system assures that many mRNAs containing IRE(s) will express themselves appropriately in relation to iron availability. Other than this workshop, there has been only a little evidence (Oshiro *et al.* 2002) that any other metal might affect this response system. Arredondo *et al.* (2002) showed that the response of IRP1 to copper was similar to its response to iron. As the effect of iron levels is thought to be mediated by alterations in the Fe-S cubane cluster, their observations raise an issue of how copper would affect IRP1. It is difficult to envision how copper could work via a similar mechanism.

Parallels between iron and copper

Both iron and copper are essential nutrients (Table 2) for most organisms. Much of their utility is based on their ability to assume at least two oxidation states so that they participate in redox reactions. Unfortunately ever since the atmosphere began to contain molecular oxygen, this same property has become a basis for life's love-hate relationship with the metals as the Fenton reaction (and Haber-Weiss reaction) lead to the formation of reactive oxygen species (ROS). Another consequence is that both ferric iron and cuprous copper are essentially insoluble in aqueous solutions at neutral pH and would precipitate under most physiological conditions. The potential toxicity and the challenge of maintaining solubility are why the metals are associated with carriers or chaperones at nearly all stages of their metabolism. When the capacity of the carrier/chaperone/storage systems is overloaded, then the metal becomes toxic.

The essentiality (in nearly all organisms) of both copper and iron combined with the toxicity of high levels in turn underlies another shared property (Table 2) – the observation that many response curves are bell shaped or U-shaped (Núñez *et al.* 2002; Hentze 2002; Youdim *et al.* 2002; Lu *et al.* 2002; Arredondo *et al.* 2002). This characteristic clearly initially reflects regulatory responses to demand then to the need for protection against toxicity but finally reflects the overwhelming of the protective mechanisms by toxicity. This workshop provided particularly clear examples of these curves, some of which were novel.

Alternate splicing of pre-mRNAs leading to protein isoforms is a frequent occurrence for expression of specific genes within mammalian genomes; this pattern applies to genes involved in iron and copper homeostasis. Two presentations (Garrick *et al.* 2002; Hentze 2002) considered the isoforms of DMT1; another briefly reviewed multiple transcripts from the Menkes' gene (Harris *et al.* 2002). On occasion a relevant protein for both genes is found in the nucleus (Garrick *et al.* 2002; Harris *et al.* 2002), raising the question of why it is there. The isoforms of proteins involved in metal metabolism appear to vary in type and abundance according to the cell that expresses them (Haile 2002; Hentze 2002). In the case of Cp the observation that the major lesions in Aceruloplasminemia are in the brain (Miyajima *et al.* 2002) surely relates to the finding of a GPI-anchored isoform in the brain (Patel *et al.* 2000).

Table 2. Parallels between iron and copper.

Essential nutrients
Useful for redox reactions
Fenton reaction → ROS
Fe^{3+} and Cu^{+} are essentially insoluble without a carrier or chaperone under physiological conditions
Overload → toxicity
Many response curves are bell shaped or 'smile'
Reflect protection/requirement over lower range then overwhelming response and/or generating ROS
Alternate splicing for relevant mRNAs
Adds another level for regulation
Nuclear targeting?
Isoforms and responses vary according to cell specificity
DMT1
MTP1
MNK
Cp
Transport motifs

Of course most transporters for metals are not located in the nucleus. The identification of motifs for localizing the Cu-ATPases was a theme for Mercer *et al.* (2002). Similarly, the finding of the -IRE form of DMT1 in the nuclei of PC12 and other neuronal cells (Garrick *et al.* 2002) raises intriguing questions on the role of metal transporters there.

Differences between iron and copper

Of course iron and copper homeostasis differ significantly too (Table 3). Excretion of copper as noted by Linder *et al.* (2002) is a major step for homeostatic regulation whereas iron is lost through processes that proceed independently relative to body iron levels except to the extent that higher iron content of sloughed cells leads to slightly greater excretion. One therefore wonders why DMT1 levels are high in the kidney, a location that one might ordinarily associate with excretion. Perhaps DMT1 is there to assure recovery of filtered iron or to manage the homeostasis of another metal.

