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Meeting report – Copper research at the top

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Abstract

In this brief paper, the author reports on a meeting on copper research (2nd International Meeting on Copper Homeostasis and its Disorders: Molecular and Cellular Aspects) recently held in Ravello, Italy (17–21 September 1999). Aimed at elucidating the diverse roles played by copper ions in biology and medicine, as they are currently intensely investigated worldwide, the meeting has been organized around a number of major topics from prominent areas of copper research. These included the molecular and cellular basis of copper transport, molecular advances in Menkes and Wilson's diseases, the involvement of copper in neurodegenerative diseases, the structure and function of copper metalloproteins.

Abbreviations: ALS – amyotrophic lateral sclerosis; CCS – copper chaperone for superoxide dismutase; Cu-Zn SOD – copper-zinc superoxide dismutase; LTQ – lysine tyrosylquinone.

Copper is one of the most prevalent biological transition metals, second only to iron, being an essential micronutrient required for survival by all organisms, from bacteria to humans. Indeed, thanks to its unique chemistry, copper plays a key role as a cofactor at the active site of many important redox enzymes that carry out a number of fundamental biological functions (Table 1). It follows that the consequences of deficiency or defective functioning of these enzymes may often be, grave ending with severe disease states. Since copper concentration in biological habitats and nutrients is usually low, appropriate mechanisms must exist that ensure its accumulation in living cells through active transport and its delivery to copper-requiring enzymes (Leone & Mercer 1999).

But copper is a Janus-faced element. While it is necessary for life, copper is also highly toxic when present in excess of cellular needs (Dameron & Harrison 1998). Paradoxically, the same redox chemistry that makes it precious when incorporated in an enzyme's frame contributes to the production of hydroxyl radicals and other highly reactive oxygen species when copper exists as a free ion in solution. To circumvent these problems, copper ions must be sequestered in non-reactive forms, usually complexed with specific proteins, to be transported into cells and moved through cellular compartments (Valentine & Gralla 1997; Radisky & Kaplan 1999).

In definitive, the maintenance of appropriate copper homeostasis depends on a delicate balance between copper uptake and distribution on one hand, and detoxification and removal on the other (Leone & Mercer 1999; Peña *et al.* 1999).

Copper metabolism and its regulation are currently the subjects of intense investigation worldwide, and our knowledge of this area has increased substantially in the last years. Despite these efforts, however, much remains to be learned. A recent meeting was organized at Ravello* (Italy) to give workers an opportunity to discuss some of the more recent developments on copper homeostasis and related diseases, and on other prominent areas of copper research. Nearly 130 scientists representing more than a dozen countries at-

*The 2nd International Meeting on Copper Homeostasis and its Disorders: Molecular and Cellular Aspects, Ravello, Italy, 17–21 September 1999.

Table 1. Biological function of selected copper-binding proteins and the known or expected consequence of their deficiency or defect*.

Common name	Biological function	Consequence of deficiency or defect
Cu-Zn SOD	Free radical detoxification	Oxidative damage of cell components
Cytochrome *c* oxidase	Terminal oxidase	Symptoms of deficiency of ATP: muscle weakness, neurologic effects, hypothermia
Lysyl oxidase	Cross-linking of collagen and elastin	Decreased strength of collagen and elastin: arterial abnormalities, bladder diverticula, loose skin and joints
Dopamine β-hydroxylase[a]	Norepinephrine synthesis	Neurologic effects, possible hypothermia
Peptidylglycine α-hydroxylating monooxygenase[b]	Bioactivation of peptide hormones	Probable widespread effects through malfunction of several peptide hormones
Tyrosinase	Melanin synthesis	Hypopigmentation
Ceruloplasmin	Ferroxidase activity, copper transport	Anemia, secondary copper deficiency
Blood clotting factors V and VIII	Blood clotting	Tendency to bleed
Angiogenin	Induction of blood vessel formation	Defective blood vessel development
Metallothionein	Copper sequestration	Copper toxicity
Prion protein	Normal function currently unknown (believed to have a role in copper uptake)	Prion diseases: Creutzfeld-Jacob disease, Kuru, Gerstmann-Straussler-Scheinker disease, Fatal familial insomnia
β-amyloid precursor protein	Normal function currently unknown	Familial Alzheimer's disease
Hephaestin	Iron egress from intestines	Sex-linked anemia
Sulfhydryl oxidase	Cross-linking of keratin (disulphide bond formation)	Pili torti

*Compiled, with minor modifications, from Harrison & Dameron (1999) and Peña *et al.* (1999).
[a]Often listed as a monooxygenase.
[b]This is one functional component of the enzyme peptidylglycine α-amidating monooxygenase.

tended this meeting. What follows is a brief account of some of the main contributions and discussion, which focused on a number of distinct yet related topics, including molecular and cellular basis of copper transport, molecular advances in Menkes and Wilson's diseases, copper related disease states, structure and function of copper metalloproteins, role of copper in cellular regulation, metabolic interactions of copper with iron and zinc, role of copper in plant physiology.

