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# The role of Ctr1 and Ctr2 in mammalian copper homeostasis and platinum-based chemotherapy

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ABSTRACT

Copper (Cu) is an essential metal for growth and development that has the potential to be toxic if levels accumulate beyond the ability of cells to homeostatically balance uptake with detoxification. One system for Cu acquisition is the integral membrane $Cu^+$ transporter, Ctr1, which has been quite well characterized in terms of its function and physiology. The mammalian Ctr2 protein has been a conundrum for the copper field, as it is structurally closely related to the high affinity Cu transporter Ctr1, sharing important motifs for Cu transport activity. However, in contrast to mammalian Ctr1, Ctr2 fails to suppress the Cu-dependent growth phenotype of yeast cells defective in $Cu^+$ import, nor does it appreciably stimulate Cu acquisition when over-expressed in mammalian cells, underscoring important functional dissimilarities between the two proteins. Several roles for the mammalian Ctr2 have been suggested both in vitro and in vivo. Here, we summarize and discuss current insights into the Ctr2 protein and its interaction with Ctr1, its functions in mammalian Cu homeostasis and platinum-based chemotherapy.



## Introduction

Copper (Cu) is an essential metal to all aerobic life that is used in a wide range of enzymatic reactions owing to its ability to undergo redox reactions (see Cu reviews [1–3]). Our knowledge of how Cu traverses cell membranes, is trafficked intracellularly by its chaperone proteins, and is transported out of cells or incorporated into Cu-dependent proteins has drastically increased over the last few decades. We now recognize that the evolutionary conserved $Cu^+$ transporter 1, Ctr1, is part of a major pathway for cellular Cu uptake [4–10] and that Cu chaperones hand over Cu to Cu, Zn superoxide dismutase (SOD1) via direct ligand-exchange interactions between the Cu chaperone CCS and SOD1 [11–14], and between the Cu chaperone Atox1 and the two Cu-transporting ATPases, Atp7A and Atp7B [15–18], which pump Cu into both the secretory- and export pathways [19,20]. The discovery of an existing protein homologous to Ctr1, presented the field with another potential player in mammalian Cu homeostasis; the Cu transporter 2, Ctr2 [5]. Although, seventeen years have passed since Zhou and Gitschier made the discovery of a cDNA encoding this protein related to Ctr1, in contrast to the wealth of information regarding the structure, physiological function and mechanism of action of mammalian Ctr1, comparatively little is known about Ctr2 in mammalian Cu homeostasis. Here, we summarize and discuss what is currently known about the roles the enigmatic mammalian Ctr2 protein plays in Cu uptake, intracellular Cu distribution, Cu utilization, and platinum-based chemotherapy and its functional interaction with Ctr1.

## Gene and protein organization and structure

By DNA sequence analysis Zhou and Gitschier [5] identified a human gene, h*CTR2* (SLC31A2), which is highly homologous to the h*CTR1* gene (SLC31A1). Both h*CTR2* and h*CTR1* are located on chromosome 9 on the positive strand, separated by approximately 60 kb that encompasses the *FKBP15* gene on the negative strand. The SLC31A2 gene covers 17 kb, with its encoded mRNA possessing four exons, while the SLC31A1 gene covers 43 kb encoding an mRNA which has five exons. However, the consensus coding DNA sequences (CDS) are 50% identical between the two genes (CCDS, Clustal Omega). Like human Ctr1, the human Ctr2 mRNA is ubiquitously expressed in all tissues evaluated, with the highest levels found in brain, spleen, placenta, pancreas, and testis, and lower levels in liver, thymus, ovary, intestine and colon [5]. Mouse Ctr2 is also ubiquitously expressed but show a somewhat different mRNA expression pattern with the highest levels found in heart, liver, kidney, and testis and lower levels in muscle and brain [21].

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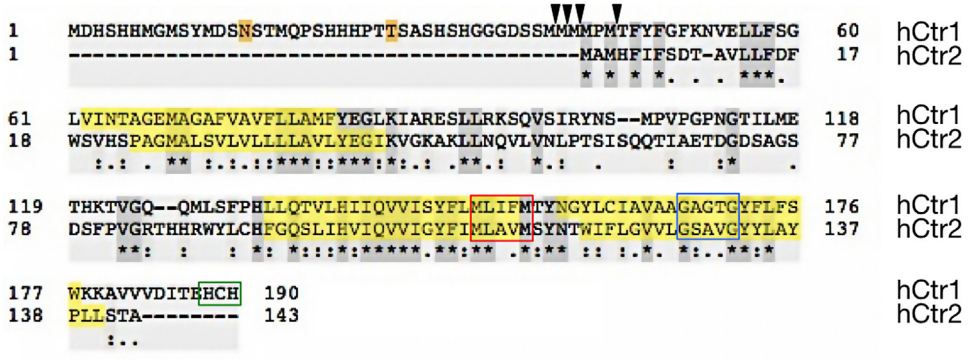


**Fig. 1.** Alignment of human Ctr1 and Ctr2 showing trans-membrane domains in yellow and glycosylation sites in the ecto-domain of Ctr1 in orange. Cleavage sites of Ctr1 protein ecto-domain are indicated with vertical black arrowheads. The Met-$X_3$-Met motif in second trans-membrane domain, crucial for $Cu^+$ transport activity, is boxed in red and the Gly-$X_3$-Gly in third trans-membrane domain, involved in helix packing, is boxed in blue. The Cys-His-Cys motif at the carboxyl-terminus of Ctr1, involved in trafficking Cu to the chaperones CCS and Atox1, is boxed in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Interestingly, the steady state levels of mouse Ctr1 mRNA has a similar expression profile as mouse Ctr2 [6,21], possibly indicating that the encoded proteins may act in the same biological process. The discrepancies between the rodent and human data can be due to species differences, but also possibly explained by alterations in Cu status. While, we know the Cu content in the standardized diet for laboratory mice, and that they are maintained in a controlled environment regarding water, pathogens, day and night cycles, and housing, we know very little about the Cu status and potential pathologies in the human tissue samples evaluated. The tissue expression profile of Ctr2 mRNA needs to be further investigated in several species under controlled conditions.

