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Mechanism and regulation of intestinal copper absorption

Yasuhiro Nose, Dennis J Thiele.

Department of Pharmacology and Cancer Biology, Duke University School of Medicine, Durham, North Carolina, USA

Copper (Cu) is an essential cofactor for a broad variety of biological functions from mitochondrial energy generation and free radical detoxification to iron acquisition and peptide hormone maturation. It has been estimated that approximately 2.0 mg dietary Cu is needed by humans each day, but the precise levels of Cu for adequate function in humans are relatively poorly understood (Milne, 1998). The ability of copper to assume two oxidation states, oxidized copper (Cu^{2+}) or reduced copper (Cu^{+}), provides the redox power to drive enzymatic reactions and the chemical variability to coordinate with distinct preferred sets of amino acid ligands to facilitate a variety of protein structures. For example, Cu^{+} prefers thiol rich ligands and Cu^{2+} is often found associated with nitrogen or oxygen ligands (Davis and O'Halloran, 2008). While Cu is essential for normal mammalian growth and development, excess Cu is thought to engage in redox chemistry that generates damaging free radicals such as hydroxyl radical (Halliwell and Gutteridge, 1990). Cu has also been demonstrated to cause toxicity due to its ability to compete for iron incorporation into iron sulfur clusters (Macomber and Imlay, 2009).

Many of the nuts and bolts of Cu metabolism have been elucidated at the cellular level through both studies in microbes and through the analysis of human genetic disorders of Cu metabolism (Lutsenko and Petris, 2002; Puig et al. 2002; De Bie et al. 2007; Kim et al. 2008). These experiments reveal an impressive conservation of structure and function with respect to the proteins that carry out Cu homeostasis and in the fundamental tenet that there are extraordinarily low levels of intracellular free Cu (Rae et al. 1999). In mammalian cells Cu import is carried out, at least in part, by the Ctr1 protein, a homotrimeric channel-like protein that facilitates Cu^{+} uptake with high affinity (Lee et al. 2002a; Puig and Thiele 2002; Nose et al. 2006b; De Feo et al. 2009). However, the observation that mouse embryonic fibroblasts lacking Ctr1 harbor an activity for Cu uptake that may have low affinity and may import Cu^{2+}, suggests that there are alternative mechanisms for Cu absorption that must be defined (Lee et al. 2002b). Intracellular Cu is routed to proteins and compartments, as an essential part of the controlled distribution process, via Cu chaperone proteins (Rosenzweig and O'Halloran 2000; Luk et al. 2003; Robinson and Winge 2010) and the delivery of Cu to the secretory lumen, or its removal from cells, makes use of two Cu^{+} transporting ATPases, ATP7A or ATP7B (Lutsenko and Petris 2002; Bartee and Lutsenko 2007). Given that Cu is essential but reactive, and there are very low levels of labile intracellular Cu, it is clear that Cu homeostasis at the cellular level is very tightly controlled. Mechanisms for controlling Cu homeostasis have been reported to occur at the level of Cu induced transcription of genes encoding the metallothionein Cu binding proteins, protein trafficking in response to changing Cu levels and through changes in protein stability in response to Cu (Bertinato and L'Abbé 2003; Petris et al. 2003; Greenough et al. 2004; Nittis and Gitlin 2004; West and Prohaska 2004; Nose et al. 2010). However, little is known about how mammals regulate systemic Cu homeostasis.

While the regulation and maintenance of systemic Cu metabolism is critical to human health, severe genetic diseases of Cu metabolism exist and copper deficiency causes myeloneuropathy, anemia, cognitive disorders and cardiac hypertrophy (Prohaska 1983; Kumar and Low 2004; Madsen and Gitlin 2007). Dietary Cu is absorbed through the intestine, stored in the liver and mobilized into the circulation for provision to peripheral organs. To begin to understand how Cu is absorbed from the diet, we and others ascertained the subcellular location of the Ctr1 high affinity Cu^{+} transporter in intestinal epithelial cell. While one study found Ctr1 on the basolateral membrane of Caco2 cells and mouse intestinal epithelial cells (Zimnicka et al. 2007) the dominant observation in the field is that Ctr1 is localized to

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the apical surface of intestinal epithelial cells of mouse, rat and pig (Bauerly et al. 2004; Nose et al. 2006a; Kuo et al. 2006; Nose et al. 2010). To begin to understand the potential role of Ctr1 in intestinal Cu absorption, we generated a Ctr1 floxed mouse to facilitate tissue-specific and temporal Ctr1 knock out mice. Consistent with a critical role for Ctr1 in dietary Cu absorption, mice bearing an intestinal epithelial cell-specific (IEC) loss of Ctr1 demonstrated Cu deficiency in all peripheral tissues examined and developed severe cardiac hypertrophy, similar to what is observed in animals reared on a Cu deficient diet (Nose et al. 2006a). To explore whether there is a cardiac-intrinsic requirement for Cu or whether cardiac hypertrophy is a response to peripheral Cu deficiency, we constructed a cardiac-specific Ctr1 knock out mouse. Indeed, both fruit flies and mice lacking Ctr1 in heart tissue develop cardiac hypertrophy, indicating a cardiac specific requirement for Cu.

Interestingly, mice with a cardiac-specific Ctr1 knock out send a signal back to intestinal epithelial cells and to the liver, the major Cu storage organ, to induce expression of the ATP7A Cu efflux transporter that moves more Cu across the IEC basolateral membrane and, presumably, out of the liver into the bloodstream (Kim et al. 2010). These results suggest the presence of a currently undefined systemic Cu homeostasis regulatory mechanism that allows the Cu status of the heart to be communicated to the Cu transport machinery expressed in tissues involved in Cu uptake and in Cu storage. Interestingly, serum from mice bearing a cardiac-specific Ctr1 knock out was able to induce ATP7A protein levels in Caco2 and other cell lines, as compared to serum from control mice, suggesting the involvement of a diffusible factor in the bloodstream for the communication of a Cu deficiency signal. Whether this signal represents a hypoxic response due to compromised cardiac function, a hormonal response or a Cu-specific sensing mechanism remains to be elucidated.

The mechanisms of intestinal Cu transport, at a minimum, involve the apical membrane Cu^+ transporter Ctr1, intracellular Cu chaperones and the basolateral ATP7A Cu^+ efflux pump. While our understanding of the fundamental mechanism of action of these proteins is being intensely investigated, we know little about how this basic machinery is regulated and how the Cu status in peripheral tissues communicates back to the intestinal absorption machinery to regulate intake.

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