Copper transport and metallochaperones

A number of recent studies have helped at elucidating the main features of copper trafficking (Peña *et al.* 1999). At first, two cytoplasmic proteins were identified in yeast that specifically bind copper and deliver it to a determinate intracellular target: Cox17 (Glerum *et al.* 1996), which gets Cu to the mithochondria where it is required for the activation of cytochrome *c* oxidase, and Atx1 (Lin *et al.* 1997; Pufahl *et al.* 1997), which carriers copper ions to a P-type copper-transporting ATPase in the secretory pathway. Since then, the picture has enlarged considerably, and it is now clear that a number of proteins, commonly referred to as 'copper chaperones', exist in bacteria, yeasts, plants and animals that function to deliver copper to specific copper-requiring proteins or subcellular compartments, while preventing cytoplasmic exposure to copper ions in transit that would result in oxidative damage (Harrison *et al.* 1999).

A major breakthrough in this field has been the recently solved X-ray structure (at 1.8 Å resolution) of the yeast CCS (Lamb *et al.* 1999), a copper chaperone that specifically inserts copper in the copper-zinc superoxide dismutase (Cu-Zn SOD) in the cytosol (Culotta *et al.* 1997).

Several groups at the meeting reported progress in delineating different aspects of the Cu transport machinery. Dennis Thiele (University of Michigan Medical School, Ann Arbor, USA) reported on recent work that provides novel, valuable insight into the biological roles and mechanisms of action of copper metalloregulatory trascription factors in the regulation of metal ion transport genes. Attendants learned also about the first demonstration of the direct transfer

of stoichiometric amounts of Cu ions from a copper chaperone to a target protein, achieved by studying the components required for chaperone-mediated copper transfer in *Enterococcus hirae* (Charles Dameron, University of Queensland, Coopers Plains, Australia).

Valeria Culotta (Johns Hopkins University School of Public Health, Baltimore, USA) presented recently collected evidence that suggest that CCS and other copper chaperones do not retrieve the metal directly from cell surface transporters but may capture the ion from specialized intracellular stores. Research summarized by Thomas O'Halloran (Northwestern University, Evanston, USA) was of relevance to the comprehension of the steps of capture and release in the metal transfer mechanism of copper chaperones, such as Atx1 and CCS, that are currently the object of extensive physical, chemical, structural and mechanistic studies.

Menkes and Wilson's diseases

Failure to maintain an appropriate copper homeostasis may lead, as underlined above, to severe illness. This is underscored by the existence of Menkes and Wilson's diseases, two well characterized disorders known to be caused by genetic defects in copper transport (Danks 1995; DiDonato & Sarkar 1997; Leone & Mercer 1999). Menkes disease is associated with copper deficiency resulting in deficient supply of copper to copper enzymes. It is an X-linked, recessively inherited disorder characterized by progressive neuronal degeneration, severe mental retardation, connective tissue disturbances, and patient mortality in early childhood. Wilson disease is an autosomal recessive disorder. Patients with Wilson disease accumulate copper in the liver and brain, with progressive liver damage, neurodegeneration, and impairment of copper incorporation into ceruloplasmin (Danks 1995; DiDonato & Sarkar 1997; Leone & Mercer 1999).

Work conducted in the last few years permitted to identify the human Menkes and Wilson's disease genes, and provided precious insight into the structure, function, and localization of their gene products. These proteins, which are defective in patients afflicted with Menkes or Wilson's diseases, have been shown to be in both cases membrane-localized copper-transporting P-type ATPases, that share a similar structure and play a key role in the redistribution of copper in the cell and tissues, and in the supply of copper to secreted copper enzymes (for review see Harrison & Dameron 1999; Peña *et al.* 1999).

A considerable part of the Ravello meeting was devoted to the discussion of recent findings on the molecular and cellular basis of Menkes and Wilson's diseases. The available animal models, molecular genetics, pathophysiology, clinical manifestations and treatments of both disorders, and a lot beside, were reviewed and updated in a number of lectures and posters. Key contributions focused on the copper-induced trafficking of the Menkes and Wilson copper ATPases (Julian Mercer, Deakin University, Burwood, Australia), the Rab proteins and the trafficking of the Menkes disease protein (Arturo Leone, Università di Salerno, Fisciano, Italy), the structure, function and regulation of the Wilson's protein (Svetlana Lutsenko, Oregon Health Sciences University, Portland, USA), the phenotype/genotype correlations and effect of the most common mutation of the hemochromatosis gene (C282Y) in patients with Wilson disease (Diane Cox, University of Alberta, Edmonton. Canada).