In contrast to Ctr1, Ctr2 is not conserved from yeast to humans, though, as described below, both the yeast and mammalian Ctr2 proteins function in pathways that serve to mobilize vesicular Cu stores into the cytoplasm [21–23]. When and how Ctr2 evolved is currently unknown. Possibly the Ctr2 gene arose from a gene duplication event, giving rise to a new protein by neofunctionalization. However, whether this is a plausible course of events remains to be further explored.

When translated, the human SLC31A2 mRNA encodes a Ctr2 protein of 143 amino acid residues, compared with the human SLC31A1 (Ctr1) protein consisting of 190 amino acid residues. The amino acid sequences between these two proteins are 30% identical (Clustal Omega, Fig. 1) and the two proteins share several common topological features that are conserved in the Ctr1 family of high affinity $Cu^+$ transporters from yeast to humans. Ctr2 is computationally predicted to harbor three trans-membrane domains, which is the same number as both predicted in silico for Ctr1 and which is supported by the cryo electron microscopy structure of hCtr1 [24,25]. Ctr1 and Ctr2 also share a conserved Met-$X_3$-Met motif in the second trans-membrane domain that is critical for efficient $Cu^+$ transport by all known members of the Ctr1 family [26,27], and the Gly-$X_3$-Gly motif in the third trans-membrane domain that is known to be important for proper helix packing, localization, and oligomerization of the Ctr1 protein [28]. Moreover, in line with Ctr1, evidence suggests that Ctr2 homo-multimerizes to form a complex with nine total trans-membrane domains [23], but whether this occurs in vivo, and the significance of Ctr2 oligomerization is currently unknown. Both proteolytic mapping and epitope-access experiments indicate that Ctr1 and Ctr2 have the same topological orientation, with the amino-termini located outside of the plasma membrane or inside of an endosomal/lysosomal vesicle, and the carboxyl-terminus facing the cytoplasm [21,26,29–31]. In contrast to Ctr2, Ctr1 has a significantly longer amino-terminus, with several metal binding motifs consisting of Met and His that, while not essential, are important for full activity of the high affinity import of $Cu^+$ [26,32]. The mammalian Ctr2 protein also lacks the His-Cys-His motif that Ctr1 harbors at the carboxyl-terminus, which is thought to act as a sink for the $Cu^+$ traversing the pore [24], and which may function in trafficking the Cu to the intracellular chaperones CCS and Atox1.

## Post-transitional modifications, expression and interactions

The Ctr1 protein undergoes post-translational modifications that may influence its function. Two glycan chains are added to the amino-terminal ecto-domain of Ctr1, one N-linked modification at Asn15 and one O-linked glycosylation at Thr27 (Fig. 1) [31,32]. The glycosylation shows differential tissue patterns in mice [21], which is in line with other membrane proteins modified by glycans. Currently it is unknown whether alterations in glycosylation can function as a regulatory mechanism for Ctr1 abundance, localization, and/or function in different tissues.

The human Ctr1 O-linked glycosylation at Threonine 27 has, by mutational studies, been suggested to protect the Ctr1 amino-terminal ecto-domain from proteolytic cleavage [32]. When this O-linked glycosylation event is prevented, the Ctr1 ecto-domain is cleaved more frequently, generating a shorter form of Ctr1. This cleaved form of Ctr1 has been observed in both cell lines and mouse tissues [32,33], and like the full length Ctr2 protein, lacks a large portion of the $Cu^+$ binding ecto-domain (Fig. 1). The Ctr1 ecto-domain Ctr1 harbors the capacity to bind multiple $Cu^+$ atoms via the Met- and His-rich motifs and through the amino-terminal copper/nickel (ATCUN) motif [34,35]. Recently it was demonstrated by mass spectrometry that the truncated form of Ctr1 initiates within the Met-rich motif closest to the first trans-membrane domain (Fig. 1) [21]. Initial studies evaluating the function of truncated Ctr1 suggested that this form is still localizes to the plasma membrane and is competent to transport Cu, albeit with approximately 50% the efficiency of the full-length protein [32]. Thus, changes in Ctr1 truncation would be predicted to alter cellular Cu import.

The mouse Ctr2 protein, in line with its mRNA, is ubiquitously expressed in a wide range of tissues, showing the highest protein expression in liver, kidney, and testis, which is very similar to the protein expression pattern for mouse Ctr1 (Unpublished data). In mammalian cells Ctr2 protein localizes to intracellular vesicular compartments including endosomes and lysosomes [21,23], and the protein also contains both di-leucine and tyrosine-based motifs, which are potential lysosomal targeting sequences. However, when overexpressed via transfection with an epitope tag attached to