The IRE/IRP paradigm dominates thinking about regulation of iron homeostasis (Table 3). It focuses attention on post-transcriptional mechanisms particularly involving initiation of translation and mRNA stabilization. Kim and Ponka (2002) and Hentze (2002) reviewed this important system; while Kim and Ponka (2002) also focused on how nitric oxide interacts with IRPs. Núñez *et al.* (2002) considered circumstances in which the IRP1 shut-off mechanism is overwhelmed by iron-mediated oxidative stress, while Garrick *et al.* (2002) and Haile (2002) mentioned transporters where the mRNA can contain an IRE but the regulatory significance is yet to be characterized. In contrast, copper homeostasis involves more reliance on transcriptional regulation. Harris *et al.* (2002) have begun to identify regulatory motifs in the Menkes' gene promoter. One should not forget that other levels of regulation are important. For copper homeostasis, Mercer *et al.* (2002) discussed how copper alters trafficking of the ATPases involved in its metabolism while Lu *et al.* (2002) showed that a copper chaperone is subject to copper-activated proteolysis. The admonition to examine other levels of regulation has perhaps not been given its due in studies of iron homeostasis heretofore; but Hentze's (2002) observation of new isoforms of DMT1 based on variation in the initial exon (reviewed in Garrick *et al.* (2002)) certainly draws attention to regulation via both the alternate promoters of this gene and its alternate splicing (subsuming alternate polyadenylation sites).

Table 3. Differences between iron and copper.

Excretion
Cu regulated but Fe is essentially constitutive
Regulation
IRE/IRP paradigm emphasizes translation and mRNA stabilization for iron
Transcriptional regulation is the major mode for copper
BUT
Copper has other modes like trafficking changes and proteolytic turnover
Should transcription and alternate splicing be studied more for iron?
Bacterial and yeast systems are closely parallel to mammalian metabolism for copper; but microbial models are less helpful for iron
DMT1/MTP1 appear to be more closely related to SMF1-3 and bacterial Mn transporters than main yeast and bacterial Fe systems

Comparing work on bacterial copper homeostasis (Table 3) e.g., that of Lu *et al.* (2002), and studies in yeast (here reviewed by De Freitas *et al.* (2002)) to presentations on mammalian copper homeostasis like that of Mercer *et al.* (2002), one is struck by the close parallels among the microbial and the mammalian systems. These parallels are weaker in the case of iron homeostasis – also reviewed by De Freitas *et al.* (2002). One sees little parallel for example for the IRE/IRP system or for the transferrin cycle. Also the transporters DMT1 and MTP1 appear to be hijacked by evolution from homologues that transport manganese in bacteria and from the SMF1, SMF2 and SMF3 proteins of yeast that are involved in iron metabolism as back-up systems while the main actors are other proteins (De Freitas *et al.* 2002).

Themes and questions – continuing and emerging

One topic that recurred was that cell responses and isoform content for particular gene products vary with the cell type (Table 4). As it has been covered well above, we will not repeat statements nor references here.

Accumulating evidence starting with Rae *et al.* (1999) indicates that free copper occurs at less than one molecule per cell. That is, copper always is bound or associated with a chaperone. Although the presumption behind many studies, particularly those on the labile iron pool, is that free iron exists in cells, could it be the case that it does not? How would one address this issue? The best experimental approach described until now is the labile iron pool assay (Epsztejn *et al.* 1997) that defines the pool of reactive iron bound with low affinity (Ka $< 10^6$) to undetermined cell components.

Metallothionein plays a major role in sequestration of copper (Cherian 2002). Does this ability to bind copper make it a functional analog of ferritin? Reversing viewpoints, one of the trainee presentations (Tapia & González 2002) demonstrated that mutant fibroblasts null for metallothionein preserved copper homeostasis despite the loss of this storage capability.

The potential ability of DMT1 to transport other divalent metals (Gunshin *et al.* 1997; Garrick *et al.* 2002) leaves us to wonder about the extent to which these metals actually interact with iron (and copper). It seems untidy of nature to leave the extent of lead toxicity dependent on the iron status of the cell or individual. Similarly Roth *et al.* (2002) demonstrate that iron status does affect manganese toxicity likely via shared transporters like DMT1.

DMT1 not only transports a multiplicity of metals, it also has a multiplicity of names (DCT1, Nramp2, SLC11A2). A similar plethora of names applies to MTP1 (Ferroportin1, Ireg1, SLC11A3). A convention agreeing on a single name for each transporter would certainly make life easier for all concerned.