Copper and neurodegenerative diseases

Copper plays a fundamental role in the biochemistry of the human nervous system. However, studies conducted in the last years have shown that copper is also implicated in the pathogenesis of neuronal injury in a number of neurodegenerative disorders, such as Alzheimer's disease, prion-mediated encephalopathies, familial amyotrophic lateral sclerosis (Familial ALS), and other age-related diseases (Waggoner *et al.* 1999). In some cases, it is now evident that copper may bind to proteins associated with neurodegeneration, including the prion protein and the β-amyloid precursor protein in Alzheimer's disease, implicating copper as mediator of oxidative stress in neurodegenerative diseases (Sayre *et al.* 1999). For sure, general agreement exists in the scientific community that our understanding of the pathophysiology and treatment of neurodegenerative disorders relies also on the elucidation of the mechanisms of copper trafficking and metabolism within the nervous system.

To this end, Gerd Multhaup (University of Heidelberg, Heidelberg, Germany) described new data that suggest that copper interferes with β-amyloid precursor protein processing, in a way that strengthens the hypothesis that the Alzheimer's protein may have a normal function in copper homeostasis.

Other contributors have informed the delegates on recent progress made studying the familial form of the motor neuron disease ALS (also known as Lou Gehrig's disease), that is often associated with mutations in the gene encoding Cu-Zn SOD that may confer new and toxic properties to the protein. Joan Valentine (UCLA, Los Angeles, USA) and colleagues have purified a series of these human Cu-Zn SOD mutants and characterized them with respect to metal-binding properties and chemical reactivities. In particular, elevated levels of peroxidase activity in the mutant proteins relative to the wild type, and the fail of zinc ions at stabilizing the mutant dimer, have been noted, as well as the presence of a new copper-binding site at the subunit interface of the H46R mutant. Reporting further work on the same human Cu-Zn SOD mutant, Giuseppe Rotilio (University of Roma 'Tor Vergata' Roma, Italy) presented sound evidence that the aberrant copper chemistry of this mutant enzyme is the actual mediator of the oxidative stress in a human cellular model of ALS, supporting the theory that this neurodegenerative disease may have an oxidative stress-linked pathogenesis (see also Gabbianelli *et al.* 1999).

Structure and biosynthesis of copper proteins

Recent progresses made towards the elucidation of the structure and biosynthetic pathways of copper proteins, as well as the research trends in this field, have also been reported at the meeting. In particular, Ivano Bertini (CERM Polo Scientifico, Sesto Fiorentino, Italy) stressed the wealth of information which is possible to gather from NMR studies of copper proteins. In addition, new data on the copper transferase properties of ceruloplasmin (Lilia Calabrese, University of Roma Tre, Roma, Italy), that harbors three different Type 1 copper centres, in addition to a Type 2/Type 3 trinuclear copper cluster, and is considered the most complex amongst blue multicopper oxidases, and a recently solved X-ray structure of bovine heart cytochrome *c* oxidase in the fully oxidized state at 2.3 Å (Shinya Yoshikawa, Himeji Institute of Tecnhology, Akoh Hyogo, Japan), a resolution which permits to better delineate some aspects of the reaction mechanism, were presented (see the concise but informative review by Malmström and Leckner, 1998, to get more information on several aspects of copper biochemistry).

Robert Rucker (University of California, Davis, USA) described the effect of Cu deprivation on the *in vivo* activation of lysyl oxidase, a crucial enzyme which catalyses the cross-linking of extracellular matrix proteins and is defective in Menkes disease (Smith-Mungo & Kagan 1998). Interestingly, Cu deficiency was found not to influence lysyl oxidase expression, but resulted in an enzyme with reduced levels of its active site redox cofactor, lysine tyrosylquinone (LTQ), which is postulated to derive from a Tyr inserted in the polypeptide chain through a copper-dependent reaction (Rinaldi *et al.* 1999).

These are only a small sample of the results reported at the meeting, and the pace of discovery is such that there are too many exciting results to describe in a short report. We look forward to the next Copper Meeting to see how further we have come towards our goal of understanding the multiple roles copper plays in biology and medicine. Results reported at the Ravello Meeting suggest that we are on our way towards achieving this goal.

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