Cutting edge research tools are often valuable because they make new vistas accessible. An unfortunate coincidence has deprived this issue of the results for three applications of one such tool to iron and copper homeostasis: Vulpe presented data from an array based analysis comparing wild-type yeast to a strain with a targeted disruption of *Mac1*, a gene encoding a critical regulator of copper metabolism. The results corresponded very well to our current knowledge of copper and iron homeostasis in yeast and yielded some

Table 4. Themes and questions – Continuing and emerging.

Responses and isoforms vary with cell type
Copper is never free; could it be the same for iron?
Does metallothionein act like a primitive ferritin for copper?
We need metallothioneins or do we?
Other metals can interact with iron and/or copper too, but which?
Let's agree on one name for each iron transporter instead of having to keep track of up to 4
Arrays are wonderful tools
When a good metal goes bad, it's sad
'What a waste it is to lose one's mind'
Restless Legs Syndrome
Menkes'
Alzheimer's
Wilson's
Parkinson's
Aceruloplasminemia
Neurodegeneration
Carcinogenesis
Need biomarkers for exposure
Kupffer cells can be poisoned
Hereditary hemochromatosis
NO problems
Pregnancy is not a disease, but it's also not always easy

novel insights. Their review for this issue (De Freitas *et al.* 2002) covers the assigned topic of yeast as a model for iron and copper homeostasis so one looks forward later to reading about the array data. Hentze (2002) and Youdim *et al.* (2002) also showed fascinating array based data but each was unable to provide a full length Ms. The former study focused on selected probes rather than thousands of cDNAs and revealed interesting profiles after varying iron levels in cell culture or comparing normal mice to HFE knockouts. The latter analysis compared Parkinson's disease models to controls and also to models after exposure to potential neuroprotective reagents. Again multiple changes in expression were found.

"What a waste it is to lose one's mind. Or not to have a mind is being very wasteful. How true that is." – Dan Quayle. Although for former Vice-President of the USA addressed these remarks to the NAACP, he managed to capture a point related to the strongest theme of this workshop. Burdo and Connor (2002) covered brain iron homeostasis and disorders where homeostasis goes awry. They reviewed data relevant to multiple disorders including Restless Legs Syndrome, Alzheimer's and Parkinson's Disease and Hereditary Hemochromatosis. Perry *et al.* (2002) addressed the potential breakdown of iron homeostasis in Alzheimer's Disease and pointed out that copper too is found in the redox active metal associated with oxidative damage in this disorder. Egaña *et al.* (2002) have found signal-alterations for tau protein in hippocampal cell cultures that are associated with iron-induced oxidative stress, an intriguing result. Copper redox activity (Opazo *et al.* 2002) also has intriguing associations with neurodegeneration. When one adds the syndromes associated with Wilson's and Menkes' Diseases (Mercer *et al.* 2002) and Aceruloplasminemia (Miyajima *et al.* 2002), it becomes strongly apparent that copper and iron are critical for proper mental development and toxicity due to involvement of one or both is closely involved when many forms of neurodegeneration set in. Untangling their role(s) in neurodegeneration is clearly a task as difficult as removing the tangles in Alzheimer's brains, but papers in this workshop have made a start in this challenging task.

Neurons are not the only cells where iron and copper management issues arise. Okada (2002) illustrated

the exquisite sensitivity of renal tissue to iron toxicity and a peculiar and specific kind of carcinogenesis. Videla *et al.* (2002) examined the role of Kupffer cells in hepatotoxicity after exposure to elevated iron or copper. The placenta is where much of the stress of pregnancy is managed; iron status of the fetus and mother depend critically on it. McArdle *et al.* (2002) reviewed how this management occurs and examined data on the efflux of iron in model systems. Biomarkers for iron status are well established (Finch *et al.* 1986), but copper particularly needs markers for overexposure. Speisky *et al.* (2002) and Araya *et al.* (2002) are in pursuit of this goal; Tapia *et al.* (2002) provide data that should help to make this pursuit fruitful.

Future workshops

This is an exciting time for research on iron and copper homeostasis. An outline of the interactions between these metals has emerged and details are coming in. Clearly more will be accomplished in the immediate future. This meeting should provide the basis for fruitful collaborations among researchers. We hope to see enough progress to schedule at least a third workshop.

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